Original Investigation

Sex differences in *TTC12/ANKK1* haplotype associations with daily tobacco smoking in Black and White Americans

Sean P. David, M.D., S.M., D.Phil.,¹ Briana Mezuk, Ph.D.,² Peter P. Zandi, Ph.D.,³ David Strong, Ph.D.,⁴ James C. Anthony, Ph.D.,⁵ Raymond Niaura, Ph.D.,⁴ George R. Uhl, M.D., Ph.D.,⁶ & William W. Eaton, Ph.D.³

¹ Division of Family & Community Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA

² Department of Epidemiology & Community Health, Virginia Commonwealth University School of Medicine, Richmond, VA

³ Department of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

⁴ Department of Psychiatry & Human Behavior, Brown Alpert Medical School, Providence, RI

⁵ Department of Epidemiology, Michigan State University College of Human Medicine, East Lansing, MI

⁶ Molecular Neurobiology Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD

Corresponding Author: Sean P. David, M.D., S.M., D.Phil., Division of Family & Community Medicine, Department of Medicine, Center for Education in Family & Community Medicine, Stanford University School of Medicine, 1215 Welch Road, Modular G, Stanford, CA 94305-5408, USA. Telephone: 401-265-2275; Fax: 650-736-7772; E-mail: spdavid@stanford.edu

Received September 3, 2009; accepted December 4, 2009

Abstract

Introduction: The 11q23.1 genomic region has been associated with nicotine dependence in Black and White Americans.

Methods: By conducting linkage disequilibrium analyses of 7 informative single nucleotide polymorphisms (SNPs) within the tetratricopeptide repeat domain 12 (*TTC12*)/ankyrin repeat and kinase containing 1 (*ANKK1*)/dopamine (D2) receptor gene cluster, we identified haplotype block structures in 270 Black and 368 White ($n = 638$) participants, from the Baltimore Epidemiologic Catchment Area cohort study, spanning the *TTC12* and *ANKK1* genes consisting of three SNPs (rs2303380–rs4938015–rs11604671). Informative haplotypes were examined for sex-specific associations with daily tobacco smoking initiation and cessation using longitudinal data from 1993–1994 and 2004–2005 interviews.

Results: There was a Haplotype $\times$ Sex interaction such that Black men possessing the GTG haplotype who were smokers in 1993–2004 were more likely to have stopped smoking by 2004–2005 (55.6% GTG vs. 22.0% other haplotypes), while Black women were less likely to have quit smoking if they possessed the GTG (20.8%) versus other haplotypes (24.0%; $p = .028$). In Whites, the GTG haplotype (vs. other haplotypes) was associated with lifetime history of daily smoking (smoking initiation; odds ratio = 1.6; 95% CI = 1.1–2.4; $p = .013$). Moreover, there was a Haplotype $\times$ Sex interaction such that there was higher prevalence of smoking initiation with GTG (77.6%) versus other haplotypes (57.0%; $p = .043$).

Discussion: In 2 different ethnic American populations, we observed man–woman variation in the influence of the rs2303380–rs4938015–rs11604671 GTG haplotype on smoking initiation and cessation. These results should be replicated in larger cohorts to establish the relationship among the rs2303380–rs4938015–rs11604671 haplotype block, sex, and smoking behavior.

Introduction

Tobacco use, particularly daily tobacco smoking, continues to be a substantial cause of morbidity and mortality globally with an estimated annual mortality of 5.4 million and projections of 8 million deaths per year by 2030 by the most recent World Health Organization (2009) projections. Substantial reductions in smoking prevalence have been observed in the United States and Europe and other more established market economic nations in the last 50 years; tobacco-associated health disparities are manifest in low- to middle-income populations from different continental origins (Centers for Disease Control and Prevention [CDC], 1998). In the United States, the overall national estimates of smoking prevalence between Black and White adults are similar for women (Black: 25.4% [95% CI = 24.1–26.7%]; White: 26.6% [95% CI = 26–27.1%]) and men (Black: 39.8% [95% CI = 39.3–40.7%]; White: 40.0% [95% CI = 39.3–40.7%]; Caraballo, Yee, Gfroerer, & Mirza, 2008). Nevertheless, there are marked ethnic/racial variations in estimates of estimated occurrence of smoking in adolescence and early adulthood and in the likelihood of smoking cessation

doi: 10.1093/ntr/ntp201

Advance Access published on February 4, 2010

© The Author 2010. Published by Oxford University Press on behalf of the Society for Research on Nicotine and Tobacco.

All rights reserved. For permissions, please e-mail: journals.permissions@oxfordjournals.org

in middle age between Blacks and Whites (CDC, 1998). According to the 1998 Surgeon General's report on "Tobacco Use Among U.S. Racial/Ethnic Minority Groups," smoking among high school seniors in the United States had become more common in Whites (25%) compared with Blacks (10%; CDC, 1998). However, the estimated occurrence of smoking cessation was found to be markedly higher among Whites (~50%) than Blacks (~35%) despite increases in access to over-the-counter nicotine replacement therapy (NRT) and other smoking cessation resources (CDC, 1998).

Twin and family studies have consistently identified a major contribution of heritability factors to smoking initiation, smoking persistence, and nicotine dependence (Li, Cheng, Ma, & Swan, 2003). Among the many nongenetic factors that may contribute to observed differences in smoking prevalence between Blacks and Whites are differences in menthol cigarette consumption, tobacco industry marketing practices, and access to smoking prevention and cessation services (CDC, 1998; Fu, Burgess, et al., 2008; Fu, Kodl, et al., 2008; Kendzor et al., 2008; Primack, Bost, Land, & Fine, 2007).

In addition to disparities in tobacco use between ethnic/racial groups, disparities exist between sex/gender subgroups in many aspects of smoking behavior. The 2002 Surgeon General's report on "Women and Smoking" highlighted divergent trends in youth smoking initiation and persistence and called for additional research into sex differences in genetic influences on smoking behavior (CDC, 2002). For example, it has been observed that the efficacy of NRT is greater for men than women (Cepeda-Benito, Reynoso, & Erath, 2004; Perkins & Scott, 2008) and that Gene $\times$ Sex interactions may influence efficacy to both NRT (Yudkin et al., 2004) and bupropion (Lerman et al., 2002) for smoking cessation. However, the degree to which Gene $\times$ Sex interactions differentially affect one ethnic group compared with another with respect to smoking phenotypes has not been established.

Very few molecular genetic studies of non-White populations for smoking-related phenotypes have been conducted to date. Nevertheless, linkage analyses have found logarithm [base 10] of odds of linkage scores for nicotine dependence on chromosome 11q23.1 (D11S908–D11S1999) approaching or greater than 2 in both Blacks and Whites, respectively (Gelernter et al., 2006; Uhl et al., 2008). This genomic region includes a cluster of four genes, dopamine (D2) receptor (*DRD2*; Grandy et al., 1989), ankyrin repeat and kinase containing 1 (*ANKK1*; Neville, Johnstone, & Walton, 2004), tetratricopeptide repeat domain 12 (*TTC12*; Katoh & Katoh, 2003), and neural cell adhesion molecule (*NCAM1*; Nguyen et al., 1986). Several biologically plausible candidate gene variants have been identified within this genomic region in genetic association studies of nicotine dependence and smoking cessation (Abrous et al., 2002; Breen et al., 1999; Jonsson et al., 1996; Jonsson et al., 1999; Katoh & Katoh; Noble, Blum, Ritchie, Montgomery, & Sheridan, 1991; Noble, Gottschalk, Fallon, Ritchie, & Wu, 1997; Yang et al., 2007, 2008). For example, functional genetic variants in the *DRD2* gene (i.e., rs6277 "exon [S] [C957T]"; rs1799732 "promoter (ins/del)" and *ANKK1* gene (i.e., rs1800497 "TaqIA") have been associated with efficacy of NRT and bupropion for smoking cessation (David, Brown, et al., 2007; David, Strong, et al., 2007; Johnstone et al., 2004; Lerman et al., 2003, 2006; Swan

et al., 2005, 2007; Yudkin et al., 2004). Of the genetic variants within this cluster, the TaqIA variant is the most widely studied for association with smoking behavior. Three meta-analyses suggest association with one or more related phenotype (e.g., smoking initiation or persistence; Li, Ma, & Beuten, 2004; Munafo, Clark, Johnstone, Murphy, & Walton, 2004; Munafo, Timpson, David, Ebrahim, & Lawlor, 2009). However, in two of the three meta-analyses (Munafo, Clark, et al.; Munafo et al.), substantial statistical heterogeneity across studies rendered associations nonsignificant when pooled using random-effects statistical modeling, and these reports also had an insufficient number of studies to incorporate non-White participants in the pooled estimates. In the most recent meta-analysis of TaqIA and smoking behavior (Munafo et al.), meta-regression indicated that the effect size of associations between the TaqIA-A1 allele and smoking initiation, smoking persistence, and cigarettes smoked per day was greater in men than women. However, the mechanism underlying an apparent Gene $\times$ Sex interaction for these phenotypes is not known.

Gelernter et al. (2006) examined 43 single nucleotide polymorphisms (SNPs) within the *NCAM1/TTC12/ANKK1/DRD2* gene cluster in 1,615 participants from 319 Black and 313 White families who had been ascertained for nicotine dependence. A distinct haplotype block was identified in Blacks and Whites containing four SNPs from the *TTC12* (rs2303380 A/G) and *ANKK1* (rs4938012 G/A, rs4938015 C/T, rs11604671 C/T) genes that were associated with nicotine dependence in both Blacks and Whites (Gelernter et al., 2006). Recently, our group published a genome-wide association study of smoking cessation that disclosed an association between the relative efficacy of bupropion versus NRT for a cluster of SNPs that overlap with nicotine dependence SNPs in the *NCAM1* gene region analyzed by Gelernter and colleagues (2006) (David & Munafo, 2008; Uhl et al., 2008). These overlapping associations of SNPs in the *NCAM1/TTC12/ANKK1/DRD2* gene cluster and smoking cessation associations with *NCAM1* SNPs are intriguing because multiple pharmacogenetic clinical trial investigations have found consistent associations between SNPs within the neighboring *ANKK1* (i.e., rs1800497 "DRD2-TaqIA") and *DRD2* (i.e., rs6277 "exon [S] C957T"; rs1799732 "promoter [-141 ins/del]") genes and efficacy of bupropion and NRT (David, Brown, et al., 2007; David, Strong, et al., 2007; Johnstone et al., 2004; Lerman et al., 2003, 2006; Swan et al., 2005, 2007; Yudkin et al., 2004). Given that SNPs associated with pharmacogenetic smoking cessation outcomes may manifest differing degrees of linkage disequilibrium (LD) depending on the population studied (e.g., *ANKK1* rs1800497 and rs6277: $D' = 0.13$ for Blacks; $D' = 0.74$ for Whites; Gelernter et al., 2006), haplotype analyses may be necessary to identify genetic variants that contribute to smoking cessation outcomes in populations of different continental origins. A separate report provides more details about SNP associations with nicotine dependence phenotypes within the *NCAM1/TTC12/ANKK1/DRD2* gene cluster (David & Munafo).

There is evidence that the associations between the *ANKK1* rs1800497 SNP and smoking initiation, smoking persistence (Munafo et al., 2009), and NRT efficacy for smoking cessation are moderated by sex (Yudkin et al., 2004). Mechanisms underlying these apparent Gene $\times$ Sex interactions are not well understood,

but there is growing evidence that there are sex differences in nicotine sensitivity and motivational properties. Sex differences have been demonstrated for nicotine reinforcement (Perkins, 1999, 2001; Perkins et al., 1996), psychomotor reactivity to environmental smoking cues (Niaura et al., 1998), and nicotine metabolism (Zeman, Hiraki, & Sellers, 2002). Furthermore, there is mixed evidence regarding whether women may be less responsive to NRT than men (Munafo, Bradburn, Bowes, & David, 2004; Perkins & Scott, 2008). If indeed, the rs1800497 associations with smoking phenotypes are proxies for haplotypes composed of SNPs, other than or in addition to rs1800497, within the *NCAM1/TTC12/ANKK1/DRD2* gene cluster, we would anticipate Haplotype $\times$ Sex interactions moderating smoking initiation and persistence and smoking cessation.

In addition to the reasons cited above to explore ethnic and sex differences in smoking behavior, there are additional justifications for the present study: There are few genetic association studies of smoking-related phenotypes in non-White populations, and the result of publication disparity in the literature could slow the development of personalized medicine for minority populations. Finally, our understanding of differing LD patterns by different ancestral groups has advanced with completion of the International HapMap project and recent independent investigations. In consequence, we now can make a more informed selection of genomic markers for investigation of smoking-related phenotypes across populations.

In a sample of Black and White men and women in Baltimore, MD, we now seek to estimate associations that link specific haplotypes from this genomic region with smoking initiation (i.e., onset of daily smoking), persistence, and cessation in this longitudinal cohort study. Genetic association studies of smoking behavior often utilize cross-sectional or retrospective data. The present analyses, however, are based on data from two assessments over a 10- to 11-year follow-up interval, precisely defining smoking initiation in terms of the onset of daily smoking. Extending our prior genetic association studies of SNPs in the 11q23.1 region, the present study examines informative haplotypes derived from stratified LD analyses in Blacks and Whites separately and examines test for theoretically important Gene $\times$ Sex interactions in both ethnic populations (Lerman et al., 2002; Yudkin et al., 2004). Given its proximity to and high degree of LD with other functional SNPs in this gene cluster, it is possible that rs1800497 may be a proxy for other genetic loci or haplotypes spanning the same genomic region (Gelernter et al., 2006).

The Baltimore site of the Epidemiologic Catchment Area (ECA) study is an appropriate population sample for genetic association studies of smoking behavior because of its diverse composition of participants, the quality of measures, and the longitudinal nature of the study (Eaton, Kalaydjian, Scharfstein, Mezuk, & Ding, 2007; Eaton, Neufeld, Chen, & Cai, 2000; Regier et al., 1984). In the present investigation, we tapped an ongoing longitudinal cohort research sample that WVE and JCA have been studying since its original recruitment wave in 1981 from the household-residing population of East Baltimore.

Recently, Johnson et al. reported results from a genome-wide association investigation of the ECA sample, which found convergence of ECA-derived estimates of SNP associations of nicotine and other drug dependence substance dependence phe-

notypes including smoking cessation other volunteer samples of Blacks and Whites (Johnson et al., 2008). In the present study, we extend the findings of our group to explore associations between informative haplotypes within the *TTC12/ANKK1/DRD2* gene cluster (Gelernter et al., 2006) and smoking behavior by additional genotyping of Baltimore ECA participants and using prospectively collected data from the most recent two assessments in 1993–2004 and 2004–2005, respectively.

Objectives and hypotheses

The present study had three objectives: First, we sought to confirm and strengthen, through replication, the findings of Gelernter et al. (2006), with respect to specific haplotype blocks composed of SNPs in the *TTC12* and *ANKK1* genes via LD analyses of seven informative SNPs selected from the *TTC12/ANKK1/DRD2* gene cluster in separate analyses for Blacks and Whites; second, we examined whether informative haplotypes in Blacks and Whites were associated with initiation and persistence of daily smoking and smoking cessation; third, we explored potential Haplotype $\times$ Sex interactions with phenotypes studied over a 10- to 11-year follow-up interval. Given the diminishing sample size of subgroup analyses by sex and haplotype, Haplotype $\times$ Sex interaction analyses were intended to be exploratory.

Methods

Sample

The Baltimore site of the ECA study was one of five sites of the National Institute of Mental Health collaboration, initiated in the late 1970s to estimate the prevalence, incidence, and natural history parameters for psychiatric disorders in the general population (Regier et al., 1984). The household-residing participants were interviewed four times over the 23-year follow-up period (1981 to 2004–2005), and DNA was collected from most of the 1,071 participants in the latest interview wave (Eaton et al., 2007; Mezuk, Eaton, & Zandi, 2008). Unlike many extant genetic association studies of nicotine dependence, the Baltimore ECA research team repeatedly measured smoking and nicotine dependence-related phenotypes, including the Fagerström Test for Nicotine Dependence (FTND; Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991) and tobacco use history.

We examined data from the next-to-latest survey, Wave 3 (W3), conducted during 1993 and 1994 and from the latest survey, Wave 4 (W4), conducted during 2004 and 2005—for which detailed smoking phenotype data were gathered. The recruitment and survey techniques and population-based sampling methods and participant characteristics of the W3 and W4 samples, as well as the methods of biospecimen and DNA preparation from this sample and the participant characteristics of those who provided biospecimens, are described in detail elsewhere (Eaton et al., 2007; Fu, Kodl, et al., 2008; Mezuk et al., 2008; Regier et al., 1984). We note that the original 1981 sample was weighted heavily with elderly (age ≥ 65 years) residents due to deliberate oversampling for research on late-life disorders (1,086 elderly among 3,481 participants); by 2005, many of the elderly had died. Hence, this is a sample of long-term survivors, as is true for all cross-sectional samples of adults, especially when follow-up assessments are made over long spans of time.

Biospecimen collection and DNA preparation

Biospecimen collection procedures—involving primarily phlebotomy for whole blood and buccal samples from those participants unable or unwilling to undergo phlebotomy—and DNA preparation and quantification are described elsewhere (Fu, Kodl, et al., 2008; Mezuk et al., 2008). Separate consent forms were used for the survey, the collection of blood samples, and collection of buccal samples (Mezuk et al.). Participants in this study gave informed consent to investigators to permit storage of DNA for unspecified future research by checking a “yes” box in response to a clause in the consent form stating: “I agree to the following: storage of my DNA for other medical research.”

The Johns Hopkins Bloomberg School of Public Health Institutional Review Board approved the study, including DNA collection and genetic association analysis.

SNP selection

The role of dopamine as a critical neural substrate for nicotine dependence is well established, and there have been many examples of replication between genotypes affecting dopamine (D2) receptor function and nicotine dependence phenotypes (David & Munafo, 2008; Munafo, Clark, et al., 2004; Munafo et al., 2009). Proximal (3') to the *DRD2* gene, the *ANKK1* gene contains the “TaqIA” polymorphism, which affects D2 receptor-binding potential and substrate-binding specificity (Jonsson et al., 1999; Neville et al., 2004). Our criteria for SNP selection were to include (a) exonic or intronic genetic variants within a defined genomic region (11q23.1) with (b) strong a priori evidence of association nicotine dependence phenotypes and (c) inclusive of SNPs constituting a haplotype that was associated with nicotine dependence in Blacks and Whites in the fine-mapping family-based genetic association study by Gelernter et al. (2006) described above (i.e., *TTC12* rs2303380; *ANKK1* rs4938012; *ANKK1* rs4938015; *ANKK1* rs1160467). Therefore, the SNPs selected for genotyping included rs2303380, rs4938012, rs4938015, and rs1160467 and three additional SNPs from this region in the *ANKK1* (rs1800497) and *DRD2* (rs6277; rs1799732) genes that have been associated with smoking cessation in clinical trials (David, Brown, et al., 2007; David, Strong, et al., 2007; Johnstone et al., 2004; Lerman et al., 2003, 2006).

Genotyping

Genotyping of these seven SNPs was conducted with a fluorogenic 5' nuclease assay using TaqMan primers and probes with the ABI PRISM 7900 Sequence Detection System (ABI, Foster City, CA; Shi, Myrand, Bleavins, & de la Iglesia, 1999).

Quality control

In addition to determining Hardy–Weinberg Equilibrium (HWE) calculations for each SNP, missing data rates per participant and per SNP are calculated as well as concordance rates for duplicate genotype calls. Any SNP deviating from HWE at $p < .05$ corrected for multiple comparisons, with missing data on 2% or more of participants or less than 100% concordance, was dropped from analyses per standard quality control practice (Zandi, Avramopoulos, et al., 2007; Zandi, Badner, et al., 2007; Zandi et al., 2008). Only one SNP, rs4938012, which deviated from HWE, was removed from the analyses as described further below.

HWE analysis

Each of the SNPs was analyzed for HWE in the White and Black populations separately. One of the SNPs, rs4938012, deviated from HWE in Blacks ($\chi^2 = 184.9$; $p = .001$) and Whites ($\chi^2 = 184.9$; $p = .001$), which was also observed by Gelernter et al. (2006) using a similar genotyping assay. Therefore, the rs4938012 SNP in the *ANKK1* gene was removed from subsequent analyses because it could not be determined whether the mean allele frequencies were accurate or represented limitations of the 5' nuclease assay for this SNP. The consequences of losing one SNP from the analyses are that the haplotypes are theoretically less informative, and we are unable to precisely replicate the analytic approach published by Gelernter et al. (2006). However, inclusion of a SNP that deviates from HWE would result in haplotypes for which analytic results are uninterpretable without major speculation.

Haplotype block determination

Once SNPs were selected and genotyped, LD was evaluated among all SNPs in order to reduce the number of markers that would be potentially informative in haplotype analysis. The LD analysis, demonstrated in Figure 1, illustrates the degree of LD between neighboring SNPs, with SNPs constituting haplotype blocks corresponding to D' 's of 0.65 or greater in our sample. Haplotype blocks were determined according to whether or not the D' between neighboring SNPs was statistically significant at conventional alpha levels.

LD analysis

The R statistical platform version 2.9.0 (R Core Development Team; <http://www.r-project.org/>) was used to examine the LD structure across the genomic region of interest. Both D' and r^2 were calculated between all pairs of SNPs and estimated haplotype block structure based on the degree of LD between the remaining six SNPs included in the analyses. The generation of LD maps was conducted separately for Blacks and Whites (Figure 1).

Genetic association analysis

To estimate haplotype associations with levels of nicotine dependence, lifetime cigarette smoking assessed in 1993–1994 (W3), and cigarette smoking at the 2003–2004 (W4), we employed generalized linear models to adjust for nongenetic cofactors, including the age at assessment and sex. Haplotypes were constructed using available SNPs constituting the haplotype block; uncertainty in haplotype assignment was accounted for in the analyses of covariates using a likelihood approach to inferences provided by the software hapassoc (Burkett, Graham, & McNeney, 2006) and R statistical platform version 2.9.0 (R Core Development Team; <http://www.r-project.org/>).

When the FTND (Heatherton et al., 1991) was completed by ECA participants, there were one or more missing items from the FTND for Blacks ($n = 109/215$; 51%) and Whites ($n = 250/374$; 67%). Therefore, FTND was not included in the analyses because the reduction in sample size markedly reduces statistical power. Rather, cigarettes per day were used as a proxy measure for nicotine dependence in the analyses—for which data were more readily available. “Lifetime history of daily smoking” status at W3 was assessed with “Have you ever smoked tobacco cigarettes?” Participants responding affirmatively that they had ever smoked daily were also asked at what age they first

Figure 1. (A) Linkage disequilibrium (LD) plots of Blacks for *TTC12* rs2303380 *ANKK1*, rs4938015, rs11604671, rs1800497, *DRD2* rs6277, and rs1799732. (B) LD plots of Whites for *TTC12* rs2303380 *ANKK1*, rs4938015, rs11604671, rs1800497, *DRD2* rs6277, and rs1799732.

Table 1. Demographic differences between participants excluded for missing genetic data and participant included in the current analyses

	Blacks			Whites		
	Included (<i>n</i> = 270)	Excluded (<i>n</i> = 204)	<i>p</i> value	Included (<i>n</i> = 368)	Excluded (<i>n</i> = 294)	<i>p</i> value
Demographics						
Women	74.1%	69.1%	.29	57.3%	59.5%	.57
Mean age W3 (<i>SD</i>)	46.43 (11.09)	45.17 (9.69)	.65	48.40 (13.00)	50.09 (14.41)	.04
Mean age W4 (<i>SD</i>)	57.28 (11.13)	55.82 (9.86)	.55	59.27 (13.11)	60.94 (14.48)	.04
Smoking history						
Lifetime smoker	62.4%	58.9%	.58	61.7%	54.4%	.08
Current smoker (W4)	31.2%	35.8%	.35	24.7%	17.7%	.03

Note. Mean and *SD* presented for age of assessments during Wave 3 (W3; 1993–1994) and Wave 4 (W4; 2004–2005). *p* values presented for differences between genotyped and nongenotyped participants in frequencies (χ^2 tests) and means (*t* tests).

started smoking daily. Participants at both waves who reported ever smoking daily and who also reported having smoked in the last 7 days were coded as being a “current smoker.” Biochemical verification of abstinence from tobacco (e.g., salivary or plasma cotinine; exhaled carbon monoxide) was not part of the survey protocol. Other psychological and behavioral covariates were assessed but are not included in the present analyses; they will be explored in future analyses of mediation of genetic associations for a subsequent paper.

Results

Participant characteristics

Extensive details regarding the characteristics of the Baltimore ECA participants who provided biospecimens are given elsewhere (Mezuk et al., 2008). Briefly, 883 (86.8%) of the 1,017 survivors interviewed in 2004–2005 provided biospecimens for DNA collection, of which 683 allowed phlebotomy for whole blood samples; 291 provided buccal samples or both. Of the 883 participants providing biospecimens, 791 consented to future studies. There were insufficient quantities of DNA remaining from previous genotyping assays or other, nongenetic missing data for 153 of the 791 participants; the effective sample for analyses included 270 Black and 368 White participants.

Table 1 illustrates available characteristics of the 638 study population (i.e., those participants providing biospecimens with complete data available for analyses [“included”] and of those lacking complete genetic and nongenetic data for the present analyses [“excluded”]). The Blacks included and excluded based on donation of biospecimens were similar in age, sex, and frequency of current smokers at both survey waves. However, Whites included in the analyses, compared with Whites excluded, were younger at W3 (aged 48.4 years [*SD* = 13.0] vs. 50.1 years [*SD* = 14.1]; *p* = .04) and W4 (aged 59.2 years [*SD* = 13.1] vs. 60.9 years [*SD* = 14.5]; *p* = .04), more likely to have reported ever smoking in their lifetimes at W3 (61.7% vs. 54.4%; *p* = .08), and more likely to have reported smoking in the last 7 days at the most recent assessment (W4; 24.7% vs. 17.7%; *p* = .03).

Mean allele frequencies and HWE analyses are presented in Table 2. None of the SNPs deviated from HWE in either

population (Table 2). In Blacks, the HWE χ^2 for rs1800497 was 5.93 (*p* = .017). When corrected for multiple comparisons (i.e., α = .05/6 = .008), rs1800497 did not deviate from HWE.

Haplotype association analysis

Similar to findings of Gelernter et al. (2006) with samples from Connecticut, Massachusetts, and South Carolina, analysis of haplotypes in the present study population from East Baltimore demonstrated that the region most likely to contain a nicotine dependence locus was bounded by *TTC12* rs2303380 centromerically and extending to *ANKK1* 11604671. While we excluded rs4938012 from the analyses, we confirmed similar haplotype block structures in Blacks and Whites in the East Baltimore sample composed of *TTC12* (rs2303380) and *ANKK1* (4938015, rs11604671) genes as seen in Figure 1. The *D*'s between rs2303380 and the other two SNPs were 0.825 and 0.780 for Blacks and 0.666 and 0.903 for Whites, respectively.

Association analysis of smoking phenotypes

Informative haplotypes in each population were then examined for association with daily smoking using longitudinal interview data in ECA W3 and W4 using the generalized linear model.

In the Black sample, using the generalized linear model, none of the haplotypes were associated with either having started to smoke or daily smoking at W3 or daily smoking at W4 among participants who smoked at W3. However, the GTG Haplotype $\times$ Sex interaction term indicated that Black male daily smokers at W3 who possessed the GTG haplotype were less likely to have remained smokers in W4 ([4/9, 44%] vs. [25/32] 78%, respectively)—implying smoking cessation (β = -2.94, *SE* = 1.34, *p* = .028), but Black women possessing the GTG haplotype (vs. other haplotypes) were more likely to be smoking at W4 if they were daily smokers at W3 ([19/24, 78.2%] vs. [57/75, 76.0%], respectively).

Among Whites, the GTG haplotype was associated with lifetime history of daily smoking (odds ratio [OR] = 1.6; 95% CI = 1.1–2.4; *p* = .013). In addition, there was a significant GTG Haplotype $\times$ Sex interaction such that men possessing the GTG haplotype (vs. other haplotypes) were more likely to have a lifetime history of daily smoking (i.e., smoking initiation; 59/76,

Table 2. Frequency of genotypes for examined markers and Hardy–Weinberg Equilibrium (HWE) Significance Test

Locus	Genotype	Blacks				Whites			
		Count	MAF	HWE χ^2	<i>p</i> value	Count	MAF	HWE χ^2	<i>p</i> value
<i>TTC12</i> gene									
rs2303380	A/A	87	0.497	0.039	.853	152	0.401	0.175	.651
	A/G	72	0.411			179	0.472		
	G/G	16	0.091			48	0.127		
	missing	199				283			
<i>ANKK1</i> gene									
rs4938015	C/C	56	0.316	2.295	.134	160	0.420	0.945	.323
	C/T	78	0.441			167	0.438		
	T/T	43	0.243			54	0.142		
	missing	197				281			
rs11604671	A/A	18	0.104	3.582	.054	86	0.227	3.724	.05
	A/G	59	0.341			169	0.446		
	G/G	96	0.555			124	0.327		
	missing	201				283			
rs1800497 “ <i>DRD2</i> -TaqIA”	A/A	22	0.124	5.933	.017	19	0.051	0.000	1.000
	A/G	60	0.339			131	0.348		
	G/G	95	0.537			226	0.601		
	NA	197				286			
<i>DRD2</i> gene									
rs6277 “C957T”	A/A	8	0.046	1.937	.118	119	0.313	0.705	.407
	A/G	45	0.259			180	0.474		
	G/G	121	0.695			81	0.213		
	missing	200				282			
rs1799732 Promoter “-141 ins/del”	0/0	33	0.191	3.995	.054	17	0.045	5.54	.023
	0/C	70	0.405			93	0.245		
	C/C	70	0.405			270	0.711		
	missing	201				282			

Note. Genotype frequencies and HWE equation statistics for Black (right) and White (left) participants. Reported frequencies reflect the entire sample (including participants with missing nongenetic data who were excluded from the haplotype analyses). Nucleotide base pairs listed according to antisense (3′–5′) sequence (www.hapmap.org)—thus, for example, conventional (5′–3′) rs1800497 T=A (TaqIA-A1); G=C (TaqIA-A2). NA = missing; MAF = mean allele frequency.

77.6% vs. 110/193, 57%; *OR* = 1.99, 95% *CI* = 1.02–5.10, *p* = .043). However, none of the haplotypes were associated with current daily smoking at the time of the W3 or W4 surveys.

Figure 2 illustrates the frequency of participants with history of daily smoking, current daily smoking at W3 and current daily smoking at W4 among individuals reporting daily smoking at W3, stratified by ethnicity and haplotype, respectively.

Discussion

These data demonstrated that in two different ethnic populations, *TTC12/ANKK1* rs2303380–rs4938015–rs11604671 haplotypes were associated with different phenotypes across a trajectory of lifetime smoking behavior. The GTG haplotype was associated with smoking initiation but only in White men. The same haplotype was associated with smoking cessation but only in Black men. Given the sample size of the stratified samples, the Haplotype $\times$ Sex interaction results are tentative and serve mainly as leads for future research.

It is notable, however, that in both populations, the GTG haplotype was associated with smoking behavior only in men. There are several, somewhat speculative but biologically plausible, explanations for the observed man–woman variation in haplotype associations.

In the larger family study, by Gelernter et al. (2006) cited above, the investigators observed that the strongest associations between SNPs in the *TTC12/ANKK1/DRD2* region with nicotine dependence were located in the *TTC12* and *ANKK1* genes and specifically with the rs2303380–rs4938012–rs4938015–rs11604671 haplotypes (Blacks: *p* = .0003; Whites: *p* = .0008). These investigators suggested that reported associations between other SNPs in this region (e.g., rs1800497/“TaqIA”) may actually be representing neighboring SNPs with *D*’s as high as 0.9–1.0 (Gelernter et al., 2006). If indeed the haplotype is associated with the same differences dopamine D2 receptor expression in the ventral striatum observed with the upstream rs1800497 SNP (Jonsson et al., 1999)—which we do not as yet know—then the observed directionality of the associations is convergent with results from our meta-analysis of rs1800497, indicating that the

Figure 2. (A) Frequency of self-reported history of ever having smoked daily assessed at Wave 3 stratified by haplotype and sex in Blacks and Whites. Vertical lines are standard error bars for proportions of ever-daily smokers. (B) Frequency of self-reported daily smoking in the last 7 days assessed at Wave 3 stratified by haplotype and sex in Blacks and Whites. Vertical lines are standard error bars for proportions of current daily smokers. (C) Frequency of self-reported daily smoking in the last 7 days assessed at Wave 4 if smoked at Wave 3 stratified by haplotype and sex in Blacks and Whites. Vertical lines are standard error bars for current daily smokers.

SNP or haplotype may “operate only in males or more strongly in males” (Munafo et al., 2009). However, given a lack of in vitro or in vivo studies of the influence of this haplotype on receptor expression or function, we cannot conclusively assert that the observed associations reflect genetically influenced neuroadaptations in the ventral striatum or elsewhere in the mesocorticolimbic system. However, if TaqIA is a proxy for the rs2303380–rs4938012–rs4938015–rs11604671 (or rs2303380–rs4938015–rs11604671) haplotypes, our observations of association in male subgroups would be consistent with the extant literature on TaqIA. As discussed in the introduction, sex differences have been demonstrated in nicotine sensitivity and cue reactivity and it is possible, especially given results of the Patch II trial finding that women with the low activity allele of TaqIA are more responsive to nicotine patch therapy for smoking cessation (Yudkin et al., 2004), that haplotypes moderate sex differences in smoking initiation and cessation. Sex differences in genetic moderation of nicotine dependence and smoking cessation, however, are an understudied domain in the field of nicotine dependence research and require extensive further study to better understand the etiology of observed man–woman variation in genetic association.

It is also possible that in addition to potentially different genetic influences on nicotine dependence and smoking cessation, cultural differences in access to smoking cessation resources, tobacco marketing, life stressors including economic and ethnic/racial disparities and prejudice and social norms for tobacco use in men and women at different times in the life cycle existed between Blacks and Whites in the ECA population under study; thereby, some of the variation in our findings may reflect variations in Gene $\times$ Environment interactions between subgroups.

It is not clear why the GTG haplotype would be associated with smoking initiation in Whites but not in Blacks and with W3–W4 smoking cessation in Blacks but not in Whites. The mean age of participants at W3 was in the late 40s and at W4 was in the late 50s (Table 1). While we do not know the motivators for participants’ smoking cessation during the course of the study, it is plausible that smokers who quit in early adulthood and early middle age are more likely to be motivated by social norms, while those quitting in late middle age may be more motivated by health concerns. One explanation is that there may be variation in Gene $\times$ Environment interactions operating between Blacks and Whites over the period of time the cohort was surveyed, which may be age related and influenced by social determinants influencing smoking behavior (e.g., life stressors or access to health care and smoking cessation resources). Furthermore, different LD patterns in the 11q23.1 region in Blacks and Whites may also portend different and untested epistatic relationships or effects on expression during neurodevelopment or neuroadaptations to chronic nicotine exposure.

Another explanation for differences in haplotype associations observed between Blacks and Whites may have to do with statistical power. Given the much smaller cell sizes in Blacks when conducting subgroup analyses by haplotype and sex, the observation of a greater reduction in smoking prevalence in one haplotype–sex stratum may either be a spurious finding resulting from chance or it may reflect an actual difference between haplotypes in smoking cessation.

As referenced above, the present study has several limitations, such as limited power to detect Gene $\times$ Sex interactions

due to the constrained ECA sample size of long-term survivors and when stratified by ethnic population, lack of sufficient data for nicotine dependence severity to incorporate in the analysis plan, and no biochemical verification of abstinence. By not including rs4938012 in the haplotype analyses, it is possible that the haplotype is less informative than the rs2303380–rs4938012–rs4938015–rs11604671 haplotypes examined by Gelernter et al. (2006). However, as in the prior work, our team observed that SNP demonstrated Mendelian inconsistencies; the 5’ nuclease results were abandoned in favor of a restriction fragment length polymorphism assay demonstrating consistency with HWE assumptions. We cannot be certain whether our observed deviation from HWE in both populations is the result of inaccurate assay results or violations of HWE assumptions. As such, it was best to remove this SNP from the analyses altogether.

However, the study also has several somewhat unique strengths, such as the sampling of Blacks and Whites, use of longitudinal data, and the empirical selection of SNPs within a gene region associated with nicotine dependence in previous studies of Blacks and Whites—which likely reflects a more precise assessment of the genetic variation within this gene cluster contributing to smoking behavior than the use of single candidate gene variants.

Given the complex interplay of potential environmental differences and social norms regarding tobacco use in different population groups, and many potential behavioral mediators of smoking, such as depression and comorbid use of alcohol and other drugs, additional studies are needed to further explore Gene $\times$ Environment interactions within this genomic region in Blacks and Whites. Moreover, replication is needed with larger sample sizes in order to confirm the generalizability of these findings to other Black and White populations.

In summary, these longitudinal ECA data suggest that the rs2303380–rs4938015–rs11604671 haplotype may be associated with becoming a daily smoker in White men and with smoking cessation in Black men, while the converse is apparent in women (i.e., GTG haplotype associated with not becoming a daily smoker in White women and with failure to quit daily smoking in Black women when compared with male counterparts, respectively). These results converge somewhat with the findings of Gelernter et al. (2006), who found associations between haplotypes consisting of these SNPs with nicotine dependence; they add to the increasing body of evidence implicating the 11q23.1 region in nicotine dependence and smoking cessation (David & Munafo, 2008; Gelernter et al., 2006; Uhl et al., 2008).

Funding

This study was supported by the National Institutes of Health–Intramural Research Program, National Institute on Drug Abuse (NIDA), Department of Health and Social Services (GRU); National Institutes on Mental Health (NIMH) grants R01-47447 and T32-14592; and Johns Hopkins Bloomberg School of Public Health Institutional Review Board H.33.01.03.26.A2 (WWE) and NIDA grants R01-DA026652 (WWE); K08-14276 and R21-DA027331 (SPD). Personal support to RN was provided by P50-CA84719.

Declaration of Interests

GRU is listed as an inventor for a patent application filed by Duke University based on genomic markers that distinguish successful quitters from unsuccessful quitters in data from other study samples.

Acknowledgments

We are also grateful to the ECA's principal collaborators (D. Regier, B. Locke, W.E., and J. Burke) and to Drs. M. Kramer, E. Gruenberg, and S. Shapiro from the Johns Hopkins site, supported by NIMH grant U01-33870. JCA directed the original Baltimore ECA sampling, recruitment, and assessment and was Co-Principal Investigator with WWE for the ECA follow-up research; his recent research is supported by a NIDA Senior Scientist award (K05-DA015799) and Michigan State University research funds. We are also grateful to G. Sullivan for editorial assistance.

References

- Abrous, D. N., Adriani, W., Montaron, M. F., Aourousseau, C., Rougon, G., Le Moal, M., et al. (2002). Nicotine self-administration impairs hippocampal plasticity. *Journal of Neuroscience*, 22, 3656–3662.
- Breen, G., Brown, J., Maude, S., Fox, H., Collier, D., Li, T., et al. (1999). -141 C del/ins polymorphism of the dopamine receptor 2 gene is associated with schizophrenia in a British population. *American Journal of Medical Genetics*, 88, 407–410.
- Burkett, K., Graham, J., & McNeney, B. (2006). hapassoc: Software for likelihood inference of trait associations with SNP haplotypes and other attributes. *Journal of Statistical Software*, 16(2), 1–19.
- Caraballo, R. S., Yee, S. L., Gfroerer, J., & Mirza, S. A. (2008). Adult tobacco use among racial and ethnic groups living in the United States, 2002–2005. *Preventing Chronic Disease*, 5, A78.
- Centers for Disease Control and Prevention. (1998). Tobacco use among U.S. racial/ethnic minority groups—African Americans, American Indians and Alaska Natives, Asian Americans and Pacific Islanders, Hispanics. A report of the Surgeon General. Executive summary. *Morbidity and Mortality Weekly Reports. Recommendations and Reports*, 47(RR-18), v–xv. 1–16.
- Centers for Disease Control and Prevention. (2002). Women and smoking: A report of the Surgeon General. Executive summary. *Morbidity and Mortality Weekly Report. Recommendations and Reports*, 51(RR-12), i–iv. 1–13.
- Cepeda-Benito, A., Reynoso, J. T., & Erath, S. (2004). Meta-analysis of the efficacy of nicotine replacement therapy for smoking cessation: Differences between men and women. *Journal of Consulting and Clinical Psychology*, 72, 712–722.
- David, S. P., Brown, R. A., Papandonatos, G. D., Kahler, C. W., Lloyd-Richardson, E. E., Munafo, M. R., et al. (2007). Pharmacogenetic clinical trial of sustained-release bupropion for smoking cessation. *Nicotine & Tobacco Research*, 9, 821–833.
- David, S. P., & Munafo, M. R. (2008). Genetic variation in the dopamine pathway and smoking cessation. *Pharmacogenomics*, 9, 1307–1321.
- David, S. P., Strong, D. R., Munafo, M. R., Brown, R. A., Lloyd-Richardson, E. E., Wileyto, P. E., et al. (2007). Bupropion efficacy for smoking cessation is influenced by the DRD2 Taq1A polymorphism: Analysis of pooled data from two clinical trials. *Nicotine & Tobacco Research*, 9, 1251–1257.
- Eaton, W. W., Kalaydjian, A., Scharfstein, D. O., Mezuk, B., & Ding, Y. (2007). Prevalence and incidence of depressive disorder: The Baltimore ECA follow-up, 1981–2004. *Acta Psychiatrica Scandinavica*, 116, 182–188.
- Eaton, W. W., Neufeld, K., Chen, L. S., & Cai, G. (2000). A comparison of self-report and clinical diagnostic interviews for depression: Diagnostic interview schedule and schedules for clinical assessment in neuropsychiatry in the Baltimore epidemiologic catchment area follow-up. *Archives of General Psychiatry*, 57, 217–222.
- Fu, S. S., Burgess, D. J., Hatsukami, D. K., Noorbaloochi, S., Clothier, B. A., Nugent, S., et al. (2008). Race and nicotine replacement treatment outcomes among low-income smokers. *American Journal of Preventive Medicine*, 35(Suppl. 6), S442–S448.
- Fu, S. S., Kodl, M. M., Joseph, A. M., Hatsukami, D. K., Johnson, E. O., Breslau, N., et al. (2008). Racial/ethnic disparities in the use of nicotine replacement therapy and quit ratios in lifetime smokers ages 25 to 44 years. *Cancer Epidemiology, Biomarkers and Prevention*, 17, 1640–1647.
- Gelernter, J., Yu, Y., Weiss, R., Brady, K., Panhuysen, C., Yang, B. Z., et al. (2006). Haplotype spanning TTC12 and ANKK1, flanked by the DRD2 and NCAM1 loci, is strongly associated to nicotine dependence in two distinct American populations. *Human Molecular Genetics*, 15, 3498–3507.
- Grandy, D. K., Litt, M., Allen, L., Bunzow, J. R., Marchionni, M., Makam, H., et al. (1989). The human dopamine D2 receptor gene is located on chromosome 11 at q22–q23 and identifies a TaqI RFLP. *American Journal of Human Genetics*, 45, 778–785.
- Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., & Fagerstrom, K. O. (1991). The Fagerstrom Test for Nicotine Dependence: A revision of the Fagerstrom Tolerance Questionnaire. *British Journal of Addiction*, 86, 1119–1127.
- Johnson, C., Dragon, T., Liu, Q. R., Zhang, P. W., Walther, D., Li, C. Y., Anthony, J. C., et al. (2008). *Biomed Central Medical Genetics*, 9, 113.
- Johnstone, E. C., Yudkin, P. L., Hey, K., Roberts, S. J., Welch, S. J., Murphy, M. F., et al. (2004). Genetic variation in dopaminergic pathways and short-term effectiveness of the nicotine patch. *Pharmacogenetics*, 14, 83–90.
- Jonsson, E., Sedvall, G., Brene, S., Gustavsson, J. P., Geijer, T., Terenius, L., et al. (1996). Dopamine-related genes and their relationships to monoamine metabolites in CSF. *Biological Psychiatry*, 40, 1032–1043.

- Jonsson, E. G., Nothen, M. M., Grunhage, F., Farde, L., Nakashima, Y., Propping, P., et al. (1999). Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Molecular Psychiatry*, 4, 290–296.
- Katoh, M., & Katoh, M. (2003). Identification and characterization of TPARM gene in silico. *International Journal of Oncology*, 23, 1213–1217.
- Kendzor, D. E., Cofta-Woerpel, L. M., Mazas, C. A., Li, Y., Vidrine, J. I., Reitzel, L. R., et al. (2008). Socioeconomic status, negative affect, and modifiable cancer risk factors in African-American smokers. *Cancer Epidemiology, Biomarkers and Prevention*, 17, 2546–2554.
- Lerman, C., Jepson, C., Wileyto, E. P., Epstein, L. H., Rukstalis, M., Patterson, F., et al. (2006). Role of functional genetic variation in the dopamine D2 receptor (DRD2) in response to bupropion and nicotine replacement therapy for tobacco dependence: Results of two randomized clinical trials. *Neuropsychopharmacology*, 31, 231–242.
- Lerman, C., Shields, P. G., Wileyto, E. P., Audrain, J., Hawk, L. H., Jr., Pinto, A., et al. (2003). Effects of dopamine transporter and receptor polymorphisms on smoking cessation in a bupropion clinical trial. *Health Psychology*, 22, 541–548.
- Lerman, C., Shields, P. G., Wileyto, E. P., Audrain, J., Pinto, A., Hawk, L., et al. (2002). Pharmacogenetic investigation of smoking cessation treatment. *Pharmacogenetics*, 12, 627–634.
- Li, M. D., Cheng, R., Ma, J. Z., & Swan, G. E. (2003). A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. *Addiction*, 98, 23–31.
- 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.
- Mezuk, B., Eaton, W. W., & Zandi, P. (2008). Participant characteristics that influence consent for genetic research in a population-based survey: The Baltimore epidemiologic catchment area follow-up. *Community Genetics*, 11, 171–178.
- Munafo, M., Bradburn, M., Bowes, L., & David, S. (2004). Are there sex differences in transdermal nicotine replacement therapy patch efficacy? A meta-analysis. *Nicotine & Tobacco Research*, 6, 769–776.
- Munafo, 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.
- Munafo, M. R., Timpson, N. J., David, S. P., Ebrahim, S., & Lawlor, D. A. (2009). Association of the DRD2 gene Taq1A polymorphism and smoking behavior: A meta-analysis and new data. *Nicotine & Tobacco Research*, 11, 64–76.
- 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 Mutation*, 23, 540–545.
- Nguyen, C., Mattei, M. G., Mattei, J. F., Santoni, M. J., Goridis, C., & Jordan, B. R. (1986). Localization of the human NCAM gene to band q23 of chromosome 11: The third gene coding for a cell interaction molecule mapped to the distal portion of the long arm of chromosome 11. *Journal of Cell Biology*, 102, 711–715.
- Niaura, R., Shadel, W. G., Abrams, D. B., Monti, P. M., Rohsenow, D. J., & Sirota, A. (1998). Individual differences in cue reactivity among smokers trying to quit: Effects of gender and cue type. *Addictive Behaviors*, 23, 209–224.
- Noble, E. P., Blum, K., Ritchie, T., Montgomery, A., & Sheridan, P. J. (1991). Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. *Archives of General Psychiatry*, 48, 648–654.
- Noble, E. P., Gottschalk, L. A., Fallon, J. H., Ritchie, T. L., & Wu, J. C. (1997). D2 dopamine receptor polymorphism and brain regional glucose metabolism. *American Journal of Medical Genetics*, 74, 162–166.
- Perkins, K. A. (1999). Nicotine self-administration. *Nicotine & Tobacco Research*, 1(Suppl. 2), S133–S137; discussion S139–S140.
- Perkins, K. A. (2001). Smoking cessation in women. Special considerations. *Central Nervous System Drugs*, 15, 391–411.
- Perkins, K. A., D'Amico, D., Sanders, M., Grobe, J. E., Wilson, A., & Stiller, R. L. (1996). Influence of training dose on nicotine discrimination in humans. *Psychopharmacology*, 126, 132–139.
- Perkins, K. A., & Scott, J. (2008). Sex differences in long-term smoking cessation rates due to nicotine patch. *Nicotine & Tobacco Research*, 10, 1245–1250.
- Primack, B. A., Bost, J. E., Land, S. R., & Fine, M. J. (2007). Volume of tobacco advertising in African American markets: Systematic review and meta-analysis. *Public Health Reports*, 122, 607–615.
- Regier, D. A., Myers, J. K., Kramer, M., Robins, L. N., Blazer, D. G., Hough, R. L., et al. (1984). The NIMH epidemiologic catchment area program. Historical context, major objectives, and study population characteristics. *Archives of General Psychiatry*, 41, 934–941.
- Shi, M. M., Myrand, S. P., Bleavins, M. R., & de la Iglesia, F. A. (1999). High throughput genotyping for the detection of a single nucleotide polymorphism in NAD(P)H quinone oxidoreductase (DT diaphorase) using TaqMan probes. *Molecular Pathology*, 52, 295–299.
- Swan, G. E., Jack, L. M., Valdes, A. M., Ring, H. Z., Ton, C. C., Curry, S. J., et al. (2007). Joint effect of dopaminergic genes on likelihood of smoking following treatment with bupropion SR. *Health Psychology*, 26, 361–368.
- Swan, G. E., Valdes, A. M., Ring, H. Z., Khroyan, T. V., Jack, L. M., Ton, C. C., et al. (2005). Dopamine receptor DRD2 genotype and smoking cessation outcome following treatment with bupropion SR. *Pharmacogenomics Journal*, 5, 21–29.
- Uhl, G. R., Liu, Q. R., Drgon, T., Johnson, C., Walther, D., Rose, J. E., et al. (2008). Molecular genetics of successful smoking cessation: Convergent genome-wide association study results. *Archives of General Psychiatry*, 65, 683–693.

World Health Organization. (2009). Tobacco key facts. Retrieved 6 April 2009, from <http://www.who.int/topics/tobacco/facts/en/index.html>

Yang, B. Z., Kranzler, H. R., Zhao, H., Gruen, J. R., Luo, X., & Gelernter, J. (2007). Association of haplotypic variants in DRD2, ANKK1, TTC12 and NCAM1 to alcohol dependence in independent case control and family samples. *Human Molecular Genetics*, 16, 2844–2853.

Yang, B. Z., Kranzler, H. R., Zhao, H., Gruen, J. R., Luo, X., & Gelernter, J. (2008). Haplotypic variants in DRD2, ANKK1, TTC12, and NCAM1 are associated with comorbid alcohol and drug dependence. *Alcoholism, Clinical and Experimental Research*, 32, 2117–2127.

Yudkin, P., Munafo, M., Hey, K., Roberts, S., Welch, S., Johnstone, E., et al. (2004). Effectiveness of nicotine patches in relation to genotype in women versus men: Randomised controlled trial. *British Medical Journal*, 328, 989–990.

Zandi, P. P., Avramopoulos, D., Willour, V. L., Huo, Y., Miao, K., Mackinnon, D. F., et al. (2007). SNP fine mapping of chromosome 8q24 in bipolar disorder. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, 144B, 625–630.

Zandi, P. P., Badner, J. A., Steele, J., Willour, V. L., Miao, K., MacKinnon, D. F., et al. (2007). Genome-wide linkage scan of 98 bipolar pedigrees and analysis of clinical covariates. *Molecular Psychiatry*, 12, 630–639.

Zandi, P. P., Belmonte, P. L., Willour, V. L., Goes, F. S., Badner, J. A., Simpson, S. G., et al. (2008). Association study of Wnt signaling pathway genes in bipolar disorder. *Archives of General Psychiatry*, 65, 785–793.

Zeman, M. V., Hiraki, L., & Sellers, E. M. (2002). Gender differences in tobacco smoking: Higher relative exposure to smoke than nicotine in women. *Journal of Women's Health and Gender-Based Medicine*, 11, 147–153.