American Journal of Medical Genetics 90:299–302 (2000)

Family-Based Study of the Association of the Dopamine D2 Receptor Gene (*DRD2*) With Habitual Smoking

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A recent study showed an association between the dopamine D2 receptor gene (*DRD2*) and smoking. The purpose of this study was to determine if the familial transmission of smoking is linked to variation at the *DRD2* locus in a genetically informative sample. Subjects were identified in alcohol treatment centers and their relatives were recruited for study. All subjects were interviewed to assess alcohol dependence, smoking habits, and psychiatric disorders. Two polymorphisms within the *DRD2* gene were analyzed, including the TaqIA polymorphism. The sample consisted of 138 nuclear families with at least one offspring with habitual smoking, and analysis was by the transmission disequilibrium test (TDT), which avoids problems due to population stratification. There was no significant difference in the frequency between *DRD2* alleles transmitted and not transmitted to habitual smokers. There also was no evidence for unequal transmission of *DRD2* alleles for the phenotypes "ever smoker" or comorbid alcohol dependence and habitual smoking. This study does not support linkage of the *DRD2* with smoking. Am. J. Med. Genet. 90: 299–302, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: smoking; dopamine; *DRD2*; genetics; family study

INTRODUCTION

Smoking clusters in families, and genetic factors are implicated [Heath and Martin, 1993]. Since a body of evidence indicates that the dopaminergic system is involved in dependence on a variety of drugs [Smith et al., 1992], the dopamine D2 receptor gene (*DRD2*) is a logical candidate to contribute to the genetic susceptibility of smoking.

A recent study by Spitz et al. [1998] reported an association between *DRD2* and "ever smokers" (those who had smoked more than 100 cigarettes in their lifetime), suggesting that allelic association at the *DRD2* locus determines, in part, genetic susceptibility to the development of nicotine dependence. However, their study included only a small number of never smokers, drawn from both case (newly diagnosed lung cancer patients) and control subjects, and their analyses pooled homozygous and heterozygous genotypes containing the rarer allele at two linked sites in the *DRD2*

Contract grant sponsor: National Institute on Alcohol Abuse and Alcoholism; Contract grant numbers: U10AA08401, U10AA08402, U10AA08403; Contract grant sponsor: U.S. Public Health Services; Contract grant numbers: AA00231, MH14677.

The Collaborative Study on the Genetics of Alcoholism (COGA) (H. Begleiter, SUNY HSCB principal investigator; T. Reich, Washington University, co-principal investigator) includes six different centers where data collection takes place. The six sites (and principal investigator and co-investigators) are Indiana University (J. Nurnberger Jr., T-K Li, P.M. Conneally, H. J. Edenberg); University of Iowa (R. Crowe, S. Kuperman); University of California at San Diego and Scripps Institute (M. Schuckit, F. Bloom); University of Connecticut (V. Hesselbrock); State University of New York, Health Sciences Center at Brooklyn (H. Begleiter, B. Porjesz); Washington University in St. Louis (T. Reich, C.R. Cloninger, J. Rice).

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Received 14 July 1999; Accepted 29 October 1999

gene. Two other studies [Comings et al., 1996; Noble et al., 1994] also reported an association between smoking and the TaqI-A1 allele of the *DRD2* gene, when analyzing pooled homozygous and heterozygous genotypes containing the rarer A1 allele. These population-based association studies can be subject to spurious findings due to population stratification, where persons affected and unaffected by a disease may come from different populations or ethnic backgrounds.

The purpose of this study was to examine the relationship between *DRD2* and habitual smoking in a large number of intensively evaluated families. We used a family-based method, the transmission disequilibrium test (TDT), which compares the number of times that a heterozygous parent transmits a specific allele to an affected offspring with the number of times the same allele is not transmitted. This within-family analysis removes the need for a matched control sample, avoids spurious findings because of population stratification, and can be used as a direct test of genetic linkage between habitual smoking and the *DRD2* locus.

METHODS

Subjects

Subjects were recruited as part of the Collaborative Study on the Genetics of Alcoholism, a multicenter family and genetic study of alcohol dependence [Reich et al., 1998]. The six sites are Indiana University, State University of New York at Brooklyn, University of California at San Diego and Scripps Institute, University of Connecticut, University of Iowa, and Washington University in St. Louis. Written informed consent was obtained from all subjects.

Index cases who met criteria for both *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IIIR) alcohol dependence [American Psychiatric Association, 1987] and Feighner definite alcoholism [Feighner et al., 1972] were identified in chemical dependency treatment settings, and all available first degree relatives were invited to participate in the study. See Reich et al. [1998] for further details.

Assessment

All subjects completed the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) [Bucholz et al., 1994], a highly reliable, semistructured interview designed to assess physical, psychological, and social manifestations of alcohol dependence, other substance dependence, smoking, and related psychiatric disorders over a lifetime. Alcoholism was defined in index cases and relatives as meeting lifetime criteria for both DSM-IIIR alcohol dependence and Feighner definite alcoholism. "Ever smoker" was defined as a person who had ever smoked daily for a month or more. "Habitual smoking" was defined as ever smoking at least one pack (20 cigarettes) daily for six months or more, and was used as a proxy for nicotine dependence. Comorbid habitual smoking and alcohol dependence required both lifetime diagnoses, and need not occur concurrently.

Genetic Sample

A subset of families with at least three interviewed members diagnosed with alcohol dependence was selected for genetic analysis. From these families, 970 interviewed individuals from 105 multigenerational families were genotyped. These families were divided into 176 nuclear families for analysis. A subset of 138 families that was informative for the genetic study of habitual smoking was examined.

Two polymorphisms within the *DRD2* gene were genotyped: the TaqI-A polymorphism and a simple tandem repeat polymorphism (STRP) in intron 2 [Edenberg et al., 1998]. The TaqI-A *DRD2* polymorphism was genotyped by polymerase chain reaction (PCR) amplification followed by TaqI restriction endonuclease digestion. The samples were then electrophoresed on agarose gels and polymorphisms were visualized. The polymorphism in intron 2 of *DRD2* was genotyped using a fluorescent detection system. Genotypic data were checked for Mendelian inheritance of markers, and allele frequencies were determined by maximum likelihood methods using USERM13 [Boehnke, 1991]. The two lowest frequency alleles for the STRP *DRD2* locus (alleles 76 and 86) were collapsed into a single category for genetic analysis to increase statistical power in the multiallelic analysis.

Genetic Analysis

In the genetic sample, differences in allele frequencies of the TaqI-A *DRD2* polymorphism and STRP *DRD2* locus were compared between habitual smokers and nonhabitual smokers using the chi-squared test with SAS [SAS Institute, Inc., 1990]. Since previous studies categorized individuals as carriers of TaqI-A1 alleles or not (that is either homozygous or heterozygous for the TaqI-A1 allele), we categorized subjects in a similar manner. For the multiallelic STRP *DRD2*, we studied allele frequencies for habitual smokers and nonhabitual smokers.

The program TDTEX (Transmission Test for Linkage Disequilibrium) of the S.A.G.E (Statistical Analysis for Genetic Epidemiology Version 3.1) suite of programs was then used. The TDT looks for unequal transmission of alleles from heterozygous parents to their affected children [Spielman et al., 1993]. Under the null hypothesis (that there is no linkage), each allele is transmitted with equal frequency to affected children, and data from related individuals in a pedigree are independent, so the TDT can be applied to multiple affected children in a family and remain a valid test for linkage [Spielman and Ewens, 1996]. Since the STRP *DRD2* polymorphism has multiple alleles, allele-wise analysis was used to test the hypothesis that alleles vary in their transmission to affected individuals [Sham and Curtis, 1995].

RESULTS

The demographic characteristics of the sample genotyped are shown in Table I. The selection of individuals for genotyping was designed to maximize sib-pairs affected with alcoholism and to include parents and un-

TABLE I. Demographic Characteristics and Lifetime Prevalences of the Genetic* Sample

	Men (N = 454) %	Women (N = 516) %
Alcohol dependent	68	26
Ever smoked	73	60
Habitual smoking	54	29
Comorbid[a]	41	11

*Mean age of sample 40.4 years (range 17–88 years); 76% Caucasian; 13% African American; 9% Hispanic; and 2% other.
[a]Comorbid alcohol dependence and habitual smoking is defined as having the lifetime diagnoses of both disorders. The disorders need not be concurrent.

affected siblings as necessary to aid in the accuracy of genetic analyses. Therefore, the high rates of alcohol dependence in the sample are by design; the data do not represent the entire families, or a population sample. Habitual smoking was not a criterion for selection; nevertheless, the rate of habitual smoking was high, especially among the men. This is consistent with increased co-occurrence of alcohol dependence and habitual smoking seen in the general population.

Table II shows the frequency of alleles for the TaqI-A *DRD2* and STRP *DRD2* polymorphisms in habitual smokers and nonhabitual smokers. Though the *Taq*I-A *DRD2* allele 1 was more common in habitual smokers than nonhabitual smokers (39.5 vs. 34.7%), this did not reach statistical significance. There was a difference in the frequency of the STRP *DRD2* alleles in habitual smokers versus nonhabitual smokers. Specifically, allele 84 was more common in habitual smokers than in non–habitual smokers. This is consistent with an association of the STRP *DRD2* locus and habitual smoking.

We next examined the familial transmission of these alleles with habitual smoking (see Table III). Each parent "transmits" one allele to every offspring, and the other parental allele is "nontransmitted." There was no significant difference in the frequency with which heterozygous parents transmitted the different *DRD2* alleles to children characterized as habitual smokers (TaqI-A, $P = 0.32$, 1 df and STRP, $P = 0.16$, 4 df). Examining only the non-Hispanic Caucasians in the sample gave similar results (TaqI-A, $P = 0.64$ and STRP, $P = 0.17$). Given these negative results, we also compared the transmitted and nontransmitted alleles for ever smokers (TaqI-A, $P = 0.86$ and STRP, $P = 0.52$) and for the combined phenotype of comorbid habitual smoking and alcohol dependence (TaqI-A, $P = 0.53$ and STRP, $P = 0.52$). Restricting the analyses to one affected child per family, another test of association also showed no difference in the frequency of transmitted or nontransmitted alleles (TaqI-A, $P = 0.25$ and STRP, $P = 0.71$). All of these tests were negative: there was no evidence for biased transmission of *DRD2* alleles to ever smokers, habitual smokers, or habitual smokers with comorbid alcohol dependence.

TABLE II. Frequency of *DRD2* Alleles in Habitual Smokers vs. Non–habitual Smokers

	Habitual smokers	Nonhabitual smokers
	(N = 388)	(N = 566)
DRD2 Taq I A polymorphism	(%)	(%)
Allele 1 (either A1A1 or A1A2)	39.5	34.7
Allele 2 (A2A2)	60.6	65.3
$\chi^2 = 2.96$; $P = 0.09$ with 1 degree of freedom		
DRD2 single tandem	(N = 680)	(N = 1006)
repeat polymorphism[a]	(%)	(%)
78	12.8	14.0
80	16.2	19.6
82	40.3	40.5
84	26.3	20.5
76/86	4.4	5.5
$\chi^2 = 10.00$; $P = 0.04$ with 4 degrees of freedom		

[a]Allele sizes are in base pairs.

CONCLUSIONS

This study used a family-based method to examine the possible relationship between smoking and alleles at the *DRD2* locus. The TDT avoids potential problems of population stratification by comparing alleles transmitted to affected individuals with other alleles that parents could have transmitted. The individuals studied were drawn from a large, U.S. study of alcohol dependence. We tested ever smokers, habitual smokers, and people who were both habitual smokers and alcohol dependent. In no case was there evidence for a biased transmission of *DRD2* alleles to affected individuals. Though we did detect increased transmission of the TaqI-A *DRD2* allele 1 (55% transmitted vs. 45% not transmitted), this did not reach statistical significance. In this sample, among heterozygous parents for the TaqI-A *DRD2* alleles (26% of subjects were heterozygous), the allele 1 needed to be transmitted 60% of the time to a habitual smoking child for the test to reach statistical significance.

Interestingly, we did find an association of the STRP *DRD2* locus with habitual smoking when habitual smokers were compared with nonhabitual smokers. However, when the sample was examined for increased transmission of specific alleles to affected children in a family, there was no evidence of biased transmission.

The studies by Spitz et al. [1998] and Comings et al. [1996] reported an association between smoking and *DRD2* alleles. Both of these earlier reports were association studies, subject to problems of population stratification. This may be true particularly when the control group is chosen from the literature, as in Comings et al. [1996]. One of the studies had only a small number of never smokers, and carried out many tests on two closely linked polymorphisms, only some of which showed evidence for association [Spitz et al., 1998]. It should be noted that the definition of smoking was somewhat different: ever smokers were defined by Spitz et al. as having smoked more than 100 cigarettes in a lifetime, whereas our definition was having smoked daily for a month or more. It seems unlikely that this would explain the difference in results.

Alcoholism and smoking often co-occur within an individual more often than expected by chance alone

TABLE III. Transmission Disequilibrium Test (TDT) for Habitual Smoking

Transmitted alleles	Nontransmitted alleles					
DRD2 Taq I A polymorphism[a]	A1	A2				
A1	21	47				
A2	37	212				
DRD2 single tandem repeat polymorphism[b]	78	80	82	84	86	Total
78	10	5	13	8	1	37
80	3	7	25	7	5	47
82	18	16	41	7	10	92
84	6	8	13	28	5	60
86	0	2	4	2	2	10
Total	37	38	96	52	23	

[a]P value = 0.32 with 1 degree of freedom.
[b]P value = 0.16 with 4 degrees of freedom; Allele sizes are in base pairs.

[Breslau, 1995]. A twin study by Carmelli supports common genetic factors in the development of heavy alcohol use and smoking [Carmelli et al., 1992]. Consistent with general population samples, data from this study show that there is a clear comorbidity of habitual smoking and alcohol dependence. Since there may be common genetic factors in the development of both alcohol and nicotine dependence, we examined comorbid habitual smoking and alcohol dependence. The possible association between *DRD2* alleles and alcoholism has been very controversial [see Edenberg et al., 1998, and references therein). This study finds no evidence that the *DRD2* gene is linked to the combined phenotype. A recent family-based study of this same sample demonstrated no evidence for either association or linkage of the *DRD2* locus with alcohol dependence [Edenberg et al., 1998].

In conclusion, although the dopamine system may play a significant role in addiction, genetic variation in the *DRD2* locus does not play a significant role in increasing the risk of smoking in this sample.

ACKNOWLEDGMENTS

We would like to acknowledge the assistance of Joseph Mullaney in data analysis. Some of the results of this paper were obtained by using the program package S.A.G.E., which is supported by a U.S. Public Health Service resource grant (1 P41 RR03655) from the National Center for Research Resources.

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