

D2 Dopamine Receptor Gene and Cigarette Smoking: A Reward Gene?

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Abstract — There is now growing evidence that the less prevalent allele (A1) of the D₂ dopamine receptor (DRD2) gene is strongly associated with severe alcoholism. Similarly, subjects who abuse illegal drugs or who are obese also show a significantly higher prevalence of the A1 DRD2 allele compared to controls. Moreover, cigarette smokers, both past and current, demonstrate significantly higher prevalence of the A1 allele than nonsmokers. In as much as alcohol, cocaine, opiates, nicotine and food are known to increase brain dopamine levels and activate the mesocorticolimbic dopaminergic reward pathways of the brain, it is hypothesized that an inherited deficit of D₂ dopamine receptor numbers in brain reward areas of A1 allelic subject predisposes them to substance abuse problems.

Introduction

While environmental factors may be important determinants of cigarette and other nicotine-containing product use, results from adoption, association, family, trait marker and twin studies indicate that acquisition and maintenance of smoking are also influenced by heredity (1). Of particular relevance are studies of twins which show that concordance rates for smoking are consistently higher in monozygotic (MZ) than dizygotic (DZ) twins (2–4). Although increases in concordance rates for MZ twins are quite reliable, other factors, such as interactions between twinning conditions and personality in MZ and DZ twins, may account for some of the differences in concordance rate. In order to examine this issue, adjustments were

made for shared variance in a recent analysis of MZ and DZ twins (5). Before and after adjustments for covariates, heritability for smoking was unchanged and remained highly significant, accounting for 52% of the variance.

Thus, hereditary factors appear to be involved in smoking, yet how might these be mediated at the neurobehavioral level? It is well acknowledged that the consumption of nicotine, and misused drugs, induce euphoria and pleasurable feelings in users. It has been hypothesized that these positive reinforcement manifestations occur by activation of the mesocorticolimbic dopaminergic reward pathways of the brain (6, 7). In support of this hypothesis is evidence that stimulation of nicotinic receptors by nicotine enhances the release of dopamine in the brain, especially in the nu-

cleus accumbens and the ventral tegmentum, regions known to be involved in reward (8–10). Inasmuch as there are nicotinic receptors on the dopamine cell bodies in these brain regions (11, 12), it is argued that these reward areas are a likely site of nicotine's reinforcing effects (6, 13).

An opportunity to link smoking behavior with a genetic factor arose with the discovery of the D₂ dopamine receptor (DRD2) gene. Bunzow et al (14) cloned and expressed rat complementary DNA (cDNA) of the DRD2 gene. Grandy et al (15) used rat cDNA to clone a human genomic fragment and mapped the DRD2 gene to the q22–q23 region of chromosome 11. Moreover, they identified two alleles in TaqI digests of human DNAs: A1, a less frequent allele, and A2, a more frequent allele. The role of this dopaminergic system was examined in current smokers, past smokers and nonsmokers by their association with the DRD2 alleles.

Methods

A sample of 354 Caucasian (non-Hispanic) subjects was recruited from two US sites: Reno, Nevada (n=286) and Los Angeles, California (n=68). Through the administration of questionnaires, smoking behavior was ascertained. Subjects were considered to be smokers if they had consumed 100 cigarettes or more in their lifetime. In the present sample, 57 were current smokers, and 115 were past smokers and 182 were nonsmokers. Of these subjects, 190 were males and 164 were females.

A blood sample was obtained from each subject. Genomic DNA was extracted and subsequently used as a template for the polymerase chain reaction. Two primers were used to amplify a 310 bp fragment spanning the polymorphic TaqI A site of the DRD2 gene (15). The 310 bp fragment obtained from each subject was digested with TaqI restriction enzyme and the products were separated by agarose gel electrophoresis and visualized with ethidium bromide. Three potential fragments were obtained. The A1/A2 genotype is revealed by three fragments: 310 bp, 180 bp and 130 bp; the A2/A2 genotype is indicated by two fragments: 180 bp and 130 bp; and the A1/A1 genotype is shown by the uncleaved 310 bp fragment.

Results

The distribution of DRD2 genotypes in the three groups of subjects studied was as follows — current smokers: A1/A1, n = 5, A1/A2, n = 21, and A2/A2, n = 31; past smokers: A1/A1, n = 5, A1/A2, n = 41, and A2/A2, n = 69; nonsmokers: A1/A1, n = 6, A1/A2, n = 45, and A2/A2, n = 131. Allelic distribution was not differentiated either by gender or age in this sample.

The Figure shows the ratio of the presence of the A1 allele (A1/A1 + A1/A2 genotypes) to the absence of this allele (A2/A2 genotype) in current and past smokers and in nonsmokers. Analysis of the data showed that DRD2 allelic prevalence was different among these three groups ($X^2 = 8.00$, $df = 2$, $P = 0.018$). Specifically, the A1 allele occurred in a larger proportion of current smokers compared to nonsmokers ($X^2 = 5.37$, $P = 0.021$, odds ratio = 2.15). The incidence of the A1 allele was also higher in past smokers compared to nonsmokers ($X^2 = 4.07$, $P = 0.044$, odds ratio = 1.71). Furthermore, smokers (past and current combined) had a significantly higher incidence of the A1 allele compared to nonsmokers ($X^2 = 6.87$, $P = 0.009$, odds ratio = 1.85). Linear trend analysis (16) of A1 allelic incidence in the nonsmoker, past smoker and current smoker groups respectively showed that as smoking severity increased, so did the incidence of the A1 allele ($X^2 = 7.69$, $df = 1$, $P = 0.006$).

Discussion

It is of interest that the higher incidence of the A1 allele in smokers is not restricted to the presence of smoking behavior. We have previously reported a strong association of the DRD2 A1 allele with severe alcoholism (17). A recent review (18) of nine independent studies of Caucasians showed that alcoholics (n = 491) had a significantly higher incidence of the A1 allele ($P < 10^{-7}$) than controls (n = 495). Similarly, a higher proportion of cocaine addicts had the A1 allele (19) as did subjects with polysubstance abuse (cocaine, amphetamine, opiates) (20, 21). Meta-analysis of the data from controlled studies available to date (22) is consistent with the proposal that DRD2 gene variants are associated with inter-individual differences in vulnerability to alcoholism and polysubstance abuse. Furthermore, in a recent study (23) of obese subjects (80 lbs greater than ideal weight), we also found a significantly higher incidence of the A1 allele of the DRD2 in this group compared to controls (odds ratio = 3.48, $P < 0.001$). Moreover, Comings et al (24) have similarly reported the DRD2 to be a major gene in obesity.

Like nicotine, the consumption of alcohol (25, 26), cocaine (27, 28), opiates (29, 30) and the food (31, 32) increase brain dopamine levels. Given that neuroanatomic, neurophysiologic and neuropharmacologic evidence suggests that dopamine, acting on dopamine receptors in the mesocorticolimbic pathways of the brain is involved in reward (6, 7, 33), it is hypothesized that individuals who carry the DRD2 A1 allele have a deficit in their reward system. By using agents that increase brain dopamine, an enhanced reward may be experienced by A1 allelic subjects

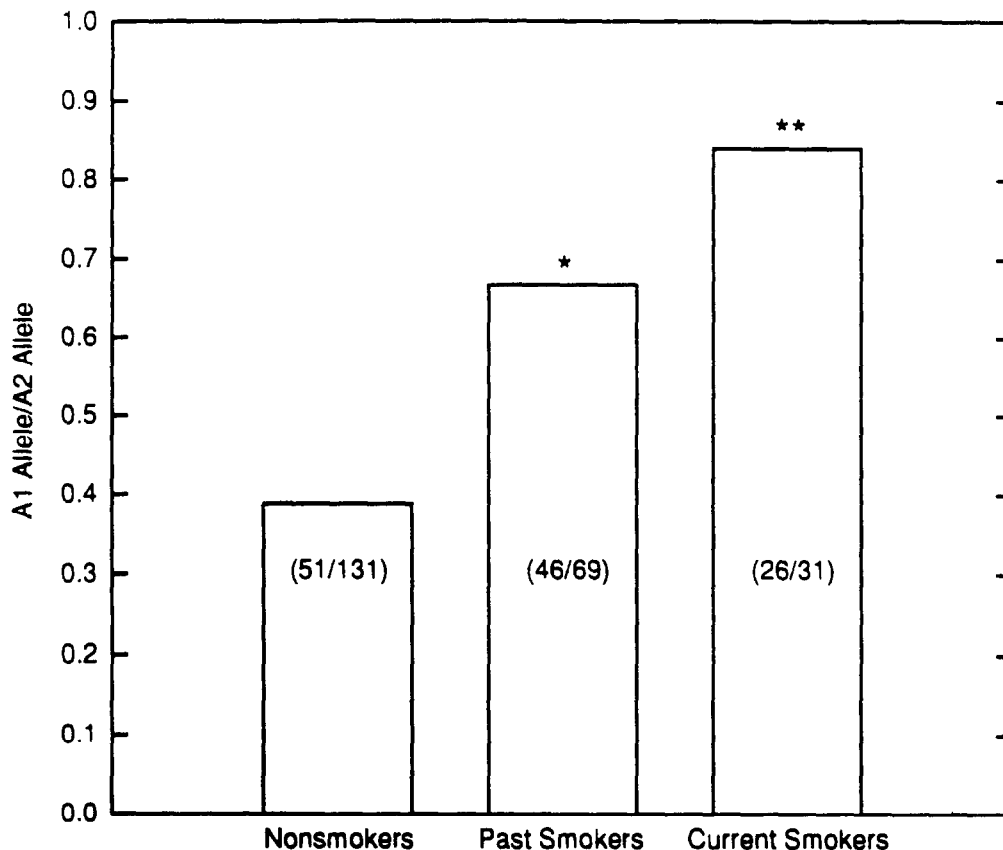


Fig. The ratio of A1 allele (A1/A1 and A1/A2 genotypes) to A2 allele (A2/A2 genotype) in nonsmokers, past smokers and current smokers. *Significantly higher in past smokers compared to nonsmokers ($X^2=4.07$, $P=0.044$, odds ratio = 1.71). **Significantly higher in current smokers compared to nonsmokers ($X^2 = 5.37$, $P = 0.021$, odds ratio = 2.15). Smokers (past and current combined) had a significantly higher prevalence of the A1 allele than nonsmokers ($X^2 = 6.87$, $P = 0.009$, odds ratio = 1.85).

which is a diathesis for developing problems associated with the excess use of these agents.

The pathophysiological basis for the molecular genetic findings presented here is as yet unclear. However, it has been suggested (20) that a plausible explanation for the apparent relationship between increased expression of symptoms and the incidence of the A1 allele is that either the mutation causing *TaqI* A polymorphism or a mutation in linkage disequilibrium with *TaqI* A polymorphism is associated with a decrease in the function of the DRD2 gene. Evidence for such a mechanism is suggested by a significant decrease in the number, without a change in the structure, of D₂ dopamine receptors in brains of individuals carrying the A1 allele compared to those who did not (34).

Conclusion

While environmental factors alone can promote the misuse of a variety of addictive substances, it is pro-

posed that a heritable deficit in the brain reward system, associated with the expression of the DRD2 gene, is another factor that contributes to the misuse of these substances.

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