ORIGINAL RESEARCH

Predictors of Relapse in a Bupropion Trial for Smoking Cessation in Recently-Abstinent Alcoholics: Preliminary Results Using an Aggregate Genetic Risk Score

John E. McGeary[1], Valerie S. Knopik[2], John E. Hayes[3], Rohan H. Palmer[2], Peter M. Monti[4] and David Kalman[5]

[1]Providence Veterans Affairs Medical Center, Division of Behavioral Genetics, Rhode Island Hospital and Center for Alcohol and Addiction Studies, Brown University. [2]Division of Behavioral Genetics, Rhode Island Hospital, Department of Psychiatry and Human Behavior, Brown University. [3]Department of Food Science, Pennsylvania State University. [4]Center for Alcohol and Addiction Studies, Brown University. [5]University of Massachusetts Medical School.
Corresponding author email: john_mcgeary@brown.edu

Abstract

Introduction: Rates of smoking in the US population have decreased overall, but rates in some groups, including alcoholic smokers, remain high. Many newly sober alcoholics are concerned about their smoking and some attempt to quit. However, quit rates in this population are low. Prior studies suggest risk for relapse in this population may be genetically influenced and that genetic factors may moderate response to treatment.

Methods: In this exploratory study, we had two specific aims: (1) to investigate associations between genetic risk and outcome; (2) to investigate whether genetic risk moderates the efficacy of a medication intervention. Data are from a subsample of 90 participants from a clinical trial of smoking cessation treatment for smokers with between 2 and 12 months of alcohol abstinence. Subjects were randomly assigned to bupropion or placebo. All subjects received counseling and nicotine patches. To examine the possibility that bupropion may have been efficacious in participants with a specific genetic profile (ie, a pharmacogenetic approach), an aggregate genetic risk score was created by combining risk genotypes previously identified in bupropion treatment studies.

Results: Although medication efficacy was not moderated by the aggregate genetic risk score, there was an interaction between nicotine dependence and genetic risk in predicting smoking abstinence rates at the end of treatment (10 weeks).

Conclusions: Results suggest an aggregate genetic risk score approach may have utility in treatment trials of alcoholics who smoke. Additionally, these findings suggest a strategy for understanding and interpreting conflicting results for single genetic markers examined as moderators of smoking cessation treatment.

Keywords: nicotine dependence, pharmacogenetics, bupropion

Public health efforts to reduce smoking have been broadly successful in the United States, but smoking rates remain high in some groups. Alcohol dependent individuals are one such group with estimated smoking prevalence between 45%–80% (see[1] for review). It is unclear whether individuals in alcohol and/or drug recovery differ from other smokers in their ability to quit smoking with some reports finding no difference (e.g.,[2,3])and others finding that it may be particularly difficult for persons who have achieved short-term abstinence from alcohol and other drugs to quit smoking.[3] This apparent discrepancy may be explained by genetic variability across study populations. Treatment efficacy may be improved by identifying factors associated with outcome and, in particular, factors that affect outcome by moderating response to treatment.

Numerous studies have demonstrated that the efficacy of smoking cessation medications is moderated by genetic variation.[4,5] These studies have focused on single genetic variants. A problem with this approach is that the examination of any single variant may be obscured by noise in other variants (eg, the effects of one isolated risk allele may be overshadowed by an aggregate effect of several risk and/or protective variants).[6]

The use of an aggregate genetic risk score (AGRS), which considers the collective impact of several variants, is a promising approach for dealing with this problem. This method assumes a simple additive model and relies on prior knowledge of risk alleles (in contrast to the atheoretical approach of genome wide association). Moreover, it has the additional benefit of providing greater statistical power than does modeling each variant individually.[7]

In this smoking cessation study of recently abstinent alcoholics receiving bupropion plus nicotine replacement therapy and counseling,[8] an AGRS was formed to examine whether response to bupropion was moderated by genetic risk. Review of the literature revealed four candidate genes that had been identified as relevant to bupropion response in at least two separate studies. The 10-repeat allele of the 3′ UTR variable number of tandem repeats polymorphism (VNTR) in the dopamine transporter (*SLC6A3*) gene has been associated with abstinence in two studies of bupropion for smoking cessation.[9,10]

The Val158Met polymorphism in the gene for Catechol-O-methyltransferase (*COMT*) has also been identified as having a moderating influence in studies of bupropion.[9,11] The single nucleotide polymorphism rs1800497 in the ankyrin repeats and kinase domain containing gene 1 (*ANKK1*) historically associated with the *DRD2* gene is associated with bupropion response.[12,13] Finally the long (7 or greater repeat) allele of the exon 3 VNTR polymorphism of the dopamine D4 receptor gene (*DRD4*) has been associated with bupropion response.[14] Bupropion is a noncompetitive antagonist of nicotinic acetylcholine receptors that also inhibits dopamine reuptake.[15] This suggests that genetic variation in the nicotinic acetylcholine system may be important to moderating bupropion's effects. However, we elected to focus on the AGRS strategy using the criteria defined above and at the time of analysis, there was not sufficient evidence in the literature to include variants in nicotine receptor genes.

Method
Participants
Participants in the present study were recruited from a Veterans Administration Medical Center to participate in a double-blind, placebo-controlled smoking cessation clinical trial of smokers with a recent history of alcohol dependence. All participants provided written informed consent, and the study was approved by the Institutional Review Board of the University of Massachusetts Institutional Review Board and the Edith Nourse Rogers Veterans Administration Hospital. Participants were randomly assigned to bupropion or placebo for eight weeks. Participants began study medication (bupropion 150 mg SR-sustained release tablets or placebo) 1 week prior to their quit day. Active and placebo medications were identical in appearance. Participants were instructed to take one tablet per day for 3 days and then one 150-mg tablet twice per day for the remainder of the treatment phase of the study. They were instructed to quit smoking 1 week after they began study medication. In addition, all participants received the nicotine patch for 7 weeks starting on their quit day. They received the 21-mg patch for 4 weeks, the 14-mg patch for 2 weeks and the 7-mg patch for 1 week. All participants received the nicotine patch for seven weeks starting on their quit day as well as eight weekly counseling sessions starting one week prior to their quit day.[8]

To be eligible for the trial, participants must have smoked at least 10 cigarettes per day, have had a history of DSM-IV alcohol abuse or dependence and have had between 2 and 12 months of abstinence from alcohol prior to enrollment. The Alcohol Disorders section of the Structured Clinical Interview for Diagnosis for DSM-IV (First, Spitzer)[16] was administered to establish a diagnosis of alcohol use disorder. Exclusion criteria were: (1) older than age 70; (2) diagnosis of schizophrenia; (3) current psychotic episode; (4) cardiac problems in the past 3 months; (5) uncontrolled hypertension; (6) history of seizure; (7) history of head injury (8) use of medications that lower the seizure threshold (additional details related to baseline characteristics and inclusion/exclusion criteria may be found in[8]). Of the 144 participants in the main trial, 90 provided a DNA sample for use in the genetic substudy.

Measures

Smoking outcomes and nicotine dependence

Days abstinent smoking were calculated based upon data collected using the Timeline Follow Back measured at end of treatment (8 weeks following initiation of bupropion/placebo treatment) and at an eleven week follow-up. Consistent with the recommendations of the Society for Research on Nicotine and Tobacco expert panel,[17,18] participants were considered abstinent if they self reported complete abstinence on each of 7 days prior to the time of assessment and had salivary cotinine levels of less than or equal to 15 ng/mL. The Fagerstrom Test for Nicotine Dependence (FTND) was administered at the baseline assessment.[19] For purposes of this report, we used the entire range of Fagerstrom scores as psychometric data collected on this measure is based upon the sum scores (e.g.,[20]).

Genotypes and aggregate genetic risk score

Genomic DNA was collected and extracted from buccal cells using methods described previously.[21] Assays for the *DRD4* exon 3 VNTR and *SLC6A3* 9/10 repeat allele were conducted as described in[22], with each *DRD4* allele ≥ 7 repeats being considered a risk allele for the AGRS and each 9 repeat allele being a risk factor for SLC6A3. The *COMT*

Val158Met and *DRD2* TaqIA genotypes were obtained using the Taqman approach for rs4680 and rs1800497, respectively. Risk alleles for the AGRS were assigned as each copy of the Met COMT allele and each copy of the *DRD2* TaqIA allele.

An aggregate genetic risk score (AGRS) was then calculated for each participant. The AGRS included genotypes of candidate polymorphisms that were identified as important candidates in bupropion response in at least two smoking cessation trials (ie, *SLC6A3* VNTR, *DRD4* exon 3 VNTR, *COMT* Val158Met polymorphism and *DRD2* TaqIA as outlined above).

The simple count method was utilized to calculate the AGRS as the meta-analyses typically used for determining relative weights (e.g.,[23]) are not available in the bupropion literature. Assumptions inherent to the count method include an additive genetic model and equivalent effects of each polymorphism on smoking cessation outcome. This AGRS model also does not allow for epistatic effects. For each identified polymorphism, a participant was given a risk allele score of 0, 1, or 2 corresponding to the number of putative risk alleles they possess. The risk allele scores across each of the four candidate genes were summed to create an AGRS following methods described in[24] (ie, AGRS = [sum of risk allele scores/number of non-missing genotypes × 2] × 8).

Demographic factors

Gender, ethnic ancestry, marital status, employment status, occupation, and income were assessed via self report.

Statistical considerations

As is typical for event occurrence data, our main outcome variable—number of days until relapse—was not normally distributed. Specifically, data obtained for this measure were found to be zero-inflated (due to those participants who did not stop smoking) and right censored (14.6% of observations were censored due to limited assessments over time that did not characterize relapse in all participants). Thus, survival methods were used to examine predictors of risk to relapse.

Kaplan–Meier estimates of the time to relapse were computed. Next, the association between time to relapse and genetic and psychiatric predictors, as

well as treatment condition, was then investigated using Cox regression models. Such models estimate a hazard ratio, which is the ratio of the estimated hazard (ie, probability of relapse) for those with the covariate (eg, genetic risk score, Fagerstrom nicotine dependence) to those without the covariate.

Results

Descriptive data

Ninety participants were included in the analyses. Participants were 84% male, 68% of mixed European ancestry, 24% African American and the remainder Hispanic/Other. The mean age of participants was 50 (SD = 6.9). Participants smoked on average 21.3 cigarettes per day (SD = 11.7) when they entered the study. With regard to the level of drinking participants reported in the month prior to achieving abstinence from alcohol, 42% reported drinking fewer than 8 drinks/day, 31% reported 8–14 drinks/day, 17% reported 15–28 drinks/day, and 10% reported more than 28 drinks/day. The mean Fagerstrom score was 5.4 (SD = 1.8).

AGRS effects on time to relapse

We fitted two multivariate Cox regression models to the data (Table 1). Model I included the main effects of AGRS, medication condition, gender, the FTND score, and the interaction between AGRS and FTND. Model II included all predictors except the interaction term, thus serving as a test comparative model that assumes no higher-order effects. Submodels were compared by examining the difference in the (-2LL) estimates that are distributed as a Chi-square with degrees of freedom equivalent to the differences in degrees of freedom between the models. Models were also compared using their

Akaike Information Criterion (AIC).[25] The results indicated that model I (-2 LL) = 368.20 degrees of freedom (df) = 5, AIC = 378.20) provided a better fit to the data than model II (-2 LL = 373.97, df = 4, AIC = 381.99) (ie, $\Delta\chi^2$ = 5.79, Δdf = 1, $P < 0.05$; ΔAIC = 3.79); that is, the removal of the interaction term worsened the fit of the model. The results of the best fitting model (Model I) indicated that increasing levels of genetic risk (as measured by the AGRS) (Hazard Ratio (HR) = 1.97, 95% Confidence Interval (CI) = 1.13–3.43), as well as increasing levels of nicotine dependence (as measured by the FTND) (HR = 1.63, CI = 1.12–2.37) are associated with a higher likelihood of relapse. Moreover, the absence of both high levels of nicotine dependence and genetic risk is associated with the best outcome (ie, decreased likelihood of relapse); see Figure 1.

Figure 1 best illustrates effects of the interaction term. For instance, close inspection of these results suggests that at low and moderate levels of nicotine dependence, possessing higher values on the genetic risk score increases risk for relapse. Conversely, at high levels of nicotine dependence, the genetic risk appears to paradoxically protect against relapse (ie, as genetic risk score increases the risk for relapse decreases). This interactive effect is supported in Figure 1 which shows the cumulative incidence of risk for relapse as a function of the median split of both the aggregate genetic risk score (low vs. high risk) and Fagerstrom scores (low vs. high scores on nicotine dependence items). Although this interaction is significant, the confidence interval for Hazard Ratio corresponding to the interaction term is very close to including one and should be interpreted with caution, at least regarding this paradoxical effect.

Table 1. Predictors of relapse to smoking after 10 weeks (70 days).

	Model I (with interaction)		Model II (without interaction)	
	Hazard ratio	95% CI	Hazard ratio	95% CI
Bupropion treatment	0.78	0.49–1.24	0.77	0.49–1.24
Gender- male	1.10	0.52–1.97	1.08	0.53–2.00
Genetic risk score	1.97**	1.13–3.43	1.06	0.91–1.24
Nicotine dependence (ND)#	1.63**	1.12–2.37	1.05	0.94–1.19
Genetic risk score by ND*	0.90**	0.82–0.98	–	–

Notes: Hazard ratios estimated from Cox regression models. #Measured by fagerstrom test for nicotine dependence; **$P \leq 0.01$.
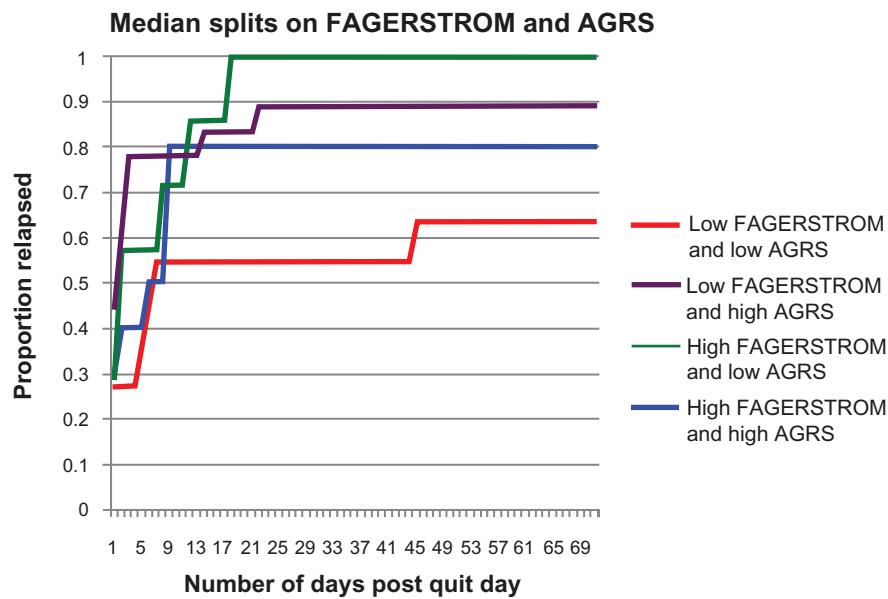
Median splits on FAGERSTROM and AGRS

Figure 1. Survival curves based upon median splits of Fagerstrom scores and AGRS used for visual representation of the data.

Nonetheless, the finding at low and medium levels of nicotine dependence is notable.

Discussion

We found no evidence that genetic variation predicts a differential response to bupropion for smoking cessation in this sample of recently abstinent alcoholics. Despite the combination of patch plus bupropion vs. patch alone being largely ineffective for promoting smoking cessation in this population, an AGRS (created by examining genetic variation previously identified in at least two bupropion trials) interacted with nicotine dependence level to be associated with smoking cessation status at the end of treatment. Given that this AGRS does not interact with the medication condition, the implication may be that this AGRS by dependence interaction may actually be associated with the platform treatment of counseling + NRT (Nicotine Replacement Therapy). It is interesting that the candidates selected for inclusion in the AGRS were identified in pharmacogenetic trials of bupropion while the AGRS by dependence interaction moderated treatment response independently of medication condition. It is possible that a particular combination of alterations in the dopaminergic system may not only put one at risk for the development of comorbid alcohol and nicotine dependence, but also be related to response to standard smoking cessation treatments for individuals at varying levels of dependence.

The tendency for both dependence level and AGRS to decrease time to relapse is consistent with the predicted directions (ie, higher dependence and higher genetic risk being associated with poorer treatment outcome). The interaction term suggests a slightly protective effect of genetic risk at high levels of dependence and may suggest that the platform treatment of counseling and nicotine patch that bupropion was added to in this trial may be optimized for those highly dependent individuals who carry high levels of dopaminergic genetic risk (conversely those participants who become highly dependent through etiological pathways not indexed by this dopaminergic AGRS may require a different treatment approach than this standard of care). This is more difficult to explain and may not be worthy of interpretation as the confidence interval for this effect was very close to including 1. We also note that this seemingly paradoxical effect could be an artifact of low power: by definition very few participants would score either very high or very low on the AGRS scale (ie, this reflects the need to multiply the minor allele frequencies of each variant together to determine the proportion of the sample that will score in the highest AGRS category, with the result that the distribution for AGRS scores in a population will necessarily be leptokurtic).

Thus, the veracity of this finding should be interpreted with caution, pending future replication.

Nonetheless, the use of an AGRS approach may provide fresh insight into the mixed findings in the smoking cessation literature when a single variant is examined in isolation. Although it has long been known in the animal literature that genetic alterations may have different effects on phenotype depending upon the strains used, this approach is among the earlier attempts to account for the genetic 'background' that may influence single polymorphism investigations in humans. This concept is nicely demonstrated in a study of the genetic contributions to pain wherein main effects of a single variant are obscured when confounding influences of other associated polymorphisms are not accounted for.[6] It is important to note however that the AGRS approach in this instance is completely dependent upon prior studies performed to examine single polymorphisms, which speaks to the need for continued efforts in this area. It is conceivable that a more broadly-based AGRS that included additional variants implicated by bupropion's pharmacology would have improved the ability of the AGRS to moderate treatment. As sufficient sample sizes are gathered to allow more atheoretical variant finding approaches such as genome wide association and next generation sequencing to be performed, the utility of the AGRS approach will be further enhanced as currently unidentified variants may be added to the score. As further refinements are made in disentangling single genetic markers with reliable effects on smoking related phenotypes (eg, bupropion response), the utility of the AGRS approach will potentially increase.

The AGRS approach has limitations in that it is not necessarily a candidate system approach (although in this case all of the included candidate polymorphisms are related to the dopamine system). Additionally, the AGRS score in this study assumed equal weights as sufficient data on pharmacogenetic predictors of bupropion response to assign relative weights were not available. Furthermore the AGRS approach used in this study assumes additive effects and does not allow for epistasis. Nevertheless, the approach has strengths in allowing for the complex genetics of smoking cessation to be modeled in a more statistically and logically tractable fashion. This study has limitations, such as a limited sample size, and a reliance on existing genetic markers related to bupropion

response. The *DRD2* TaqIA marker, for example is unlikely to be a causal variant but may be in linkage disequilibrium with other putatively functional variants nearby (e.g.,[26]). Similarly, variation in the *DRD4* gene may be more complex than the traditional binning methods and merit alternative coding strategies.[21] Moreover population stratification of individual variants may artificially inflate or reduce the AGRS with the potential consequence of Type 1 or Type 2 errors. Additional research is indicated to determine whether these findings are supported in larger trials of both alcoholic and nonalcoholic samples. Interestingly, these replications attempts may be possible by leveraging existing bupropion trials, either via additional genotyping, or by creating an aggregate risk score using existing data.

Author Contributions

Conceived and designed the experiments: JEM, VSK, JEH, PMM, DK. Analysed the data: JEM, VSK, JEH, PMM. Wrote the first draft of the manuscript: JEM, VSK, JEH, PMM, DK. Contributed to the writing of the manuscript: JEM, VSK, JEH, RHP, PMM, DK. Agree with manuscript results and conclusions: JEM, VSK, JEH, RHP, PMM, DK. Jointly developed the structure and arguments for the paper: JEM, VSK, JEH, RHP, PMM, DK. Made critical revisions and approved final version: JEM, VSK, JEH, RHP, PMM, DK. All authors reviewed and approved of the final manuscript.

Funding

Competing Interests

Author(s) disclose no potential conflicts of interest.

Acknowledgments

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

References

1. Kalman D, Kim S, Digirolamo G, Smelson D, ZiedonisD. Addressing tobacco use disorder in smokers in early remission from alcohol dependence: The case for integrating smoking cessation services in substance use disorder treatment programs. *Clinical Psychology Review*. 2009;30:12–24. doi:10.1016/j.cpr.2009.08.009.

2. Hughes JR & Kalman D. Smokers with alcohol problems have more difficulty quitting? *Drug and Alcohol Dependence*. 2006; 82:91–102.

3. Prochaska J, Delucchi K, Hall, S. A meta-analysis of smoking cessation interventions with individuals in substance abuse treatment or recovery. J. Consult. Clin. Psychol. 2004; 72:1144-56.

4. Heitjan DF, Guo M, Ray R, Wileyto EP, Epstein LH, Lerman C. Identification of pharmacogenetic markers in smoking cessation therapy. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2008;147B(6):712–9.doi:10.1002/ajmg.b.30669.

5. Kortmann GL, Dobler CJ, Bizarro L, Bau CH. Pharmacogenetics of smoking cessation therapy. *American Journal* of *Medical Genetics Part B*: *Neuropsychiatric Genetics*. 2010;153B(1):17–28. doi:10.2217/PGS.09.176.

6. Lötsch J, Flühr K, Neddermayer T, Doehring A, Geisslinger G. The consequence of concomitantly present functional genetic variants for the identification of functional genotype-phenotype associations in pain. *Clinical Pharmacology and Therapeutics*. 2009;85(1):25–30. doi:10.1038/clpt.2008.103.

7. Plomin R, Haworth CM, Davis OS. Common disorders are quantitative traits. *Nature Reviews Genetics*. 2009;10(12):872–8. doi:10.1038/nrg2670.

8. Kalman D, Herz L, Monti P, et al. Incremental efficacy of adding bupropion to the nicotine patch for smoking cessation in smokers with a recent history of alcohol dependence: results from a randomized, double-blind, placebo-controlled study. *Drug Alcohol Depend*. Nov 1, 2011;118(2–3):111–8.

9. Han DH, Joe KH, Na C, Lee YS. Effect of genetic polymorphisms on smoking cessation: a trial of bupropion in Korean male smokers. *Psychiatric Genetics*. 2008;18(1):11–6. doi:10.1097/YPG.0b013e3282df0939.

10. Lerman C, Shields PG, Wileyto EP, et al. Effects of dopamine transporter and receptor polymorphisms on smoking cessation in a bupropion clinical trial. *Health Psychology*. 2003;22(5):541–8. doi:10.1037/0278-6133.22.5.541.

11. Berrettini WH, Wileyto EP, Epstein L, et al. Catechol-O-methyltransferase (COMT) gene variants predict response to bupropion therapy for tobacco dependence. *Biological Psychiatry*. 2007;61(1):111–8. doi:10.1016/j.biopsych.2006.04.030.

12. David SP, Strong DR, Munafò MR, et al. Bupropion efficacy for smoking cessation is influenced by the DRD2 Taq1A polymorphism: analysis of pooled data from two clinical trials. *Nicotine and Tobacco Research*. 2007;9(12):1251–7. doi:10.1080/14622200701705027.

13. Swan GE, Valdes AM, Ring HZ, et al. Dopamine receptor DRD2 genotype and smoking cessation outcome following treatment with bupropion SR. *Pharmacogenomics Journal*. 2005;5(1):21–9.

14. Leventhal AM, David SP, Brightman M, et al. Dopamine D4 receptor gene variation moderates the efficacy of bupropion for smoking cessation. *Pharmacogenomics J*. Jul 27, 2010. [Epub ahead of print] PubMed PMID: 20661272.

15. Slemmer JE, Martin BR, Damaj MI. Bupropion is a nicotinic antagonist. *J Pharmacol Exp Ther*. Oct 2000;295(1):321–7. PubMed PMID: 10991997.

16. First MB, Spitzer RL, Williams JB, Gibbon M. Structured Clinical Interview for DSM-IV (SCID). New York State Psychiatric Institute, New York. 1995.

17. SRNT Subcommittee on Biochemical Verification, Biochemical verification of tobacco use and cessation. *Nicotine Tob Res*. 2004;4(2):149–59.

18. Hughes JR, Keely JP, Niaura RS, et al Measures of abstinence in clinical trials: issues and recommendations. *Nicotine and Tobacco Research*. 2003;5(1):13–25. doi:10.1080/1462220031000070552.

19. Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO. The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire. *British Journal of Addiction*. 1991;86(9):1119–27. doi:10.1186/1471-2458-9-493.

20. Buckley TC, Mozley SL, Holohan DR, Walsh K, Beckham JC, Kassel JD. A psychometric evaluation of the Fagerstrom Test for Nicotine Dependence in PTSD smokers. *Addictive Behaviors*. 2005;30(5):1029–33. doi:10.1016/j.addbeh.2004.09.005.

21. McGeary, JE. The DRD4 exon 3 VNTR polymorphism and addiction-related phenotypes: A review. Pharmacology, Biochemistry and Behavior, Sep 2009;93(3):222-9.

22. Anchordoquy HC, McGeary C, Liu L, Krauter KS, Smolen A. Genotyping of three candidate genes after whole-genome preamplification of DNA collected from buccal cells. *Behavior Genetics*. 2003;33(1):73–8. doi:10.1023/A:1021007701808.

23. Lin X, Song K, Lim N, et al. Risk prediction of prevalent diabetes in a Swiss population using a weighted genetic score—the CoLaus Study. *Diabetologia*. 2009;52(4):600–8. doi:10.1007/s00125-008-1254-y.

24. Cornelis MC, Qi L, Zhang C, et al. Joint effects of common genetic variants on the risk for type 2 diabetes in US men and women of European ancestry. *Annals of Internal Medicine*. 2009;150(8):541–50. doi:150:541–550.

25. Akaike H. Factor analysis and AIC. *Psychometrika*. 1987;52:317–32.

26. Dick DM, Wang JC, Plunkett J, et al. Family-based association analyses of alcohol dependence phenotypes across DRD2 and neighboring gene ANKK1. *Alcoholism: Clinical and Experimental Research*. 2007;31(10):1645–53. doi:10.1111/j.1530-0277.2007.00470.x.