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Genetic ancestry as an effect modifier of naltrexone in smoking cessation among African Americans: an analysis of a randomized controlled trial

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Abstract

Objectives—To determine if there were differential quit rates between AA and European Americans (EA) with the experimental treatment naltrexone, and examine the role of genetic ancestry on these outcomes among AAs.

Methods—Data from a previous randomized trial of 315 smokers to naltrexone vs. placebo were reanalyzed using West African (WA) genetic ancestry to define sub-populations. Logistic regression models were used to estimate treatment effects on early and end of treatment quit rates, by race and WA ancestry.

Results—Among EAs (n=136), naltrexone significantly increased quit rates at four weeks (62% vs. 43%, p=0.03) with directional, but not statistically significant effects at 12 weeks (30% vs. 18%, p=0.12). In contrast, among the AAs (n=95), quit rates did not differ between naltrexone and placebo groups at either interval (four weeks: 43% vs. 32%, p=0.27; 12 weeks: 22% vs. 18%, p=0.60). A median split was conducted in AAs for WA ancestry. Among AAs with low WA ancestry, quit rates were significantly higher with naltrexone compared with placebo (60% vs. 27%, p=0.03). There was no advantage in quit rates with naltrexone for the high WA ancestry group.

Conclusions—Naltrexone efficacy for smoking cessation varies across AA subjects with different levels of WA ancestry. These results suggest that genetic background may partially explain racial differences in drug response.

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Keywords

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INTRODUCTION

Nearly 44 million Americans currently smoke cigarettes and over 450,000 individuals are estimated to die from tobacco-related causes each year in the United States alone.¹ African Americans (AA) are disproportionally affected by smoking induced illness. For example, they have higher rates of smoking-related cancer and smoking-related mortality than European Americans (EA).^{2–5} Several pharmacotherapies are FDA approved for smoking cessation, including nicotine replacement, bupropion, and varenicline.⁶ However, the majority of smokers using these pharmacotherapies are not successful, prompting the need for research on patient factors affecting response and on other pharmacological targets for smoking cessation, particularly in racial minority populations. Response to pharmacotherapy for smoking cessation may differ in AAs compared with other racial/ethnic groups for a range of reasons, including racial variation in smoking patterns and socioeconomic status, pharmacokinetic and pharmacodynamics factors, and other biological and genetic factors.^{7, 8} Few studies examining smoking cessation pharmacotherapies have enrolled sufficient numbers of AAs to make meaningful direct comparisons of outcomes relative to EAs. However, several recent studies in other therapeutic areas have highlighted differential or reduced treatment response among AA subgroups, including in depression and heart failure.^{9–12}

Naltrexone, a mu opioid receptor antagonist FDA-approved for alcohol and opioid dependence, has demonstrated smoking cessation efficacy in some studies^{13–16} but not in others.^{17, 18} The rationale for using naltrexone in smoking cessation is based on evidence of a biological interaction between nicotinic and opioid receptor systems.^{19, 20} However, most prior clinical research with naltrexone in addictive disorders has included primarily EA patients.²¹ A recent secondary analysis of data from Project COMBINE²² demonstrated that, relative to EAs, AAs were not as responsive to naltrexone (vs. placebo) in reducing percent-days abstinent, time to first heavy drinking day, and global clinical outcome.²¹ The authors concluded that this differential response might be in part due to pharmacogenetic variants that affect response to naltrexone and differ between AAs and EAs. For example the Asp40 allele (rs1799971) in the mu-opioid receptor gene (*OPRM1*), the primary drug target of naltrexone, has been shown in some studies of alcohol dependence to be associated with enhanced response to naltrexone (increased days abstinent and decreased heavy drinking days).^{23, 24} This genetic polymorphism occurs much less frequently in persons of African Ancestry (<5%) than those of European Descent (20%).^{23, 27, 28}

Variance in the genetic background of study subjects (in particular, admixed groups such as AAs and Hispanic Americans) may impact the power and reliability of genetic association studies.²⁹ Methods to detect and control for differences in ancestry in genetic association studies utilize ancestry informative markers (called AIMs). These AIMs are single nucleotide polymorphisms (SNPs) which have large allele frequency differences between

continental groups such as Europeans and West Africans and are powerful for estimating genetic ancestry.^{30, 31} Genetic ancestry, as measured by AIMS, represents an approach to characterizing biological contributions to observed racial differences in treatment response. Estimating genetic ancestry using AIMS is important, as genetic heterogeneity within racial and ethnic groups may bias associations in genetic association studies, generating both false-positive and false-negative results.^{29, 32–35} The genetic ancestry proportions assigned to each individual (i.e., % west African and % European), as opposed to membership of one racial group, serves as a proxy for the genetic ancestral background of the individual and can be used to assess the relationship between genetic ancestry and drug response. The finding of an association between genetic ancestry and a particular outcome may implicate genetic factors which may be differentially expressed among groups. AIMS have been used to test the potential role of genetics in disease disparities and pharmacogenomics within admixed populations.^{33, 36–39} To date, they have not been used in the context of racial differences in the effects of smoking cessation pharmacotherapy.

To our knowledge, the current study is the first to compare smoking cessation outcomes with naltrexone in AAs versus EAs. We analyzed data from our previously published randomized placebo-controlled clinical trial of naltrexone in adult smokers who desired to quit smoking and included genetic ancestry in the analysis.⁴⁰ Analyses on the full sample (N=315) showed that naltrexone increased smoking quit rates (30% naltrexone vs. 17% placebo), and also reduced the number of cigarettes smoked and delayed the time to first cigarette during the active treatment phase through 12 weeks.⁴⁰ The trial was unusual for oversampling for AAs: 35% of the sample self-identified as African American/Black. Thus, this study has the largest sample of AAs within a randomized pharmacological smoking cessation trial. In this study, we compared smoking cessation quit rates in AAs and EAs, and examined the extent to which West African ancestry modified response to treatment among the AAs. Based on prior research, we hypothesized that AAs would be less responsive to naltrexone compared to EAs. We also hypothesized, based on previous research showing decreased drug response in AAs compared to EAs, that AAs with higher WA genetic ancestry would experience less benefit from naltrexone on quit rates than AAs with lower levels of WA genetic ancestry.

SUBJECTS AND METHODS

Data for this study were culled from the previously mentioned trial of naltrexone versus placebo in 315 adult smokers recruited in Chicago, IL.⁴⁰ In the present analyses, only participants self-identified as non-Hispanic AA (n=110) or EA/Caucasian (n=181) who provided a biological sample for genetic analysis were considered (92% of original sample). After examination of the data, three AAs were further excluded from analyses because they were deemed outliers based on their estimated ancestry (percent WA ancestry <30%, >5 SDs from the mean). Further, DNA samples were not of sufficient quantity or quality in an additional 12 AA and 45 EA participants, thus resulting in a final sample size of n=95 AA and n=136 EA participants in analyses.

Eligibility criteria for the clinical trial were: 18–65 years of age; smoked 12–40 cigarettes daily for at least 2 years and reported a desire to quit smoking; had a body mass index of 19–

38 kg/m²; had hepatic transaminase concentrations within normal range (<2.5 times normal); were able to read and write English; had a breath carbon monoxide reading of at least 10 ppm; had a urinary Nicalert reading greater than 500 ng/mL cotinine, confirming regular smoking; were not currently taking opioid or psychotropic medications; did not have a past-year history of a major medical or psychiatric disorder, or a lifetime diagnosis of opioid abuse or dependence; and were not nursing or pregnant. Screening measures and procedures are described in detail in King et al.⁴⁰ The study was approved by The University of Chicago Institutional Review Board.

Treatments and Procedures

Participants were randomized to either naltrexone or placebo conditions at their second study visit, and instructed to begin taking their study medication the next day (one week prior to the designated quit date). Both study participants and staff were blinded to treatment assignment. Participants were enrolled between June 2006 and March 2009. All participants also received open-label nicotine patches and weekly behavioral counseling during the first post-quit month (for details, see King et al 2012).⁴⁰ The nicotine patch was intended to alleviate tobacco withdrawal, which may be exacerbated with opioid antagonist treatment.⁴¹ During the pre-quit week, naltrexone was titrated with 12.5 mg on day one, 25 mg daily on days two and three, and 50 mg on days four to six, and continued for 12 weeks after the quit date. Adherence to naltrexone was confirmed by urine and saliva test to determine naltrexone and its main metabolite, 6-β-naltrexol. Quit status was defined as prolonged abstinence which is not smoking even a puff of a cigarette allowing for a 1-week grace period⁴², confirmed by expired air carbon monoxide reading of 10 ppm. Outcomes were assessed at four weeks to ascertain naltrexone augmentation of patch and counseling and at the end of treatment at 12 weeks, with naltrexone or placebo only given from week five to 12.

Genotyping and Ancestry Estimation

DNA from blood was genotyped for 100 carefully selected, and previously published, SNPs forming the AIMs panel using the Sequenom MassARRAY iPLEX platform.^{36, 43} These autosomal markers have previously been identified and validated and are used to extract continental ancestry information in admixed populations.^{44–46} From a study of over 4,000 autosomal single nucleotide polymorphisms (SNPs) spanning 22 chromosomes, an enriched panel of 100 unlinked AIMs was selected to provide individual ancestry assessment based on several measures of marker informativeness (F_{ST} , F_{IC} , and δ) and confirmed in several parental populations consisting of 60 CEPH Europeans, 56 Yoruban sub-Saharan Africans, 19 Bini sub-Saharan Africans, 23 Kanuri West Africans, 50 Mayan Amerindians, 26 Quechuan Amerindians, 29 Nahua Amerindians.^{47, 48} Previous work shows that the correlation coefficient between estimated ancestry scores and true individual ancestral proportions is greater than 0.9 when the estimated ancestry scores are derived from approximately 100 ancestry informative markers (AIMS).^{35, 47}

All 100 AIMs were genotyped using the Sequenom MassARRAYTM genotyping platform with iPLEXTM chemistry according to manufacturer's recommendations. Briefly, iPLEXTM assays were designed utilizing the Sequenom Assay Design software, allowing for single

base extension designs used for multiplexing. Multiplex assays were performed to amplify 5 ng of genomic DNA by polymerase chain reaction (PCR). PCR reactions were treated with shrimp alkaline phosphatase (SAP) to neutralize unincorporated dNTPs. Subsequently, a post-PCR single base extension reaction was performed for each multiplex reaction using concentrations of 0.625 μ M for low mass primers and 1.25 μ M for high mass primers. Reactions were diluted with 16 μ l of H₂O and fragments were purified with resin, spotted onto Sequenom SpectroCHIP™ microarrays and scanned by MALDI-TOF mass spectrometry. Individual SNP genotype calls were generated using Sequenom TYPER™ software, which automatically calls allele specific peaks according to their expected masses. Genotyping quality control for all SNPs was assessed using blinded duplicate genotyping for 60 DNA samples. A genotype concordance rate of 99% was observed for all markers. Genotyping call rates exceeded 98.5% for all individuals included in the analyses. The *OPRM1* A118G genotype (rs1799971) was determined as previously described.²⁸

Individual admixture estimates for each study participant were calculated using a model-based clustering method as implemented in the program STRUCTURE v2.3.⁴⁹ STRUCTURE 2.3 was run using parental population genotypes from west Africans and Europeans⁴⁴ under the admixture model using the Bayesian Markov chain Monte Carlo method (K=2, assuming two founding populations) and a burn-in length of 30 000 for 70 000 repetitions.

The distribution of the genetic ancestry among the self-reported AAs and EA participants is shown in Figure 1. The AAs in the sample had a mean WA ancestry score of 80% (SD 9%;IQR [73% to 87%]). Among EA participants, the mean WA ancestry score was 2.6% (SD 2.9%, IQR 1.1% to 2.6%). AAs with high and low WA ancestry were defined via a median split of the WA ancestry variable (high WA ancestry, 80% AIMS, low WA ancestry <80%).

Statistical Analysis

Demographic and smoking categorical characteristics were compared between naltrexone and placebo groups using Chi Square or Fishers Exact tests and continuous variables were compared by Student's t-test or by Mann-Whitney U test (for non-normally distributed) as appropriate. Logistic regression analyses were used to compare quit rates for naltrexone versus placebo in AAs and EAs at four weeks and twelve weeks. Similar logistic regression models were used to investigate the effect of naltrexone on quit rates within the EA and AA groups using a product term. Covariates in these models included characteristics that were different between AAs and EAs, including age, sex, body mass index, Fagerström Test for Nicotine Dependence (FTND) scores, socioeconomic status (Hollingshead score⁵⁰), mentholated cigarette preference, alcohol use and marital status. These variables have also been shown to relate to smoking outcomes in other studies.⁵¹ Models were repeated without these covariates, and the main results remained. Confidence intervals and p-values were estimated using the bootstrap.⁵² Similar logistic regression models were used to investigate the effect of medication on quit rates comparing AA high and low WA ancestry subgroups using product terms. All tests were two-sided and p value of less than 0.05 was considered as statistical significance. Statistical analyses were performed with the SAS software

package, version 9.2 (SAS Institute, Cary, NC, USA), and Stata/SE software, Version 12.1 (StataCorp, College Station, TX, USA).

RESULTS

Table 1 shows that among the EAs in the sample, naltrexone significantly improved quit rates at four weeks (62% vs. 43%, risk ratio (RR) 1.4 95%CI 1.03 to 2.1, $p=0.03$) but not at 12 weeks (30% vs. 18%, RR 1.6 95%CI 0.91 to 3.6, $p=0.12$). In contrast, among the AAs, quit rates were not statistically different between naltrexone and placebo groups at four weeks (43% vs. 32%, RR 1.3 95%CI 0.77 to 2.6, $p=0.27$) or at 12 weeks (22% vs. 18%, RR 1.2 95%CI 0.51 to 3.5, $p=0.60$). The p value for interaction between race and treatment group on 4 and 12 week quit rates was 0.43 and 0.41, respectively. The coefficient estimates from this model are in supplementary Table 1. Covariates were well balanced across the naltrexone and placebo groups. However, as stated earlier, there were several demographic and smoking characteristics that differed between AAs and EAs, which were accounted for as covariates in the logistic regression models (Supplementary Table 2). The AA group was older, had higher proportions of females, higher FTND scores, higher years smoked, and a higher proportion of menthol preference. The AAs group also had lower a lower Hollingshead index and lower proportion of college education.

Within the AAs, the high and low WA ancestry groups were well balanced with respect to clinical and smoking characteristics as well as education and socioeconomic status (Table 2). When we allowed the treatment effect to vary across WA genetic ancestry levels, we found that the four week quit rates were significantly different between naltrexone and placebo groups in the low WA ancestry group but not in the high WA ancestry group (Figure 2). Table 3 shows that among AAs with low WA Ancestry, naltrexone increased four week quit rates by 33 percentage points (60% vs 27%, RR 2.2 95%CI 1.1 to 5.9, $p=0.037$). In contrast, among AAs with high WA ancestry, quit rates were not significantly different between naltrexone and placebo groups. The p value on the treatment by ancestry interaction term was statistically significant, $p=0.03$. The coefficient estimates from this model are in supplementary Table 3. Supplementary Table 4 shows the difference in twelve week quit rates between naltrexone and placebo groups across levels of WA genetic ancestry. Naltrexone did not generate a statistically significant effect on 12 week quit rates in either of these models. However, the direction of the effects is consistent with the four week results in that naltrexone quit rates were higher in the low WA ancestry group.

DISCUSSION

Our analysis showed that naltrexone increased quit rates at four weeks in EAs but not in AAs, and that this difference was moderated and partly explained by differences in WA ancestry among AAs. Naltrexone improved quit rates among AAs with low WA ancestry but it did not affect quit rates among AAs with high WA ancestry. In the pooled sample these heterogeneous effects led to null findings. The low WA ancestry group of AAs showed a 33-percentage point increase in quit rates with a 60% quit rate with naltrexone but the high WA group were not responsive to naltrexone with a 28% quit rate, i.e., 11% lower than with placebo. Uncovering effect modification by genetic ancestry suggests a genetic explanation

for the reduced naltrexone response observed within the self-reported AA group. Should our preliminary results be replicated, individualized treatment strategies based on ancestry could be developed. This, in turn, could increase quit rates and reduce racial disparities in smoking related adverse health outcomes.

There are several potential explanations for our findings. Evidence that naltrexone response among AAs depends on WA genetic ancestry suggests a genetic explanation beyond potential socio-economic factors. For example, we investigated the *OPRM1* A118G variant (rs1799971), which lies in exon 1 of the *OPRM1* gene, the primary drug target of naltrexone (supplementary Table 5 and supplementary Table 6). The G allele causes the normal amino acid asparagine to be replaced with aspartic acid. Some studies have shown that one or both copies of the G allele are associated with higher alcohol induced reward and better treatment response to naltrexone in alcohol dependence^{23, 27, 28, 53} but other studies have shown lack of such association in substance dependence.^{25, 26} In our sample, the 118G allele frequency was significantly lower in AAs compared to the EAs (6% vs. 15%, $p=0.004$), which is consistent with previous reports. Moreover, within the African Americans, the G allele frequency was significantly lower (10% vs 2%, $p=0.03$) in the low West African Ancestry group. The low frequency of this allele in our small population precluded a robust analysis of this SNP in our sample. However, including the *OPRM1* A118G in our regression models did not change our results and west African ancestry remained a significant effect modifier of naltrexone treatment. There are likely many more variants that are closely linked with ancestry, and that could affect drug absorption, distribution, metabolism, and excretion.

Our data are consistent with two other studies that found variable drug response across different levels of genetic ancestry. The Study of African-Americans, Asthma, Genes and Environments (SAGE) investigators found that albuterol response was modified by ancestral background in Latinos, but no such modification was found in Puerto Rican or AA asthmatic patients.⁵⁴ Among Latinos in that study, increased European ancestry was associated with decreased response to albuterol. Another study in depression pharmacotherapy found that African ancestry, estimated using multidimensional scaling, predicted lower treatment response in major depression.⁵⁵ However, unlike our study, they did not examine drug treatment effect heterogeneity across different levels of genetic ancestry.

Our study also makes a methodological contribution. The collection of the genetic information used to construct genetic ancestry estimates provides a way to conduct exploratory research using a method that occupies a middle ground between theoretically grounded candidate gene studies and agnostic genome wide association studies. To obtain more definitive answers on ancestry as an effect modifier, researchers should consider building genetic ancestry information into the design of randomized clinical trials by preemptively blocking on levels of genetic ancestry and self-reported race. A block design may work particularly well in AA population where admixture is high.^{39, 56} Another issue is the statistical handling and coding of the ancestry variable which ranges from 0 to 1 for each subject. We chose to conduct a median split for simplicity in our interaction analysis. However, one could handle the variable as continuous under the assumption that a linear function provided a good approximation to its effect on the dependent variable. One also could categorize ancestry into categories, such as high, medium, and low based on quantiles.

Depending on the sample size flexible methods such as splines could be used to analyze the data. Previous studies have treated ancestry as a continuous variable,^{38, 39, 55, 57–63} both as continuous and categorical,^{64, 65} and as a spline term.⁶³ Further research is needed to clarify the proper handling of genetic ancestry in genetic association and pharmacogenomic studies.

Overall, our work represents a contribution in elucidating methods that uncover biological contribution to drug treatment effect heterogeneity. Nevertheless, it is important to interpret our results in the context of its strengths and limitations. The key strengths of our study flow from the original randomized, double blind, placebo controlled study design. The original study also measured a rich set of smoking predictors and patient characteristics that allowed for statistical adjustment, which was important for the subgroup analysis. The most important limitation arise because our study is based on a secondary analysis of a data set with a modest sample size. Unplanned subgroup analysis of clinical trials can be misleading, especially when then number of sub groups tested is high. However, we examined only one sub group (ancestry within AAs) which was theory driven. Still given this risk, our findings should be considered provisional and warrant replication in an independent sample. We were limited to existing study measures; however, these included a rich set of characteristics that are theoretically related to the quitting outcome. Genetic ancestry represents a reliable proxy for biological and or genetic difference amongst the AAs, however, the variable could also be correlated with unmeasured differences in socio-economic status or other patient characteristics that could confound our results. We found that ancestry modified naltrexone response on the four week quit rates but not at 12 weeks. This could reflect low statistical power. More speculatively, it may be evidence that naltrexone effects dissipate over time due to compliance reductions or receptor down-regulation over time. For example, quitting event rates are lower at 12 weeks than they are at 4 weeks.⁴⁰

Conclusion

In the study as a whole, naltrexone increased quit rates in EAs but not in AAs. However, among AAs with low WA ancestry, naltrexone significantly increased quit rates at four weeks. The drug had no statistically detectible effects among AAs with high WA ancestry. Our study attempts to disentangle socio-economic and genetic contributions to heterogeneous drug effects across racial groups. By stratifying on self-reported race, and then examining heterogeneity by genetic ancestry we can learn about potential biological differences that may contribute to the heterogeneity observed across self-reported racial groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Distribution of West African genetic ancestry in subjects who self-identified as either African American (Blue) or European American (Red)

Figure 2.
Four week quit rates in the AAs between naltrexone and placebo groups in the overall sample and within ancestry groups

Table 1

Naltrexone vs. Placebo for Smoking Cessation in European and African Americans

Quit Rates (%)	European Americans (n=136)					African Americans (n=95)					P Value for Interaction	
	Naltrexone	Placebo	QRD (95%CI)	RR (95%CI)	P Value	Naltrexone	Placebo	QRD (95%CI)	RR (95%CI)	P Value		
Four week	62	43	19 (2.2 to 34)	1.4 (1.03 to 2.1)	0.03	43	32	11 (-10 to 32)	1.3 (0.77 to 2.6)	0.27		
Twelve Week	30	18	12 (-5.1 to 26)	1.6 (0.91 to 3.6)	0.12	22	18	4.3 (-12 to 22)	1.2 (0.51 to 3.5)	0.60		

QRD= Quit rate differences; difference in quit rates between naltrexone and placebo groups

RR = Risk ratio

95%CI = 95% Confidence interval

P values are Wald Tests from the logistic regression models

Covariates include age, sex, BMI, FTND, Hollingshead score, menthol preference, marital status and alcohol use status

Table 2

Baseline Demographic and Smoking Characteristics by High and Low WA Ancestry Groups

Variable	High WA Ancestry (n=47)	LowWA Ancestry (n=48)	P value
Age, y	45 ± 10.7	47 ± 9	0.36
Male Gender	14 (30)	22 (46)	0.11
Body Mass Index, kg/m ²	28 ± 4.6	29 ± 4	0.77
Nicotine Dependence (FTND)	5 ± 1.8	6 ± 1.9	0.21
Cigarettes per day	18 ± 5.9	18 ± 6.3	0.69
Carbon monoxide level, ppm	21 ± 9.4	23 ± 10.7	0.28
Age of smoking initiation	18 ± 3.9	18 ± 4.8	0.64
Smoking urge (BSQU)	34 ± 13	34 ± 13.8	0.96
No. of past quit attempts (>12hr)	3 [2–5]	2 [0–4]	0.92
Number of years smoking	28 ± 11.2	29 ± 9.7	0.51
Menthol preference	40 (85)	42 (88)	0.74
Married or living with partner	10 (21)	16 (33)	0.19
Hollingshead index	41 ± 13	41 ± 14	0.80
Current alcohol drinker	28 (60)	32 (67)	0.48
Education			
High school	13 (28)	13 (27)	0.95
Some College	23 (49)	19 (40)	0.36
More than college	11(23)	16 (33)	0.29
Naltrexone	26 (55)	21 (44)	0.26

Data are mean ± SD or counts (%) or Median [IQR].

Current alcohol drinker defined as consuming at least one drink in past six months.

BQSU indicates Brief Questionnaire of Smoking Urges; FTND, Fagerstrom Test of Nicotine Dependence.

Table 3

Differences in Four Week Quit Rates Between Naltrexone and Placebo Groups in African Americans

Variables	<u>Model 1 Main Effects Model</u>			<u>Model 2 Interaction Model</u>		
	RR	95%CI	P value	RR	95%CI	P value
Naltrexone	0.11	0.76 to 2.5	0.27	-	-	-
Naltrexone and Low WA	-	-	-	2.2	1.1 to 5.9	0.037
Naltrexone and High WA	-	-	-	0.73	0.26 to 1.9	0.48
N	95			95		

RR= Risk ratio

Covariates include age, sex, BMI, FTND, Hollingshead score, menthol preference, marital status and alcohol use status

P values are Wald Tests from the logistic regression models