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Stress-related genes and heroin addiction: A role for a functional *FKBP5* haplotype

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GAL

Summary
Background: Stress is a critical risk factor affecting both the development of and the relapse to drug addictions. Drug addictions are caused by genetic, environmental and drug-induced factors. The objective of this hypothesis-driven association study was to determine if genetic variants in stress-related genes are associated with heroin addiction.
Methods: 112 selected genetic variants in 26 stress-related genes were genotyped in 852 case subjects and 238 controls of predominantly European ancestry. The case subjects are former heroin addicts with a history of at least one year of daily multiple uses of heroin, treated at a methadone maintenance treatment program (MMTP). The two most promising SNPs were subsequently tested in an African-American sample comprising of 314 cases and 208 control individuals.
Results: Nineteen single nucleotide polymorphisms (SNPs) in 9 genes (*AVP, AVPR1A, CRHR1, CRHR2, FKBP5, GAL, GLRA1, NPY1R and NR3C2*) showed nominally significant association with heroin addiction. The associations of two *FKBP5* SNPs that are part of one haplotype block, rs1360780 (intron 2) and rs3800373 (the 3′ untranslated region), remained significant after correction for multiple testing ($P_{corrected}$ = 0.03; OR = 2.35, $P_{corrected}$ = 0.0018; OR = 2.85, respectively). The two SNPs also showed nominally significant association ($P < 0.05$) with heroin addiction in an independent African-American cohort. FKBP5 is a co-chaperone that regulates

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glucocorticoid sensitivity. These *FKBP5* SNPs were previously associated with diverse affective disorders and showed functional differences in gene expression and stress response. This study also supports our and others' previous reports of association of the *GAL* SNP rs694066 and the *AVPR1A* SNPs rs11174811, rs1587097 and rs10784339 with heroin and general drug addiction, respectively. *Conclusions:* This study suggests that variations in the *FKBP5* gene contribute to the development of opiate addiction by modulating the stress response. These findings may enhance the understanding of the interaction between stress and heroin addiction.

1. Background

Addiction to opiates and the illicit abuse of prescription opioids is a growing epidemic. Addiction to drugs is a chronic relapsing brain disease caused by a combination of genetic, epigenetic, environmental and drug-induced factors. Stress is a critical risk factor affecting both the development of addictive disorders, by promoting drug seeking and excessive drug intake, and the relapse to addictive behaviors, since drug withdrawal can increase stress response, and stress increases reward-seeking behavior, such as reinstatement of drug-taking behavior (Koob and Kreek, 2007; Sinha, 2008; Ulrich-Lai and Herman, 2009; Kreek et al., 2012). Studies showed a high rate of various types of childhood trauma exposure and affective disorders comorbidity among individuals with opioid dependence (Mills et al., 2005; Nelson et al., 2006; Sansone et al., 2009). The response to stress is influenced by genetic and environmental factors and has high inter-individual variability. A plastic neural circuitry that includes the hippocampus, amygdala, hypothalamus, brainstem and prefrontal cortex coordinates the response systems (McEwen and Gianaros, 2011).

Adrenal secretion of glucocorticoids is one of the mechanisms of response to stress. Stress exposure, as well as endogenous opioids and drugs of abuse, activate the hypothalamic—pituitary—adrenal (HPA) axis. Consequently, corticotropin-releasing hormone (CRH, CRF) and arginine vasopressin (AVP) are released from the hypothalamic paraventricular nucleus (PVN) and are transported to the anterior pituitary and stimulate adrenocorticotropic hormone (ACTH) secretion, which in turn stimulates glucocorticoid synthesis and release from the adrenal cortex. CRF is also produced in other brain regions and activates the sympathetic nervous system to release epinephrine and norepinephrine from the adrenal glands. It also stimulates the mesocorticolimbic dopamine system that mediates the rewarding effects associated with drug use. High levels of glucocorticoids can "sensitize" CRF systems in the extra hypothalamic brain stress systems (extended amygdala) (Koob, 2010).

Glucocorticoids regulate the activity of the HPA axis through negative feedback via the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). These receptors regulate the expression of genes necessary for coping with stress. The functions of the GR and the MR are partly moderated by chaperone proteins including the heat shock protein 90 (Hsp90) co-chaperone FKBP5 (FKBP51, FK506-binding protein 51). Glucocorticoids have many other effects when bound to glucocorticoid receptors (e.g., modulation of cardiovascular function, immunologic status, arousal, learning and memory). They can also alter the methylation patterns of genes (Stephens and Wand, 2012).

Numerous molecular genetic studies have evaluated the association between polymorphisms in stress-related genes and affective disorders (Domschke and Reif, 2012), but only a few studies have reported an association of variants in these genes with specific drug addictions. We have previously performed association studies of heroin addiction that include several genes related to stress response (Levran et al., 2008; Proudnikov et al., 2008; Levran et al., 2009). These studies identified, in these genes, association of SNPs in the galanin gene (*GAL*) in European Americans, the AVP receptor gene (*AVPR1A)* in African Americans, and the ACTH receptor gene (*MC2R*) in Hispanics. A different *AVPR1A* SNP was shown to be associated with general drug use disorders by another group (Maher et al., 2011), and *NPY2R* SNPs were associated with alcohol and cocaine dependence (Wetherill et al., 2008).

Here, we report the results of a case—control hypothesis-driven association study of 112 SNPs from 26 genes related to stress response, with heroin addiction, in a sample of 1090 subjects of predominantly European ancestry. The study is a major expansion of our previous study (Levran et al., 2008) to which 517 samples, 12 additional stress-related genes, and several new SNPs in genes included in the previous study were added. The study employed more stringent inclusion criteria for ancestry, based on biographic ancestry scores obtained by STRUCTURE analysis of 155 Ancestry Informative Markers (AIMs). This study included a validation sample of different ethnicity (African-American) for the most significant results.

2. Methods

2.1. Subjects

2.1.1. Discovery sample

The 1090 subjects of this study are part of a larger cohort recruited by the Kreek laboratory for the study of the genetics of specific drug addictions. There were 852 cases (33% female; mean age 40 ± 12) and 238 controls (49% female; mean age 42 ± 16). The subjects were selected based on phenotype (history of severe heroin addiction, normal controls) and self-identified European ancestry (including a Middle-Eastern contribution). Other ethnicities were excluded (e.g., Africans, Hispanics, Asians, Native Americans or mixed ancestry). Ancestry was verified by a family history questionnaire and STRUCTURE analysis (see below), and specific inclusion criteria were employed to obtain relative homogeneity and to limit population stratification. To be