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Overlapping Dopaminergic Pathway Genetic Susceptibility for Heroin and Cocaine Addictions in African Americans

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Summary

Drugs of abuse activate the mesolimbic dopaminergic pathway. Genetic variations in the dopaminergic system may contribute to vulnerability to drug addiction. Several processes are shared between cocaine and heroin addictions but some neurobiological mechanisms and environmental influence may be specific. This study examined the association of 98 single nucleotide polymorphisms (SNPs) in 13 dopamine pathway-related genes with heroin and/or cocaine addiction in a sample of 801 African Americans (315 subjects with heroin addiction (OD), with or without cocaine (CD) or alcohol addiction (AD), 279 subjects with CD, with or without AD, and 207 controls). Single-marker analyses provided nominally significant evidence for associations of 24 SNPs in *DRD1*, *ANKK1/DRD2*, *DRD3*, *DRD5*, *DBH*, *DDC*, *COMT* and *CSNK1E* with OD and/or CD. A *DRD2* 7-SNPs haplotype block that includes SNPs rs1075650 and rs2283265, which were shown to alter D2S/D2L splicing, was indicated in both addictions. The Met allele of the *COMT* Val158Met functional variant was associated with protection from OD. None of the signals remained significant after correction for multiple testing. The study results are in accordance with the results of several previous studies, including our report of association of *DRD1* SNP rs5326 with OD in a smaller sample from this cohort. The findings

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Conflict of interest The authors declare no conflict of interest

Supporting Information Additional supporting information may be found at the publisher's web-site:

Supplement Table 1. SNP list

Supplement Table 2. LD data

Supplement Table 3. ANOVA

Supplement Table 4. *COMT* Val158Met genotype frequencies

Supplement Fig 1. Pairwise Linkage Disequilibrium of all the genes analyzed

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suggest the presence of an overlap in the genetic liability for heroin and cocaine addictions, as well as shared and distinct liability for OD in subjects of African and European descent.

Keywords

dopaminergic pathway; polymorphism; heroin addiction; cocaine addiction; African Americans; *DRD1*, *DRD2*, *COMT Val158Met*, rs5326, rs1075650, rs2283265

INTRODUCTION

Drug addiction is a chronic brain disease that is the product of genetic, environmental and drug-induced factors (Kreek et al., 2012). The genetic determinants likely include interactions among multiple neurochemical systems, including the dopaminergic pathway. Drugs of abuse activate the mesolimbic dopaminergic pathway and dopamine (DA) has been implicated in all stages of drug addiction (Di Chiara et al., 2004). Cocaine inhibits DA reuptake by blocking the dopamine transporter thus directly increasing DA synaptic levels. Heroin increases DA levels indirectly by inhibiting GABA neurons in the ventral tegmental area (VTA). Twin studies indicated that a large proportion of variance in the vulnerability for drug dependence is heritable (Kendler et al., 2000). Although cocaine and heroin addiction share psychological processes and neurobiological substrates, they differ in their behavioral, psychological and neurobiological mechanisms and have unique environmental influences (Badiani et al., 2011; Peters et al., 2013). In addition, many individuals are addicted to both drugs.

This study examined the association of variations in dopamine pathway-related genes with heroin and/or cocaine addiction in African Americans. Although there is a similar risk to develop drug addiction among different ancestral populations, differences in linkage disequilibrium (LD) and allele frequencies may suggest the existence of some distinct genetic risk factors. African Americans are significantly underrepresented in association studies and this study should help to address this gap.

The genes selected for this study are encoding DA receptors (*DRD1-5*), a DA transporter (*DAT1*, *SLC6A3*), enzymes involved in DA synthesis and degradation (*TH*, *DBH*, *DDC* and *COMT*) and regulators of DA signaling (*ANKK1*, *CSNK1E* and *PPP1R1B*). Dopamine is the endogenous ligand for two classes of G protein-coupled dopamine receptors; the D-like receptors (D1 and D5) and the D2-like receptors (D2, D3, and D4). Tyrosine hydroxylase (TH) produces L-DOPA that is in turn catalyzed to dopamine by DOPA decarboxylase (DDC). Dopamine is degraded by catechol-*O*-methyltransferase (COMT), which is a modulator of prefrontal dopaminergic tone. The dopamine transporter (*DAT1*, *SLC6A3*) mediates the synaptic reuptake of dopamine. Dopamine beta-hydroxylase (DBH) converts dopamine to norepinephrine thus regulating the ratio of the two neurotransmitters. DARPP-32 (*PPP1R1B*, dopamine- and cAMP-regulated phosphoprotein, 32 kDa) is a key regulatory molecule in the dopaminergic signaling pathway. The casein kinase 1 epsilon isoform (*CSNK1E*) is a serine/threonine-selective phosphotransferase that regulates dopaminergic signaling through DARPP-32. *ANKK1* is located in close proximity to *DRD2*, and there is high LD between SNPs in both genes. The *Taq1A* polymorphism (rs1800497) is

located in the coding region of *ANKK1*. There is a potential relationship between *ANKK1* and the dopaminergic system (Hoenicka et al., 2010).

Several association studies of DA-related genes with heroin or cocaine addiction have been reported. *ANKK1/DRD2* SNPs were associated with heroin or cocaine addiction/abuse in different populations (Al-Eitan et al., 2012; Clarke et al., 2014; Jacobs et al., 2013a; Lawford et al., 2000; Li et al., 2006; Nelson et al., 2013; Perez de los Cobos et al., 2007; Shahmoradgoli Najafabadi et al., 2005; Vereczkei et al., 2013; Xu et al., 2004). In addition, a *DRD1* SNPs were associated with heroin abuse (Jacobs et al., 2013a,b), a *DBH* SNP was associated with progressive OD in Han Chinese (Xie et al., 2013), and *CSNK1E* SNPs were associated with OD in subjects with European descent (Levran et al., 2008) and Han Chinese (Wang et al., 2014). *COMT* variants have been associated with cocaine addiction in African Americans (AA) (Lohoff et al., 2008), and cocaine-induced paranoia in European Americans (EA) and AA (Ittiwut et al., 2011). Two association studies of cocaine dependence in a Spanish Caucasian sample that included dopaminergic genes reported nominal significant association of SNPs in *DBH*, *SLC6A3* and *TH* (Fernandez-Castillo et al., 2010; Fernandez-Castillo et al., 2013). A recent GWAS of cocaine dependence in EA and AA did not identify association with SNPs in DA-related genes (Gelernter et al., 2013).

This study examined the association of variations in 13 dopamine pathway-related genes with heroin and/or cocaine addiction in a sample of 801 American subjects of predominantly African ancestry. The study is an extension of our previous studies in this population with a larger sample, modified SNP content and an additional cocaine addiction sample (Levran et al., 2009; Levran et al., 2014b) and follows our association studies in subjects with European ancestry (Levran et al., 2008; Levran et al., 2014a).

MATERIALS AND METHODS

Subjects

The study sample (n = 801) was divided into three main samples based on addiction status and drug of addiction (heroin or cocaine) (Table 1). The control sample included 207 subjects. All subjects (n = 315) in the heroin addiction sample (#1, “OD±CD”) were current addicts in methadone maintenance treatment programs and reported heroin as their drug of choice. This sample is composed of two subsamples: (#1a) “OD only”; including subjects with no cocaine addiction (CD) and current or past alcohol addiction (AD) (5%) (n = 138, 44%), and (#1b) “OD+CD”; including subjects with current or past CD, with or without AD (n = 177, 56%). All subjects (n = 279) in the cocaine addiction sample (#2, “CD only”) had current or past CD and reported cocaine as their drug of choice. Some of them (29%) also had current or past AD and none of them had OD. The sex ratios were 0.52, 0.37, and 0.37 females in the controls, “OD±CD” and “CD only” samples, respectively. The mean ages (± SD) when blood was obtained were 33 ±12, 49 ±10, and 43 ± 7 years in the control, “OD ±CD” and “CD only” samples, respectively. The age range was 18-76 years. Cannabis use/abuse was assessed but was not taken into account in the present analysis.

This study is an expansion of our previous study of OD in AA (Levran et al., 2009) for which we added 481 new subjects. Most subjects were self-identified as African Americans

(AA) and showed > 50% African ancestry contribution by STRUCTURE analysis (see below). A total of 46 subjects who self-identified as AA were excluded because they showed < 50% African ancestry contribution. Seventy subjects whose self-identified ancestry was different from AA (ambiguous, Caribbean African non-Hispanic, European, or Native American) were included based on > 50% African ancestry contribution. Hispanic subjects were not included.

Ascertainment was made by extensive personal interview, using the following instruments: the Addiction Severity Index (McLellan et al., 1992), the Kreek-McHugh-Schluger-Kellogg Scale (KMSK) (Kellogg et al., 2003) and the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV).

Subjects were excluded from the control category with (1) >1 instance of drinking to intoxication or any illicit drug use in the last month; (2) a history of alcohol drinking to intoxication or illicit drug use more than twice a week for more than 6 consecutive months and (3) cannabis use for more than 12 days in the last month or past cannabis use for > 2 days/week for > 4 years. The former heroin addicts had a history of > 1 year of daily multiple uses of heroin and were treated at a methadone maintenance treatment program (MMTP).

Subjects were recruited at the Rockefeller University Hospital, the Manhattan Campus of the VA New York Harbor Health Care System and the Dr. Miriam and Sheldon G. Adelson Clinic for Drug Abuse Treatment and Research in Las Vegas (LV), NV. The Institutional Review Boards of the Rockefeller University Hospital and the VA New York Harbor Healthcare System approved the study. The Rockefeller University IRB also reviews the Adelson Clinic, LV. All subjects signed informed consent for genetic studies.

Genes/SNPs and Genotyping

A new custom array (GS0013101-OPA) was designed based on the “addiction” array (GS0007064-OPA; Illumina, San Diego, CA, USA) (Hodgkinson et al., 2008) that was used in our previous studies (Levran et al., 2008; Levran et al., 2009). The original design included tagging SNPs with a minor allele frequency (MAF) > 0.005 that aimed to capture the full haplotype information of the HapMap Yoruban population (Hodgkinson et al., 2008).

The 13 genes associated with the dopaminergic pathway in this array were included in this analysis (Table 2). X chromosome genes (e.g., *MAOA*) were not included. Based on current HapMap data for the African YRI sample, the mean gene coverage for 10 of the genes is ~70%. For 3 genes (*DRD4*, *DRD5* and *PPP1R1B*) there is not enough data to calculate % coverage.

Fourteen SNPs from these genes were excluded from the new array based on low quality in the original array, and 19 SNPs were excluded based on low MAF in the population of this study. Nine SNPs were added, based on functionality or reports of association with related phenotypes, including three *ANKK1* SNPs, with a total of 118 SNPs (Supplement Table 1).

Genotyping was performed using an Illumina GoldenGate Custom Panel of 1536 SNPs at the Rockefeller University Genomics Resource Center. Random samples were genotyped in duplicate. Analysis was performed with BeadStudio software v2.3.43 (Illumina) and all cluster plots were manually inspected.

Assessment of percentage of African Ancestry Using Ancestry Informative Markers (AIMs)

A set of 155 AIMs was used for STRUCTURE 2.2 analysis (Pritchard et al., 2000). Biographic Ancestry Scores (e.g., fractions of genetic affiliation of the individual in each cluster) were estimated under the assumption of seven clusters (k). Each subject was anchored against genotypes of 1051 samples from the Human Genome Diversity Cell Line Panel (<http://www.cephb.fr/HGDP-CEPH-Panel>), as described (Ducci et al., 2009). To be included in the study, an individual had to show > 50% African ancestry contribution.

Statistical analysis

The statistical power was estimated for the different case samples with the standard test for difference between proportions, under additive, dominant or recessive logistic regression model, assuming odds ratios (OR) between 1.2 and 2.2 and MAF within the range 0.05-0.45, at a fixed significance level of 0.05 and power = 0.80 (Sham & Purcell 2014). The same control sample size ($n = 207$) was used in each calculation. All samples have sufficient power to detect ORs as low as 1.2 for a SNP with MAF as high as 0.45. For SNPs with MAF as low as 0.05, the combined sample (“OD±CD” & “CD only”, $n = 594$) can detect ORs as low as 2.0, the “OD±CD” sample ($n = 315$) can detect OR as low as 2.2, and the “CD only” sample ($n = 279$) can detect OR as low as 1.8. The “OD only” sample of ($n = 138$) can detect OR as low as 2.1 for a SNP with MAF as low as 0.1.

Deviation from Hardy–Weinberg equilibrium (HWE) was examined in the control sample by the exact test using the PLINK program (Purcell et al., 2007). Pairwise LD and LD blocks were estimated using Haploview 4.2 (Barrett et al., 2005) based on ‘Solid Spine of LD’ algorithm. Association analyses were performed by two methods:

1. ANOVA with the data as a categorical variable: (a) “OD only” #1a, (b) “OD+CD” #1b, (c) “CD only” #2, and (d) control (see Table 1), followed by *Post hoc* pair comparisons for the significant associations; The group is used as a fixed factor in a logistic regression model and the genotype as the binary response variable according to the following codification. We fitted logistic regression models for all SNPs and used the chi-square to test the significance of the model (the alternative hypothesis that the genotypes are different across the 4 groups). *P*-values were corrected according to the Benjamin-Hochberg methodology (FDR).
2. Independent logistic regression analyses for the “OD±CD”, “OD only”, “CD only”, and “OD±CD” + “CD only” samples. Logistic regression was performed for each SNP separately using the PLINK program, and as two groups reflecting dominant or recessive inheritance model. The direction of the regression coefficient represents the minor allele. Sex was included as a covariate in the initial model but had no significant effect on the results so it was not included in the final analysis.

Adjustment for multiple testing was also performed by permutation test ($n = 100,000$) for each model of inheritance, using PLINK.

RESULTS

Case control association analysis was conducted in 801 subjects (315 subjects with heroin addiction, with or without cocaine or alcohol addiction, 279 subjects with cocaine addiction, with or without alcohol addiction, and 207 controls)(Table 1). The heroin sample (#1) is comprised of two subsamples: #1a, “OD only”; including subjects with no past or current CD ($n = 138$), and #1b, “OD+CD”; including subjects with current or past CD ($n = 177$).” One hundred and eighteen SNPs from 13 genes related to the dopaminergic pathway were genotyped (Table 2, Supplement Table 1). Twenty SNPs were excluded based on low quality. The remaining 98 SNPs were analyzed separately for association with heroin or cocaine addiction. One SNP (*ANKK1* rs7118900) showed significant deviation from HWE in controls ($P = 0.007$) and the rest of the SNPs showed no significant deviation ($P > 0.01$). There was no evidence for population substructure among the three samples (heroin, cocaine and controls) based on Structure analysis of 155 AIMs. There was an average of $81 \pm 10\%$ African ancestry and similar admixture pattern among the samples, as was shown in our previous study of this cohort (Levran et al., 2014b). Analysis of LD indicated 26 haplotype blocks, including 17 highly correlated ($r^2 > 0.7$) SNPs (7 pairs and one triplet) (Supplement Table 2 & Supplement Figure 1).

ANOVA assuming four categories: (#1a) “OD only”, (#1b) “OD+CD”, (2) “CD only”, and controls, revealed 16 SNPs with nominally significant differences among the groups under at least one model of inheritance (Supplement Table 3). None remain significant after correction for multiple testing. *Post hoc* pairwise comparisons of these SNPs revealed nominally significant differences of 11 SNPs, including five SNPs (*ANKK1* rs7118900, *DRD2* rs1079596 and rs2587548 *DRD3* rs167771, and *COMT* rs4818) between the control sample and the “OD only” sample, one SNP (*CSNK1E* rs5757037) between “CD only” and “OD only”, and two SNPs (*COMT* rs2239393 and rs4818) between the “OD only” sample and “OD+CD” sample (Supplement Table 3). None remained significant after correction for multiple testing.

Logistic regression under two models of inheritance (dominant/recessive) for “OD only” (1a), “OD±CD” (1a & 1b), the “CD only” (2), and “OD±CD” & “CD only” (1 & 2) samples revealed nominally significant differences in genotype frequencies for 24 SNPs in nine genes (*DRD1*, *ANKK1*, *DRD2*, *DRD3*, *DRD5*, *DBH*, *DDC*, *COMT* and *CSNK1E*)(Table 3). No signal survived correction for multiple testing. Of those, four SNPs (in *ANKK1*, *DBH* and *COMT*) are coding SNPs (two non-synonymous and two synonymous). All the SNPs indicated by ANOVA were also indicated by the logistic regression analyses.

LD analysis showed that some of these SNPs are part of three haplotype blocks, including seven *DRD2* SNPs, *DRD1* SNPs rs686 and rs5326, as well as *COMT* SNPs rs2239393 and rs4818, respectively. Among the *DRD2* haplotype block there are two SNP pairs and one triplet in strong correlation ($r^2 > 0.72$) (Table 3, Figure 1, Supplement Table 2, & Supplement Figure 1).

The comparison of results of the different analyses indicated several SNPs that were only detected in a specific sample (e.g., “OD only”), and SNPs for which the *P*-values were lower in the “OD only” or “CD only” compared with other samples, despite the smaller sample size (Table 3). Other SNPs showed lower signals in the combined addiction group including *DRD2* and *DRD5* SNPs.

The association signal, under recessive model, of the functional *COMT* SNP rs4680 (Val158Met) was driven by lower frequency (0.04) of the AA genotype (encoding Met/Met) in the “OD only” group than in the control group (0.11) indicating protective effect (OR = 0.38; 95% CI 0.15-0.97) (Supplement Table 4).

DISCUSSION

This study provides evidence for nominally significant associations of several SNPs in genes involved in the dopaminergic pathway with heroin and/or cocaine addiction in African Americans. This study can be viewed as a major expansion of our previous study of OD in African Americans (Levran et al., 2009). The genes included in the current study, except for *ANKK1*, were included in the previous study, although the SNP content was modified to exclude redundant and non-informative markers and to include additional SNPs. We have not previously studied cocaine addiction in this cohort.

The two *DRD1* SNPs identified in association with heroin and/or cocaine addictions are located in the 5' and the 3' UTR of the gene, respectively. They are in the same haplotype block, but they are not strongly correlated ($r^2 < 0.2$) in both African and European populations. SNP rs5326 has a comparable allele frequency in different populations (0.14-0.2 in HapMap CEU, YRI and CHB). *DRD1* rs686 is located in a sequence complementary to the seed sequence of miR-504 and showed differential expressions of the two variants (Huang & Li, 2009). The study results are in accordance with our previous finding of association of *DRD1* SNP rs5326 with OD in AA (Levran et al., 2009). It is also in line with a study showing association of this SNP with time to develop to heroin dependence in Han Chinese (Peng et al., 2013). *DRD1* SNP rs686 was associated with nicotine dependence in AA (Huang et al., 2008) and alcohol dependence in Europeans (Batel et al., 2008). It was also associated with duration of transition from the first use to opioid dependence in Han Chinese (Zhu et al., 2013), and with opioid abuse in AA (Jacobs et al., 2013b).

One *ANKK1* SNP and 10 *DRD2* SNPs showed association with heroin and/or cocaine addiction, including seven SNPs that are part of one haplotype block, suggesting a related signal. The non-synonymous *ANKK1* SNP rs7118900 (Ala239Thr) identified in this study is in perfect LD with SNP rs1800497 (TaqIA) in the HapMap European population (CEU), but in low LD ($r^2 < 0.2$) with this SNP in the HapMap African population (YRI) suggesting a potentially different effect of this SNP on *DRD2* expression. SNP rs1800497 was excluded from this analysis based on low quality. The association of *ANKK1* SNP rs7118900 should be interpreted with caution since the SNP showed significant deviation from HWE in the control sample, although there was no indication of low genotyping quality and this SNP was in HWE in another control sample of European ancestry that was genotyped with the same array (Levran et al. 2014c). An *in vitro* study showed differential gene expression of

the two variants of SNP rs7118900 at baseline and in response to stimulation with apomorphine. The variant 239Thr is predicted to create a new phosphorylation site (Garrido et al., 2011). DRD2 has two major splice isoforms with distinct functions: D2 long (D2L) and D2 short (D2S). The *DRD2* SNPs rs1076560 and rs2283265 that are in perfect LD, were shown to shift splicing from D2S to D2L changing their expression ratio. These variants were associated with differences in brain functions and psychiatric disorders (Zhang et al. 2007, Moyer et al., 2011).

Previous association studies of *DRD2* with heroin and cocaine addictions produced mixed results that may be partly explained by small sample size, population substructure and suboptimal ascertainment (for additional references and discussion see Moyer et al., 2011). The results of this study are compatible with several studies. *DRD2* SNPs rs2283265 and rs10765560 were associated with OD in a Jordanian sample (Al-Eitan et al., 2012), and in Europeans (Jacobs et al., 2013a). They were also associated with cocaine abuse and dependence in European Americans (Moyer et al., 2011). SNP rs10765560 has been recently associated with opioid dependence, but not with cocaine dependence, in European and African Americans (Clarke et al., 2014). Of note, the OD sample used by Clarke *et al.* includes a substantive number of DNA samples from our laboratory that are part of the NIDA Center for Genetic Studies DNA Repository, so it cannot be considered an independent sample. *DRD2* SNP rs1079597 (also called “TaqIB”) has been previously associated with heroin dependence in a Hungarian sample (Vereczkei et al., 2013). Although this SNP was not included in the study, it is in strong LD, in the HapMap African sample (YRI), with SNP rs1076560. *DRD3* SNP rs167771 indicated in this study was previously associated with nicotine dependence (Wei et al., 2012), smoking behavior (Yu et al., 2006), and autism (Toma et al., 2013).

The functional rs4680 (Val158Met) identified in this study in association with OD, is one of the most a well-studied SNP (Montag et al., 2012)(Tammimaki & Mannisto 2010). The frequency of the A allele (encoding Met) and the AA genotype was lower in subjects with OD (without CD), compared with controls or subjects with CD or OD+CD. This allele is associated with lower enzymatic activity, higher dopamine levels, enhanced vulnerability to stress, as well as increased susceptibility to white matter structural alterations in the context of addiction (Zhang et al., 2013). It was associated with CD in AA (Lohoff et al., 2008), and cocaine-induced paranoia in EA and AA (Ittiwut et al., 2011). It has been suggested that environmental factors impact the detection of small effects of this variant on alcohol addiction vulnerability (Schellekens et al., 2012). *COMT* SNPs rs4680 and rs933271, indicated in this study, were predicted to have potential functionality based on a signature of positive selection in EA and AA (Ittiwut et al., 2011). SNP rs737866 that was associated with earlier age of opiate onset in Chinese (Li et al., 2012) is in strong LD ($D' = .85$) with SNP rs933271 in this sample.

The suggested association of a SNP upstream of *CSNK1E* with OD is intriguing since we have found association of another *CSNK1E* SNP and haplotype with OD, in EA (Levran et al., 2014c) and yet another SNP was associated with OD in Han Chinese (Wang et al., 2014). There is no LD between these SNPs in this sample and no information on the SNP identified. *CSNK1E* interacts with circadian rhythms and DARPP-32 and has been

implicated in negative regulation of sensitivity to opioids in rodents (Bryant et al., 2012; Cheong & Virshup 2011).

DDC SNP rs2329341 that was indicated in this study was recently shown to be associated with cocaine dependence subtype, by interacting with *GABRG3* SNP (Bi et al., 2014). A recent report suggested an association of *DBH* SNP rs1611115 with progressive OD in Han Chinese (Xie et al., 2013). This SNP was included in this study but did not show significant association.

Cocaine vs. heroin addiction

The model of common (non-drug specific) liability to addiction hypothesizes the presence of a non-specific liability to all drug addictions based on shared biological mechanisms and high genetic correlations between liabilities to specific drug addictions (Vanyukov et al., 2012). Decrements in *DRD2* receptors in the striatum were observed in addicts to several drugs of abuse (Koob & Volkow, 2010). Although cocaine and heroin addiction share psychological processes and neurobiological substrates, they differ in neurobiological mechanisms and environmental influence (Badiani et al., 2011; Peters et al., 2013). For example, chronic heroin or cocaine abusers showed unique gene expression profiles in the post-mortem nucleus accumbens (Albertson et al., 2006). In this study, we have analyzed different combinations of heroin and cocaine addiction, in order to identify SNPs with either drug-specific or non-drug-specific effect. Comparison of the association signals in the different samples suggests several shared signals (e.g. *DRD2* SNPs rs1079596 and rs1125394) that might reflect an association with a general phenotype of substance addiction, and also signals that might be unique to the CD or OD (e.g. *COMT* rs4680 Val158Met).

European vs. African Americans (heroin addiction)

Although heroin and/or cocaine addiction have similar prevalence in different ancestral populations, these populations may have distinct as well as shared genetic risk factors. The causes for the distinct factors may include different allele frequencies, LD patterns, modifier genes and/or gene-environment interactions.

We have reported association analyses of OD using similar marker set in subjects of predominantly European ancestry (Levran et al., 2008, Levran et al. 2014c). Comparison of the findings in the two ancestral populations reveals mostly distinct polymorphisms. We have reported an association of the casein kinase 1 ϵ gene *CSNK1E* SNP rs1534891, and related haplotype, with OD in subjects with European ancestry. This SNP was not indicated in this study, although that may be partly explained by the low MAF of this SNP in African populations. This study indicated the common SNP rs5757037 in association with OD, but this SNP was not identified in EA. There is no LD between these SNPs.

Nevertheless, in both populations there were common association signals in *DRD2* (rs1076563, rs2587548). Although the two *DRD2* SNPs are in strong LD in both populations, their allele frequency is different and their minor alleles in EA are the major alleles in AA. Another potential common association is the *DRD2* SNP rs1076560. This

SNP (or SNPs in strong LD with this SNP) were associated with OD in subjects with European ancestry by other groups (Jacobs et al., 2013a, Vereczkei et al., 2013, Clarke et al., 2014).

The general LD pattern of the *ANKK1/DRD2* region is different between the two populations. Thus, the *ANKK1* SNP rs7118900, which was identified in this study, is in strong LD with the two *DRD2* SNPs mentioned above and the *ANKK1* SNP rs1800497 (TaqIA) only in population of European ancestry, suggesting a potentially different effect of this SNP on gene expression. There is no LD between the *DRD3* SNPs indicated in EA and AA.

Some limitations should be considered when interpreting the results of this study. Although nominally significant, the associations may be due to chance and further testing in independent populations and/or meta-analysis are needed for confirmation. The study is underpowered to detect variants with small effects and it is possible that significant associations were not detected because of limited power due to the sample size and partial gene coverage. Although there was no indication of population substructure between cases and controls, the admixed nature of the African American populations could be a source of error for SNPs that differ in their allele frequencies between the two ancestral populations. In addition, because of the high substance-related comorbidity and the study design, the labeling of shared or common liability may not be accurate. Despite these limitations, the study has important strengths, including rigorous ascertainment, reliable phenotype data and stringent inclusion/exclusion criteria.

In summary, this study provides evidence for nominally significant associations of specific variants in DA-related genes with heroin and cocaine addictions that are common and/or specific to the African American population. African Americans are significantly under-represented in association studies and this study helps to address this gap. The findings suggest the presence of both shared and distinct genetic liability for heroin and cocaine addictions as well as for African and European Americans. Several SNPs of potential importance were indicated and further studies are warranted to confirm the results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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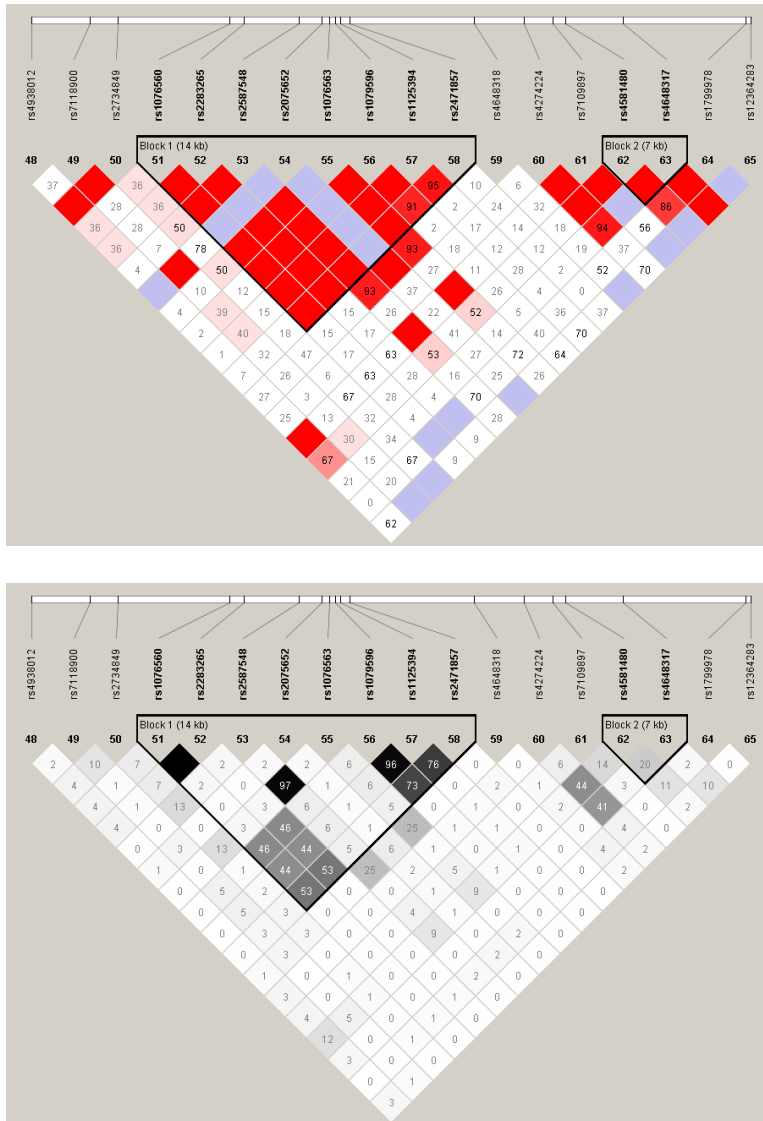


Figure 1. DRD2 pairwise Linkage Disequilibrium
 The pairwise correlation between SNPs was measured as D' (a) or r^2 (b). The values are shown (x100) in each box. The color scheme indicates the magnitude of the value. When the value is equal to 1.0 no number is given.

Table 1

Groups description

Sample			Diagnosis		n	f
#	Drug "symbol"	Symbol	1	2		
1	Heroin "OD±CD"				315	
	1a	"OD only"			138	0.44
			OD#	-	123	0.39
			OD#	AD*	15	0.05
	1b	"OD+CD"			177	0.56
			OD#	CD*	76	0.24
			OD#	CD*/AD*	101	0.32
2	Cocaine "CD only"				279	
			CD*	-	198	0.71
			CD*	AD*	81	0.29
3	Control		-	-	207	

Abbreviation: OD, heroin addiction; CD, cocaine addiction; AD, Alcohol addiction; n, number of subjects; f,

frequency; current,

* past and current

Table 2

Dopaminergic pathway genes analyzed in the study

Symbol	Name
<i>ANKK1</i>	ankyrin repeat and kinase domain containing 1
<i>COMT</i>	catechol-O-methyltransferase
<i>CSNK1E</i>	casein kinase 1, epsilon
<i>DBH</i>	dopamine beta-hydroxylase
<i>DDC</i>	DOPA decarboxylase
<i>DRD1</i>	dopamine receptor D1
<i>DRD2</i>	dopamine receptor D2
<i>DRD3</i>	dopamine receptor D3
<i>DRD4</i>	dopamine receptor D4
<i>DRD5</i>	dopamine receptor D5
<i>PPP1R1B</i>	protein phosphatase 1, regulatory (inhibitor) subunit 1B (DARPP-32)
<i>SLC6A3</i>	solute carrier family 6 member 3 (dopamine transporter, DAT)
<i>TH</i>	tyrosine hydroxylase

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Table 3

Nominally significant associations using logistic regression

Gene	SNP	Location	P I				Model	OR ²	L95 ³	U95 ⁴
			“CD only”	“OD only”	“OD±CD”	“OD±CD” & “CD only”				
			2	1a	1a & 1b	1 & 2				
<i>DRD1</i>	rs686	3' UTR	0.014			0.031	D	1.61	1.10	2.35
	rs5326	5' UTR	0.006				D	1.87	1.16	3.02
<i>ANKK1</i>	rs7118900 ⁵	Ala239Thr	0.008	0.006		0.010	R	0.49	0.29	0.81
<i>DRD2</i>	rs1076560	intron		0.038		0.047	D	1.78	1.03	3.08
	rs2283265	intron		0.038		0.047	D	1.78	1.03	3.08
	rs2587548 ⁶	intron	0.022	0.005		0.018	D	0.51	0.32	0.81
	rs1076563 ⁶	intron	0.032	0.011		0.043	D	0.55	0.35	0.87
	rs1079596	intron	0.007	0.006	0.019	0.005	D	1.63	1.16	2.29
	rs1125394	intron	0.011	0.010	0.028	0.009	D	1.58	1.12	2.21
	rs2471857	intron	0.045	0.029		0.029	D	1.48	1.04	2.10
	rs4648318	intron	0.039				R	1.58	1.02	2.45
	rs4274224	intron	0.025				R	2.14	1.10	4.16
	rs1799978	intron	0.017			0.029	D	1.64	1.09	2.46
<i>DRD3</i>	rs3773678	intron		0.016			R	2.17	1.16	4.07
	rs167771	intron		0.007	0.012	0.048	R	4.83	1.53	15.31
<i>DRD5</i>	rs2867383	downstream	0.031		0.013	0.010	D	0.64	0.46	0.90
<i>DBH</i>	rs1108580	Glul62=		0.042		0.046	R	0.58	0.34	0.98
<i>DDC</i>	rs2122822	intron		0.046			D	0.67	0.45	0.99
	rs2329341	intron		0.031			D	0.68	0.48	0.97
<i>COMT</i>	rs933271	intron	0.039				R	1.68	1.03	2.75

Gene	SNP	Location	P ¹			Model	OR ²	L95 ³	U95 ⁴	
			“CD only”	“OD only”	“OD±CD”					“OD±CD” & “CD only”
		intron	2	1a	1a & 1b	1 & 2				
	rs2239393			0.047			D	1.65	1.01	2.71
	rs4818	Leu126=		0.002			D	1.98	1.27	3.07
	rs4680	Val158Met		0.043			R	0.38	0.15	0.97
CSNK1E	rs5757037	upstream		0.012			D	1.78	1.14	2.77

P-Value in bold are the lowest obtained for the specific SNP; Dash lines box represents haplotype block

- ¹The lowest nominal P value obtained from testing two models of inheritance: dominant and recessive. Empty cells indicate P = 0.05.
- ²OR for the lowest P value for this SNP. OR < 1 represent protective effect of the minor allele, OR > 1 represent risk effect of the minor allele
- ³95% confidence interval lower value
- ⁴95% confidence interval upper value
- ⁵This SNP showed significant deviation from HWE in controls (P = 0.007)
- ⁶These SNPs were associated with heroin addiction in subjects with European ancestry (Levran et al. 2014c)

Abbreviations: D, dominant; R, recessive; Chr, chromosome; OR, odds ratio