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Glutamatergic and GABAergic susceptibility loci for heroin and cocaine addiction in subjects of African and European ancestry

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ABSTRACT

Background: Drug addiction, a leading health problem, is a chronic brain disease with a significant genetic component. Animal models and clinical studies established the involvement of glutamate and GABA neurotransmission in drug addiction. This study was designed to assess if 258 variants in 27 genes of these systems contribute to the vulnerability to develop drug addiction.
Methods: Four independent analyses were conducted in a sample of 1860 subjects divided according to drug of abuse (heroin or cocaine) and ancestry (African and European).
Results: A total of 11 SNPs in eight genes showed nominally significant associations ($P < 0.01$) with heroin and/or cocaine addiction in one or both ancestral groups but the associations did not survive correction for multiple testing. Of these SNPs, the *GAD1* upstream SNP rs1978340 is potentially functional as it was shown to affect GABA concentrations in the cingulate cortex. In addition, SNPs *GABRB3* rs7165224; *DBI* rs12613135; *GAD1* SNPs rs2058725, rs1978340, rs2241164; and *GRIN2A* rs1650420 were previously reported in associations with drug addiction or related phenotypes.
Conclusions: The study supports the involvement of genetic variation in the glutamatergic and GABAergic systems in drug addiction with partial overlap in susceptibility loci between cocaine and heroin addiction.

1. Introduction

Drug addiction, a leading health problem, is a chronic relapsing brain disease with a significant genetic component (Kendler et al., 2000; Kreek et al., 2012). Though most drugs of abuse increase dopamine (DA) release in the striatum, the diversity of drug effects is mediated by multiple neurotransmitters (Di Chiara et al., 2004). Opioids indirectly disinhibit DA neurons by inhibition of gamma-aminobutyric acid (GABA) release through binding opioid receptors on GABA interneurons in the ventral tegmental area (VTA) (Dilts and Kalivas, 1989; Johnson and North, 1992). Cocaine increases DA availability in the striatum through the blockade of transporter-mediated reuptake. Cocaine was shown to alter $GABA_A$ receptor subunit expression in the nucleus accumbens (NAc) through chromatin remodeling (Kennedy et al., 2013). Rodent's studies provided evidence for an essential role for the glutamatergic and the GABAergic systems in drug addiction and indicated an effect of genetic background and impairments in synaptic plasticity (Gipson et al., 2014; Kalivas, 2009; Miguens et al., 2013; Schlussman et al., 2013; Xi and Stein, 2000).

GABA and glutamate are the major inhibitory and excitatory neurotransmitters in the central nervous system, respectively. These systems are involved in memory, learning and synaptic plasticity, and they are modulated by drugs of abuse (Pinheiro and Mulle, 2008). GABA also inhibits the hypothalamic–pituitary–adrenal axis (HPA) responses to stress and may be important in addiction-associated stress and relapse

Abbreviations: AA, African Americans; AIMs, Ancestry informative markers; CA, California; CD, cocaine dependence; CI, confidence interval; DA, dopamine; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; GABA, gamma-aminobutyric acid; HWE, Hardy-Weinberg equilibrium; HPA, Hypothalamic-pituitary-adrenal axis; KMSK, Kreek-McHugh-Schluger-Kellogg Scale; LD, Linkage disequilibrium; MAF, Minor allele frequency; ME, Middle East; NSFC, Natural Science Foundation of China; NA,c, nucleus accumbens; NY, New York; OD, opioid dependence/heroin addiction; OR, odds ratio; SNP, Single nucleotide polymorphism; VA, Veteran Affairs; VTA, ventral tegmental area.

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(Herman et al., 2004). The GABA system is targeted as a potential pharmacotherapeutic target for the treatment of alcohol and drug abuse disorders (Addolorato et al., 2012). Glutamate receptors have a role in forming associative memories that are important for drug craving, and several glutamatergic medications have been investigated for reducing the vulnerability to relapse (Olive et al., 2012).

GABA acts via ionotropic ($GABA_A$) and metabotropic ($GABA_B$) receptors. $GABA_A$ receptors are ligand-gated chloride-ion channels that confer fast synaptic inhibition (Olsen and Sieghart, 2009). The $GABA_B$ receptors are linked to potassium and calcium channels via G-proteins (Pinard et al., 2010). Glutamate receptors can be divided into ionotropic glutamate receptors (iGluR; NMDA, AMPA, and kainate) and metabotropic receptors (mGluR). The AMPA receptors comprise of four subunits (GluR1-4) that assemble in different combinations. NMDA receptors are heterotetramer composed of two NR1 subunits, and two of the four isoforms' NR2 subunits. There are five types of kainate receptor subunits ($GluK_{1-5}$) that are arranged in different ways to form a tetramer. Activation of the metabotropic glutamate receptors (mGluR1-8) modulates ion channels and other signaling proteins through G-proteins. mGluR1 is predominantly expressed post-synaptically and modulates NMDA, AMPA and GABA receptor activity (Ferraguti et al., 2008). The group I mGluRs (mGluR1 and mGluR5) are associated with the synaptic scaffolding protein, HOMER1.

The HOMER family belong to a complex network that converges dopamine and glutamate signaling to appropriate nuclear targets (de Bartolomeis and Tomasetti, 2012). Cocaine withdrawal was shown to create an imbalance of Homer1/2 isoforms' expression, which disturbed glutamate function and heightened cocaine preference in rodents (Ary et al., 2013; Szumlinski et al., 2006).

Activation of the NMDA receptor leads to postsynaptic calcium entry that binds to calmodulin, which activates calcium calmodulin-dependent protein kinase (CaMKII). CaMKII is composed of four different chains and has an important role in the plasticity of glutamatergic synapses (Giese and Mizuno, 2013). Expression and phosphorylation of alpha-CaMKII (encoded by *CaMK2A*) is increased following cocaine exposure and a *CaMK2A* SNP was reported to confer rapid progression to severe cocaine use (Easton et al., 2014).

A number of studies have reported associations between polymorphisms in genes of these systems and drug addictions. We have reported an association of *GRIN2A* SNPs with heroin addiction (opioid dependence/OD) in a subsample of the current sample of African Americans (AA) (Levran et al., 2009). *GRIN2A* SNPs were also associated with OD in Han Chinese (Zhao et al., 2013; Zhong et al., 2014). *GABRA2*, *GABRB3*, *GABRG2*, and *GAD1* SNPs were associated with OD in different populations (Chen et al., 2014; Enoch et al., 2010; Li et al., 2014; Loh et al., 2007; Wu et al., 2012; Yang et al., 2014). *HOMER1* SNPs were associated with opiate abuse in Caucasians (Jacobs et al., 2013) and with cocaine dependence (CD) in AA (Dahl et al., 2005). Alcohol dependence (AD) was associated with SNPs in *GRIN1*, *GRIN2B*, *GRIK1*, *GABRA1*, *GABRA2*, *GABRA6*, *GABRG1*, *GABRG2*, and *GAD2* SNPs in different populations (e.g., Kim et al., 2006; Kranzler et al., 2009; Enoch et al., 2010; Zintzaras, 2012; Li et al., 2014).

This study focuses on 27 genes including eight glutamate and nine $GABA_A$ receptors' subunits, and ten related proteins. It was designed to determine whether polymorphism in these genes contribute to the susceptibility to heroin and/or cocaine addiction in two populations of distinct ancestry (European and African). The study is an extension of our previous studies of OD with a larger sample, modified SNP content and an additional cocaine group (Levran et al., 2008, 2009). The sample analyzed in the study was independently analyzed for genes in other systems (e.g. stress, dopaminergic) (Levran et al., 2014a,b,c, 2015).

2. Methods

2.1. Subjects

This study follows our previous studies (Levran et al., 2008, 2009), for which we added 481 new African American (AA) subjects and 465 new European/Middle Eastern (EA) subjects. The study included 1860 subjects (38% females) divided into two major ancestry groups. A subject was defined as EA if he/she shows >70% European, Middle Eastern (ME) or combined EA/ME ancestry contributions based on *Structure* analysis (see below). A subject was defined as AA if he/she shows >50% African ancestry contribution. Self-identified Hispanics and AA subjects with >25% contribution of any major ancestry other than European, Middle Eastern or African were not included.

Ascertainment of cases and controls was made by personal interview performed in a similar manner at the recruiting places, using several instruments: the Addiction Severity Index (McLellan et al., 1992); Kreek–McHugh–Schluger–Kellogg Scale (KMSK) (Kellogg et al., 2003); and Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV). Drug addiction diagnosis was based on life-time DSM-IV criteria. Subjects with active major psychotic mental illnesses were excluded. Subjects were recruited at the Rockefeller University Hospital, the Manhattan campus of the VA NY Harbor Health Care System, and the Dr. Miriam and Sheldon G. Adelson Clinics for Drug Abuse Treatment and Research in Las Vegas, USA and Tel Aviv, Israel.

The two ancestry groups were further divided into five groups based on addiction status and drug of abuse (heroin or cocaine) as follows: (1) EA heroin ± cocaine (OD ± CD), (2) AA OD ± CD, (3) AA cocaine (CD, without OD), (4) EA control, and (5) AA control (Table 1).

Subjects in groups 1 and 2 were former heroin addicts in methadone maintenance treatment with a history of at least one year of daily multiple uses of heroin, and about half of them also had past or current cocaine addiction. The subjects in group 3 had past or current cocaine addiction, had no heroin addiction, and about a third of them also had past or current alcohol addiction. The EA "CD without OD" group was not included in this study due to small sample size. Subject could not be defined as controls if they had (1) ≥1 instance of drinking to intoxication or any illicit drug use in the last month; (2) alcohol drinking to intoxication or illicit drug use, ≥2 times per week, for ≥6 consecutive months, and (3) cannabis use for ≥12 days in the previous month or past cannabis use for ≥2 times per week for ≥4 years.

The Institutional Review Boards of the Rockefeller University Hospital, the VA New York Harbor Healthcare System and the Tel Aviv Sourasky Medical Center (Helsinki Committee) approved the study. All subjects signed informed consent for genetic studies.

2.2. Genes and SNPs

A total of 27 genes were selected based on the original "addiction" array (Hodgkinson et al., 2008) with an addition of SNPs in six new genes (Table 2). After exclusion of 10 SNPs, in these genes, due to failure or low frequency in our previous studies, a total of 272 SNPs including tagging SNPs from the original array and 23 new SNPs that were added based on functionality or reported association with related phenotypes, were genotyped with a modified GoldenGate Custom Panel (GS0013101-OPA, Illumina, San Diego, CA) (Supplement Table 1). The X chromosome genes from these systems are included in the array (*GRIA3*, *GABRA3*, *GABRE*, and *GABRQ*) but were not included in the current analysis. Analysis was performed with BeadStudio software v2.3.43 (Illumina) and cluster plots were also visually inspected.

Table 1
Groups description.

Ancestry	Heroin addiction (OD ± CD)	Cocaine addiction (CD without OD)	Controls	Total
	n	n	n	n
EA	827 (1)	–	232 (4)	1059
AA	315 (2)	279 (3)	207 (5)	801
Total	1142	279	439	1860

Group assigned numbers are in parenthesis.

Table 2
Genes List.

Symbol		Description	Chr.	# of SNPs
Glutamate receptors subunits				
Ionotropic	*GRIA1*[*]	AMPA 1 (GluR1)	5	6
	GRIA2[*]	AMPA 2 (GluR2)	4	4
	GRIN1	NMDA 1 (NR1)	9	3
	GRIN2A	NMDA 2A (NR2A)	16	16
	GRIN2B	NMDA 2B (NR2B)	12	23
	GRIN2C	NMDA 2C (NR2C)	17	3
	GRIK1	Kainate 1 (GluK5)	21	13
Metabotropic	*GRM1*	Metabotropic 1	6	17
$GABA_A$ receptors subunits				
	GABRA2	Alpha 2	4	11
	GABRA4	Alpha 4	4	10
	GABRA6	Alpha 6	5	4
	GABRB1	Beta 1	4	17
	GABRB2	Beta 2	5	15
	GABRB3	Beta 3	15	17
	GABRG2	Gamma 2	5	11
	GABRG3	Gamma 3	15	16
	GABRD	Delta	1	1
Related enzymes, transporters and modulators				
	CaMK2A[*]	Calcium/calmodulin-dependent protein kinase II alpha	5	2
	DBI	Diazepam binding inhibitor (GABA receptor modulator)	2	3
	GAD1	Glutamate decarboxylase 1 (67 kDa)	2	9
	GAD2	Glutamate decarboxylase 2 (65 kDa)	2	13
	HOMER1[*]	Homer homolog 1 (Drosophila)	5	5
	HOMER2[*]	Homer homolog 2 (Drosophila)	15	2
	HOMER3[*]	Homer homolog 3 (Drosophila)	19	2
	SLC6A11	Solute carrier family 6 (neurotransmitter transporter), member 11	3	15
	SLC6A13	Solute carrier family 6 (neurotransmitter transporter), member 13	12	16
	SLC32A1	Solute carrier family 32 (GABA vesicular transporter), member 1	20	4

[*] These genes were not included in the original "addiction" array (Hodgkinson et al., 2008; Levran et al., 2008, 2009). The number of SNPs does not include the 14 SNPs excluded from analyses.

2.3. Assessment of ancestry contribution using ancestry informative markers (AIMs)

Biographic Ancestry Scores (e.g., fractions of affiliation of an individual in each cluster) were estimated by *Structure* 2.2 with seven clusters (K) using data from 155 AIMs with high quality (Hodgkinson et al., 2008). Each subject was anchored against genotypes of 1051 samples from 51 worldwide populations represented in the Human Genome Diversity Cell Line Panel (Ducci et al., 2009). The European and Middle-Eastern clusters were combined based on their low population differentiation (Atzmon et al., 2010; Tian et al., 2009). There was no evidence for substructure among the case/control subgroups for each ancestry.

2.4. Statistical analysis

Pairwise linkage disequilibrium (LD) (D′ and r^2) was estimated using Haploview 4.2. LD blocks were identified using confidence intervals (Gabriel et al., 2002). Exact tests for deviation from Hardy-Weinberg equilibrium (HWE) were performed with the PLINK program in the control samples. Association analyses were conducted only for the genes of these two pathways using PLINK for each SNP separately by logistic regression, under dominant or recessive model assumptions. Association analyses were performed independently for EA OD ± CD (1), AA OD ± CD (2), AA CD (3), and AA OD ± CD + CD (2 + 3). Correction for multiple testing was also performed by permutation test (n = 100,000) for each model of inheritance, using PLINK.

3. Results

Four case–control association analyses of selected SNPs in genes of the glutamatergic and GABAergic pathways were performed under two different models of inheritance (dominant or recessive) as follows: OD ± CD in EA (1 vs. 4), OD ± CD in AA (2 vs. 5), CD ± AD in AA (3 vs. 5), and OD ± CD + CD in AA (2 + 3 vs. 5) (Table 1). A total of 272 SNPs spanning 27 genes were genotyped in 1860 subjects (Tables 1, 2 & Supplement Table 1). Fourteen SNPs were excluded from analysis due to technical reasons and 258 SNPs were analyzed. In addition, 34 SNPs were excluded from the EA analysis and 10 SNPs were excluded from the AA analysis, based on low MAF (<0.05) in the respective control samples, including one SNP (*SLC6A11* rs11720592) that was excluded from both analyses (Supplement Table 1). Large deviation from HWE ($P = 0.005$) was detected for *GABRB3* SNP rs7165224 (EA), *SLC6A13* SNP rs10848623 (AA), and *GABRA4* SNP rs3792208 (AA). LD analysis revealed 43 LD blocks including 15 SNP pairs and five triplets in almost complete LD ($r^2 > 0.95$) in EA, of which five pairs are also in strong LD in AA, reducing the effective number of independent SNPs in the analyses (Supplement Table 1).

Of the 224 variants that passed quality control in the EA sample, 13 SNPs in ten genes showed nominally significant associations ($P < 0.05$) with OD ± CD in EA, including *GRIA1* SNP pair in strong LD (Supplement Table 2). Only the synonymous *GRIN2C* SNP rs689730 survived the $P < 0.01$ cutoff (Table 3). None of the signals survived correction for multiple testing.

Of the 248 variants that passed quality control in the AA sample, 34 SNPs in 15 genes showed nominally significant associations ($P < 0.05$) with OD ± CD and/or CD (Supplement Table 2), including two nonsynonymous SNPs (*GRIK1* rs363504 and *GAD1* rs769402). The ten SNPs with the most stringent associations ($P < 0.01$) are listed in Table 3. The two *GABRB3* SNPs indicated are in strong LD ($D' = 1$, $r^2 < 0.1$ in EA and AA). *GRIN2C* SNP rs689730 that showed significant associations in EA showed less stringent association with OD ± CD in AA in the opposite direction ($P = 0.032$, OR = 0.66, Table 3). The upstream *GABRB3* rs7165224 that showed significant association in AA showed less stringent association in EA ($P = 0.035$, Table 3). None of the signals survived correction for multiple testing.

Under the more stringent cutoff ($P < 0.01$) there was no gene or SNP in common for EA and AA. Under the less stringent cutoff ($P < 0.05$) there were eight genes and two SNPs (*GRIN2C* rs689730 and *GABRB3* rs7165224) in common although the association of *GRIN2C* rs689730 was in the opposite direction suggesting a protection effect in AA (Supplement Table 2, Table 3). Comparison of the results for the different AA addiction groups (OD ± CD, CD or both) revealed three SNPs (*GAD1* rs2058725, *GRM1* rs1997766, *GABRB3* rs7165224) that showed associations ($P < 0.01$) in the same direction and model, in more than one analyses (Table 3).

4. Discussion

The study suggests specific genetic contributions to heroin and cocaine addictions in the GABA and glutamate systems. This study follows our studies of heroin addiction in EA and AA (Levran et al., 2008, 2009) and was conducted after the recruitment of additional subjects to approximately double the size of the cohort. We have also added a cocaine addiction AA group, modified the SNP content and changed the analysis process to include only one or two pathways at a time instead of an analysis of the whole array, to reduce multiple testing. The study follows our recent independent studies of other pathways (e.g. stress, dopaminergic) in the current sample (Levran et al., 2014a,b,c, 2015).

Since the associations were only nominally significant and did not survive correction for multiple testing, they should be considered

Table 3
Association results ($P < 0.01$).

Gene	SNP	Chr	Position	Location	Alle–les	MAF		P				Model	OR (95% CI)
						EA	AA	EA	AA				
								OD ± CD	OD ± CD	CD	OD ±CD +CD		
DBI	**rs12613135**	2	119364764	Upstream[b]	C/T	0.27	0.30		**0.006**		(0.02)	R	2.48 (1.3–4.7)
GABRB2	rs3816596	5	161548326	Upstream	C/T	0.34	0.50		**0.009**		(0.02)	R	0.56 (0.4–0.9)
	rs6882041	5	161515394	Intron	C/T	0.27	0.31		(0.01)		**0.008**	R	0.47 (0.3–0.8)
GABRB3	**rs7165224**	15	26779189	Upstream	C/T	0.07	0.21	(0.04)	**0.003**	(0.04)	0.005	D	1.71 (1.2–2.5)
	rs1863456	15	26728885	Intron	A/G	0.48	0.15			**0.008**	(0.02)	D	0.56 (0.4–0.9)
GAD1	**rs1978340**[c]	2	170813611	Upstream	C/T	0.27	0.09			**0.006**	(0.01)	D	1.85 (1.2–2.9)
	rs2058725	2	170833611	Intron	A/G	0.27	0.37		0.004	(0.01)	**0.001**	R	0.47 (0.3–0.7)
GRIN2A	rs1833161	16	9791170	Intron	T/C[a]	0.32	0.42			(0.04)	**0.009**	R	1.77 (1.2–2.7)
GRIN2B	rs2284416	12	13766280	Intron	T/G	0.49	0.44		(0.01)	(0.04)	**0.006**	R	0.57 (0.4–0.9)
GRIN2C	rs689730	17	74854994	Ala33=	C/T	0.09	0.21	**0.004**	(0.03)[d]			D	1.76 (1.2–2.6)
GRM1	rs1997766	6	146180786	Intron	T/C	0.006	0.18		0.008	(0.03)	**0.006**	D	0.62 (0.4–0.9)

SNPs with P values < 0.01 in at least one analysis are listed. For these SNPs, P values < 0.05 in the other analyses are also listed. Blank cells represent $P > 0.05$.
The complete results ($P < 0.05$) are listed in Supplement Table 2.
Alleles are listed with the major allele (in EA) first.
The model and OR refers to the minor allele. OR is listed for the lowest P value (in bold) but the other analyses for the same SNP showed the same direction, model and similar OR range unless indicated. OR > 1 represent risk effect of the minor allele (in bold), OR < 1 represent protective effect of the minor allele.
The bolded SNP was previously associated with OD in AA (Levran et al., 2009) and in Han Chinese (Zhao et al., 2013; Zhong et al., 2014).
Box represent LD ($D' = 1$, $r^2 < 0.1$ in EA and AA).
[a]The minor allele in AA is the major allele in EA.
[b]This SNP is also located at an intron of *C2orf76* (chromosome 2 open reading frame 76).
[c]This SNP showed an effect on GABA levels in prefrontal cortex.
[d]The association of OD ± CD in AA was in the opposite direction compared to the OD ± CD in EA (OR (95% CI) 0.66 (0.45–0.96)).
Chr, Chromosome; MAF, minor allele frequency; EA, European/Middle Eastern ancestry; AA, African Americans; OD, opioid dependence; CD Cocaine dependence; OR, Odds ratio; CI, confidence interval; D, dominant; R, recessive.

tentative until further verification. Nevertheless, a hypothesis-driven study of genes with known or potential addiction-related functionality may not require as stringent a threshold for significance as a hypothesis-free study.

One of the main finding is the association of SNP rs1978340 upstream of *GAD1* with CD without OD in AA. This SNP was previously associated with drug addictions and related phenotypes (Kuo et al., 2009; Wu et al., 2012; Yang et al., 2014) and showed an effect on GABA concentrations in the cingulate cortex (Marenco et al., 2010). Glutamic acid decarboxylase (GAD_{67}, encoded by *GAD1*) is one of two major isoforms of GAD that converts glutamate to GABA and is critical for the maintenance of GABA reserves. It has an important role in the pathogenesis of anxiety disorders and decreased expression of GAD1 in hippocampus GABAergic interneurons was observed in subjects with schizophrenia and bipolar disorder that was associated with cognitive deficits (Benes et al., 2007). Studies have shown that chronic administration of drugs of abuse alters the expression and activity of GAD1 in the brain (Enoch et al., 2012).

The study corroborates the association of SNPs *GAD1* rs2058725, *DBI* rs12613135, and *GABRB3* rs7165224 that were identified in our previous study of OD in a subsample of the AA group (Levran et al., 2009). *GRIN2A* SNP rs1650420 that was indicated in this study in association with OD in AA by the less stringent cutoff ($P = 0.019$), was previously associated with OD in Han Chinese (Zhao et al., 2013; Zhong et al., 2014) and in our previous study of in AA (Levran et al., 2009).

Some of the findings may be population-specific. Comparison of the results obtained in EA and AA reveals no SNP in common under the more stringent cutoff and only two SNPs in common under the less stringent cutoff, one of them with an opposite effect. Interestingly, the upstream *GABRB3* SNP rs7165224 indicated in the current study, was not detected in Han Chinese in a study that reported association with another upstream *GABRB3* SNP rs4906902 with OD (Chen et al., 2014). *GABRB3* SNP rs4906902 is very rare in African populations and was not included in the current study. This data suggests a potentially different profile of the *GABRB3* regulatory region across different populations.

Comparison of the results obtained in the analyses of the two addictions (CD and OD), in AA, reveals several genes and SNPs in common that support the existence of both shared and specific vulnerability between the two addictions. For example, the P values for the associations of SNPs *GAD1* rs1978340 and *GABRB3* rs1863456 were lower in the analysis of CD without OD than in the combined OD ± CD, in spite of the smaller sample size, suggesting a cocaine-specific vulnerability. The P values for the associations of SNPs *DBI* rs12613135 and *GABRB2* rs3816596 were lower in the OD ± CD analysis than in the combined analysis suggesting a heroin-specific vulnerability. However, this comparison is limited due to substance-related comorbidity, and a limited power to detect small effects.

The GABA system is a potential pharmacotherapeutic target for the treatment of drug abuse disorders (Addolorato et al., 2012) and variants in genes of this system may also affect the response to treatment, as was shown for *GABRA2* genotype and the treatment of alcohol drinking (Bauer et al., 2007).

4.1. Conclusions

This study suggests numerous potential susceptibility loci (or markers that tag them) in the glutamatergic and GABAergic pathways for heroin and cocaine addiction, in subjects of African and European ancestry. There was partial overlap in susceptibility loci between populations and between addictions to different drugs. Future studies are required to corroborate the results and to assess the relevance of the findings for diagnosis and treatment.

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Author contributions

O. Levran: project design, data collection, analysis and interpretation, manuscript writing; M.J Kreek: principal investigator who oversaw all aspects of the study including review of the final manuscript; M. Randesi sample preparation and data acquisition; J. Ott and J. Correa da Rosa: statistical analysis. E. Peles, M. Adelson, and J. Rotrosen: subjects' ascertainment, study samples providers. All authors have approved the final manuscript.

Disclosure

None of the authors have any conflicts of interest to declare with respect to this manuscript.

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