Research Article

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Pharmacogenomics

Susceptibility loci for heroin and cocaine addiction in the serotonergic and adrenergic pathways in populations of different ancestry

Background: Drug addiction is influenced by genetic factors. **Aim:** To determine if genetic variants in the serotonergic and adrenergic pathways are associated with heroin and/or cocaine addiction. **Subjects & methods**: The study examined 140 polymorphisms in 19 genes in 1855 subjects with predominantly European or African ancestries. **Results:** A total of 38 polymorphisms (13 genes) showed nominal associations, including novel associations in *S100A10* (p11) and *SLC18A2* (VMAT2). The association of *HTR3B* SNP rs11606194 with heroin addiction in the European ancestry subgroup remained significant after correction for multiple testing ($p_{corrected}$ = 0.04). **Conclusion:** The study strengthens our previous findings of association of polymorphisms in *HTR3A, HTR3B* and *ADRA1A*. The study suggests partial overlap in genetic susceptibility between populations of different ancestry and between heroin and cocaine addiction.

Keywords: *ADRA1A* • adrenergic pathway • African–American • association study • cocaine addiction • genetic variants • heroin addiction • *HTR3A* • *HTR3B* • *S100A10* • serotonergic pathway • *SLC18A2* • *SLC6A4* • stress

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Drug addiction is a chronic compulsive and relapsing brain disease caused by a combination of genetic, epigenetic, environmental and drug-induced factors. Addictions to different drugs are related and are also connected to other psychiatric diseases by shared neurobiological pathways, including reward modulation and stress response [1,2]. Although the dopamine pathway is generally thought to be the common pathway for the reinforcing properties of drugs, other neurotransmitters are involved as well, either as modulators of dopamine function or independent of dopamine. Cocaine binds to monoamine transporters thereby blocking transmitter reuptake, and its psychostimulant properties are the result of increase in synaptic levels of dopamine, serotonin and norepinephrine [3]. This study focuses on two of these systems; the serotonergic and the noradrenergic system, as dysfunction of these systems provoked by genetic polymorphism may contribute to the vulnerability to drug addiction.

Serotonin (5-HT) functions as both a short-range neurotransmitter and a long-range signaling modulator via peripheral system. Serotonin is essential for the maintenance of synaptic plasticity, motivational and reinforcement processes, and for learning and memory. The central serotonergic system regulates mood and has been implicated in several neuropsychiatric conditions and behavior. Addictive drugs have acute effects on extracellular 5-HT activity and tissue levels [4]. Cocaine withdrawal is accompanied by impairments in serotonin function [5] and a long period of reduced sensitivity of serotonin receptors was shown in heroin addicts even after drug cessation [6]. Serotonergic signaling is mediated via several G protein-coupled receptors and an ion channel (the 5-HT3 receptor) that activate the mesolimbic reward circuitry. This study examined polymorphisms in genes encoding seven 5-HT receptors (HTR1A and HTR1B, HTR2A and HTR2B, HTR3A and HTR3B, and

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HTR4), three 5-HT transporters (SLC6A4, SLC6A7, SLC18A2 and S100A10) and two enzymes involved in 5-HT biosynthesis (TPH1 and 2).

The noradrenergic (NA) system serves multiple brain functions that are relevant to drug actions and addiction including mood, memory and stress response [7,8]. The NA system is able to influence the hypothalamic-pituitary-adrenal stress axis [9]. Norepinephrine (NE) effects are mediated by three families of nine adrenergic receptors. Hyperactivity of brain NE has been implicated in opiate withdrawal [10,11]. The NA system underlies the neurobiology for stress-induced reinstatement of drug-seeking behavior in animal models [12]. There is a strong interaction between stress and drug use in human addicts [13]. This study examined polymorphisms in genes encoding five noradrenergic receptors (ADRA1A, ADRA2A, ADRA2B, ADRA2C and ADRB2) and a transporter (SLC6A2).

Numerous genetic studies have evaluated the association between polymorphisms in NE and 5-HT genes and drug addiction (e.g., [14–16]). Associations of polymorphisms of the serotonin transporter (5-HTT) with heroin addiction (opioid dependence, OD) were reported [17–19]. Five association studies of OD that included NE and/or serotonin were previously performed by our laboratory in samples of different ancestries [20–24]. Of these, associations of OD with *HTR1B* SNP rs6297 [21], as well as *HTR3B* SNPs rs3758987 and rs11606194 [20] were detected in subjects with European ancestry. *ADRA1A* SNP [23] and *TPH1*/*TPH2* SNP interaction were indicated in African–Americans [22].

This case–control hypothesis-driven study was designed to determine whether variations in genes of the serotonergic and adrenergic pathways contribute to the susceptibility to OD and/or cocaine dependence (CD) in two populations of distinct ancestry (European and African). While several studies have analyzed SNPs in these systems for association with heroin or cocaine addiction previously, they may have used different variants, different phenotypes (e.g., multiple dependencies, less stringent criteria for defining specific addiction or different criteria for controls definition), and/or on self-described ancestry. This study relies on rigorous ascertainment with stringent inclusion/exclusion criteria, and the control for population stratification using ancestry informative markers (AIMs). The study extends our previous studies [20,23] with increased statistical power, modified SNP content and an additional cocaine group that was not analyzed previously.

Subjects & methods

Subjects

The study included 1855 subjects (38% females) that were divided into five groups according to their predominant ancestry (European/Middle Eastern or African), addiction status and preferred drug (heroin or cocaine): EA/ME OD ± CD; AA OD ± CD; AA CD; EA/ME control; AA control (Table 1). The abbreviation EA/ME will be used for the sample with predominant European/Middle Eastern ancestry and the abbreviation AA will be used for the sample with predominant African ancestry. This study is a major expansion of our previous studies [20,23] for which we added 481 new AA subjects and 465 new EA/ME subjects. To be included in the EA/ME sample, an individual had to have greater than 75% European, Middle-Eastern or combined ancestry contributions. A subsample of EA/ME OD ± CD group that includes only subjects from the USA (1a, n = 542, abbreviation EA) was used to rule out an effect of population substructure on the results. To be included in the AA sample, an individual had to have greater than 50% African ancestry contribution by Structure analysis (see below). Self-identified Hispanics and subjects with greater than 25% contribution of any other major ancestry 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 including: the Addiction Severity Index [25], Kreek–McHugh–Schluger–Kellogg Scale [26] and *Diagnostic and Statistical Manual of Mental Disorders (4th Edition)*. Subjects for the case samples were recruited at the Rockefeller University Hospital (n = 733), the Manhattan campus of the VA NY Harbor Health Care System (n = 122)

Table 1. Groups descriptions.

Ancestry	Heroin addiction (n; OD ± CD)	Cocaine addiction (n; CD)	Controls (n)	Total (n)
EA/ME	824 (1)	–	232 (4)	1056
AA	314 (2)	279 (3)	206 (5)	799
Total	1138	279	438	1855

Values provided are given as number of subjects. Numbers in parentheses are the group assigned numbers.
AA: African–American; CD: Cocaine dependence; EA/ME: European/Middle Eastern; OD: Opioid dependence.

and the Dr Miriam and Sheldon G Adelson Clinics for Drug Abuse Treatment and Research in Las Vegas, NV, USA (n = 277) and Israel (n = 285).

The heroin addiction case subjects were former heroin addicts in methadone maintenance treatment with a history of at least a year of daily multiple uses of heroin. About half of them also had past or current cocaine abuse or addiction. The abbreviation OD ± CD will be used for this sample. All subjects in the cocaine group had current or past cocaine addiction, and reported cocaine as their drug of choice. Some of them (29%) also had current or past alcohol addiction, and none of them had heroin addiction. The abbreviation CD will be used for this sample. European/Middle-Eastern CD subjects were not included in this study due to small sample size. The controls sample was mainly recruited at the Rockefeller University Hospital with an addition of 30 EA/ME samples from Israel. Subjects were not included as controls if they had at least an instance of drinking to intoxication or any illicit drug use in the previous 30 days; a history of alcohol drinking to intoxication or illicit drug use, more than twice a week, for more than six consecutive months; and, cannabis use for more than 12 days in the previous 30 days or past cannabis use for more than twice a week for more than 4 years. Subjects with active *Diagnostic and Statistical Manual of Mental Disorders (4th Edition)* axis I disorder were not included in the study. 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.

Genes, SNPs & genotyping

A total of 140 SNPs spanning 19 genes (13 serotonergic and six adrenergic pathway genes) were selected as described [27]. The original array included tagging SNPs with a minor allele frequency (MAF) greater than 0.005 that aimed to capture the maximum haplotype information. All the genes associated with the two pathways on this array were included in this analysis, except for X chromosome genes. The modified array includes some modifications based on functionality or reported association with related phenotypes (Table 2 & Supplementary Table 1 [for Supplementary Material, please see online at www.futuremedicine.com/doi/full/10.2217/PGS.15.86]). Two genes from these pathways (*S100A10* and *HTR4*) were not present in the original array and were added to the modified array. Thirty-two SNPs in these genes were excluded from the modified array based on predicted low-design score or low frequency (MAF lesser than 0.05) in the relevant populations.

SNPs were genotyped using the Illumina GoldenGate Custom Panel (GS0013101-OPA). DNA (700 ng) was precipitated as described [20]. Genotyping was performed at the Rockefeller University Genomics Resource Center. Twenty-five random samples were regenotyped with 0.003% error rate. Analysis was performed with BeadStudio software v2.3.43 (Illumina, CA, USA). The cluster plots were visually inspected.

Assessment of ancestry contribution using ancestry informative markers

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 [27]. Each subject was anchored against genotypes of 1051 samples from 51 worldwide populations represented in the Human Genome Diversity Cell Line Panel, as described [28]. The decision to include both European and Middle-Eastern clusters was based on their low-population differentiation [29,30].

Statistical analysis

Pairwise linkage disequilibrium (LD; D' and r^2) was estimated using Haploview 4.2. LD blocks were identified using the D' CI bound of 0.7–0.98 [31]. Exact tests

Table 2. Gene list.

Symbol	Description	Other symbol
Serotonergic		
HTR1A	5-hydroxytryptamine (serotonin) receptor 1a, G protein coupled	5-HT1A
HTR1B	5-hydroxytryptamine (serotonin) receptor 1b, G protein coupled	5-HT1B
HTR2A	5-hydroxytryptamine (serotonin) receptor 2a, G protein coupled	5-HT2A
HTR2B	5-hydroxytryptamine (serotonin) receptor 2b, G protein coupled	5-HT2B
HTR4	5-hydroxytryptamine (serotonin) receptor 4, G protein coupled	5-HT4
HTR3A	5-hydroxytryptamine (serotonin) receptor 3a, ionotropic	5-HT3A
HTR3B	5-hydroxytryptamine (serotonin) receptor 3b, ionotropic	5-HT2B
S100A10	S100 calcium binding protein a10	P11
SLC6A4	Solute carrier family 6 (neurotransmitter transporter), member 4	5-HTT, SERT
SLC6A7	Solute carrier family 6 (neurotransmitter transporter), member 7	PROT
SLC18A2	Solute carrier family 18 (vesicular monoamine transporter), member 2	VMAT2
TPH1	Tryptophan hydroxylase 1	
TPH2	Tryptophan hydroxylase 2	
Adrenergic		
ADRA1A	Adrenoceptor α 1a	
ADRA2A	Adrenoceptor α 2a	
ADRA2B	Adrenoceptor α 2b	
ADRA2C	Adrenoceptor α 2c	
ADRB2	Adrenoceptor β 2, surface	
SLC6A2	Solute carrier family 6 (neurotransmitter transporter), member 2	NET1

for deviation from Hardy-Weinberg equilibrium were performed with the PLINK program, with SNPs to be rejected based on threshold of $p \leq 0.001$ in controls. Association analyses were conducted using PLINK for each SNP separately by logistic regression, under dominant or recessive model assumptions. Association analyses were performed independently for EA/ME OD ± CD and for AA OD ± CD, AA CD and AA OD ± CD+CD. Association analysis was also performed for the EA OD ± CD subsample of US subjects. Correction for multiple testing was performed by permutation test (n = 100,000) for each model of inheritance, using PLINK. Ridge regression was performed using the R software, to identify the most likely explanatory SNP when two or more SNPs in strong LD showed significant associations under the same model of inheritance [32].

Results

A total of 140 SNPs from 19 genes related to the serotonergic and the adrenergic pathways were genotyped in 1855 subjects (Tables 1, 2 & Supplementary Table 1). The ancestry of all subjects (more than 75% European/Middle-Eastern or more than 50% African ancestry) was verified using Structure analysis of 155 AIMs and there was no evidence for substructure among the case/control subgroups for each ancestry group. The three AA groups (cases and control) had an average range of 80–82% (SD = 0.1) African ancestry and 10–11% (SD = 0.08) European/Middle Eastern ancestry and are described in more details in our recent study [33]. Nineteen SNPs were excluded from the EA/ME analysis and eight SNPs were excluded from the AA analyses based on low MAF (lesser than 0.05), including two SNPs (*ADRA2B* rs3813662; *HTR3B* rs10502180) that were excluded from all analyses. *ADRA2C* SNP rs7434444 was also excluded from all analyses because it does not map to any genome assembly (Supplementary Table 1). The minor allele of 21 SNPs in EA/ME was the major allele in AA. No SNP showed significant deviation from Hardy–Weinberg equilibrium in the two control samples. LD analysis in the control AA sample revealed 20 LD blocks of 53 SNPs (Supplementary Figure 1). LD analysis of the control EA/ME sample revealed 30 LD blocks of 89 SNPs (Supplementary Figure 2).

Four independent case–control association analyses were performed under two different models of inheritance (dominant or recessive): EA/ME OD ± CD (1

Table 3. Information of associated SNPs.

Gene	SNP	Chromosome	Position GRCh38	Location	Alleles	MAF†	
						EA	AA
Serotonergic							
HTR1B	rs6296	6	77462543	Val287=	G/C	0.24	0.26
HTR2A	rs1923882	13	46837526	Intron	G/A	0.24	0.38
	rs9567735		46845069	Intron	A/G	0.13	0.09
	rs6561333		46846177	Intron	G/A	0.40	0.17
HTR2B	rs6437000	2	231112813	Intron‡	A/C	0.34§	0.19
	rs17586428		231124141	Intron‡	A/G	0.07	0.38
HTR3A	rs1150226	11	113974819	Upstream	C/T	0.07	0.34
	rs897687		113985089	Intron	A/G	0.01	0.41
	rs1176713		113989703	Leu497=	A/G	0.23	0.24
HTR3B	rs3758987	11	113904553	-381	A/G	0.24	0.41
	rs11606194		113910259	Intron	T/C	0.04¶	0.01
	rs1176744		113932306	Tyr129Ser	A/C	0.29	0.39
S100A10	rs3791153	1	151985668	Intron	T/C	0.39	0.35
	rs12083193		151990213	Intron	G/C	0.39	0.35
SLC18A2	rs363271	10	117267900	Intron	T/G	0.13	0.35
	rs2244249		117272764	Intron	G/A	0.16	0.34
	rs363276		117274298	Intron	C/T	0.17	0.38
SLC6A4	rs2020942	17	30219896	Intron	A/G	0.34	0.24
	rs2066713		30224647	Intron	C/T	0.34	0.24
	rs16965628		30228407	Intron	G/C	0.08	0.27
	rs2020933		30234737	Intron	T/A	0.07	0.29
TPH1	rs10741734	11	18023101	Intron	A/G	0.45	0.20
	rs1799913		18025708	Intron	C/A	0.45	0.20
	rs1607395		18028189	Intron	T/C	0.45	0.20
TPH2	rs6582078		71981111	Intron	T/G	0.41	0.41
	rs1487275		72016512	Intron	T/G	0.32	0.29
	rs2220159		72112507	Downstream	A/C	0.22	0.34
Adrenergic							
ADRA1A	rs2291776	8	26754119	Intron/3′UTR#	G/A	0.05	0.18
	rs11135955		26781393	Intron	T/G	0.12	0.15
	rs6990485		26826850	Intron	G/C	0.00	0.24
	rs472151		26834850	Intron	G/A	0.47§	0.29
	rs10503800		26838100	Intron	G/A	0.36	0.46
	rs486179		26866417	Intron	G/A	0.04	0.27
	rs2644627		26869124	5′ UTR/upstream#	C/G	0.44	0.36

Genes are listed in alphabetical order for each pathway. Alleles are listed with the major allele first.
†In controls.
‡This SNP is also located at the overlapping *PSMD1*.
§The minor allele in African–Americans is the major allele in European ancestry.
¶This SNP was not excluded based on MAF of 0.08 in cases.
#The position depends on the isoform.
AA: African–American; EA/ME: European/Middle Eastern; MAF: Minor allele frequency.

Table 3. Information of associated SNPs (cont.).

Gene	SNP	Chromosome	Position GRCh38	Location	Alleles	MAF†	
						EA	AA
Adrenergic (cont.)							
ADRA2B	rs2312955	2	96111420	Downstream	G/T	0.27	0.18
SLC6A2	rs36024	16	55672479	Intron	C/T	0.45§	0.45
	rs10521329		55686546	Intron	C/A	0.17	0.33
	rs3785155		55688478	Intron	G/A	0.13	0.24

Genes are listed in alphabetical order for each pathway. Alleles are listed with the major allele first.
†In controls.
‡This SNP is also located at the overlapping *PSMD1*.
§The minor allele in African–Americans is the major allele in European ancestry.
¶This SNP was not excluded based on MAF of 0.08 in cases.
#The position depends on the isoform.
AA: African–American; EA/ME: European/Middle Eastern; MAF: Minor allele frequency.

vs 4), AA OD ± CD (2 vs 5), AA CD (3 vs 5) and AA OD ± CD+CD (2+3 vs 5) (Table 1). The EA OD ± CD subgroup that includes only subjects from the USA was subsequently analyzed to rule out an effect of population substructure on the results. The nominally significant associations ($p < 0.05$) are presented in Tables 3 & 4.

EA/ME

A total of 120 SNPs were analyzed for association with OD ± CD in EA/ME and subsequently in the EA OD ± CD subgroup (Supplementary Tables 1 & 2), including two triplets and nine SNP pairs in complete LD (r^2 >0.98) (Supplementary Figure 2). Eight SNPs in four genes (*HTR3B*, *TPH2*, *ADRA1A* and *SLC6A2*) showed nominally significant association of genotype with OD ± CD in both EA/ME and EA subgroup (Tables 3 & 4), including a nonsynonymous SNP (*HTR3B* rs1176744 Tyr129Ser). Two SNPs (*HTR3B* rs11606194 and *ADRA1A* rs2291776) are relatively rare (MAF ~0.05) in this population and the rest are common (MAF = 0.17–0.45). Of these SNPs, there was LD between *HTR3B* SNPs rs3758987, rs11606194 and rs1176744 (D' greater than 0.91) with strong correlation between SNPs rs3758987 and rs1176744 (r^2 = 0.67), as well as between *ADRA1A* SNPs rs472151 and rs10503800 (D' = 0.95, r^2 = 0.44) (Tables 3, 4, Figure 1A & Supplementary Figure 2). Ridge regression suggested that the most likely explanatory SNPs are *HTR3B* rs11606194 ($p = 0.003$) and *ADRA1A* rs10503800 ($p = 0.014$) (Table 4 & Supplementary Table 3).

Notably, although none of the signals survived correction for multiple testing in the analysis of the EA/ME group, the association signal of the intronic *HTR3B* SNP rs11606194 remained significant after correction for multiple testing ($p_{corrected} = 0.04$) in the analysis of the EA subgroup. Four SNPs showed significant associations in the EA/ME group that were not significant in the EA subgroup, and four SNPs showed significant associations in the EA subgroup that were not significant in the EA/ME group (Supplementary Table 2).

AA

A total of 131 SNPs were analyzed for association with OD ± CD and/or CD in AA (Supplementary Table 1). Thirty-one SNPs in 13 genes showed association of genotype with OD ± CD and/or CD, including two coding synonymous SNPs (*HTR1B* rs6296 and *HTR3A* rs1176713) (Tables 3 & 4). Three SNPs (*HTR3A* rs897687, *SLC18A2* rs363276 and *SLC6A4* rs2066713) showed association with both OD ± CD and CD. Three SNPs (*HTR3A* rs1150226, *SLC6A4* rs2020942 and *TPH2* rs2220159) were indicated in the combined OD ± CD + CD but were not identified in at least one of the separate analyses. The *HTR3B* SNP rs3758987 that was indicated in our previous study [23], showed association with OD ± CD with a protective effect of the minor G allele. Of the SNPs that showed associations in AA, the three *TPH1* SNPs and the two *S100A10* SNPs are in complete LD in this sample (r^2 = 1). Linkage disequilibrium was detected among the *HTR3A, SLC18A2, SLC6A4* and *SLC6A2* SNPs (Tables 3, 4, Figure 1B & Supplementary Figure 1). Ridge regression suggested that the most likely explanatory SNPs among the SNPs in LD that were associated under the same model of inheritance are *HTR3A* rs897687 ($p = 0.009$ for OD ± CD and CD), and *SLC6A4* rs2066713 ($p = 0.038$) (Table 4 & Supplementary Table 3).

HTR3A 3-SNP genotype

Three *HTR3A* SNPs in strong LD (D' greater than 0.7, r^2 lesser than 0.41) showed association with OD ± CD and/or CD in AA, under the dominant model (Figure 1B, Tables 3 & 4). SNP rs1176713 showed risk

Table 4. Summary of association analyses results.

Gene	SNP	p-value				OR†	L95	U95	Model
		EA	AA						
		(OD ± CD)	(OD ± CD)	CD	(OD ± CD) + CD				
Serotonergic		1	2	3	2 + 3				
HTR1B	rs6296		0.032		0.047	0.68	0.47	0.97	D
HTR2A	rs1923882		0.028		0.041	0.67	0.47	0.96	D
	rs9567735			0.019		**1.73**	1.10	2.72	D
	rs6561333		0.042			**3.63**	1.05	12.6	R
HTR2B	rs6437000			0.047		**3.59**	1.02	12.7	R
	rs17586428		0.026		0.024	0.61	0.40	0.94	R
HTR3A	rs1150226				0.046	0.72	0.52	0.99	D
	rs897687*		0.007	0.013	0.004	0.62	0.44	0.85	D
	rs1176713		0.010			**1.60**	1.12	2.28	D
HTR3B	**rs3758987**	0.013	0.002		0.014	**1.45**‡	1.08	1.94	D
	rs11606194*	0.002				**2.39**	1.39	4.11	D
	rs1176744	0.038				**1.34**	1.02	1.83	D
S100A10	rs3791153			0.031	0.027	0.57	0.35	0.94	R
	rs12083193			0.031	0.022	0.56	0.34	0.92	R
SLC18A2	rs363271		0.017		0.012	0.54	0.34	0.87	R
	rs2244249			0.021	0.019	0.56	0.35	0.91	R
	rs363276*		0.020	0.006	0.003	0.52	0.33	0.80	R
SLC6A4	rs2020942				0.047	**2.17**	1.01	4.67	R
	rs2066713*		0.022	0.019	0.014	**2.75**	1.23	6.15	R
	rs16965628			0.038		**1.47**	1.02	2.11	D
	rs2020933			0.026		**1.51**	1.05	2.17	D
TPH1	rs10741734			0.036		0.29	0.09	0.92	R
	rs1799913			0.037		0.29	0.09	0.93	R
	rs1607395			0.035		0.28	0.09	0.92	R
TPH2	rs6582078			0.048		0.69	0.47	1.00	D
	rs1487275	0.030				0.59	0.37	0.95	R
	rs2220159				0.035	**1.41**	1.03	1.95	D
Adrenergic									
ADRA1A	rs2291776	0.040				0.09	0.01	0.90	R
	rs11135955			0.029	0.032	**1.54**	1.05	2.27	D
	rs6990485			0.039	0.028	0.40	0.18	0.90	R
	rs472151	0.015				0.66	0.47	0.92	R
	rs10503800*	0.003				0.53	0.35	0.80	R

SNPs in bold were previously identified by Levran *et al.* [20,23].
SNPs with asterisk are most likely the explanatory SNPs among the SNPs in linkage disequilibrium in the relevant analyses based on ridge regression (see Supplementary Table 3).
Double solid lined boxes represents complete correlation ($r^2 = 1$) in the EA/ME and the AA control samples. Solid lined boxes represents LD (D' greater than 0.7) in EA/ME control sample only and dash lined boxes represents strong LD (D' greater than 0.7) in the two control samples.
†OR is listed for the lowest p-value except where indicated. The complete OR data is presented in Supplementary Table 4. OR greater than 1 represents risk effect of the minor allele (in bold), OR less than 1 represents protective effect of the minor allele.
‡In this case, the OR is for EA/ME OD. The OR for the AA samples was in the opposite direction.
AA: African–American; CD: Cocaine dependence; D: Dominant; EA/ME: European/Middle Eastern; L95: 95% CI lower value; OD: Opioid dependence; OR: Odds ratio; R: Recessive; U95: 95% CI upper value.

Table 4. Summary of associations analyses results (cont.).

Gene	SNP	p-value				OR†	L95	U95	Model
		EA	AA						
		(OD ± CD)	(OD ± CD)	CD	(OD ± CD) + CD				
Adrenergic (cont.)									
ADRA1A (cont.)	**rs486179**		0.042			**1.98**	1.03	3.83	R
	rs2644627		0.045		0.032	**1.67**	1.05	2.66	R
ADRA2B	rs2312955			0.031		0.65	0.44	0.96	D
SLC6A2	rs36024	0.043				0.68	0.47	0.99	R
	rs10521329		0.0006		0.007	0.32	0.17	0.62	R
	rs3785155			0.033	0.024	0.69	0.50	0.95	D

SNPs in bold were previously identified by Levran *et al.*[20,23].
SNPs with asterisk are most likely the explanatory SNPs among the SNPs in linkage disequilibrium in the relevant analyses based on ridge regression (see Supplementary Table 3).
Double solid lined boxes represents complete correlation ($r^2 = 1$) in the EA/ME and the AA control samples. Solid lined boxes represents LD (D′ greater than 0.7) in EA/ME control sample only and dash lined boxes represents strong LD (D′ greater than 0.7) in the two control samples.
†OR is listed for the lowest p-value except where indicated. The complete OR data is presented in Supplementary Table 4. OR greater than 1 represents risk effect of the minor allele (in bold), OR less than 1 represents protective effect of the minor allele.
‡In this case, the OR is for EA/ME OD. The OR for the AA samples was in the opposite direction.
AA: African–American; CD: Cocaine dependence; D: Dominant; EA/ME: European/Middle Eastern; L95: 95% CI lower value; OD: Opioid dependence; OR: Odds ratio; R: Recessive; U95: 95% CI upper value.

effect of the minor G allele (OR = 1.6) while SNPs rs897687 and rs1150226 showed protective effect of the minor alleles (G and T alleles; OR = 0.6, 0.7, respectively). To assess the cumulative effect of the three SNPs with the contradicting effects, we have created 3-SNP genotype groups based on the frequency and the direction of the effect, assuming a dominant model, as follows: 'reference' (no variant alleles); 'risk' (at least one rs1176713 G allele); 'protection' (at least one of the rs897687 G and the rs1150226 T alleles); 'mixed' (at least a risk and a protective allele) (Table 5). There was no significant difference in genotype frequencies between the OD ± CD and the CD AA groups therefore only the combined group (OD ± CD + CD) was analyzed. The results indicated a higher frequency of the 'risk' genotype group and lower frequency of the 'protective' genotype group in the combined addictions AA group, compared with the control AA group (p = 0.024). There were no significant difference in genotype frequencies between the control and the OD ± CD in EA, in which there was no indication of associations of these SNPs, and the MAFs of the two protective alleles are very small.

Discussion

The study suggests shared and unique genetic contributions to heroin and cocaine addictions in the serotonergic and the adrenergic pathways. Overlapping contributions on the gene level, but not on SNP level, were also found between subjects with European and African ancestry. Although except for the association of *HTR3B* SNP rs11606194 with heroin addiction in the EA US subgroup, none of the associations survived correction for multiple testing and may not reflect true associations, a hypothesis-driven study may not require as stringent a threshold for significance as a hypothesis-free study. In addition, some of the SNPs, have known functional consequences, were associated with related phenotypes in previous studies, or were shown to be the most likely explanatory SNPs when several SNPs in LD were indicated. The study's nominally significant results may be of importance for future studies and meta-analyses.

Comparison of the results of this study to our previous studies of heroin addiction [20,23] that used approximately half of the current sample, revealed corroboration of two SNPs in strong LD in EA/ME (*HTR3B* rs3758987 and rs11606194) and two SNPs in AA (*ADRA1A* rs486179 and *HTR3A* rs897687). A previous study of cocaine dependence and *HTR1B* SNPs from our laboratory [34] did not detect associations, albeit the sample size was small. Another study of heroin addiction from our laboratory [22] indicated association of *TPH2* haplotype in AA, but this haplotype was not analyzed in the current study.

Serotonin receptors

Seven genes encoding subtypes of 5-HT were included in this study. The transcripts of the 5-HT3A and 5-HT3B subunits are coexpressed in areas implicated in drug addictions, and form heteropentameric receptors [35] and the *HTR3A* and *HTR3B* genes are colocalized within a 90-Kb region on chromosome 11. The main finding is of the *HTR3B* intronic SNP rs11606194 that was associated with heroin addiction in the EA US subgroup

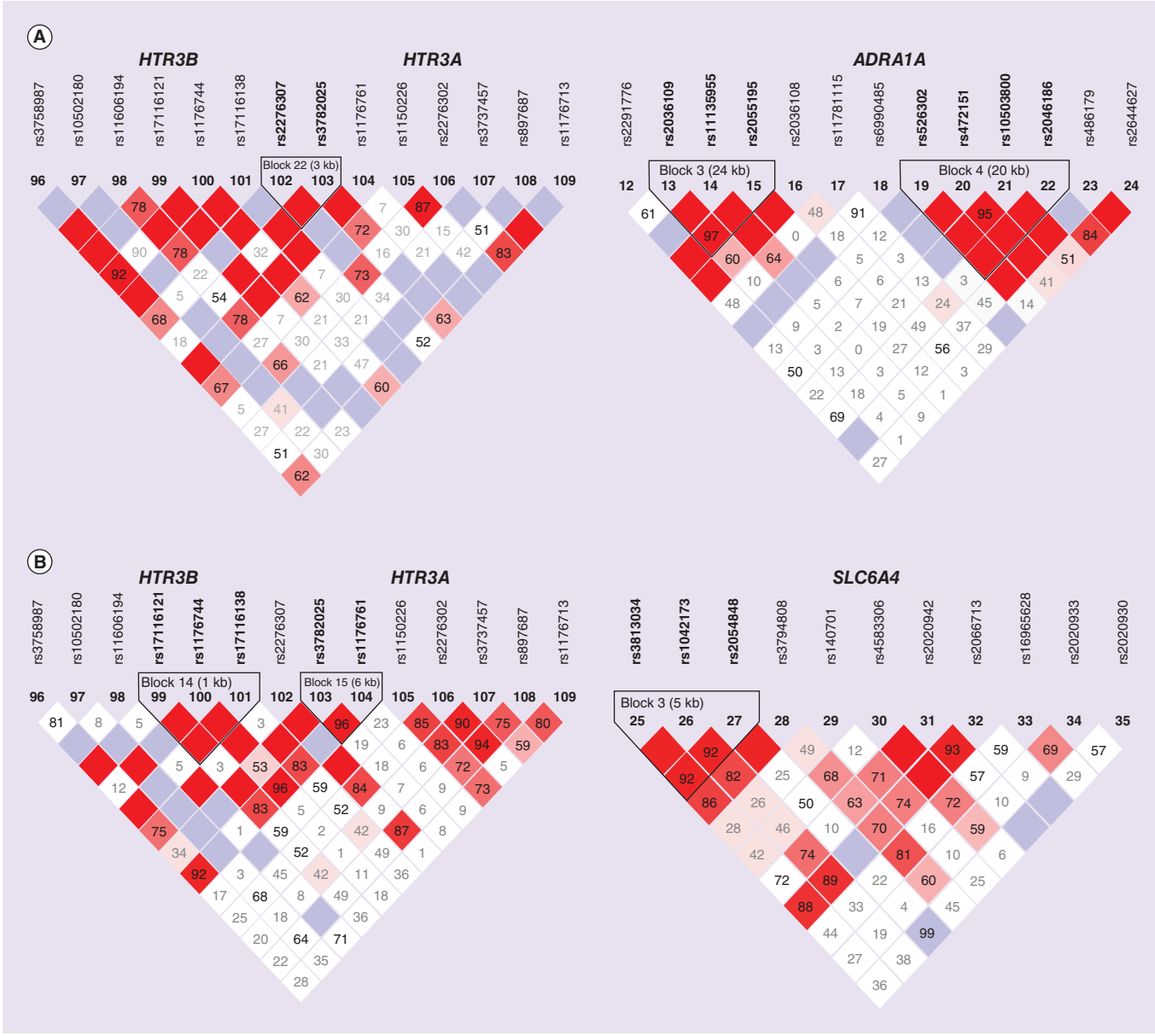

Figure 1. Pairwise linkage disequilibrium analysis. Linkage disequilibrium between SNPs was derived from genotypes of controls. The pairwise correlation between SNPs was measured as D′ and is shown (x100) in each box. The color scheme indicates the magnitude of D′. Dark red indicated D′ greater than 0.80, with D′ .1.0 when no number is given. Haplotypes were generated using the Gabriel rule and haplotype blocks are marked. **(A)** EA/ME. **(B)** AA.
AA: African–American; EA/ME: European/Middle Eastern.
For color figures, please see online at www.futuremedicine.com/doi/full/10.2217/PGS.15.86

after correction for multiple testing. Several SNPs were previously associated with related phenotypes including *HTR3B* rs11606194 with time to relapse after quitting smoking and with nicotine dependence [36,37], *HTR1B* rs6296 with substance abuse disorder and behavioral problems (e.g., [38–40]), *HTR2A* rs6561333 with CD [41], and *HTR3B* rs1176744 with alcohol and nicotine dependence [14,16,37]. Few SNPs may have or are in LD with SNP with potential functionality, including the synonymous *HTR1B* rs6296 that is in LD with SNP rs13212041 and promoter SNPs that were shown to affect gene expression [42,43], the nonsynonymous *HTR3B* rs1176744 (Tyr129Ser) that was shown to augmented signaling, *in vitro* [44], and the synonymous *HTR3A* rs1176713. Among SNPs in LD, the most likely explanatory SNPs were *HTR3A* rs897687 and *HTR3B* rs11606194.

Serotonin transporters

Polymorphisms in two transporter genes and an adaptor protein gene were indicated in this study: *SLC6A4*

Table 5. *HTR3A* 3-SNP genotype.

Genotype group	rs1150226 Upstream	rs897687 Intron	rs1176713 Leu497=	AA					EA			
				Controls		OD + CD			Controls		OD	
				N	F	N	F		N	F	N	F
Reference	CC	AA	AA	21	0.10	80	0.14		115	0.50	380	0.46
Risk[†]	CC	AA	AG/GG	36	0.17	145	0.25	↑	84	0.36	322	0.39
Protection[‡]	CT	AG	AA	101	0.49	223	0.38	↓	26	0.11	97	0.12
Mixed[§]	CT	AG	AG	49	0.24	143	0.24		6	0.03	28	0.03
Incomplete				0		3			1		1	
Total				207		594			232		829	

AA: $\chi^2 = 9.48$, df = 3, p = 0.024; EA: $\chi^2 = 1.26$, df = 3, p = 0.74.
[†]This group includes two 3-SNP genotypes in which there is at least one of the minor G allele for SNP rs1176713.
[‡]This group includes two major 3-SNP genotypes and six rare genotypes in which there is at least one T allele for SNPs rs1150226 or one G allele for rs897687.
[§]This group includes one major 3-SNP genotype and ten rare genotypes in which there is at least one of the minor allele for either of the three SNPs.
AA: African–American; EA: European ancestry; F: Frequency; N: Number.

(5-HTT, SERT), *SLC18A2* (VMAT2), and S100A10 (p11). Serotonin transporters regulates serotonin availability in the synaptic cleft through reuptake. Cocaine is a potent 5-HT reuptake inhibitor. Most of the published studies of behavioral traits and drug addiction concentrated on the upstream *SLC6A4* polymorphism (5-HTTLPR), and some of them found an association with cocaine or opiate addiction [4,16,45–47]. This polymorphism was not included in this study. Vesicular monoamine transporter 2 (VMAT2) is responsible for the uptake of cytosolic monoamines into synaptic vesicles that release their contents in response to an action potential. The addictive properties of cocaine and other psychostimulants have been attributed, in part, to their interaction with VMAT2, and VMAT2 was suggested as a target in cocaine addiction treatment [48]. Upregulation of platelet VMAT2 was observed in former heroin addicts in methadone treatment compared with controls [49]. *VMAT2* KO heterozygote ($^{+/-}$) mice showed increased sensitivity to the psychomotor effects of stimulants [50].

S100A10 (p11) is an adaptor protein for receptors targeted to the plasma membrane that was shown to interact with several serotonin receptors and to modulate depressive-like behaviors [51]. p11 expression is reduced in the NAc of subjects with major depression. Chronic cocaine administration to mice was shown to reduce p11 expression in the NAc, and p11 KO mice exhibit an increased sensitivity to the rewarding effects of cocaine [52]. To the best of our knowledge, the association of *S100A10* SNPs with CD in AA is the first report of association of *S100A10* SNPs with any phenotype [53–55].

Among the SNPs indicated, several SNPs were previously associated with related phenotypes including *SLC6A4* rs2066713 with nicotine dependence in AA [14] and with psychiatric conditions [56], as well as *SLC18A2* rs363276 with post-traumatic stress disorder (PTSD) in EA and AA [57]. Few SNPs have known or potential functional consequence including *SLC6A4* rs16965628 and rs2020933 that were reported to be correlated with allelic transcript ratio imbalance [58]. Among SNPs in LD, the most likely explanatory SNPs were *SLC6A4* rs2066713 and *SLC18A2* rs363276.

Serotonin synthesizing enzymes

TPH is the rate-limiting enzyme in the biosynthesis of 5-HT. TPH2 encodes for the main 5-HT-synthesizing enzyme in the brain, whereas TPH1 is mainly expressed in the pineal gland and the periphery. *TPH2* alternative splicing and RNA editing were found in postmortem brains [59]. Among the SNPs indicated, the upstream *TPH2* rs4570625 that is located in the putative regulatory region has known functional consequence. The T allele showed greater activity in the amygdala compared with the G allele in MRI studies [60,61]. Several SNPs were previously associated with related phenotypes including *TPH2* rs4570625 with smoking status, neuropsychiatric disorders, higher reward dependence and personality traits (e.g., [61–65]), *TPH2* rs6582078, with Beck's hopelessness scale [66] and *TPH1* rs1799913 with OD in Hispanics [22].

Adrenergic pathway

Of the six adrenergic pathway genes included in this study, polymorphism in the genes encoding subtypes of the α 1 and β 2 receptors, as well as the transporter *SLC6A2* (NET1), showed nominal association with at least one addiction in at least one ancestry group. Several SNPs were previously associated with related phenotypes including *ADRA1A* rs486179, with OD in AA in our previous study [23], and *SLC6A2* SNPs with stronger response to D-methamphetamine [15].

SLC6A2 SNPs rs36020 and rs36029 that were associated with alcoholism [67], and the loss-of-function

ADRA2B indel rs28365031 polymorphism which was shown to increase the availability of noradrenaline [68], were not included in the current study.

Many incidents of relapse in cocaine addicts are stress-related, and understanding the neurobiological processes through which stress contributes to drug abuse is important for the development of effective medications for relapse prevention. Central noradrenergic signaling has been implicated in stress-induced relapse and medications, such as alpha-2 adrenoceptor agonist clonidine, that suppress stress-induced increases in noradrenergic transmission that may be used to prevent relapse in cocaine addiction [8,69]. Polymorphisms in related genes may also affect the response to treatment, as was shown for *ADRA1A* SNP rs1048101 T allele that enhanced treatment response to disulfiram in cocaine and opioid codependent subjects [70].

Cocaine versus heroin addiction

Predisposition to addiction may be due to polymorphisms common to all addictions or specific to an addiction to a particular drug. The unified theory of addiction assumes the presence of shared psychological processes and biological mechanisms, like decrease in dopamine D2 receptors [71,72]. However, despite the similarities, cocaine and heroin addiction differ in their underlying neurobiology [73,74]. Although adrenergic signaling is probably necessary for stress-induced drug seeking across a range of drugs, the adrenergic receptor involved may vary. In this study, we have attempted to analyze heroin and cocaine addiction separately in the AA sample, although this dichotomy is limited, since many subjects were addicted to both drugs. Comparison of the association signals for CD and OD, and the combined case groups, in AA, reveals several genes and SNPs in common that support a shared susceptibility, but also shows drug-specific results.

European versus African–Americans

Different ancestral populations may have distinct and shared genetic risk factors for addiction. The reasons for the distinct factors may include different allele frequencies, LD patterns, modifier genes and/or gene–environment interactions. Since there was no CD EA group in this study, this discussion is limited to the OD ± CD groups. The findings support the presence of both shared and distinct genetic liability for OD ± CD in AA and EA. On a gene level, the four genes indicated in EA were also indicated in AA, however, nine genes were only indicated in AA. On an SNP level, only one SNP (*HTR3B* rs3758987) showed association signal in the two ancestry groups but in the opposite direction (the minor G allele was the risk allele in EA and the protective allele in AA). This SNP is common in both populations but more frequent in African populations (MAF = 0.24 in EA and 0.41 in AA). Notably, there is a difference in the LD pattern of this SNP between EA and AA. While this SNP is in strong LD with the functional nonsynonymous SNP rs1176744 in EA, it is in low LD with this SNP in AA. Seven of the SNPs indicated in AA had low MAF in EA (< 0.1) but there is no explanation for the distinct results of the other SNPs.

Conclusion

This study suggests partial overlap in susceptibility loci in the serotonergic and the adrenergic pathways for heroin and cocaine addiction, in African and European ancestry cohorts. Several SNPs of potential importance were indicated including SNPs with known functionality and SNPs in genes suggested as treatment targets that may affect response to treatment. Identifying SNPs associated with different addictions in different ancestry groups is relevant for population-specific diagnosis and treatment. Further studies are needed to confirm the associations and assess their clinical significance.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- Drug addiction is a chronic disease influenced by environmental, genetic and drug-induced factors.

Aim

- To determine if genetic variants in the serotonergic and adrenergic pathways are associated with heroin and/or cocaine addiction.

Methods

- The study examined 140 polymorphisms in 19 genes in 1855 subjects with predominantly European or African–American ancestries.

Results

- A total of 38 SNPs in 13 genes showed nominal associations, including novel associations in *S100A10* (p11) and *SLC18A2* (VMAT2). The association of *HTR3B* SNP rs11606194 with heroin addiction in the EA subgroup remained significant after correction for multiple testing ($p_{corrected} = 0.04$). Four of the SNPs indicated have functional effects.

Conclusion

- The study suggests partial overlap in genetic susceptibility between populations of different ancestry and between opioid dependence and cocaine dependence.
- The study strengthens our previous studies of opioid dependence in smaller samples, including SNPs in *HTR3A, HTR3B* and *ADRA1A*.

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