impact of basic research on tomorrow's medicine

The Genetic Determinants of Smoking*

Vikas Batra, MD; Ashwin A. Patkar, MD; Wade H. Berrettini, MD, PhD; Stephen P. Weinstein, PhD; and Frank T. Leone, MD, MS, FCCP

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.   (CHEST 2003; 123:1730–1739)

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

A nnually, tobacco smoking is responsible for approximately 3 million deaths worldwide,[1] with > 430,000 occurring in the United States alone.[2] 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,[3,4]

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.[5] 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,[6] 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-

ronmental 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[6] (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[7] and replicated in studies from the United States, Scandinavian countries, Australia, Britain, and Japan in populations of adults, elderly, and adolescent smokers.[8-20] 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[11,16] and persistence of smoking and number of cigarettes smoked[12] in both men and women.[13,17] The heri-

tability estimates for smoking (ie, 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.[15,18-20] 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[13,14] 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 regular tobacco use respectively.[17] 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.[21] 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.[12-15]

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.[4] Once regular smoking is established, the two important predictors of persistent smoking are dependence on nicotine and lower educational levels.[3] 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.[18] 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.[19] 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.[22,23]

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 al[26] 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 α [^{125}I]-bungarotoxin as a ligand for nicotinic receptors,[27,28] Miner et al[29] (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 al[30] demonstrated that the β-2 subunit neuronal nicotinic acetylcholine receptor may mediate the reinforcing properties of nicotine while the α-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-limit-

ing, 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 cotinine[34] (Fig 1). Along with the normal functional allele CYP2A6*1, two
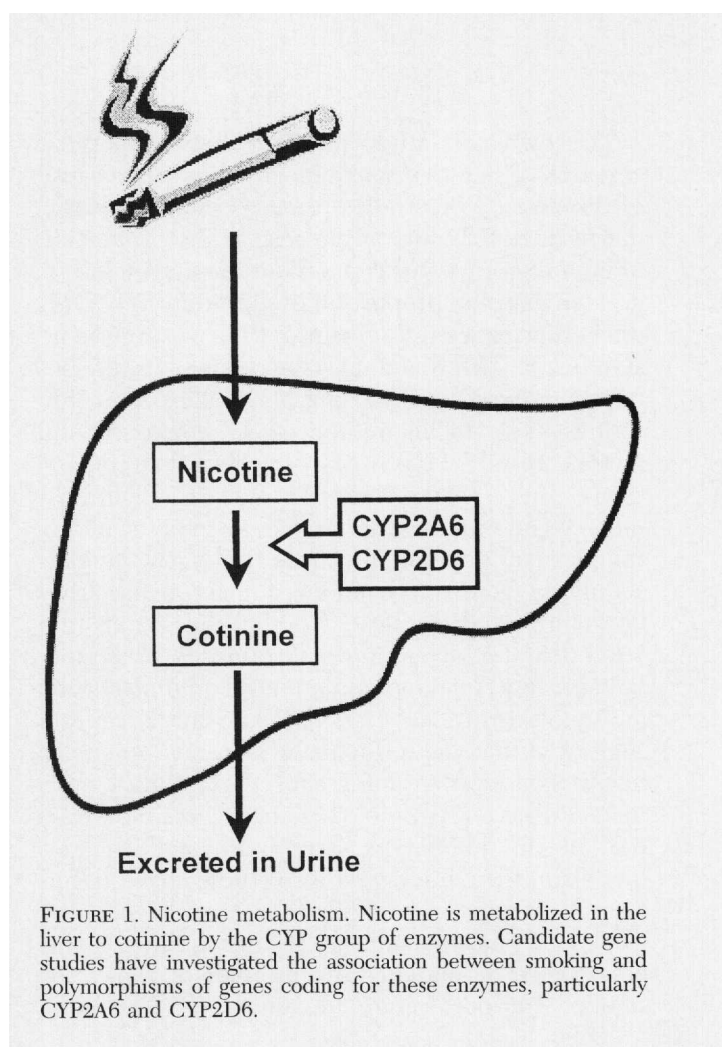

FIGURE 1. Nicotine metabolism. Nicotine is metabolized in the liver to cotinine by the CYP group of enzymes. Candidate gene studies have investigated the association between smoking and polymorphisms of genes coding for these enzymes, particularly CYP2A6 and CYP2D6.

variants of the CYP2A6 gene have been identified: CYP2A6*2 and *3; both are associated with reduced activity of the enzyme. Pianezza et al[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.[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*.[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*.[39] Several studies have investigated this polymorphism in tobacco dependence with conflicting results. Turgeon et al[40] (1995) reported that poor metabolizers were underrepresented among smokers compared to nonsmokers, supporting the hypothesis that individuals who metabolize nicotine slowly are less likely to become addicted. However, Cholerton et al[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.[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.[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.[44-47]

Dopamine Genes

The mesolimbic dopaminergic system is critical to the reinforcing effects of several addictive drugs including nicotine.[48] As shown in Figure 2, nicotine has been shown to stimulate dopamine release in the nucleus accumbens,[49] possibly through activation of
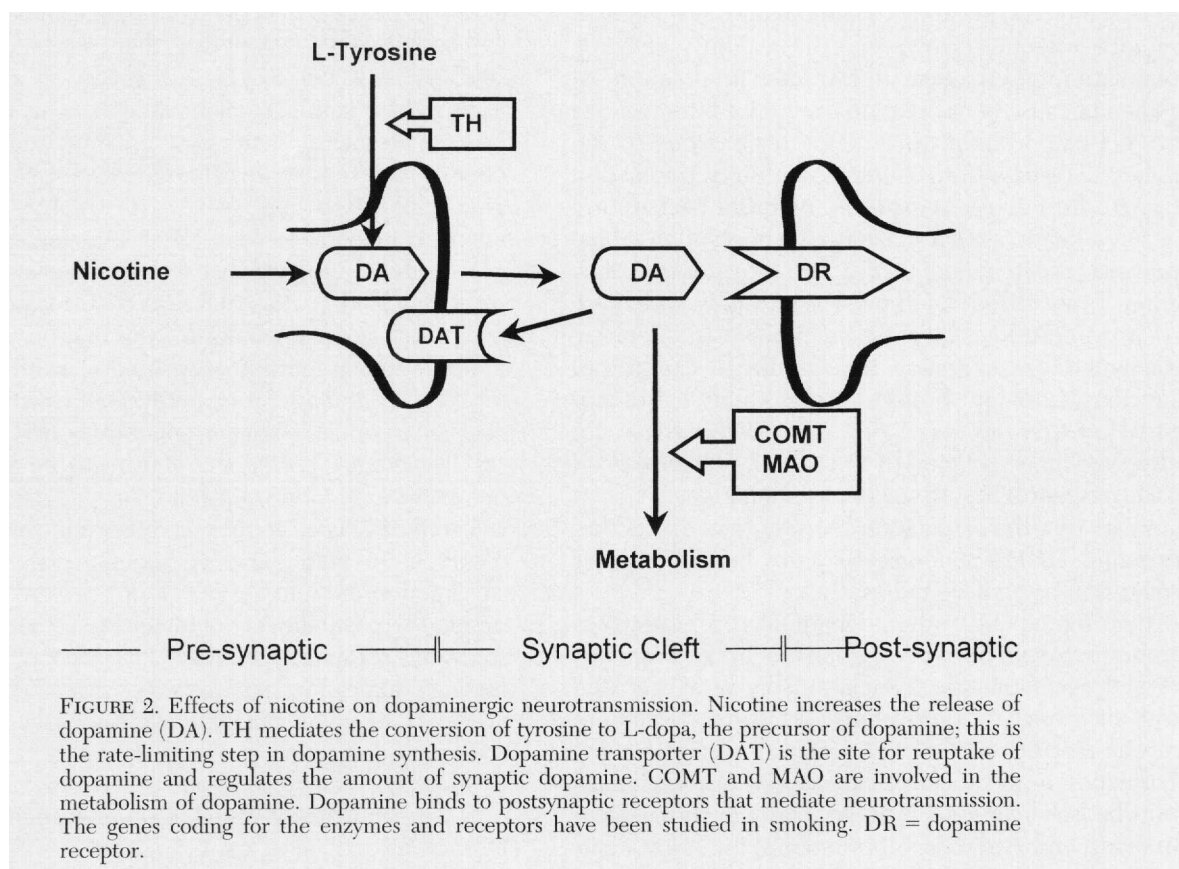
FIGURE 2. Effects of nicotine on dopaminergic neurotransmission. Nicotine increases the release of dopamine (DA). TH mediates the conversion of tyrosine to L-dopa, the precursor of dopamine; this is the rate-limiting step in dopamine synthesis. Dopamine transporter (DAT) is the site for reuptake of dopamine 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.[50] 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.[56,57] 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,"[59] 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 described[61]; 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 SCL6A3–9 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 sample[67] 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.[68] Although these genes have been investigated in several disorders such as alcoholism, depression, and schizophrenia,[69–71] 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.[72,73] Consistent with these findings, Costa-Mallen et al[74] 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.[75] The only study that investigated polymorphisms in the TH gene among smokers reported negative findings.[76]

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.[77] 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.[78,79] 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[80] 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.[81] 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,[82,83] 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,[78] 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[84] detected five novel single nucleotide polymorphisms in the gene for the α_2 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[86] performed a sibling-pair linkage analysis of two smoking-related traits, ever/never smokers and pack-years. 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[87] 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[88]; 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-

Table 1—*Candidate Gene Association Studies in Smoking**

Association Studies	Sample Size	Current Smokers, %	Nonsmokers, %	Odds Ratio (95% CI)	p Value
Nicotine metabolism CYP2A6 polymorphism		Proportion of poor metabolizers			
Pianezza et al[35] (1998)	428 (Canada)	12.3	19.6	0.57 (0.34–0.98)	< 0.05
Tyndale and Sellers[36] (2001)	400 (Canada)	NA	NA	0.52	< 0.01
London et al[85] (1999)†	460 (US)	22.1	24.2	0.88 (0.56–1.39)	NS
Nicotine metabolism CYP2D6 polymorphism		Proportion of poor metabolizers			
Turgeon et al[40] (1995)	258 (whites, Canada)	0	10	NA	NA
Cholerton et al[41] (1996)	204 (Europe)	50	45.2	1.21 (0.70–2.10)	NS
Saarikoski et al[43] (2000)	976 (Finland)	2.1	2.3	0.91 (0.30–2.74)	NS
DRD2 polymorphism		Subjects (%) with A1 allele			
Noble et al[53] (1994)	354 (whites, US)	45.6	28	1.84 (1.19–2.88)	< 0.01
Comings et al[54] (1996)	1026 (whites, US)	49	26	2.71 (2.05–3.58)	< 0.001
Singleton et al[56] (1998)	221 (whites, UK)	31	43	0.59 (0.34–1.04)	< 0.1
Wu et al[55] (2000)‡	222 (AA and HA)	68.1	63.2	1.24 (0.61–2.53)	NS
Bierut et al[57] (2000)§	954 (US)	39.5	34.7	1.23 (0.94–1.60)	NS
Dopamine transporter SLC6A3 polymorphism		Subjects (%) with SLC6A3-9 allele			
Lerman et al[65] (1999)‖	522 (mixed, US)	46.7	55.8	0.52 (0.32–0.82)	< 0.01
Sabol et al[66] (1999)‖	1107 (US)	42	43.5	NA	< 0.05
Jorm et al[67] (2000)¶	861 (Australia)	46.5	48.2	0.93 (0.66–1.32)	NS

*CI = confidence interval; US = United States; AA = African American; HA = Hispanic American; UK = United Kingdom; NA = not available; NS = not significant.
†Data from patients enrolled in a case-control study for lung cancer.
‡Control population from a lung cancer case-control study.
§Family-based association approach.
‖Volunteer sample.
¶Nonvolunteer sample.

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.[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.[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.[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.[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

1 Peto R, Lopez AD, Boreham J, et al. Mortality from smoking worldwide. Br Med Bull 1996; 52:12–21
2 Smoking-attributable mortality and years of potential life lost–United States, 1984. MMWR Morb Mortal Wkly Rep 1997; 46:444–451

3 Cigarette smoking among adults–United States, 1997. MMWR Morb Mortal Wkly Rep 1999; 48:993–996

4 Cigarette smoking among adults–United States, 1999. MMWR Morb Mortal Wkly Rep 2001; 50:869–873

5 Dani JA, Heinemann S. Molecular and cellular aspects of nicotine abuse. Neuron 1996; 16:905–908

6 Fisher RA. Lung cancer and cigarettes [letter]. Nature 1958; 182:108

7 Fisher RA. Cancer and smoking [letter]. Nature 1958; 182:596

8 Todd GF, Mason JI. Concordance of smoking habits in monozygotic and dizygotic twins. Hereditary 1859; 13:417–444

9 Raaschou-Nielsen E. Smoking habits in twins. Dan Med Bull 1960; 7:82–88

10 Williams JS, Crumpaccker DW, Krier M. Genetic and environmental variance fractions and correlation estimates for smoking behavior in a Swedish population. In: Gedda L, Parisi P, Nance WE, eds. Twin research 3: Part C; epidemiology and clinical studies. New York, NY: Alan R. Liss, 1981; 53–59

11 Eaves LJ, Eysenck HJ. New approaches to the analysis of twin data and their application to smoking behavior. In: Eysenck HJ, ed. The causes and effects of smoking. London, UK: Maurice Temple Smith, 1980; 140–314

12 Kaprio J, Hammar N, Koskenvuo M, et al. Cigarette smoking and alcohol use in Finland and Sweden: a cross- national twin study. Int J Epidemiol 1982; 11:378–386

13 Carmelli D, Swan GE, Robinette D, et al. Heritability of substance use in the NAS-NRC Twin Registry. Acta Genet Med Gemellol (Roma) 1990; 39:91–98

14 Carmelli D, Swan GE, Robinette D, et al. Genetic influence on smoking: a study of male twins. N Engl J Med 1992; 327:829–833

15 Edwards KL, Austin MA, Jarvik GP. Evidence for genetic influences on smoking in adult women twins. Clin Genet 1995; 47:236–244

16 Hannah MC, Hopper JL, Mathews JD. Twin concordance for a binary trait: II; Nested analysis of ever-smoking and ex-smoking traits and unnested analysis of a "committed-smoking" trait. Am J Hum Genet 1985; 37:153–165

17 Sullivan PF, Kendler KS. The genetic epidemiology of smoking. Nicotine Tob Res 1999; 1:S51–S57

18 Heath AC, Martin NG. Genetic models for the natural history of smoking: evidence for a genetic influence on smoking persistence. Addict Behav 1993; 18:19–34

19 Kendler KS, Neale MC, Sullivan P, et al. A population-based twin study in women of smoking initiation and nicotine dependence. Psychol Med 1999; 29:299–308

20 True WR, Heath AC, Scherrer JF, et al. Genetic and environmental contributions to smoking. Addiction 1997; 92:1277–1287

21 Kendler KS, Thornton LM, Pederson NL. Tobacco consumption in Swedish twins reared apart and reared together. Arch Gen Psychiatry 2001; 57:886–892

22 Fabsitz RR, Garrison RJ, Feinleib M, et al. A twin analysis of dietary intake: evidence for a need to control for possible environmental differences in monozygotic and dizygotic twins. Behav Genet 1978; 8:15–25

23 Austin MA, King MC, Bawol RD, et al. Risk factors for coronary heart disease in adult female twins: genetic heritability and shared environmental influences. Am J Epidemiol 1987; 125:308–318

24 Morrison CF, Lee PN. A comparison of the effects of nicotine and physostigmine on a measure of activity in the rat. Psychopharmacologia 1968; 13:210–221

25 Hatchell PC, Collins AC. The influence of genotype and sex on behavioral sensitivity to nicotine in mice. Psychopharmacology (Berl) 1980; 71:45–49

26 Robinson SF, Marks MJ, Collins AC. Inbred mouse strains vary in oral self-selection of nicotine. Psychopharmacology (Berl) 1996; 124:332–339

27 Marks MJ, Collins AC. Characterization of nicotine binding in mouse brain and comparison with the binding of α-bungarotoxin and quinuclidinyl benzilate. Mol Pharmacol 1982; 22:554–564

28 Marks MJ, Stitzel JA, Collins AC. Genetic influences on nicotine responses. Pharmacol Biochem Behav 1989; 33:667–678

29 Miner LL, Marks MJ, Collins AC. Classical genetic analysis of nicotine-induced seizures and nicotinic receptors. J Pharmacol Exp Ther 1984; 231:545–554

30 Picciotto MR, Zoli M, Rimondini R, et al. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature 1998; 391:173–177

31 Orr-Urtreger A, Goldner FM, Saeki M, et al. Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack α-bungarotoxin binding sites and hippocampal fast nicotinic currents. J Neurosci 1997; 17:9165–9171

32 Nabeshima T, Itoh A, Kobayashi K, et al. Effects of subacute administration of methamphetamine and nicotine on locomotor activity in transgenic mice expressing the human tyrosine hydroxylase gene. J Neural Transm Gen Sect 1994; 97:41–49

33 Hiremagalur B, Nankova B, Nitahara J, et al. Nicotine increases expression of tyrosine hydroxylase gene: involvement of protein kinase A-mediated pathway. J Biol Chem 1993; 268:23704–23711

34 Messina ES, Tyndale RF, Sellers EM. A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. J Pharmacol Exp Ther 1997; 282:1608–1614

35 Pianezza ML, Sellers EM, Tyndale RF. Nicotine metabolism defect reduces smoking [letter]. Nature 1998; 393:750

36 Tyndale RF, Sellers EM. Variable CYP2A6-mediated nicotine metabolism alters smoking behavior and risk. Drug Metab Dispos 2001; 29(4 pt 2):548–552

37 Alvan G, Bechtel P, Iselius L, et al. Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. Eur J Clin Pharmacol 1990; 39:533–537

38 Daly AK, Fairbrother KS, Andreassen OA, et al. Characterization and PCR-based detection of two different hybrid CYP2D7P/CYP2D6 alleles associated with the poor metabolizer phenotype. Pharmacogenetics 1996; 6:319–328

39 Lovlie R, Daly AK, Molven A, et al. Ultrarapid metabolizers of debrisoquine: characterization and PCR-based detection of alleles with duplication of the CYP2D6 gene. FEBS Lett 1996; 392:30–34

40 Turgeon J, Labbe L, Lefez C, et al. Debrisoquine metabolic ratio distribution differs between smokers and nonsmokers [abstract]. Clin Pharmacol Ther 1995; 57:150

41 Cholerton S, Boustead C, Taber H, et al. CYP2D6 genotypes in cigarette smokers and non-tobacco users. Pharmacogenetics 1996; 6:261–263

42 Boustead C, Taber H, Idle JR, et al. CYP2D6 genotype and smoking behaviour in cigarette smokers. Pharmacogenetics 1997; 7:411–414

43 Saarikoski ST, Sata F, Husgafvel-Pursiainen K, et al. CYP2D6 ultrarapid metabolizer genotype as a potential modifier of smoking behaviour. Pharmacogenetics 2000; 10:5–10

44 London SJ, Daly AK, Leathart JB, et al. Genetic polymorphism of CYP2D6 and lung cancer risk in African-Americans and Caucasians in Los Angeles County. Carcinogenesis 1997; 18:1203–1214

45 Tefre T, Daly AK, Armstrong M, et al. Genotyping of the CYP2D6 gene in Norwegian lung cancer patients and controls. Pharmacogenetics 1994; 4:47–57

46 Bouchardy C, Benhamou S, Dayer P. The effect of tobacco on lung cancer risk depends on CYP2D6 activity. Cancer Res 1996; 56:251–253

47 Agundez JA, Gallardo L, Ledesma MC, et al. Functionally active duplications of the CYP2D6 gene are more prevalent among larynx and lung cancer patients. Oncology 2001; 61:59–63

48 Koob GF. Neural mechanisms of drug reinforcement. Ann N Y Acad Sci 1992; 654:171–191

49 Pontieri FE, Tanda G, Orzi F, et al. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 1996; 382:255–257

50 Clarke PB. Mesolimbic dopamine activation: the key to nicotine reinforcement? Ciba Found Symp 1990; 152:153–162

51 Blum K, Noble EP, Sheridan PJ, et al. Allelic association of human dopamine D2 receptor gene in alcoholism. JAMA 1990; 263:2055–2060

52 Thompson J, Thomas N, Singleton A, et al. D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. Pharmacogenetics 1997; 7:479–484

53 Noble EP, St Jeor ST, Ritchie T, et al. D2 dopamine receptor gene and cigarette smoking: a reward gene? Med Hypotheses 1994; 42:257–260

54 Comings DE, Ferry L, Bradshaw-Robinson S, et al. The dopamine D2 receptor (DRD2) gene: a genetic risk factor in smoking. Pharmacogenetics 1996; 6:73–79

55 Wu X, Hudmon KS, Detry MA, et al. D2 dopamine receptor gene polymorphisms among African-Americans and Mexican-Americans: a lung cancer case-control study. Cancer Epidemiol Biomarkers Prev 2000; 9:1021–1026

56 Singleton AB, Thomson JH, Morris CM, et al. Lack of association between the dopamine D2 receptor gene allele DRD2*A1 and cigarette smoking in a United Kingdom population. Pharmacogenetics 1998; 8:125–128

57 Bierut LJ, Rice JP, Edenberg HJ, et al. Family-based study of the association of the dopamine D2 receptor gene (DRD2) with habitual smoking. Am J Med Genet 2000; 90:299–302

58 Shields PG, Lerman C, Audrain J, et al. Dopamine D4 receptors and the risk of cigarette smoking in African-Americans and Caucasians. Cancer Epidemiol Biomarkers Prev 1998; 7:453–458

59 Benjamin J, Li L, Patterson C, et al. Population and familial association between the D4 dopamine receptor gene and measures of novelty seeking. Nat Genet 1996; 12:81–84

60 Comings DE, Gade R, Wu S, et al. Studies of the potential role of the dopamine D1 receptor gene in addictive behaviors. Mol Psychiatry 1997; 2:44–56

61 Vandenbergh DJ, Persico AM, Hawkins AL, et al. Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics 1992; 14:1104–1106

62 Gelernter J, Kranzler HR, Satel SL, et al. Genetic association between dopamine transporter protein alleles and cocaine-induced paranoia. Neuropsychopharmacology 1994; 11:195–200

63 Cook EH Jr, Stein MA, Krasowski MD, et al. Association of attention-deficit disorder and the dopamine transporter gene. Am J Hum Genet 1995; 56:993–998

64 Comings DE, Wu S, Chiu C, et al. Polygenic inheritance of Tourette syndrome, stuttering, attention deficit hyperactivity, conduct, and oppositional defiant disorder: the additive and subtractive effect of the three dopaminergic genes–DRD2, DβH, and DAT1. Am J Med Genet 1996; 67:264–288

65 Lerman C, Caporaso NE, Audrain J, et al. Evidence suggesting the role of specific genetic factors in cigarette smoking. Health Psychol 1999; 18:14–20

66 Sabol SZ, Nelson ML, Fisher C, et al. A genetic association for cigarette smoking behavior. Health Psychol 1999; 18:7–13

67 Jorm AF, Henderson AS, Jacomb PA, et al. Association of smoking and personality with a polymorphism of the dopamine transporter gene: results from a community survey. Am J Med Genet 2000; 96:331–334

68 Elsworth JD, Roth RH. Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson's disease. Exp Neurol 1997; 144:4–9

69 Geijer T, Jonsson E, Neiman J, et al. Tyrosine hydroxylase and dopamine D4 receptor allelic distribution in Scandinavian chronic alcoholics. Alcohol Clin Exp Res 1997; 21:35–39

70 Todd RD, Lobos EA, Parsian A, et al Manic-depressive illness and tyrosine hydroxylase markers: Bipolar Disorder Working Group [letter]. Lancet 1996; 347:1634

71 Thibaut F, Ribeyre JM, Dourmap N, et al. Association of DNA polymorphism in the first intron of the tyrosine hydroxylase gene with disturbances of the catecholaminergic system in schizophrenia. Schizophr Res 1997; 23:259–264

72 Fowler JS, Volkow ND, Wang GJ, et al. Brain monoamine oxidase A inhibition in cigarette smokers. Proc Natl Acad Sci U S A 1996; 93:14065–14069

73 Fowler JS, Volkow ND, Wang GJ, et al. Inhibition of monoamine oxidase B in the brains of smokers. Nature 1996; 379:733–736

74 Costa-Mallen P, Costa LG, Smith-Weller T, et al. Genetic polymorphism of dopamine D2 receptors in Parkinson's disease and interactions with cigarette smoking and MAO-B intron 13 polymorphism. J Neurol Neurosurg Psychiatry 2000; 69:535–537

75 McKinney EF, Walton RT, Yudkin P, et al. Association between polymorphisms in dopamine metabolic enzymes and tobacco consumption in smokers. Pharmacogenetics 2000; 10:483–491

76 Lerman C, Shields PG, Main D, et al. Lack of association of tyrosine hydroxylase genetic polymorphism with cigarette smoking. Pharmacogenetics 1997; 7:521–524

77 Ribeiro EB, Bettiker RL, Bogdanov M, et al. Effects of systemic nicotine on serotonin release in rat brain. Brain Res 1993; 621:311–318

78 Lesch KP, Balling U, Gross J, et al. Organization of the human serotonin transporter gene. J Neural Transm Gen Sect 1994; 95:157–162

79 Heils A, Teufel A, Petri S, et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996; 66:2621–2624

80 Lerman C, Shields PG, Audrain J, et al. The role of the serotonin transporter gene in cigarette smoking. Cancer Epidemiol Biomarkers Prev 1998; 7:253–255

81 Ishikawa H, Ohtsuki T, Ishiguro H, et al. Association between serotonin transporter gene polymorphism and smoking among Japanese males. Cancer Epidemiol Biomarkers Prev 1999; 8:831–833

82 Lerman C, Caporaso NE, Audrain J, et al. Interacting effects of the serotonin transporter gene and neuroticism in smoking practices and nicotine dependence. Mol Psychiatry 2000; 5:189–192

83 Hu S, Brody CL, Fisher C, et al. Interaction between the serotonin transporter gene and neuroticism in cigarette smoking behavior. Mol Psychiatry 2000; 5:181–188

84 Silverman MA, Neale MC, Sullivan PF, et al. Haplotypes of four novel single nucleotide polymorphisms in the nicotinic acetylcholine receptor beta2-subunit (CHRNB2) gene show no association with smoking initiation or nicotine dependence. Am J Med Genet 2000; 96:646–653

85 London SJ, Idle JR, Daly AK, et al. Genetic variation of CYP2A6, smoking, and risk of cancer [letter]. Lancet 1999; 353:898–899
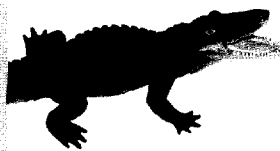
86 Bergen AW, Korczak JF, Weissbecker KA, et al. A genome-

wide search for loci contributing to smoking and alcoholism. Genet Epidemiol 1999; 17(Suppl 1):S55–S60

87 Duggirala R, Almasy L, Blangero J. Smoking behavior is under the influence of a major quantitative trait locus on human chromosome 5q. Genet Epidemiol 1999; 17(Suppl 1):S139–S144

88 Straub RE, Sullivan PF, Ma Y, et al. Susceptibility genes for nicotine dependence: a genome scan and followup in an independent sample suggest that regions on chromosomes 2, 4, 10, 16, 17 and 18 merit further study. Mol Psychiatry 1999; 4:129–144

89 Arinami T, Ishiguro H, Onaivi ES. Polymorphisms in genes involved in neurotransmission in relation to smoking. Eur J Pharmacol 2000; 410:215–226

90 Merikangas KR, Stolar M, Stevens DE, et al. Familial transmission of substance use disorders. Arch Gen Psychiatry 1998; 55:973–979

91 Swan GE, Carmelli D, Cardon LR. The consumption of tobacco, alcohol, and coffee in Caucasian male twins: a multivariate genetic analysis. J Subst Abuse 1996; 8:19–31

92 Uhl GR, Liu QR, Walther D, et al. Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. Am J Hum Genet 2001; 69:1290–1300