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## A variant in *ANKK1* modulates acute subjective effects of cocaine: a preliminary study

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### Abstract

This study aimed to evaluate whether functional variants in the *ankyrin repeat and kinase domain-containing 1* gene (*ANKK1*) and/or the *dopamine receptor D2* gene (*DRD2*) modulate the subjective effects (reward or non-reward response to a stimulus) produced by cocaine administration. Cocaine-dependent participants ( $N = 47$ ) were administered 40 mg of cocaine or placebo at time 0, and a subjective effects questionnaire (visual analog scale) was administered 15 minutes prior to cocaine administration, and at 5, 10, 15, and 20 minutes following administration. The influence of polymorphisms in the *ANKK1* and *DRD2* genes on subjective experience of cocaine in the laboratory was tested. Participants with a T allele of *ANKK1* rs1800497 experienced greater subjective ‘high’ ( $p = 0.00006$ ), ‘any drug effect’ ( $p = 0.0003$ ), and ‘like’ ( $p = 0.0004$ ) relative to the CC genotype group. Although the variant in the *DRD2* gene was shown to be associated with subjective effects, LD analysis revealed this association was driven by the *ANKK1* rs1800497 variant. A participant’s *ANKK1* genotype may identify individuals who are likely to experience greater positive subjective effects following cocaine exposure, including greater ‘high’ and ‘like’, and these individuals may have increased vulnerability to continue using cocaine or they may be at greater risk to relapse during periods of abstinence. However, these results are preliminary and replication is necessary to confirm these findings.

### Keywords

*ANKK1*; subjective effects; cocaine

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## Introduction

The reward machinery of the brain is a critical component in the development of substance addiction. Cocaine and methamphetamine increase the levels of dopamine (DA) in synapses of the nucleus accumbens (NA) (Nestler, 2001, Nestler, 2005, Wise & Bozarth, 1987). Dopamine is one of the main neurotransmitters that mediates reward and was observed to be associated with the availability of natural reinforcers (Agmo & Berenfeld, 1990, Pfaus *et al.*, 1990, Small *et al.*, 2003).

Cocaine is one of the most commonly abused drugs with approximately 1.4 million individuals (aged 12 years and older) currently using cocaine in the United States 2011 (Samhsa, 2012). Cocaine acts, in part, on the dopaminergic pathway by blocking the DA transporter (encoded by the *DAT1/SLC6A3* gene) and allowing more DA to remain in the synapse to activate DA receptors (Nestler, 2001, Nestler, 2005, Volkow *et al.*, 1996). There is inter-individual variation in how people experience the effects of cocaine and some of this variation may be due to genetic influences [reviewed in (Hart *et al.*, 2012)]. There is evidence that the nature and intensity of these subjective effects may lead to increased or decreased use, and may lead to dependence or abuse particularly when the effects are positive (Fergusson *et al.*, 2003, Haertzen *et al.*, 1983). With alcohol, for example, it has been shown that flushing and increased heart rate (e.g., negative reactions) after consumption of alcohol by individuals with aldehyde dehydrogenase II deficiency lead to reduced rates of use and alcoholism (Edenberg, 2007). Another study showed that positive reactions to cannabis early on (e.g., feeling happy or relaxed) led to an increased odds ratio of future dependence (Fergusson *et al.*, 2003).

In this study, we examined rs1800497 (C→T, also known as *DRD2/ANKK1 TaqIA*), a functional variant that codes for a substitution of glutamate to lysine at position 713 in the ankyrin repeat and kinase domain-containing 1 (*ANKK1*) protein. The rs1800497 variant in the *ANKK1* gene has been found to be associated with DA D2 receptor density, with the minor T allele found to be associated with lower receptor density (Jonsson *et al.*, 1999). The T allele of rs1800497 has been shown to be associated with various psychiatric disorders, including alcohol and cocaine dependence (e.g. (Blum *et al.*, 1991, Connor *et al.*, 2007, Dubertret *et al.*, 2001, Noble *et al.*, 1993)).

Variants in the *DA receptor D2* gene (*DRD2*) have been found to be associated with several neurological and psychiatric conditions including substance addiction and/or dependence, migraine, and schizophrenia (Blum *et al.*, 1991, Blum *et al.*, 1990, Comings *et al.*, 1991, Connor *et al.*, 2007, Glatt *et al.*, 2009, Golimbet *et al.*, 2003, Noble, 1994, Noble *et al.*, 1991, Voisey *et al.*, 2012, Yang *et al.*, 2007, Yang *et al.*, 2008). The rs2283265 T allele of the *DRD2* gene occurs more frequently in cocaine addicts than in controls (Moyer *et al.*, 2011), and has been shown to be associated with decreased expression of the short form (D2S) form of the D2 receptor (Moyer *et al.*, 2011, Zhang *et al.*, 2007).

Given the involvement of *ANKK1* and *DRD2* in the dopaminergic pathway, and given cocaine's direct effect on the dopaminergic pathway, we hypothesized that genetic variation

in the *ANKK1* and *DRD2* genes may be associated with subjective effects produced by cocaine in the laboratory.

## Materials and methods

### Participants

Non-treatment-seeking, cocaine-dependent participants were recruited between March 2010 - July 2012 via advertisements and were paid for their participation as part of ongoing research trials conducted by Drs. De La Garza's and Newton's Stimulant Addiction Research Program at the Baylor College of Medicine. A total of 47 participants signed informed consent documents in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki), had blood samples for genetic analysis collected, and were enrolled in this component of the study. All participants were administered the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan *et al.*, 1998) and Addiction Severity Index (ASI) (McLellan *et al.*, 1992), and met Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) criteria for cocaine-dependence. Participants were between 18 and 55 years of age, had a history of using cocaine by the smoked or intravenous route, and had normal laboratory evaluation (comprehensive metabolic panel and complete blood count), electrocardiogram, and vital signs. Exclusion criteria included a history of head trauma, epilepsy, dependence on drugs other than cocaine and nicotine, inability to detect effects of cocaine, or the presence of any other axis I psychiatric disorder. Serious medical condition such as heart disease, acquired immunodeficiency syndrome (AIDS), and asthma were also exclusionary. Women were required to provide a negative pregnancy test prior to enrollment. Concomitant use of psychotropic medications or medications affecting blood pressure was not allowed. This study was approved by the Institutional Review Board of the Baylor College of Medicine as well as the Research and Development committee of the Michael E. DeBakey Veteran Affairs Medical Center.

### Subjective effects testing

Using a double-blind, placebo-controlled, within-subjects study design, participants were administered 0 (saline) or 40 mg cocaine intravenously. Each individual participated in both conditions, one dose being administered in the morning (~9 AM) and one in the afternoon (~1 PM). Subjective effects of cocaine were measured using a visual analog scale anchored at 0 for "no effect" to a maximum of 100 for "most ever". Ratings were obtained for 'high' ("How high are you right now?"), 'any drug effect' ("Do you feel any drug effect right now?"), 'stimulated' ("How stimulated do you feel right now?"), 'good effect' ("Does the drug have any good effects right now?"), 'like' ("How much do you like the drug right now?"), 'bad effect' ("Does the drug have any bad effects right now?"), 'anxious' ("How anxious do you feel right now?"), 'desire' ("How much do you desire the drug right now?"), 'likely to use cocaine if had access' ("If you had access to the drug right now how likely would you be to use it right now?"), and 'depressed' ("How depressed do you feel right now?"). Subjective effects ratings were obtained 15 minutes prior to dosing and at 5, 10, 15, and 20 minutes following dosing.

## Genotyping

DNA was isolated from blood using the Genra Puregene blood kit (Qiagen, Germantown, MD) according to the manufacturer's recommendations. Genotypes were determined by a researcher who was unaware of the clinical status of the participants using 5'-fluorogenic exonuclease assays (TaqMan®, Applied Biosystems, Foster City, CA). The *ANKK1* rs1800497 variant was genotyped using the TaqMan® primer-probe sets (Applied Biosystems) assay ID C\_7486676\_10, and the *DRD2* rs2283265 variant with assay ID C\_16070796\_10. PCR amplifications were performed in duplicate using Platinum® quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, CA) on a ViiA7 and ViiA 7 Software v1.1 was used for data analysis (Applied Biosystems). *SRY* was genotyped to determine sex (Kosten *et al.*, 2013). Ten ancestry-informative markers were genotyped to determine population structure, and the data from our cohort were compared against CEPH-HGDP samples (1,035 subjects of 51 populations) as previously described (Kosten *et al.*, 2013).

## Statistical Analyses

A repeated measures analysis of variance (ANOVA) was used to analyze the subjective effects scores over time for each subject. Subjective effect values following cocaine or saline administration were normalized to baseline values (–15 minute time point), and the saline administration subjective effect values (Supplementary Figure 2, right panels) were then subtracted from the 40 mg cocaine subjective effect values (Supplementary Figure 2, left panels) for each individual to yield the final subjective effect scores (Figure 1 and Supplementary Figure 1). Covariates included in the statistical model were gender, population structure, and years of nicotine use. Data from the two genotype groups over time were analyzed to determine if the subjective effect scores were moderated by the *ANKK1* locus or the *DRD2* locus using R version 2.9.1 (R\_Development\_Core\_Team, 2009). We compared *ANKK1* genotype (0 = CC genotype, 1 = CT/TT), or *DRD2* genotype (0 = GG genotype, 1 = GT/TT), time, and interactions between genotypes and time. ANOVA was also used to analyze the continuous variables in the demographic data, while a Fisher's exact test was used to analyze any categorical variables. We calculated effect size as a partial eta-squared statistic using condition or SNP variance over residual variance. The three general cut-offs for effect size are the following: a large effect is 0.14, a medium effect is 0.06, and a small effect is 0.01. Power analyses revealed that with a 47-participant sample divided into four groups and measured over six time points, and assuming a similar effect size to what we observed for *ANKK1* and *DRD2*, there is 80% power to detect a difference within our samples at a Bonferroni-corrected alpha level of 0.05.

To determine population structure from the AIMS data, our cohort was compared to the Centre d'Etude du Polymorphisme Humain–Human Genome Diversity Panel (CEPH-HGDP) samples as outlined in Lao *et al.* (Lao *et al.*, 2006). The CEPH-HGDP cohort consists of 1035 subjects from 51 different regional populations including America, Europe, the Middle East, Central and East Asia, Oceania, and sub-Saharan Africa. Proportion of population substructure for each individual was determined using the STRUCTURE 2.3.3 software (Hubisz *et al.*, 2009, Pritchard *et al.*, 2000) using four ancestral populations ( $K = 4$ ), a burn-in period of 100,000 iterations, and 1 million Markov chain Monte Carlo

replications after burn-in. The proportion of population substructure represented in each individual was included in the analysis as a covariate to eliminate population stratification effects.

## Results

### Demographic characteristics by treatment and *ANKK1/DRD2* genetics

The genotypes of the participants included 26 CC, 18 CT, and three TT genotypes for *ANKK1*, and 37 GG, ten GT, and zero TT genotypes for *DRD2* (see Table 1). The participants were mostly African American males (68%) with a mean age of 43.5 years. Participants used cocaine for a mean of 16 years and for a mean of 17 days in the month prior to participating in this study. The only significant difference among the demographic variables was years of nicotine use for *ANKK1* ( $p = 0.0432$ ) and *DRD2* ( $p = 0.0133$ ) (see Table 1). There were no other significant baseline differences among the genotype groups in any demographic characteristics after adjusting for multiple testing (Bonferroni,  $p < 0.05$ ).

### Linkage disequilibrium (LD) analyses

LD analysis revealed that the *ANKK1* and *DRD2* genes are almost in complete LD according to the  $D'$  measure based on allele frequencies where  $D' = 0.999$  ( $r^2 = 0.35$ ). The distribution of participants among the genotype groups varies and is as follows: *ANKK1*; TT = 3, TC = 18, and CC = 26, and *DRD2*; TT = 0, TG = 10, and GG = 37. The CC genotype for *ANKK1* only is observed in the presence of the GG genotype of *DRD2*. When both variants were included in the statistical model, the *ANKK1* variant was the only significant result, indicating the effects of the variants were not independent, and the statistical significance of the *DRD2* rs2283265 variant was result of the *ANKK1* C-allele on which it arose.

### *ANKK1* genotype and subjective effects

A dominant model was used for statistical analysis that grouped participants based on the presence or absence of the *ANKK1* rs1800497 minor T allele. For the subjective scores of 'high' there was a significant main effect of genotype ( $F = 16.99$ ;  $df = 1, 179$ ;  $p = 0.00006$ , with an effect size of 0.0949) and time ( $F = 11.87$ ;  $df = 1, 179$ ;  $p = 0.0007$ , with an effect size of 0.0663), without a significant interaction ( $p = 0.53$ ). The difference from baseline between the genotype groups was the largest for 'high' at 5 minutes into the trial where the CT/TT genotype group had values of  $70.86 \pm 8.99$  (s.e.m.) and the CC genotype group had values of  $43.08 \pm 8.31$  (s.e.m.) (see Figure 1A).

For the subjective score of 'any drug effect' there was a significant main effect of genotype ( $F = 13.82$ ;  $df = 1, 175$ ;  $p = 0.0003$ , with an effect size of 0.0772) and time ( $F = 14.71$ ;  $df = 1, 179$ ;  $p = 0.00017$ , with an effect size of 0.0822), and the interaction term was not significant ( $p = 0.07$ ). The largest difference from baseline between the genotype groups was at 5 minutes into the trial for 'any drug effect' where the CT/TT genotype group had values of  $73.48 \pm 8.56$  (s.e.m.) and the CC genotype group had values of  $48.85 \pm 9.30$  (s.e.m.) (see Figure 1B).

Finally, for the subjective scores of ‘like’ there was a significant main effect of genotype ( $F = 12.98$ ;  $df = 1,179$ ;  $p = 0.0004$ , with an effect size of 0.0725), but neither time ( $p = 0.26$ ) nor the interaction term ( $p = 0.93$ ) were significant. The difference from baseline between the genotype groups was the largest for ‘like’ at 5 minutes into the trial where the CT/TT genotype group had values of  $66.81 \pm 13.36$  (s.e.m.) and the CC genotype group had values of  $35.58 \pm 11.80$  (s.e.m.) (see Figure 1C). The other subjective effects variables did not reach the  $p < 0.05$  level of significance and are therefore not reported.

## Discussion

Analyses revealed that the *ANKK1* and *DRD2* genes were in almost complete LD ( $D' = 0.999$ ) and that the *ANKK1* variant was the only significant result when both variants were included in the statistical model. The statistical significance of the *DRD2* rs2283265 variant is a result of the *ANKK1* genotype on which it arose and, therefore, this discussion will focus on the *ANKK1* rs1800497 variant found to be associated with subjective effects of cocaine.

In this preliminary study, we found that individuals who carry the T allele of rs1800497 (originally referred to as the *DRD2/ANKK1/TaqIA1* allele: (Blum *et al.*, 1990)) in the *ANKK1* gene reported higher subjective effects in response to acute cocaine administration in the laboratory. Specifically, participants with genotypes reflecting lower dopamine D2 receptor density had greater subjective effects after cocaine administration than participants with genotypes associated with higher D2 receptor density.

The dopaminergic pathway in the brain, also known as the ‘reward’ or ‘motivational’ pathway, is integral to addictive and psychiatric conditions. More recently, the role of the dopaminergic pathway has been clarified as the ‘motivational’ machinery of the brain as DA release is associated with anticipation of reward, instead of being associated with the reward administration itself (Berridge, 2007). In 2007, Berridge and colleagues described the effect as DA mediating the ‘wanting’ (also known as “incentive salience”) of pleasurable effects, more than the ‘liking’ of pleasurable effects.

DA D2 receptor density has been shown to impact cocaine addiction and self-administration. Non-human primates with low DA D2 receptor density show higher frequency of cocaine self-administration (Nader *et al.*, 2006). The brain tissues of cocaine addicts show reduced D2 receptor availability via PET scan after chronic cocaine addiction (Volkow *et al.*, 1990), and healthy volunteers with lower dopamine D2 receptor density reported liking the effects of methylphenidate (a stimulant with some structural and pharmacologic similarities to cocaine), whereas individuals with higher dopamine D2 receptor density reported more intense unpleasant effects of methylphenidate (Volkow *et al.*, 1999).

The *ANKK1* rs1800497 T allele has been found to associate with lower DA receptor tone (Jonsson *et al.*, 1999, Pohjalainen *et al.*, 1998, Ritchie & Noble, 2003, Thompson *et al.*, 1997). Low density of DA receptors also has been described in relation to the “reward deficiency syndrome” in which individuals with increased risk for substance addiction have defects in the dopaminergic pathway such as rapid degradation of DA, low dopamine receptor density, or high clearance of DA from the synapse (Comings & Blum, 2000). The

hypothesis is that these individuals experience a lower level of ‘reward’ due to these factors and therefore are more habitual in seeking “artificial” means of increasing the ‘reward’ sensation.

Given its presumed function in the dopaminergic pathway, it is not surprising that the *ANKK1* gene has been linked to several addictive disorders, polysubstance addiction (Blum *et al.*, 1991, Blum *et al.*, 1990, Cobos *et al.*, 2007, Comings *et al.*, 1996, Lawford *et al.*, 2000, Munafo *et al.*, 2004, Noble, 1994, Persico *et al.*, 1996), and several psychiatric disorders (Comings *et al.*, 1991, Denys *et al.*, 2006, Glatt *et al.*, 2009, Golimbet *et al.*, 2003, Nisoli *et al.*, 2007). In 1990, the *ANKK1/DRD2 TaqIA* variant also examined in this study (rs1800497) was one of the first genetic variants found to be associated with alcoholism (Blum *et al.*, 1991, Blum *et al.*, 1990), and since that time has been one of the most-studied variants in relation to neurological and psychiatric conditions, including substance abuse and dependence disorders. Initially identified as a polymorphism in the promoter region of *DRD2* (as it is located 10,000 bases upstream from the *DRD2* transcription start site), *ANKK1/DRD2 TaqIA* was later found to be a non-synonymous variant in the final exon of the subsequently discovered *ANKK1* gene (Neville *et al.*, 2004). The functional significance of this variation has not been identified although it changes an amino acid (Glu713Lys) in a concatenation of ankyrin repeats, for which this kinase is partially named.

In this preliminary study, we have shown that genetic variation in the *ANKK1* gene may modify the subjective effects of cocaine, given that, in this study, individuals carrying the rs1800497 minor T allele reported greater subjective response. Since the T allele of rs1800497 in the *ANKK1* gene is associated with lower DA D2 receptor tone, we speculate that when cocaine blocks the DA transporter and DA concentration in the synapse is increased, it might take less DA to saturate the reduced number DA receptors in individuals with these genetic variants, causing an increased response to the drug. More studies will be required in order to test this hypothesis.

A potential limitation of this study is that the cohort is relatively small. This should be considered a pilot study and replication in a separate cohort is needed.

In summary, individuals with the rs1800497 minor T allele in the *ANKK1* gene reported increased ratings on some subjective effects of cocaine such as ‘high’ and ‘like’ in this preliminary study. These findings upon replication in future studies may help to better treat individuals who may be at higher risk of continuing to use cocaine or those at greater risk to relapse during periods of abstinence, and may help to provide insight into the mechanism underlying cocaine addiction.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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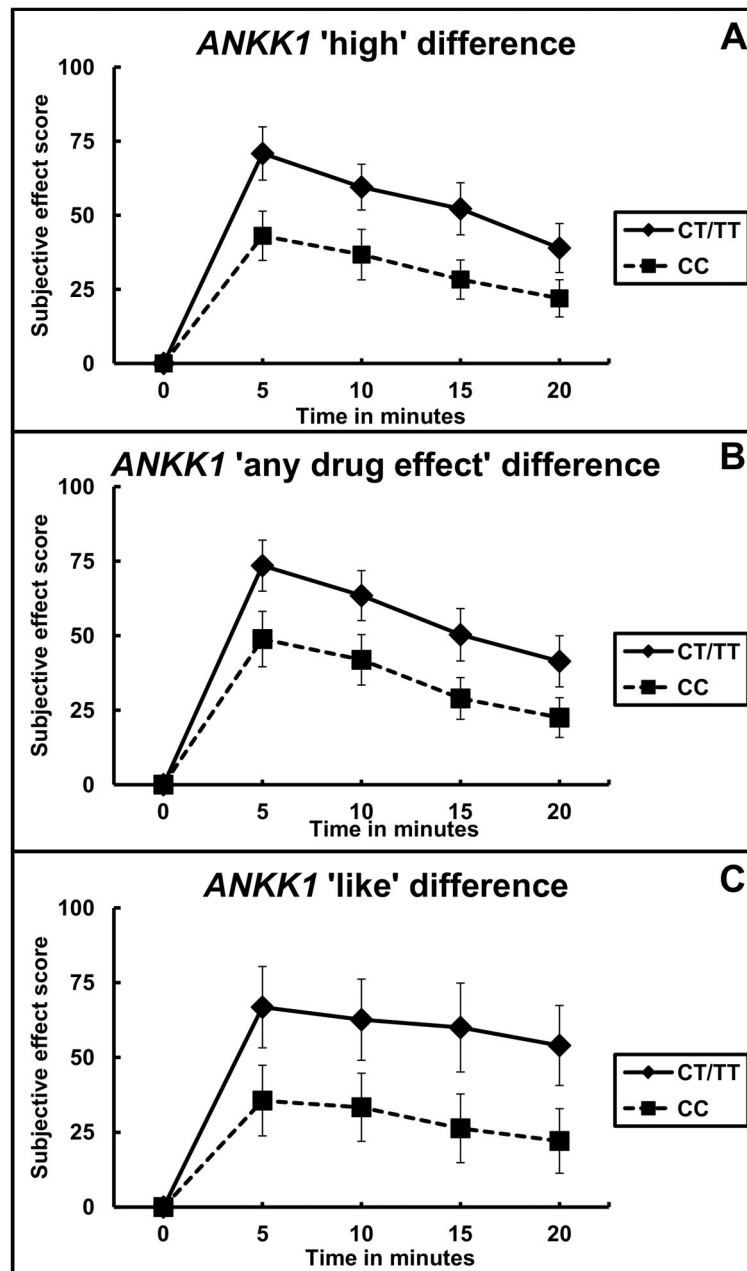
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**Figure 1. Subjective effect scores by ANKK1 genotype**

Subjective effect scores for 'high' (panel A), 'any drug effect' (panel B), and 'like' (panel C) are shown for each time point starting with baseline at time zero. Scores for each time point in the CT/TT genotype group ( $N = 21$ ) are represented by the solid line, and time points for the CC genotype group ( $N = 26$ ) are represented by the dashed line. Error bars indicate standard error of the mean.

**Table 1**Demographic and clinical characteristics by *ANKK1* and *DRD2* genotype

Gene	<i>ANKK1</i>		<i>DRD2</i>	
	TT/TC	CC	TT/TG	GG
<b>Characteristic</b>				
N	21	26	10	37
Male (%)	81	92.3	70	91.9
African American (%)	76.2	61.5	50	73
Caucasian (%)	9.5	11.5	20	8.1
Hispanic (%)	4.8	11.5	10	8.1
Other (%)	9.5	15.5	20	10.8
Education, years (SD)	12.5 (2)	13 (1.6)	13.2 (2.4)	12.7 (1.6)
Age (SD)	42.3 (6.1)	44.5 (7)	40.4 (7)	44.4 (6.3)
Weight (SD)	183.4 (38)	190.9 (30)	181.3 (41)	189.2 (32)
Cocaine use, years (SD)	16.3 (6.7)	16.2 (8)	17.5 (7.3)	15.9 (7.4)
Cocaine use, daily (SD)	4.9 (6.4)	4.1 (4)	6.5 (8.7)	3.9 (3.7)
Cocaine use, past 30 days (SD)	17.7 (7.4)	16.1 (7.9)	17.3 (7.3)	16.7 (7.8)
Grams used per day (SD)	2.7 (3.1)	1.6 (1.2)	3.5 (3.8)	1.6 (1.3)
Nicotine use, years (SD)*	17.4 (8.5)	22.7 (7.7)	14.9 (5.4)	21.5 (8.6)

\*The only significant difference among the demographic variables is for years of nicotine use for *ANKK1*  $p = 0.0432$  and *DRD2*  $p = 0.0133$ . No significant baseline differences in any clinical characteristics were observed after adjusting for multiple testing ( $p > 0.05$ ).