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Alpha-5 and -3 nicotinic receptor gene variants predict nicotine dependence but not cessation: Findings from the COMMIT cohort

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Abstract

Smoking many cigarettes per day (CPD) and short interval to first cigarette (TTF) after waking are two of the most heritable smoking phenotypes and comprise the Heavy Smoking Index (HSI). These phenotypes are often used as proxies for nicotine dependence (ND) and are associated with smoking cessation outcomes. Case-control and genome-wide association studies have reported links between single nucleotide polymorphisms (SNPs) in the alpha-5 and -3 nicotinic receptor subunit (CHRNA5 and CHRNA3) genes and CPD but few have examined TTF or cessation outcomes. In this study we longitudinally assessed 1301 European-American smokers at four time-points from 1988 to 2005. One CHRNA5 (rs16969968) and two CHRNA3 (rs1051703, rs6495308) SNPs were examined for their ability to predict smokers who 'ever' reported ND based on three phenotypic classifications: 1) 25+ CPD, 2) TTF < 10 minutes, and 3) HSI ≥ 4. In a subsample of 1157 quit attempters, we also examined each SNP's ability to predict 'ever' quitting for a period of >6 months. Demographically adjusted logistic regressions showed significant allelic and genotypic associations between all three SNPs and CPD but not TTF, HSI, or smoking cessation. Carriers of both the rs16969968-AA and rs6495308-TT genotypes had approximately two-fold greater odds for ND defined using CPD or TTF. Results suggest nicotinic receptor variants are associated with greater odds of ND according to CPD and to a lesser extent TTF. Research examining the effect of nicotinic receptor genetic variation on ND phenotypes beyond CPD is warranted.

Keywords

Cholinergic; Nicotinic; Allele; Dependence; Cessation

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Declaration of Interest: None

INTRODUCTION

Cigarette smoking is one of the most preventable causes of illness and premature death. Although public health efforts have made significant progress in reducing consumption, it is estimated that 1.6-1.9 billion people worldwide will be smokers by 2025 (Guindon, 2003) and by 2030 the death toll will rise to eight million per year (Tobacco key facts, 2011). A large proportion of these deaths will be attributed to lung cancer; and smoking significantly increases this risk (Samet, 1993). Thus, research aimed at elucidating the mechanisms that lead to nicotine dependence and/or cessation could aid in preventing and treating cigarette smoking and subsequently reduce premature illness and death.

Previous research suggests that in addition to environmental factors, genetic variation contributes significantly to smoking behavior (e.g. consumption, cessation) (Batra et al., 2003; Heath and Martin, 1993; Kendler et al., 1999; Sullivan and Kendler, 1999; True et al., 1997). Recently, several case-control and genome-wide association studies have reported associations between single nucleotide polymorphisms (SNPs) on chromosome 15 in the alpha5-alpha3-beta4 (CHRNA5-CHRNA3-CHRNA4) family of nicotinic receptor genes and smoking behavior phenotypes (Tobacco and Genetics Consortium, 2010; Berrettini et al., 2008; Bierut, 2009; Caporaso et al., 2009; Chen et al., 2009; Saccone et al., 2009a; Saccone et al., 2009b; Saccone et al., 2007; Stevens et al., 2008; Thorgeirsson et al., 2008; Weiss et al., 2008). In these studies, a non-synonymous SNP (rs16969968) in CHRNA5 as well as one synonymous (rs1051730) and one intronic SNP (rs6495308) in CHRNA3 have consistently been associated with a variety of smoking phenotypes, particularly cigarettes per day (CPD). CPD is one of two items included in the commonly used Heaviness of Smoking Index (HSI) (Heatherton et al., 1989). Interestingly, time to first cigarette (TTF), which is the other item of the HSI, has received limited examination in gene association studies. This is despite evidence that the CPD measure used alone under-classifies persons with nicotine dependence (ND), (de Leon et al., 2003) and that TTF is a well-established, reliable, and valid indicator of ND and cessation (Baker et al., 2007; Fagerstrom, 2003) with high heritability (Haberstick et al., 2007; Lessov et al., 2004).

This report describes results of a study that examined three putative risk SNPs for smoking behavior in European-American smokers from the Community Intervention Trial for Smoking Cessation (COMMIT). Specifically, we assessed one CHRNA5 (rs16969968) and two CHRNA3 (rs1051703, rs6495308) SNPs for their association with three ND phenotypes: 1) CPD, 2) TTF, and 3) HSI as well as one cessation phenotype; adjusting for key demographic and behavioral predictors of smoking behavior. Given the strong evidence in the literature for an association between genetic variation in nicotinic receptor genes and smoking behavior, we hypothesized that SNPs in the CHRNA5 and CHRNA3 genes would be associated with all three ND phenotypes (CPD, TTF, and HSI) as well as cessation.

METHODS

Study Population

Participants were selected from the multicenter COMMIT cohort project, which is explained in detail elsewhere (1991). Briefly, the COMMIT cohort was identified by a telephone survey in 1988 and re-interviewed in 1993, 2001, and 2005. In 1988, all participants were current smokers (at least 100 cigarettes in lifetime) aged 25 to 64 years who lived in communities that were located in California, Iowa, Massachusetts, New Mexico, New Jersey, New York, North Carolina, Oregon, Washington, and Ontario, Canada. In 2005, saliva samples were collected from 1658 cohort participants by mail.

Sample Collection Procedures

Upon completion of the 2005 follow-up survey, 4607 eligible participants (those who participated in telephone surveys as part of the COMMIT project in 1988 and 1993, completed follow-up surveys in both 2001 and 2005) were invited to participate in the saliva sample collection. Consenting participants were sent by mail a saliva collection kit and asked to brush their teeth and then wait for 30 minutes during which time no food or drink was consumed. Participants were then asked to vigorously swish 10ml mouthwash for 60 seconds, deposit the mouthwash into the collection vessel, and mail back to the Roswell Park Cancer Institute.

A total of 1658 collection kits were returned. For the current study, we only included participants who were current smokers aged 25-64 years at baseline and who had complete genotypic as well as key demographic and behavioral data available, resulting in 1385 participants. Due to previous research showing ethnic heterogeneity at the CHRNA5-CHRNA3-CHRNA4 locus (Saccone et al., 2009b), we excluded participants (n=84) found to have <85% European ancestry based on 27 unlinked ancestry informative markers (AIMs) (Tsai et al., 2005) specific to the HapMap Central European population (hapmap.ncbi.nlm.nih.gov). As a result, 1301 participants were included in statistical analysis. Comparison of these 1301 participants with the 1658 participants who returned collection kits, revealed no differences in demographic characteristics other than ancestry due to the exclusion of all non-European participants. Comparison of the 1301 participants with all 4607 eligible participants, revealed a smaller proportion of included participants (25% versus 31%; $p=0.001$) were under the age of 35; however, the included sample was similar to the eligible sample in regards to sex, income, and education. The Institutional Review Board at the Roswell Park Cancer Institute approved all study procedures.

Smoking Phenotypes

Nicotine Dependence—We used the two HSI items (CPD and TTF) derived from the Fagerstrom Tolerance Questionnaire (Fagerstrom, 1978) because they have been shown to be the two most heritable smoking phenotypes (Lessov et al., 2004) and CPD is the most widely used in previous genetic research (Tobacco and Genetics Consortium, 2010; Berrettini et al., 2008; Bierut, 2009; Caporaso et al., 2009; Chen et al., 2009; Saccone et al., 2009a; Saccone et al., 2009b; Saccone et al., 2007; Stevens et al., 2008; Thorgeirsson et al., 2008; Weiss et al., 2008). Information available on CPD and TTF from the 1988 (n=1301), 1993 (TTF not measured; n=988), 2001 (n=662), and 2005 (n=542) interviews was used to determine if each participant ‘ever’ or ‘never’ reported ND according to three common ND phenotypic classifications: 1) 25+ (Ever; n=651) vs. <25 (Never; n=650) CPD, 2) TTF < 10 (Ever; n=467) vs. ≥ 10 (Never; n=834) minutes, and 3) HSI ≥ 4 (Ever; n=916) vs. HSI < 3 (Never; n=385). We selected a cut-off 25+ CPD based on recent finding by Berrettini et al. (Berrettini et al., 2008) that showed specificity for DSM-IV diagnosis of ND improved to 90% when persons with 25+ CPD were compared to those reporting 20+ CPD (specificity 65%). The TTF cut-off of <10 minutes was used instead of the commonly used ≤ 30 minutes cut-off based on previous research suggesting a relatively low specificity (63%) for ND for the ≤ 30 minutes cut-off (de Leon et al., 2003). The HSI is a summary score of CPD and TTF that ranges from 0-6, with higher scores indicative of greater ND. For this study, CPD was categorized as <5, 5-14, 15-24, and 25+ and was recoded 0, 1, 2, 3, respectively. TTF included six categories ranging from <10 to >180 minutes that were recoded to: <10 = 3; 10-30 = 2; 31-60 = 1; 61+ = 3. We used the commonly applied cut-off score of HSI ≥ 4 based on previous research demonstrating good sensitivity (80%), specificity (97%) and high concordance ($\kappa=0.74$) with the Fagerstrom Questionnaire (Chabrol et al., 2005).

Smoking Cessation—Smoking cessation was examined in a subset of 1157 participants who reported making at least one attempt to quit smoking during the study period. Cessation was coded as “ever” or “never” based on self-report data. An “ever” quitter was defined as a cohort member who, at any of the four assessment points, reported not smoking any cigarettes for the preceding six months or longer.

DNA Extraction, quantification and genotyping

Qiamp DNA blood kit (Qiagen catalog # 51106) was used to isolate DNA from the mouthwash samples received from study participants. The concentration of DNA isolated was determined using the Quant-iT PicoGreen dsDNA Assay kit (Invitrogen/Molecular Probes catalog # P7589). In a subsample of 344 of the 1,943 total samples, the ND-1000 (NanoDrop Spectrophotometer) was used to calculate the OD260/280 ratio, a measure of overall quality of the DNA, prior to genotyping. SNPs for ND in the CHRNA5-CHRNA3-CHRNA4 locus (rs16969968, rs1051703, rs6495308) were selected *a priori* based upon previous findings and genotyped as part of a larger project, using Illumina GoldenGate assay and Sentrix Array Matrices (SAMs) as per manufacturer’s instructions (Illumina, Inc.).

Demographic and Behavioral Factors

Additional variables that have been associated with smoking behavior in the literature and previous studies with this cohort (Hymowitz et al., 1997) were selected from the baseline interview to adjust associations between the three selected SNPs and ND and cessation phenotypes. These variables included: sex (male or female), age in 1988 (25-34 years, 35-44, 45-54, 55-64), education in 1988 (<12 years, 12, 13-15, 16+), gross household income in 1988 (<\$10,000/year, \$10,000-\$25,000, \$25,001-\$40,000, >\$40,000), alcohol use in 1988 (daily, 3-4 times/week, 1-2 times/week, 1-3 times/month, <1 time/month or never), age started smoking (<16 years, 16-19, >19), and number of quit attempts reported during the study period.

Statistical Analysis

All analyses were conducted using PASW Statistics 18.0.2 (SPSS, Inc.). Proportional differences related to demographic and behavioral factors for the three ND and one cessation phenotype were examined using Pearson chi-square analysis. SNP associations with each of the three ND phenotypes assumed an additive genetic model using a per allele odds ratio (OR) and were determined using unconditional logistic regression adjusted for age, sex, education, gross annual income, alcohol use, and age started smoking. SNP associations with cessation used the same method but additionally adjusted for number of quit attempts in 1988 and lowest TTF over the study period. Finally, a continuous variable for the number of ‘risk’ alleles (0, 1, or 2) was created for each of the three SNPs. The A-allele for rs16969968 and rs1051703 and T-allele for rs6495308 were set as the ‘risk’ alleles based on previous studies of these SNPs (Tobacco and Genetics Consortium, 2010; Berrettini et al., 2008; Bierut, 2009; Caporaso et al., 2009; Chen et al., 2009; Saccone et al., 2009a; Saccone et al., 2009b; Saccone et al., 2007; Stevens et al., 2008; Thorgeirsson et al., 2008; Weiss et al., 2008).

RESULTS

Characteristics of the study population

Demographic and behavioral characteristics by each of the three ND phenotypes and smoking cessation are shown in Table 1. For all of the ND phenotypes examined, having less than 12 years of education, and/or starting smoking prior to 16 years of age was associated with heavy smoking. Males were more likely to ever be ND defined by our 25+

CPD or HSI ≥ 4 phenotypes. At baseline participants aged 35-44 years were more likely to ever be ND defined by 25+ CPD and HSI ≥ 4 as well as more likely to ever quit smoking. Participants with ND defined by TTF < 10 minutes were more likely to have an annual household income less than \$10,000. Alcohol use did not significantly differ between ever and never ND for any of the measured ND phenotypes or cessation. Examination of demographic and behavioral factors by each of the three SNPs showed no significant differences by genotype with the exception that participants aged 55-64 were more likely to be carriers of the GG genotype for the rs1051730 and rs16969968 SNPs (data not shown).

SNP associations with indicators of nicotine dependence

Genotypic and allelic frequencies and adjusted odds ratios (OR_{adj}) for each of the three examined SNPs by the three ND phenotypes are shown in Table 2. All three SNPs were in Hardy-Weinberg Equilibrium ($p > 0.10$). The A-allele of rs1051730 and rs16969968 as well as T-allele of rs6495308 were significantly associated with 1.21, 1.21, and 1.22 greater odds of ever consuming 25+ CPD, respectively. AA genotypes for rs1051730 or rs16969968 were respectively associated with a 1.61 and 1.60 greater odds of ever consuming 25+ CPD. Post-hoc examination of ND using a relaxed CPD cut-off (15+ vs. <15 CPD), revealed nearly identical allelic (ORs range = 1.20 – 1.27) and genotypic (ORs range = 1.67 – 2.10) effects, albeit not statistically significant (data not shown). None of the SNPs were associated with ND defined as TTF < 10 minutes or HSI ≥ 4 at the allelic or genotypic level of analysis.

To explore whether an allelic dose effect could be observed for ND, we created a summary variable representing the total number of rs16969968 A-alleles and rs6495308 T-alleles (range 0-4 alleles). The A and T alleles for rs16969968 and rs6495308, respectively, were selected based on our results and previous studies (Tobacco and Genetics Consortium, 2010; Berrettini et al., 2008; Chen et al., 2009; Stevens et al., 2008) showing that these alleles confer significant risk for ND. Concordant with previous studies, (Tobacco and Genetics Consortium, 2010; Caporaso et al., 2009; Chen et al., 2009; Stevens et al., 2008) in our sample the rs1051730 and rs16969968 were in strong linkage disequilibrium (LD) ($r^2 = 0.99$) so we selected rs16969968 to represent the two SNPs. Table 2 shows that carriers of two A- and two T-alleles (total 4 ‘risk’ alleles) have an approximate two-fold increased odds of ND defined as ever consuming 25+ CPD as well as ever TTF < 10 minutes but not for the HSI ≥ 4 phenotype. Due to the substantial LD between rs1051730 and rs16969968, we performed the same set of analyses with rs1051730 as a technical validation of our rs16969968 results. Results were identical for rs1051730 (data not shown).

SNP associations with cessation

Genotypic and allelic frequencies along with adjusted odds ratios (OR_{adj}) for each of the three examined SNPs and total number of rs16969968 A-alleles and rs6495308 T-alleles by smoking cessation phenotype are shown in Table 3. None of the examined SNPs or total number of rs16969968 A-alleles and rs6495308 T-alleles was significantly associated with cessation.

DISCUSSION

In this study, we examined one CHRNA5 (rs16969968) and two CHRNA3 (rs1051703, rs6495308) SNPs for their association with CPD, the most common measure of ND in the genetic literature, as well as two additional ND (TTF, HSI) phenotypes and one cessation phenotype frequently used in epidemiological studies but rarely in gene association studies.

All three SNPs significantly predicted ND defined as ever consuming 25+ CPD, which is concordant with previous case-control and genome-wide association studies (Tobacco and

Genetics Consortium, 2010; Berrettini et al., 2008; Caporaso et al., 2009; Chen et al., 2009; Stevens et al., 2008; Thorgeirsson et al., 2008). The A-allele of rs16969968 has been suggested to be the most likely biological contributor to ND (Bierut, 2010; Saccone et al., 2007) based on recent work showing that expression levels of CHRNA5 in the brain are rs16969968 dependent and associated with ND (Wang et al., 2009). We found that the A-allele and AA genotype of rs16969968 was associated with 20% ($OR_A=1.21$) and 60% ($OR_{AA}=1.61$) greater odds of ever consuming 25+ CPD, respectively. Only the AA genotype finding withstood correction for multiple testing. However, our effect sizes are similar to that reported in a recent meta-analysis of 34 studies (Saccone et al., 2010) that showed heavy smokers (CPD = 30+; $n=7,495$) had 1.40 greater odds of carrying the A-allele compared to light smokers (CPD ≤ 10 ; $n=10,355$). More recently, the Tobacco and Genetics Consortium (2010) reported the A-allele was significantly associated ($\beta=-1.00$, $se=0.06$, $p=5.57 \times 10^{-72}$) with an increase in CPD among a combined sample of 73,853 subjects.

In addition to rs16969968, we also showed that rs1051703 in the CHRNA3 gene significantly predicted subjects who ever consumed 25+ CPD. As previously noted, rs1051703 and rs16969968 were in strong LD in our sample and thus it was not surprising that near identical results were observed for the two SNPs. Previous research that examined both of these SNPs have also demonstrated strong LD and subsequent near identical effect sizes for ND (Tobacco and Genetics Consortium, 2010; Chen et al., 2009; Stevens et al., 2008). Therefore, it may be advantageous for future research to focus on rs16969968, given previous and current findings as well as the functional relevance of the rs16969968 described above.

The other SNP (rs6495308) in CHRNA3 that we examined has also been shown in previous research to be in a LD block containing rs16969968 and rs1051703. (Chen et al., 2009) However, in our sample rs6495308 does not appear to be in strong LD ($r^2 = 0.15$) with rs16969968 or rs1051703. In fact, for rs6495308 we only observed a significant allelic difference between ever and never 25+ CPD in which subjects that ever consumed 25+ CPD had approximately 20% greater odds of being a T-allele carrier; albeit only nominally significant. This finding is aligned with previous results by Berrettini et al (Berrettini et al., 2008) who showed in a pooled sample of nearly 8,000 subjects that carriers of the T-allele were more likely to report consumption of 25+ CPD compared to carriers of the C-allele.

Surprisingly, none of the SNPs we examined were associated with the TTF or HSI phenotypes at the allelic or genotypic level of analysis. However, analysis looking at the combined allelic dose effect of rs16969968 and rs6495308 did show an association with TTF in which homozygotes for both the rs16969968-A allele and rs6495308-T allele had almost twice the odds of ever reporting TTF < 10 minutes compared to homozygotes for both the rs16969968-G allele and rs6495308-C allele; albeit this finding did not withstand correction for multiple testing. To our knowledge, only one cross-sectional study has examined the association between nicotinic receptor SNPs and TTF and found no association (Weiss et al., 2008). However, Weiss and colleagues only examined the association between TTF and a five-marker haplotype including rs16969968 and rs1051703 rather than the markers independently. Nevertheless, our longitudinal findings should be judged as preliminary and warrant replication.

Our combined allelic analysis also showed that homozygotes for both the rs16969968-A allele and rs6495308-T allele had 1.99 greater odds of ever reporting 25+ CPD compared to homozygotes for both the rs16969968-G allele and rs6495308-C allele. Although these results are only nominally significant, they suggest an allelic dose effect whereby the rs16969968-A allele and rs6495308-T allele in combination rather than independently are

robust genetic risk markers for ND. In fact, due to the likely polygenic nature of ND and modest effect of any single SNP, our results support the notion that combined examination of several SNPs may be more advantageous than single SNP approaches. However, we did not observe a significant association with the HSI phenotype, albeit the effect observed was near significant ($p < 0.15$). One potential reason for this is that the HSI phenotype included 243 and 427 more subjects in the 'ever' group compared to the CPD and TTF phenotypes, respectively. Thus, the HSI phenotype represents a more relaxed definition of ND, which may have attenuated our ability to detect an effect due to the inclusion of potential false-positives in the 'ever' group. Post-hoc analysis using a relaxed definition of CPD (15+ vs. <15) also showed an attenuation of the examined SNPs effects; suggesting the three SNPs examined may confer a risk for more severe definitions of ND.

None of the three SNPs examined were associated with smoking cessation among those who made at least one attempt at some point during the study period. Importantly, cessation was not biochemically verified and our cessation definition only covered the 6-months prior to assessment. Thus, our cessation group may include an unknown proportion of false-positives and negatives, respectively. Nevertheless, this finding is in agreement with previous studies (Caporaso et al., 2009; Conti et al., 2008; Ray et al., 2010; Tobacco and Genetics Consortium, 2010) suggesting that the CHRNA5-CHRNA3 locus is not associated with smoking cessation, albeit a recent study by Freathy and colleagues (Freathy et al., 2009) showed the rs1051730-A allele was associated with continued smoking during pregnancy after adjusting for pre-pregnancy smoking quantity. Thus, it would appear advantageous for future cessation research to expand their investigations to other genes as well as interactions between nicotinic receptors and genetic pathways associated with metabolism and elimination of nicotine (e.g. CYP2A6) as well as mechanisms of nicotine action (e.g. dopamine receptors) (Quaak et al., 2009).

Our findings are predominately limited by the size of the sample and as a consequence suboptimal power to detect significant genotypic effects for ND and smoking cessation. Future longitudinal studies should consider collection of DNA at baseline to avoid lower participation rates in later stages as a result of losses to follow-up. Despite this main limitation, these findings significantly contribute to our current knowledge of nicotinic receptor gene variation and smoking behavior in two important ways. First, all of our results were based on longitudinal data and adjusted for key demographic and behavioral factors of smoking, a considerable strength. To date, studies of nicotinic receptors and smoking behavior have been cross-sectional in nature and have not adjusted for possible confounding demographic and behavioral factors. In fact, studies that have made adjustments have been limited to age and/or sex (Berrettini et al., 2008; Stevens et al., 2008; Thorgeirsson et al., 2008), with the exception of Caporaso et al. (Caporaso et al., 2009) who also adjusted for education and marital status. Our findings suggest associations between SNPs in the CHRNA5-CHRNA3 locus and CPD are relatively robust and withstand adjustments for key demographic and behavioral factors associated with ND. Second, as stated previously, TTF is generally considered a more stable measure and stronger predictor of ND than CPD (Baker et al., 2007). Yet, CPD has been the phenotype of choice in the gene association literature. It is difficult to ascertain why the majority of previous research has not reported gene associations with TTF. One could speculate that it is a result of lags in knowledge translation, data availability, and/or publication bias. Regardless, it is apparent that use of CPD as the predominant ND phenotype in the current genetic association literature is not aligned with previous evidence in tobacco research and clinical practice. Our data suggest that previous gene association studies between nicotinic receptor variants and ND may have been attenuated if the TTF rather than CPD phenotype were utilized. However, as stated above, sample sizes in previous studies were considerably larger than ours, so it is possible we did not have the power required to detect an effect between our candidate SNPs and

TTF. Nevertheless, until larger gene association studies using TTF are conducted the answer to this query remains unknown. Importantly, we are not suggesting that previous research utilizing CPD is not useful but are suggesting that future research aligned with the most current knowledge on ND phenotypes is required.

In conclusion, our longitudinal findings support, further strengthen, and expand on previous results from numerous case-control and genome-wide association studies showing genetic variation in nicotinic receptors independently predict ND (25+ CPD) after adjustment for key demographic and behavioral factors commonly associated with smoking behavior. In addition, our results provide preliminary evidence for a combined rs16969968 and rs6495308 allelic dose effect on ND defined as 25+ CPD or TTF < 10 minutes. Our results strongly encourage examination of TTF in addition to CPD in large, well-characterized genetic studies of smoking behavior.

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References

- Baker TB, Piper ME, McCarthy DE, Bolt DM, Smith SS, Kim SY, Colby S, Conti D, Giovino GA, Hatsukami D, et al. Time to first cigarette in the morning as an index of ability to quit smoking: implications for nicotine dependence. *Nicotine Tob Res.* 2007; 9(Suppl 4):S555–70. [PubMed: 18067032]
- Batra V, Patkar AA, Berrettini WH, Weinstein SP, Leone FT. The genetic determinants of smoking. *Chest.* 2003; 123(5):1730–9. [PubMed: 12740294]
- Berrettini W, Yuan X, Tozzi F, Song K, Francks C, Chilcoat H, Waterworth D, Muglia P, Mooser V. Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. *Mol Psychiatry.* 2008; 13(4):368–73. [PubMed: 18227835]
- Bierut LJ. Nicotine dependence and genetic variation in the nicotinic receptors. *Drug Alcohol Depend.* 2009; 104(Suppl 1):S64–9. [PubMed: 19596527]
- Bierut LJ. Convergence of genetic findings for nicotine dependence and smoking related diseases with chromosome 15q24-25. *Trends Pharmacol Sci.* 2010; 31(1):46–51. [PubMed: 19896728]
- Caporaso N, Gu F, Chatterjee N, Sheng-Chih J, Yu K, Yeager M, Chen C, Jacobs K, Wheeler W, Landi MT, et al. Genome-wide and candidate gene association study of cigarette smoking behaviors. *PLoS ONE.* 2009; 4(2):e4653. [PubMed: 19247474]
- Chabrol H, Niezborala M, Chastan E, de Leon J. Comparison of the Heavy Smoking Index and of the Fagerstrom Test for Nicotine Dependence in a sample of 749 cigarette smokers. *Addict Behav.* 2005; 30(7):1474–7. [PubMed: 16022945]
- Chen X, Chen J, Williamson VS, An SS, Hettaema JM, Aggen SH, Neale MC, Kendler KS. Variants in nicotinic acetylcholine receptors alpha5 and alpha3 increase risks to nicotine dependence. *Am J Med Genet B Neuropsychiatr Genet.* 2009; 150B(7):926–33. [PubMed: 19132693]
- COMMIT Research Group. Community Intervention Trial for Smoking Cessation (COMMIT): summary of design and intervention. *J Natl Cancer Inst.* 1991; 83(22):1620–8. [PubMed: 1749015]
- Conti DV, Lee W, Li D, Liu J, Van Den Berg D, Thomas PD, Bergen AW, Swan GE, Tyndale RF, Benowitz NL, et al. Nicotinic acetylcholine receptor beta2 subunit gene implicated in a systems-based candidate gene study of smoking cessation. *Human molecular genetics.* 2008; 17(18):2834–48. [PubMed: 18593715]
- de Leon J, Diaz FJ, Becona E, Gurpegui M, Jurado D, Gonzalez-Pinto A. Exploring brief measures of nicotine dependence for epidemiological surveys. *Addict Behav.* 2003; 28(8):1481–6. [PubMed: 14512071]

- Fagerstrom K. Time to first cigarette; the best single indicator of tobacco dependence? *Monaldi Arch Chest Dis.* 2003; 59(1):91–4. [PubMed: 14533289]
- Fagerstrom KO. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. *Addict Behav.* 1978; 3(3-4):235–41. [PubMed: 735910]
- Freathy RM, Ring SM, Shields B, Galobardes B, Knight B, Weedon MN, Smith GD, Frayling TM, Hattersley AT. A common genetic variant in the 15q24 nicotinic acetylcholine receptor gene cluster (CHRNA5-CHRNA3-CHRNA4) is associated with a reduced ability of women to quit smoking in pregnancy. *Hum Mol Genet.* 2009; 18(15):2922–7. [PubMed: 19429911]
- Guindon, G.; Boisclair, D. Past, current and future trends in tobacco use. The World Bank; Washington: 2003.
- Haberstick BC, Timberlake D, Ehringer MA, Lessem JM, Hopfer CJ, Smolen A, Hewitt JK. Genes, time to first cigarette and nicotine dependence in a general population sample of young adults. *Addiction.* 2007; 102(4):655–65. [PubMed: 17309537]
- Heath AC, Martin NG. Genetic models for the natural history of smoking: evidence for a genetic influence on smoking persistence. *Addict Behav.* 1993; 18(1):19–34. [PubMed: 8465673]
- Heatherton TF, Kozlowski LT, Frecker RC, Rickert W, Robinson J. Measuring the heaviness of smoking: using self-reported time to the first cigarette of the day and number of cigarettes smoked per day. *Br J Addict.* 1989; 84(7):791–9. [PubMed: 2758152]
- Hymowitz N, Cummings KM, Hyland A, Lynn WR, Pechacek TF, Hartwell TD. Predictors of smoking cessation in a cohort of adult smokers followed for five years. *Tob Control.* 1997; 6(Suppl 2):S57–62. [PubMed: 9583654]
- Kendler KS, Neale MC, Sullivan P, Corey LA, Gardner CO, Prescott CA. A population-based twin study in women of smoking initiation and nicotine dependence. *Psychol Med.* 1999; 29(2):299–308. [PubMed: 10218922]
- Lessov CN, Martin NG, Statham DJ, Todorov AA, Slutske WS, Bucholz KK, Heath AC, Madden PA. Defining nicotine dependence for genetic research: evidence from Australian twins. *Psychol Med.* 2004; 34(5):865–79. [PubMed: 15500307]
- Quaak M, van Schayck CP, Knaapen AM, van Schooten FJ. Genetic variation as a predictor of smoking cessation success. A promising preventive and intervention tool for chronic respiratory diseases? *Eur Respir J.* 2009; 33(3):468–80. [PubMed: 19251795]
- Ray R, Mitra N, Baldwin D, Guo M, Patterson F, Heitjan DF, Jepson C, Wileyto EP, Wei J, Payne T, et al. Convergent evidence that choline acetyltransferase gene variation is associated with prospective smoking cessation and nicotine dependence. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.* 2010; 35(6):1374–82. [PubMed: 20147892]
- Saccone NL, Culverhouse RC, Schwantes-An TH, Cannon DS, Chen X, Cichon S, Giegling I, Han S, Han Y, Keskitalo-Vuokko K, et al. Multiple independent loci at chromosome 15q25.1 affect smoking quantity: a meta-analysis and comparison with lung cancer and COPD. *PLoS genetics.* 2010; 6(8)
- Saccone NL, Saccone SF, Hinrichs AL, Stitzel JA, Duan W, Pergadia ML, Agrawal A, Breslau N, Grucza RA, Hatsukami D, et al. Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. *Am J Med Genet B Neuropsychiatr Genet.* 2009a; 150B(4):453–66. [PubMed: 19259974]
- Saccone NL, Wang JC, Breslau N, Johnson EO, Hatsukami D, Saccone SF, Grucza RA, Sun L, Duan W, Budde J, et al. The CHRNA5-CHRNA3-CHRNA4 nicotinic receptor subunit gene cluster affects risk for nicotine dependence in African-Americans and in European-Americans. *Cancer Res.* 2009b; 69(17):6848–56. [PubMed: 19706762]
- Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau O, et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet.* 2007; 16(1):36–49. [PubMed: 17135278]
- Samet JM. The epidemiology of lung cancer. *Chest.* 1993; 103(1 Suppl):20S–29S. [PubMed: 8416770]

- Stevens VL, Bierut LJ, Talbot JT, Wang JC, Sun J, Hinrichs AL, Thun MJ, Goate A, Calle EE. Nicotinic receptor gene variants influence susceptibility to heavy smoking. *Cancer Epidemiol Biomarkers Prev.* 2008; 17(12):3517–25. [PubMed: 19029397]
- Sullivan PF, Kendler KS. The genetic epidemiology of smoking. *Nicotine Tob Res.* 1999; 1(Suppl 2):S51–7. discussion S69–70. [PubMed: 11768187]
- Thorgeirsson TE, Geller F, Sulem P, Rafnar T, Wiste A, Magnusson KP, Manolescu A, Thorleifsson G, Stefansson H, Ingason A, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature.* 2008; 452(7187):638–42. [PubMed: 18385739]
- Tobacco and Genetics Consortium. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nat Genet.* 2010; 42(5):441–7. [PubMed: 20418890]
- Tobacco key facts. World Health Organization; 2011.
- True WR, Heath AC, Scherrer JF, Waterman B, Goldberg J, Lin N, Eisen SA, Lyons MJ, Tsuang MT. Genetic and environmental contributions to smoking. *Addiction.* 1997; 92(10):1277–87. [PubMed: 9489045]
- Tsai HJ, Choudhry S, Naqvi M, Rodriguez-Cintron W, Burchard EG, Ziv E. Comparison of three methods to estimate genetic ancestry and control for stratification in genetic association studies among admixed populations. *Human genetics.* 2005; 118(3-4):424–33. [PubMed: 16208514]
- Wang JC, Cruchaga C, Saccone NL, Bertelsen S, Liu P, Budde JP, Duan W, Fox L, Grucza RA, Kern J, et al. Risk for nicotine dependence and lung cancer is conferred by mRNA expression levels and amino acid change in CHRNA5. *Hum Mol Genet.* 2009; 18(16):3125–35. [PubMed: 19443489]
- Weiss RB, Baker TB, Cannon DS, von Niederhausern A, Dunn DM, Matsunami N, Singh NA, Baird L, Coon H, McMahon WM, et al. A candidate gene approach identifies the CHRNA5-A3-B4 region as a risk factor for age-dependent nicotine addiction. *PLoS Genet.* 2008; 4(7):e1000125. [PubMed: 18618000]

Table 1

Demographic and behavioral factors by nicotine dependence and cessation phenotypes

Factor	25+ CPD, n (%)		TTF < 10 mins, n (%)		χ^2/t	HSI ≥ 4 , n (%)		Cessation > 6 mos, n (%)		χ^2/t
	Ever	Never	Ever	Never		Ever	Never	Ever	Never	
OVERALL	651 (50.0)	650 (50.0)	467 (35.9)	834 (64.1)		894 (68.7)	407 (31.3)	755 (65.0)	402 (35.0)	
Baseline Age (yrs)					13.59*					10.15*
25-34	138 (21.2)	187 (28.8)	114 (24.4)	211 (25.3)		205 (22.9)	120 (29.5)	170 (22.5)	120 (29.9)	
35-44	247 (37.9)	204 (31.4)	176 (37.7)	275 (33.0)		330 (36.9)	121 (29.7)	247 (32.7)	152 (37.8)	
45-54	183 (28.1)	163 (25.1)	115 (24.6)	231 (27.7)		242 (27.1)	104 (25.6)	230 (30.5)	88 (27.7)	
55-64	83 (12.7)	96 (14.8)	62 (13.3)	117 (14.0)		117 (13.1)	62 (15.2)	108 (14.3)	42 (10.4)	
Sex					36.34*					7.79*
Male	359 (55.1)	250 (38.5)	230 (49.3)	379 (45.4)		437 (48.9)	172 (42.3)	371 (49.1)	163 (40.5)	
Female	292 (44.9)	400 (61.5)	237 (50.7)	455 (54.6)		457 (51.1)	235 (57.7)	384 (50.9)	239 (59.5)	
Baseline Education (yrs)					21.14*					19.02*
<12	74 (11.4)	46 (7.1)	65 (13.9)	55 (6.6)		90 (10.1)	30 (7.4)	63 (8.4)	50 (12.5)	
12	131 (20.2)	126 (19.4)	95 (20.4)	162 (19.5)		195 (21.9)	62 (15.3)	152 (20.2)	74 (18.5)	
13-15	305 (47.1)	273 (42.1)	212 (45.5)	366 (44.0)		399 (44.8)	179 (44.1)	345 (45.8)	168 (42.0)	
16+	138 (21.2)	204 (31.4)	94 (20.2)	248 (29.8)		207 (23.2)	135 (33.3)	194 (25.7)	108 (27.0)	
Baseline Income (\$USD)					3.85					15.99*
<10,000	32 (5.1)	25 (4.0)	30 (6.7)	27 (3.4)		48 (5.6)	9 (2.3)	22 (3.1)	27 (6.9)	
10,000-25,000	138 (22.0)	165 (26.4)	116 (25.9)	187 (23.3)		205 (23.8)	98 (25.2)	161 (22.4)	110 (28.1)	
25,001-40,000	224 (35.7)	213 (34.1)	162 (36.2)	275 (34.2)		303 (35.2)	134 (34.4)	258 (35.8)	132 (33.7)	
>40,000	233 (37.2)	221 (35.4)	140 (31.3)	314 (39.1)		306 (35.5)	148 (38.0)	279 (38.8)	123 (31.4)	
Baseline Alcohol use					7.34					1.11
Daily	89 (13.8)	74 (11.4)	59 (12.7)	104 (12.5)		113 (12.7)	50 (12.3)	96 (12.8)	47 (11.8)	
3-4 times/wk	59 (9.1)	67 (10.3)	42 (9.1)	84 (10.1)		86 (9.7)	40 (9.9)	70 (9.3)	40 (10.0)	
1-2 times/wk	154 (23.9)	153 (23.6)	93 (20.1)	214 (25.8)		203 (22.9)	104 (25.7)	173 (23.1)	94 (23.5)	
1-3 times/mo	88 (13.6)	119 (18.4)	73 (15.8)	134 (16.1)		134 (15.1)	73 (18.0)	128 (17.1)	61 (15.3)	
<1/mo or never	255 (39.5)	235 (36.3)	196 (42.3)	294 (35.4)		352 (39.6)	138 (34.1)	283 (37.7)	158 (39.5)	
Age started smoking					25.51*					24.94*
≤ 15	168 (25.8)	105 (16.2)	138 (29.6)	135 (16.2)		218 (24.4)	55 (13.5)	143 (18.9)	104 (25.9)	

Factor	25+ CPD, n (%)			TTF < 10 mins, n (%)			HSI $\geq$ 4, n (%)			Cessation > 6 mos, n (%)		
	Ever	Never	χ^2/t	Ever	Never	χ^2/t	Ever	Never	χ^2/t	Ever	Never	χ^2/t
16-19	344 (52.8)	345 (53.1)		240 (51.4)	449 (53.8)		468 (52.3)	221 (54.3)		400 (53.0)	207 (51.5)	
20+	139 (21.4)	200 (30.8)		89 (19.1)	250 (30.0)		208 (23.3)	131 (32.2)		212 (28.1)	91 (22.6)	
Total Quit attempts			1.94			3.21*			2.94*			3.09*
Mean (sd)	5.8 (10.8)	4.8 (7.9)		6.5 (13.1)	4.7 (6.6)		5.8 (10.8)	4.2 (5.1)		5.4 (10.5)	7.2 (8.4)	
Highest CPD (88-05)			1301*			223*			634*			44.38*
<5	0 (0.0)	50 (7.7)		0 (0.0)	50 (6.0)		0 (0.0)	50 (12.3)		38 (5.0)	4 (1.0)	
5-14	0 (0.0)	178 (27.4)		15 (3.2)	163 (19.5)		15 (1.7)	163 (40.0)		123 (16.3)	31 (7.7)	
15-24	0 (0.0)	422 (64.9)		94 (20.1)	328 (39.3)		259 (29.0)	163 (40.0)		266 (35.2)	122 (30.3)	
25+	651 (100)	0 (0.0)		358 (76.7)	293 (35.1)		620 (69.4)	31 (7.6)		328 (43.4)	245 (60.9)	
Lowest TTF (88-05)			296*			1300*			927*			77.44*
<10 mins	358 (55.0)	109 (16.8)		467 (100)	0 (0.0)		467 (52.2)	0 (0.0)		211 (27.9)	198 (49.3)	
10-30 mins	205 (31.5)	209 (32.2)		0 (0.0)	414 (49.7)		370 (41.4)	44 (10.8)		246 (32.6)	131 (32.6)	
31-60 mins	63 (9.7)	136 (21.0)		0 (0.0)	199 (23.9)		57 (6.4)	142 (35.0)		130 (17.2)	45 (11.2)	
61-90 mins	15 (2.3)	49 (7.6)		0 (0.0)	64 (7.7)		0 (0.0)	64 (15.8)		54 (7.2)	5 (1.2)	
91-180 mins	6 (0.9)	67 (10.3)		0 (0.0)	73 (8.8)		0 (0.0)	73 (18.0)		57 (7.5)	12 (3.0)	
>180 mins	4 (0.6)	79 (12.2)		0 (0.0)	83 (10.0)		0 (0.0)	83 (20.4)		57 (7.5)	11 (2.7)	

CPD=cigarettes per day; TTF=time to first cigarette in the morning; HSI=Heavy Smoking Index

* p<0.05

CHRNA3 rs1051730, rs6495308, and CHRNA5 rs16969968 genotype and allele frequencies and associations with nicotine dependence phenotypes 1988-2005

Table 2

N=1301	25+ CPD, count (frequency)			TTF < 10 mins, count (frequency)			HSI ≥ 4, count (frequency)		
	Ever (n=651)	Never (n=650)	OR _{adj} (95% CI) ¹	Ever (n=467)	Never (n=834)	OR _{adj} (95% CI) ¹	Ever (n=894)	Never (n=407)	OR _{adj} (95% CI) ¹
<i>rs1051730</i>									
GG	247 (.38)	281 (.43)	1.00 (reference)	186 (.40)	342 (.41)	1.00 (reference)	359 (.40)	169 (.42)	1.00 (reference)
AG	315 (.51)	302 (.47)	1.19 (0.93-1.52)	216 (.46)	401 (.48)	1.01 (0.78-1.30)	424 (.47)	193 (.47)	1.03 (0.80-1.34)
AA	89 (.14)	67 (.10)	1.61 (1.10-2.36)**	65 (.14)	91 (.11)	1.35 (0.91-1.98)	111 (.12)	45 (.11)	1.24 (0.82-1.87)
A-allele ²	493 (.38)	436 (.34)	1.21 (1.03-1.42) ⁴	346 (.37)	583 (.35)	1.10 (0.93-1.29)	646 (.36)	283 (.35)	1.06 (0.89-1.26)
<i>rs6495308</i>									
CC	27 (.04)	43 (.07)	1.00 (reference)	18 (.04)	52 (.06)	1.00 (reference)	41 (.05)	29 (.07)	1.00 (reference)
TC	219 (.34)	231 (.35)	1.38 (0.79-2.41)	159 (.34)	291 (.35)	1.53 (0.83-2.81)	305 (.34)	145 (.36)	1.29 (0.74-2.25)
TT	405 (.62)	376 (.58)	1.53 (0.88-2.64) ⁴	290 (.62)	491 (.59)	1.59 (0.88-2.89)	548 (.61)	233 (.57)	1.47 (0.85-2.53) ⁴
T-allele ³	1029 (.79)	983 (.76)	1.22 (1.01-1.46)*	739 (.79)	1273 (.76)	1.18 (0.97-1.43)	1401 (.78)	611 (.75)	1.20 (0.99-1.46)
<i>rs16969968</i>									
GG	247 (.38)	282 (.43)	1.00 (reference)	185 (.39)	344 (.41)	1.00 (reference)	360 (.40)	169 (.42)	1.00 (reference)
AG	316 (.49)	301 (.46)	1.09 (0.96-2.34)	218 (.47)	399 (.48)	1.04 (0.80-1.34)	424 (.47)	193 (.47)	1.03 (0.79-1.34)
AA	88 (.14)	67 (.10)	1.60 (1.09-2.34)**	64 (.14)	91 (.11)	1.34 (0.91-1.97)	110 (.12)	45 (.11)	1.22 (0.81-1.85)
A-allele ²	492 (.38)	435 (.35)	1.21 (1.03-1.42)*	346 (.37)	581 (.35)	1.10 (0.93-1.30)	644 (.36)	283 (.35)	1.06 (0.89-1.26)
number of alleles ⁵									
0	27 (.04)	43 (.06)	1.00 (reference)	18 (.04)	52 (.06)	1.00 (reference)	41 (.05)	29 (.07)	1.00 (reference)
1	109 (.17)	119 (.18)	1.33 (0.74-2.42)	83 (.18)	145 (.17)	1.61 (0.85-3.05)	157 (.18)	71 (.17)	1.33 (0.73-2.42)
2	221 (.34)	231 (.36)	1.33 (0.76-2.33)	161 (.35)	291 (.35)	1.46 (0.80-2.69)	309 (.35)	143 (.35)	1.33 (0.76-2.32)
3	205 (.32)	190 (.29)	1.56 (0.89-2.74)*	140 (.30)	255 (.31)	1.55 (0.84-2.86)	276 (.31)	119 (.29)	1.44 (0.82-2.53)
4	89 (.14)	67 (.10)	1.99 (1.07-3.72)*	65 (.14)	91 (.11)	1.96 (1.01-3.81)*	111 (.12)	45 (.11)	1.63 (0.87-3.08)

CPD=cigarettes per day; TTF=time to first cigarette in the morning; HSI=Heavy Smoking Index

* raw p-value range: 0.02 – 0.04

** significant after Bonferroni adjustment (0.05/9 tests = 0.005)

¹ adjustment for age, sex, education, income, alcohol use, and age started smoking

² reference G-allele

³ reference C-allele

⁴ significant (p-value range: 0.03 - 0.04) when unadjusted

⁵ risk alleles= A (rs16969968) + T (rs6495308)

Table 3

CHRNA3 and CHRNA5 association with smoking cessation (N=1157)

	Ever (n=755)	Never (n=402)	OR _{adj} (95% CI) ¹
<i>rs1051730, n (frequency)</i>			
GG	300 (.40)	160 (.40)	1.00 (reference)
AG	359 (.48)	196 (.49)	1.07 (0.80-1.41)
AA	96 (.13)	46 (.11)	1.25 (0.81-1.93)
A-allele ²	551 (.36)	288 (.31)	1.03 (0.86-1.23)
<i>rs6495308</i>			
CC	40 (.05)	22 (.06)	1.00 (reference)
TC	254 (.34)	142 (.35)	1.39 (0.75-2.59)
TT	461 (.61)	238 (.59)	1.51 (0.83-2.78)
T-allele ³	1176 (.77)	618 (.76)	1.05 (0.86-1.30)
<i>rs16969968</i>			
GG	300 (.40)	161 (.40)	1.00 (reference)
AG	359 (.48)	196 (.49)	1.09 (0.82-1.44)
AA	96 (.13)	45 (.11)	1.30 (0.84-2.00)
A-allele ²	551 (.36)	286 (.36)	1.04 (0.87-1.24)
<i>number of alleles⁴</i>			
0	40 (.05)	22 (.06)	1.00 (reference)
1	129 (.17)	67 (.16)	1.52 (0.78-3.00)
2	256 (.34)	147 (.37)	1.30 (0.70-2.41)
3	234 (.31)	121 (.30)	1.57 (0.84-2.94)
4	96 (.13)	45 (.11)	1.75 (0.87-3.51)

¹ adjustment for age, sex, education, income, alcohol use, age started smoking, number of quit attempts, lowest TTF² reference G-allele;³ reference C-allele⁴ risk alleles= A (rs16969968) + T (rs6495308)