Nicotine & Tobacco Research Volume 12, Number 9 (2007) 1–5

informa
healthcare

DRD2/ANKK1 TaqI polymorphism and smoking behavior of Egyptian male cigarette smokers

Ghada N. Radwan, Maged Setouhy, Mostafa K. Mohamed, Mohamed Abdel Hamid, Ebenezer Israel, Salwa Abdel Azem, Omima Kamel, Christopher A. Loffredo

Received 27 July 2006; accepted 14 February 2007

Little is known about the genetic contribution to cigarette smoking and nicotine addiction in Egypt. The dopamine D2 receptor gene contains a TaqI repeat fragment length polymorphism creating two alleles with functional significance, DRD2*A1 and DRD2*A2. We investigated the relationship between these alleles and tobacco use in a study of 389 Egyptian male current smokers (mean age=40 years; *SD*=12). Participants were interviewed in 2004 on their smoking behaviors and quit attempts, and were given the Fagerström Test for Nicotine Dependence (FTND). Blood samples were obtained and genotyped for DRD2 A1and A2 alleles. The frequencies of A1/A2, A1/A2, and A2/A2 genotypes were 6%, 29%, and 65%, respectively. We found no statistically significant association between genotype and age at onset of smoking, years of smoking, FTND score, or average number of cigarettes smoked per day. DRD2 genotype was associated with the number of cigarettes smoked in the past 48 hr (42.2 in A1 carriers vs. 37.6 in A2, $p=.03$), the previous quit duration (28% in A1 vs. 40% in A2 quit for more than 1 month, $p=.05$), and the depth of inhalation (82% in A1 vs. 72% in A2 inhaled the smoke deeply, $p=.03$). Logistic regression analysis including DRD2 genotype, FTND score, age at smoking initiation, marital status, and education as predictors showed that maximum duration of quit time was associated with FTND score ($p=.003$), DRD2 genotype ($p=.01$), marital status ($p=.03$), and age at smoking initiation ($p=.04$). These findings suggest a modest association between DRD2 genotype and quitting behavior in male cigarette smokers in Egypt.

Introduction

Nicotine addiction has been identified as the primary contributor to tobacco use, and few smokers successfully quit on a long-term basis (Fiore, 1992). This lack of quitting success reflects the multiple genetic, environmental, and social components of smoking (Tyndale, 2003).

Ghada N. Radwan, Ph.D., Salwa Abdel Azem, Omima Kamel, Department of Public Health, Cairo University, Cairo, Egypt; Maged El Setouhy, Department of Community, Environmental and Occupational Medicine, Ain Shams University, Egypt; Mostafa K. Mohamed, Ph.D., Department of Community, Environmental and Occupational Medicine, Ain Shams University, Egypt; Mohamed Abdel Hamid, Ph.D., Department of Microbiology, Minia University, Al Minia, Egypt; Ebenezer Israel, Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD, Christopher A. Loffredo, Ph.D., Department of Oncology, Lombardi Cancer Center, Georgetown University, Washington, DC.

Correspondence: Dr., Christopher A. Loffredo, Georgetown University, Box 571472, Washington, DC 20007-1472, USA. Tel: +1 (202) 687-3758; Fax: +1 (202) 784-3034; E-mail: cal9@georgetown.edu

Candidate genes have been examined in the dopaminergic reward system pathway (Munafò, Clark, Johnstone, Murphy, & Walton, 2004). The D2 dopamine receptor gene is located on chromosome 11 (q22–23) and has multiple allelic forms (Grandy et al., 1989). Blum et al. (1990) described a restriction fragment length polymorphism (TaqI) in the 3′ untranslated region, later localized to the neighboring ANKK1 gene (Neville, Johnstone, & Walton, 2004), with alleles designated as DRD2*A1 and *A2. The A1 allele has been associated with a reduced number of dopamine binding sites in the brain and is hypothesized to influence addiction by causing a hypodopaminergic state that is alleviated by chronic exposure to nicotine (Blum et al., 1990).

Numerous association studies reported that DRD2 polymorphisms are associated with ever smoking (Comings et al., 1996; Hamajima et al., 2002; Noble et al., 1994; Spitz et al., 1998; Yoshida et al., 2001), with earlier onset and heavy smoking (Comings et

ISSN 1462-2203 print/ISSN 1469-994X online
DOI: 10.1080/14622200701704889

al., 1996; Wu, Hudmon, Detry, Chamberlain, & Spitz, 2000), and with length of previous abstinence among current smokers (Comings et al., 1996; Spitz et al., 1998). However, it is not accepted universally that DRD2 modulates smoking behavior, given that some studies found no association (Bierut et al., 2000; Johnstone et al., 2004; Singleton et al., 1998). Two recent meta-analyses reported contradictory findings. Li, Ma, and Beuten (2004) reported higher prevalence of the A1 allele in smokers than in nonsmokers, and Munafò et al. (2004) found no effect of DRD2 on any measures of smoking. However, most studies were carried out in Whites, and Munafò et al. (2004) recommended further investigation of molecular mechanisms of tobacco dependence in other populations.

In response to the need for such information, we extended our previous study of smoking behavior among Egyptian male smokers to search for possible influences of genetic variations in DRD2. Egypt has the highest cigarette consumption rate in the Middle East and North Africa region. In 1997, smoking prevalence among adults was estimated to be 43.4% in males and 4.7% in females (Nassar, 2003). This is the first study in Egypt to investigate the possible associations of DRD2 genotypes with tobacco use and addiction.

Method

Recruitment and interviews

A household smoking prevalence survey was carried out in nine villages in Qalyubia governorate in the Nile delta region in 2004 by the Egyptian Smoking Prevention Research Institute. A systematic random sample of 300 households in each village was included. Eligible cigarette smokers for the present study were recruited from the participants in that baseline survey. All participants were of Arab-Egyptian descent. Only males were eligible, since fewer than 1% of rural women smoke. Only those who had at least 5 years of smoking history and averaged at least 10 cigarettes/day in the past year were selected. Those who consumed other types of tobacco were excluded. After obtaining signed informed consent, we interviewed the participants using a questionnaire that elicited information on demographics and smoking history. Participants also were asked about the usual pattern of inhalation of tobacco smoke by the following question: "When you smoke, do you usually inhale the smoke into the mouth and throat, or inhale the smoke deeply into the chest?" To quantify nicotine dependence, we administered the six-item Fagerström Test for Nicotine Dependence (FTND; Heatherton, Kozlowski, Frecker, & Fagerström, 1991).

Genetic testing

Blood samples were obtained by a finger prick and stored on blood spot cards. Genomic DNA was extracted using Gentra kits. DRD2 was assayed at the National Hepatology and Tropical Medicine Institute in Cairo using polymerase chain reaction (PCR), followed by restriction fragment length polymorphism enzymatic digestion and gel electrophoresis. Technicians were initially trained at the Shields Laboratory at Georgetown University, in Washington, DC. The PCR method and primers are published elsewhere (Lerman et al., 1999). Three DRD2 genotypes were identified: (a) homozygote A2/A2, indicated by two fragments, 180 and 130 bp; (b) heterozygote A1/A2, revealed by three fragments, 310, 180, and 130 bp; and (c) homozygote A1/A1, shown by the uncleaved 310-bp fragment.

Data management and analysis

Data were entered into a Microsoft Access database using duplicate data entry. Data analysis was performed with SPSS version 12. Given the low frequency of the A1/A1 genotype (6%), and consistent with categorizations in previous research (Comings et al., 1996; Lerman et al., 1999), DRD2 was classified as the presence or absence of A1 allele (A1/A1 or A1/ A2 vs. A2/A2). Genotype frequency was assessed for Hardy–Weinberg equilibrium using the chi-square test. Smokers were classified as being highly addicted if they had an FTND score of at least 7 (Moolchan et al., 2002; Rustin, 2000). Independent-samples *t*-tests (continuous variables) and chi-square tests (categorical variables) were used to examine the differences in demographics and smoking behavior by DRD2 genotype without correction for multiple comparisons. A logistic regression analysis was performed to determine if the DRD2 genotype was associated with the quit duration, controlling for potential confounders. The quit duration was defined as 1 month or less versus more than 1 month.

Results

Participation of subjects

A total of 620 adult male subjects were identified in the baseline survey and were scheduled to be contacted again in the present study. Of those scheduled, 437 (70.5%) were eligible to participate. Among eligible subjects, 389 (89%) agreed to participate in the present study.

Genotype prevalence and demographic characteristics

DRD2 genotyping was completed on 386 subjects (three subjects had DNA that could not be

genotyped despite repeated attempts). More than one-third (35%) of the subjects had at least one A1 allele, and the remainder had the A2/A2 genotype. The alleles were in Hardy–Weinberg equilibrium ($p>.05$). We found no significant differences in the distributions of the A1 and A2 genotypes by age, marital status, educational level, or occupation (Table 1). The mean age of the subjects was 40 years ($SD=12$, range=19–81). Almost all of these rural males (88%) were currently or formerly married, and the majority (66%) was employed in either agriculture or manual labor.

Smoking, addiction, and quitting behaviors by DRD2 genotype

The mean FTND score was 4.3 ($SD=3$, range=0–10). One out of five smokers had an addiction score of at least 7 points. We found no significant differences between the two genotypes in mean age at smoking initiation or in mean duration of smoking (Table 1). The A1 carriers had a significantly higher mean consumption of cigarettes in the past 48 hr (42.2% vs. 37.7%, $p=.03$). We found no significant difference between the two genotypes in mean number of cigarettes consumed daily, addiction score, or individual FTND items. A significantly higher proportion of subjects with the A1 allele reported that they usually inhale cigarette smoke deeply compared with those with A2/A2 (82% vs. 72%, $p=.03$). We observed a modest association between DRD2 and quitting attempts. A higher proportion of the subjects with A2/A2 genotype reported that they attempted to quit smoking for more than 24 hr (72% vs. 65%, $p=.14$). They also reported a longer duration of the maximum quit time (>30 days), compared with those with A1 alleles (40% vs. 28%, $p=.05$).

To further examine the association between DRD2 genotype and quit duration, we performed a logistic regression analysis that also included age, age at onset of smoking, FTND score, marital status, and educational level. Smokers with the A2/A2 genotype were two times more likely to quit for more than 1 month compared with smokers with A1 alleles ($OR=2.0$, 95% $CI=1.16$–3.54). FTND score, marital status, and age at smoking initiation also were associated with duration of quitting (Table 2).

Discussion

The mean FTND score in this study of male smokers in Egypt was 4.3 ($SD=3$), consistent with other populations (Park, Young, Joon, Hee-Choon, & Ji Ho, 2004; Spitz et al., 1998). One-third of our smokers were highly or very highly addicted, comparable with other studies (Hughes, Gust, & Pechacek, 1987; Niu et al., 2000; Schoberberger, Kunze, & Schmeiser-Rieder, 1997). Large differences in the prevalence of the A1 allele in different population groups have been reported, with a range of 9%–75% (Barr & Kidd, 1993). Studies of White

Table 1. Smoking and quitting behaviors in male cigarette smokers by DRD2 genotype ($N=386$).

	Genotype				
Characteristic[a]	A1/A1 or A1/A2 ($n=134$)		A2/A2 ($n=252$)		p value[b]
Age, years; *M* (*SD*)	39.4	(10.6)	40.3	(12.3)	.47
Marital status; *n* (%)					
Single	12	(9.0%)	32	(12.8%)	.27
Ever married	121	(91.0%)	218	(87.2%)	
Education; *n* (%)					
Illiterate	44	(33.1%)	92	(36.8%)	.63
Read and write	28	(21.1%)	54	(21.6%)	
Education below secondary	20	(15.0%)	42	(16.8%)	
Education secondary and above	41	(30.8%)	62	(24.8%)	
Age at smoking initiation, years; *M* (*SD*)	17.3	(4.1)	17.2	(4.3)	.92
Duration of smoking, years; *M* (*SD*)	22.4	(11)	23.7	(12)	.29
Number of cigarettes smoked in the past 48 hr; *M* (*SD*)	42.2	(25.0)	37.6	(17.8)	.03
Number of cigarettes smoked/ day; *M* (*SD*)	20.9	(9.9)	20.1	(9.1)	.39
FTND score; *M* (*SD*)	4.3	(2.2)	4.3	(2.4)	.89
Degree of inhalation of tobacco smoke; *n* (%)					
Into the mouth and throat	24	(17.9%)	70	(27.9%)	.03
Into the chest	110	(82.1%)	181	(72.1%)	
Any previous quit attempt; *n* (%)					
No	47	(35.3%)	71	(28.2%)	.14
Yes	86	(64.7%)	181	(71.8%)	
Duration of the maximum quit time; *n* (%)[c]					
1–30 days	63	(72.4%)	108	(60%)	.05
>30 days	24	(27.6%)	72	(40%)	

Note. FTND, Fagerström Test for Nicotine Dependence. [a]Two subjects were missing data for educational level and marital status. [b]The p values are from chi-square comparisons for categorical variables or t-tests for continuous variables. [c]The number of individuals who attempted to quit=277.

Table 2. Logistic regression analysis for predictors of the duration of the maximum quit time.

Variable	*OR* (95% *CI*)	*p* value
FTND score (⩾7 vs. <7[a])	0.27 (0.12–0.64)	.003
Genotype (A2/A2 vs. A1 carriers [a])	2.00 (1.16–3.54)	.01
Marital status (married vs. single [a])	3.00 (1.07–8.80)	.03
Age at smoking initiation (⩾20 vs. <20 [a])	1.72 (1.00–2.96)	.04
Education level		.11
Illiterate[a]	1.00	
Read and write	1.36 (0.69–2.70)	
Below secondary	1.13 (0.50–2.52)	
Above secondary	2.16 (1.13–4.50)	

Note. OR, odds ratio; CI, confidence interval; FTND, Fagerström Test for Nicotine Dependence. [a]Reference for each variable.

Americans reported A1 allele frequencies ranging from 6% to18% (Blum et al., 1990; Comings et al., 1991; Parsian et al., 1991). The allele frequency for A1 (21%) in our study was comparable with the range of 18%–26% found in several other White populations (Cinciripini et al., 2004; Comings et al., 1996; Gelernter, Kranzler, Cubells, Ichinose, & Nagatsu, 1998; Johnstone et al., 2004; Singleton et al., 1998; Swan et al., 2005; Zhao, Pakstis, Kidd, & Kidd, 1999).

We examined associations between DRD2 and several behavioral variables. We found no significant associations between DRD2 and age at smoking initiation, cigarettes/day, duration of smoking, or FTND score. These findings agreed with those of several studies (Bierut et al., 2000; Singleton et al., 1998; Wu et al., 2000) but conflicted with others that suggested that DRD2 influences tobacco consumption and dependence (Comings et al., 1996; Wu et al., 2000). This lack of consistency, including the recent assignment of the TaqI SNP to the ANKK1 locus, has been attributed to several factors, including differences between populations, linkage disequilibrium, methods of classification of smoking, and population stratification (Johnstone et al., 2004; Munafò et al., 2004). We recruited only current smokers and applied a standardized definition for their selection (according to the Centers for Disease Control and Prevention [1996] definition of current smokers) to reduce potential misclassification of smoking phenotypes. The problem of multiple ethnicity does not apply to this homogeneous Arab-Egyptian population.

We observed a significant association between DRD2 and number of cigarettes per day in the past 2 days but not mean number of cigarettes per day. This finding may be attributable to inaccuracy in self-reported measures of cigarette consumption (Spitz et al., 1998). For example, some smokers usually share the pack with others but report the whole pack as what they used. This reporting error may be reduced when they are asked about very recent consumption. Also, smokers with a genetically determined dopamine deficit might compensate by increasing nicotine intake through inhaling more deeply, without increasing the number of cigarettes (Johnstone et al., 2004). Thus precise characterization of the level of tobacco consumption using biochemical markers such as cotinine in blood might be beneficial.

Nicotine dependence is the primary reason why smokers cannot quit (U.S. Department of Health and Human Services, 1988), and it is a major predictor of making a quit attempt and of quitting success (Borland, Owen, Hill, & Schofield, 1991; Hyland et al., 2006). Our results revealed associations of addiction with quit attempts and quit duration. Those who tried to quit smoking had significantly lower addiction scores than those who never attempted to quit (3.9 vs. 5.1, $p<.001$). Furthermore, some aspects of quitting behavior may be influenced by genetic variations in DRD2. Those who carried the A1 allele were more likely to experience a shorter duration of quitting compared with those who carried the A2/A2 genotype. This finding is in concordance with previous reports of an inverse relationship between the length and number of previous abstinence attempts and the A1 allele (Comings et al., 1996; Spitz et al., 1998). In addition, several researchers have suggested that the influence of DRD2 on smoking cessation is particularly important during a pharmacological challenge or treatment that targets the specific neurotransmitter influenced by this gene (Cinciripini et al., 2004; David et al., 2003; Lerman et al., 2003; Swan et al., 2005).

In conclusion, our findings suggest that the putative DRD2 TaqI polymorphism may exert some influence on quitting behavior in current male smokers. Weaknesses of the study include the cross-sectional design and its focus on a single gene from a complex pathway; the smoking phenotype needs to be better characterized with biochemical and behavioral markers. Thus future research efforts should focus on an integrative approach that incorporates psychological, social, cultural, behavioral, pharmacological, and genetic influences, as well as interactions among these factors.

References

Barr, C. L., & Kidd, K. K. (1993). Population frequencies of the A1 allele at the dopamine D2 receptor locus. *Biological Psychiatry*, *34*, 204–209.

Bierut, L. J., Rice, J. P., Edenberg, H. J., Goate, A., Foroud, T., Cloninger, C. R., Begleiter, H., Conneally, P. M., Crowe, R. R., Hesselbrock, V., Li, T. K., Nurnberger, J. I., Jr., Porjesz, B., Schuckit, M. A., & Reich, T. (2000). Family-based study of the association of the dopamine D2 receptor gene (DRD2) with habitual smoking. *American Journal of Medical Genetics*, *90*, 299–302.

Blum, K., Noble, E. P., Sheridan, P. J., Montgomery, A., Ritchie, T., Jagadeeswaran, P., Nogami, H., Briggs, A. H., & Cohn, J. B. (1990). Allelic association of human dopamine D2 receptor gene in alcoholism. *The Journal of the American Medical Association*, *263*, 2055–2060.

Borland, R., Owen, N., Hill, D., & Schofield, P. (1991). Predicting attempts and sustained cessation of smoking after the introduction of workplace smoking bans. *Health Psychology*, *10*, 336–42.

Centers for Disease Control and Prevention. (1996). *Definition of current smoker*. National Center for Health Statistics. Retrieved from www.cdc.gov.

Cinciripini, P., Wetter, D., Tomlinson, G., Tsoh, J., De Moor, C., Cinciripini, L., & Minna, J. (2004). The effects of the DRD2 polymorphism on smoking cessation and negative affect: Evidence for a pharmacogenetic effect on mood. *Nicotine & Tobacco Research*, *6*, 229–239.

Comings, D. E., Comings, B. G., Muhleman, D., Dietz, G., Shahbahrami, B., Tast, D., Knell, E., Kocsis, P., Baumgarten, R., Kovacs, B. W., Levy, D. L., Smith, M., Borison, R. L., Evans, D. D., Klein, D. N., MacMurray, J., Tosk, J. M., Sverd, J., Gysin, R., & Flanagan, S. D. (1991). The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. *The Journal of the American Medical Association*, *266*, 1793–1800.

Comings, D. E., Ferry, L., Bradshaw-Robinson, S., Burchette, R., Chiu, C., & Muhleman, D. (1996). The dopamine D2 receptor (DRD2) gene: A genetic risk factor in smoking. *Pharmacogenetics*, *6*, 73–79.

David, S. P., Niaura, R., Papandonatos, G. D., Shadel, W. G., Burkholder, G. J., Britt, D. M., Day, A., Stumpff, J., Hutchison, K., Murphy, M., Johnstone, E., Griffiths, S. E., & Walton, R. T. (2003). Does the DRD2-Taq1 A polymorphism influence treatment response to bupropion hydrochloride for reduction of the nicotine withdrawal syndrome? *Nicotine & Tobacco Research*, *5*, 935–942.

Fiore, M. C. (1992). Trends in cigarette smoking in the United States. The epidemiology of tobacco use. *Medical Clinics of North America*, *76*, 289–303.

Gelernter, J., Kranzler, H., Cubells, J. F., Ichinose, H., & Nagatsu, T. (1998). DRD2 allele frequencies and linkage disequilibria, including the _141CIns/Del promoter polymorphism, in European-American, African-American, and Japanese subjects. *Genomics*, *51*, 21–26.

Grandy, D. K., Marchionni, M. A., Makam, H., Stofko, R. E., Alfano, M., Frothingham, L., Fischer, J. B., Burke-Howie, K. J., Bunzow, J. R., Server, A. C., & Civelli, O. (1989). Cloning of the cDNA and gene for a human D2 dopamine receptor. *Proceedings of the National Academy of Sciences USA*, *86*, 9762–9766.

Hamajima, N., Ito, H., Matsuo, K., Saito, T., Tajima, K., Ando, M., Yoshida, K., & Takahashi, T. (2002). Association between smoking habits and dopamine receptor D2 taqI A A2 allele in Japanese males: A confirmatory study. *Journal of Epidemiology*, *12*, 297–304.

Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., & Fagerström, K. O. (1991). The Fagerström Test for Nicotine Dependence: A revision of the Fagerström Tolerance Questionnaire. *British Journal of Addiction*, *86*, 1119–1127.

Hughes, J. R., Gust, S. W., & Pechacek, T. F. (1987). Prevalence of tobacco dependence and withdrawal. *American Journal of Psychiatry*, *144*, 205–208.

Hyland, A., Borland, R., Li, Q., Yong, H. H., McNeill, A., Fong, G. T., O'Connor, R. J., & Cummings, K. M. (2006). Individual-level predictors of cessation behaviours among participants in the International Tobacco Control (ITC) Four Country Survey. *Tobacco Control*, *15*(Suppl. 3), iii83–iii94.

Johnstone, E. C., Yudkin, P., Griffiths, S. E., Fuller, A., Murphy, M., & Walton, R. (2004). The dopamine D2 receptor C32806T polymorphism (DRD2 Taq1A RFLP) exhibits no association with smoking behaviour in a healthy UK population. *Addiction Biology*, *9*, 221–226.

Lerman, C., Caporaso, N. E., Audrain, J., Main, D., Bowman, E. D., Lockshin, B., Boyd, N. R., & Shields, P. G. (1999). Evidence suggesting the role of specific genetic factors in cigarette smoking. *Health Psychology*, *18*, 14–20.

Lerman, C., Shields, P. G., Wileyto, E. P., Audrain, J., Hawk, L. H., Jr., Pinto, A., Kucharski, S., Krishnan, S., Niaura, R., & Epstein, L. H. (2003). Effects of dopamine transporter and receptor polymorphisms on smoking cessation in a bupropion clinical trial. *Health Psychology*, *22*, 541–548.

Li, M. D., Ma, J. Z., & Beuten, J. (2004). Progress in searching for susceptibility loci and genes for smoking-related behaviour. *Clinical Genetics*, *66*, 382–392.

Moolchan, E. T., Radzius, A., Epstein, D. H., Uhl, G., Gorelick, D. A., Cadet, J. L., & Henningfield, J. E. (2002). The Fagerström Test for Nicotine Dependence and the Diagnostic Interview Schedule. Do they diagnose the same smokers? *Addictive Behaviors*, *27*, 101–113.

Munafò, M., Clark, T., Johnstone, E., Murphy, M., & Walton, R. (2004). The genetic basis for smoking behavior: A systematic review and meta-analysis. *Nicotine & Tobacco Research*, *6*, 583–597.

Nassar, H. (2003). *The economics of tobacco in Egypt, a new analysis of demand*. Retrieved from www.emro.who.int/TFI/tobaccoeconomics-egypt.pdf

Neville, M. J., Johnstone, E. C., & Walton, R. T. (2004). Identification and characterization of ANKK1: A novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Human Mutations*, *6*, 540–545.

Niu, T., Chen, C., Ni, J., Wang, B., Fang, Z., Shao, H., & Xu, X. (2000). Nicotine dependence and its familial aggregation in Chinese. *International Journal of Epidemiology*, *29*, 248–252.

Noble, E. P., St Jeor S., T., Ritchie, T., Syndulko, K., St Jeor, S. C., Fitch, R. J., Brunner, R. L., & Sparkes, R. S. (1994). D2 dopamine receptor gene and cigarette smoking: A reward gene? *Medical Hypotheses*, *42*, 257–260.

Park, S. M., Young, S. K., Joon, L. Y., Hee-Choon, S. L., & Ji Ho, K. (2004). A preliminary investigation of early smoking initiation and nicotine dependence in Korean adults. *Drug and Alcohol Dependence*, *74*, 197–203.

Parsian, A., Todd, R. D., Devor, E. J., O'Malley, K. L., Suarez, B. K., Reich, T., & Cloninger, C. R. (1991). Alcoholism and alleles of the human D2 dopamine receptor locus. Studies of association and linkage. *Archives of General Psychiatry*, *48*, 655–663.

Rustin, T. A. (2000). Assessing nicotine dependence. *American Family Physician*, *62*, 579–584.

Schoberberger, R., Kunze, U., & Schmeiser-Rieder, A. (1997). [Diagnosis and therapy of nicotine dependence.]. *Versicherungsmedizin*, *49*, 25–29.

Singleton, A. B., Thomson, J. H., Morris, C. M., Court, J. A., Lloyd, S., & Cholerton, S. (1998). Lack of association between the dopamine D2 receptor gene allele DRD2*A1 and cigarette smoking in a United Kingdom population. *Pharmacogenetics*, *8*, 125–128.

Spitz, M. R., Shi, H., Yang, F., Hudmon, K. S., Jiang, H., Chamberlain, R. M., Amos, C. I., Wan, Y., Cinciripini, P., Hong, W. K., & Wu, X. (1998). Case-control study of the D2 dopamine receptor gene and smoking status in lung cancer patients. *Journal of the National Cancer Institute*, *90*, 358–363.

Swan, G. E., Valdes, A. M., Ring, H. Z., Khroyan, T. V., Jack, L. M., Ton, C. C., Curry, S. J., & McAfee, T. (2005). Dopamine receptor DRD2 genotype and smoking cessation outcome following treatment with bupropion SR. *Pharmacogenomics Journal*, *5*, 21–29.

Tyndale, R. F. (2003). Genetics of alcohol and tobacco use in humans. *Annals of Medicine*, *35*, 94–121.

U.S. Department of Health and Human Services. (1988). *The health consequences of smoking: Nicotine addiction. A report of the surgeon general*. Rockville, MD: Author, Centers for Disease Control and Prevention.

Wu, X., Hudmon, K. S., Detry, M. A., Chamberlain, R. M., & Spitz, M. R. (2000). D2 dopamine receptor gene polymorphisms among African-Americans and Mexican-Americans: A lung cancer case-control study. *Cancer Epidemiology, Biomarkers, and Prevention*, *9*, 1021–1026.

Yoshida, K., Hamajima, N., Kozaki, K., Saito, H., Maeno, K., Sugiura, T., Ookuma, K., & Takahashi, T. (2001). Association between the dopamine D2 receptor A2/A2 genotype and smoking behavior in the Japanese. *Cancer Epidemiology, Biomarkers, and Prevention*, *10*, 403–405.

Zhao, H., Pakstis, A. J., Kidd, J. R., & Kidd, K. K. (1999). Assessing linkage disequilibrium in a complex genetic system. I. Overall deviation from random association. *Annals of Human Genetics*, *63*(Part 2), 167–179.