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Neuronal calcium sensor-1 and cocaine addiction: A genetic association study in African-Americans and European Americans

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Abstract

Genes involved in drug reward pathways are plausible candidates for susceptibility to substance use disorders. Given the prominent role of dopamine in drug reward, dopamine receptor-interacting proteins (DRIPs) such as the neuronal calcium sensor-1 (NCS-1) protein have been hypothesized to play a role in the pathophysiology of cocaine addiction (CA). In this study, we investigated whether genetic variants in the *NCS-1* gene confer risk to CA. We genotyped 8 SNPs (rs4837479, rs7849345, rs3824544, rs10819611, rs947513, rs2277200, rs7873936 and rs1342043) in our discovery sample (cases $n = 796$, controls $n = 416$) of African descent. Confirmation of associated or trending SNPs (rs7849345, rs10819611, rs1342043) was attempted using a replication sample of African American (AA) ethnicity (cases $n = 335$, controls $n = 336$) and European-American (EA) ancestry (cases $n = 336$, controls $n = 656$). Secondary sex specific analysis was also carried out for each SNP in both AA and EA individuals. Genotyping of the discovery cohort showed significant genotypic ($p = 0.0005$, corrected q -value) as well as allelic ($p = 0.005$, corrected q -value) associations of rs1342043 with CA in AAs; however, this marker could not be confirmed in either the AA or EA replication sample. Combined analysis of all AA samples ($n = 1883$) for rs1342043 showed a significant association with CA (genotypic $p = 0.0001$, allelic $p = 0.002$) with a gender specific effect for males (allelic $p = 0.005$, genotypic $p = 0.0003$). Our data suggest that genetic variants in the *NCS-1* gene contribute to susceptibility of CA in individuals of African descent.

Keywords

Addiction; Cocaine; NCS-1; African Americans; European Americans

1. Introduction

Cocaine addiction (CA) is characterized by compulsive cocaine use despite adverse consequences. The profound loss of behavioral control is the hallmark of CA and

contributes to the high risk of relapse. Despite continuous research efforts to disentangle the exact pathophysiology of CA in the last several decades, the exact mechanisms of cocaine's addicting properties remains unknown and currently there are no FDA approved pharmacological treatments available. Studies have estimated that approximately two thirds of an individual's risk for developing cocaine dependence is heritable [13,14], thus, identifying genetic susceptibility factors for CA may provide important new insights into its pathophysiology and eventually may lead to effective therapies.

Cocaine's pleasurable and addictive effects are thought to be principally mediated via its blockage of dopamine transporters (DAT) which substantially increases the concentration of extracellular dopamine (DA), resulting in elevated stimulation of brain regions involved in reward and reinforcement behavior [16,24]. In line with this is evidence from human genetic, transgenic/knockout mouse, and inbred rodent studies that show that genetic differences in dopaminergic pathways mediate cocaine's addictive or reinforcing properties [10]. The recent identification of a group of DA receptor-interacting proteins (DRIPs) [12,15,17] suggests that the intracellular activity of individual DA receptor subtypes is regulated by the concerted actions of signaling proteins [3] known as neuronal calcium sensors (NCS) [4] encoded by a set of 14 genes in humans [7]. NCS-1 is neuron specific [19] and exhibits an affinity for Ca^{2+} [8]. It inhibits receptor phosphorylation mediated by G protein-coupled receptor kinase 2 (GRK2) in a Ca^{2+} -dependent manner [12]. Chronic cocaine exposure reduces the α -subunits of dopamine D2 receptor (D2R)-coupled Gi/o protein [21], Ca^{2+} influx [Hu, 2004 #155;Hu, 2004 #197] and activity of the adenylate cyclase calcineurin pathway in the nucleus accumbens medium spiny neurons (NAc MSN). Single nucleotide polymorphisms (SNPs) in genes encoding dopamine interacting proteins such as *NCS-1* may alter the rewarding and reinforcing behavior following cocaine abuse. Therefore, *NCS-1* is an interesting candidate gene for addiction risk susceptibility and the current study was designed with following aims (1) to investigate the genetic association between the *NCS-1* gene and cocaine addicted individuals in AA and EA populations (2) to test the hypothesis that *NCS-1* polymorphisms increase the risk for addiction in a sex specific manner.

2. Materials and methods

2.1. Ethnicity of population for the current study

African American (AA) and European American (EA).

2.2. Sample collection

For the AA individuals, DNA was collected during clinical and pharmacological trials of cocaine addicted individuals at University of Pennsylvania Treatment Research Center (TRC $n = 796$, discovery sample) as well as requested from the Rutgers University Cell and DNA Repository ($n = 335$, replication sample). DNA samples from EA cocaine addicted patients ($n = 336$) were requested and obtained from the Rutgers University Cell and DNA Repository. Detailed information on the total number of cases and controls for AA and EA populations is summarized in Table 1.

Subjects who were at least 18 years of age were assessed with the structured clinical interview for DSM disorders and screened for drugs in urine. All patients had a clinical diagnosis of CA as defined by DSM-IV. All psychiatric axis I disorders except alcohol dependence and nicotine dependence were used as exclusion criteria [5]. In addition, participants were excluded if they had a history of a seizure disorder (except cocaine-induced seizures) or a severe medical illness, including a history of AIDS. DNA samples for the control populations were collected from University of Pennsylvania, Thomas Jefferson

University [9] and the National Institute of Mental Health Genetics Initiative (NIMH-GI). Control individuals were screened for history of substance use disorders and other psychiatric illnesses. They were not assessed with a urine drug screen and ethnicity was determined by self-report.

2.3. Ethical clearance

All protocols were approved by the Institutional Review Boards at Thomas Jefferson University and the University of Pennsylvania. All subjects provided written informed consent before DNA sample collection.

2.4. SNP selection and genotyping

The *NCS-1* gene is located on chromosome 9 spanning 65,114 bp (UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly) and 8 exons (Fig. 1A). Seven intronic SNPs (rs4837479, rs7849345, rs3824544, rs10819611, rs947513, rs2277200, rs7873936) and one SNP located in the alternative promoter (rs1342043) were genotyped. These SNPs were selected using the Tagger algorithm as part of the Haploview software based upon HapMap data with a minor allele frequency (MAF) cut-off set at 20% [2]. These SNPs captured 42% of AA (ASW) alleles (Fig. 1B) and only 12% of EA (CEU) alleles (3 SNPs) with r^2 of 0.8 (Fig. 1C). Genotyping of the populations in the current study was divided into two phases. In the first phase of genotyping, all 8 SNPs were genotyped in our AA discovery sample collected from TRC. The second phase of genotyping included genotyping our replication sample of AA and EA populations using only the 3 SNPs (rs7849345, rs10819611, rs1342043) which had shown a trend toward significance in the first phase. SNP genotyping was performed using Applied Biosystems Taqman[®] SNP Genotyping Assays. For quality control purposes, 10% of samples were genotyped in duplicates.

2.5. Statistical analysis

The allelic and genotypic association of SNPs with CA was determined using the Chi-square test in the software package PLINK v1.07 [23]. For each SNP, deviation from Hardy–Weinberg was assessed in the total population and also in cases and controls individually. A sex specific analysis in cases and controls was done separately using Chi-squared tests for allelic and genotypic association. The program QVALUE 1.0 was used [25–27] to correct for multiple testing on all reported p -values. This includes allelic and genotypic p -values for the total population, males and females separately across both ethnicities (experiment-wise correction). A q -value <0.05 was considered statistically significant.

3. Results

Of the 8 intronic SNPs analyzed during phase 1 (discovery) genotyping, all were found to be in HWE except rs4837479 ($p = 0.002$) and rs947513 which deviated in controls ($p = 6.39E-12$), in the AA population. Phase 1 genotyping included AA population (TRC) consisting of 796 cases and 416 controls. Single marker Chi-squared analysis for the SNP rs1342043 in AA (TRC population) showed a highly significant allelic ($p = 0.005$, OR = 0.78) as well as genotypic association ($p = 0.0005$) and both of these tests withstood correction for multiple testing (q -value < 0.05). The MAF of the C allele in controls for rs1342043 (40% controls vs 34% in cases) was found to be higher and therefore may act as a protective allele against CA (Table 2A). When comparing genotypic and allelic distributions between cocaine-addicted and control samples, none of the other SNPs remained statistically significant after correction for multiple testing (Table 2A). Furthermore, we carried out sex specific analyses on the phase 1 genotyping. rs1342043 showed significant genotypic association ($p = 0.005$) in males. The MAF of C allele for rs1342043 was higher in controls (40%) as compared to cases (35%) thereby conferring protection for CA in males (Table

2B). Also, the SNP rs7849345 showed significant association at the allelic ($p = 0.002$) as well as the genotypic level ($p = 0.005$) in females (Table 2B). The MAF of the T allele (rs7849345) was higher in controls (49%) when compared to cases (38%), thus conferring protection against CA. The associations of rs1342043 in males and rs7849345 in females withstood correction for multiple testing ($p < 0.05$).

Next, we performed phase 2 (replication) genotyping of the SNPs rs7849345, rs10819611 and rs1342043 which showed a trend towards significance in phase 1 genotyping using an independent replication sample, consisting of AA cocaine addicted individuals and healthy controls (samples obtained from Rutgers University Cell and DNA Repository as described in Section 2) and an independent sample of cases and controls of EA ancestry. None of these SNPs showed any statistical significance in the replication samples that survived multiple testing (Table 3A and B); however, combined analysis of the discovery and replication sample showed that rs1342043 was associated on the genotypic ($p = 0.0001$) as well as on the allelic level ($p = 0.002$), which withstood correction for multiple testing ($p < 0.05$) (Table 3C). The MAF of the rs1342043 C allele was higher in controls (40% vs 36%) than in cases, therefore potentially providing protection against CA in the total AA population. Sex specific combined analysis revealed an allelic association of rs7849345 in AA females ($p = 0.006$) whereas rs1342043 showed a significant allelic ($p = 0.002$) and genotypic association in AA males ($p = 0.0003$, data not shown).

4. Discussion

In this study we investigated whether polymorphisms in the *NCS-1* gene confer risk to CA using a discovery sample of AA subjects and replication samples of individuals of both AA and EA ancestry. While we found a strong association of rs1342043 with CA in AA individuals in the discovery sample, our replication attempt failed to demonstrate a statistically significant association; however, combining AA discovery and replication sample showed a statistically significant effect (Table 3C). Furthermore, our results provide evidence for a gender-specific association between *NCS-1* polymorphisms and CA. The variant rs1342043 was associated with cocaine dependence in AA males (genotypic $p = 0.005$), while the rs7849345 was associated significantly with CA in AA females (genotypic $p = 0.005$, allelic $p = 0.0016$), suggesting a differential influence of these SNPs in males and females. The C allele in rs1342043 in the total population as well as in males and the T allele of rs7849345 in females demonstrated a protective effect against cocaine addiction in the AA populations. No significant associations were observed in EA population.

The dopaminergic brain system is important for drug reward [11], thus making genes involved in these circuits plausible candidates for influencing susceptibility to substance use disorders. In fact, several genes coding for the dopaminergic system have been investigated in CA including genes for the dopamine receptor D2 (*DRD2*) [20], D3 [6], D4 [1] the (*DAT*) [22] and the catechol-O-methyl-transferase (*COMT*) gene [18]. The results of all these studies have been conflicting with some positive reports and some negative findings, possibly due to small sample sizes and the complex genetic nature of CA. In this study, we focused on dopamine receptor-interacting proteins, since they represent a novel target in the investigation of the genetics of CA. Genetic variation in the *NCS-1* gene may alter NCS-1 protein levels and/or function and thus have an effect on the rate of D2 receptor desensitization and internalization, consistent with data in human abstinent cocaine abusers that show decreased D2 receptor availability in the striatum [28]. To our knowledge, this is the first study investigating *NCS-1* as a risk gene for CA. Earlier, Dahl et al. (2006) reported an interesting pharmacogenetic interaction between polymorphisms in two genes encoding components of *DRD2* and *NCS-1* in a smoking cessation trial with nicotine replacement therapy (NRT) [9]. They observed a statistically significant interaction between a common

variant in the *NCS-1* gene (rs1054879) and a functional variant in the DRD2 promoter and treatment outcome. Those individuals with the DRD2-141Ins/Del and the A allele of *NCS-1* rs1054879 showed greater abstinence at end of treatment, strengthening the potential role of dopamine interacting proteins and substance use phenotypes. It is likely that many of our cocaine addicted individuals are co-addicted to nicotine as this was not used as an exclusion criterion for this study. Therefore, our findings may reflect an association with nicotine addiction or addiction in general, an important aspect that should be considered in the interpretation of results.

Despite the significant finding of SNPs in *NCS-1* being associated with CA in the AA population (discovery and combined but not replication sample) there are a number of limitations to this study. One limitation is that population stratification has not been addressed in this study. This is particularly pertinent for the AA population and might explain the failure to confirm the rs1342043 association in the replication sample. Different AA populations may have different degrees of population admixture and unless ancestry informative markers are genotyped in this sample it is not possible to conclusively state that the associations we are observing are due to population stratification or association with CA. The initial evidence of the protective role of the C allele in rs1342043 and the T allele in rs7849345 against CA in the AA population requires genotyping in additional samples in order to confirm this finding. Another limitation is the limited power of our replication sample for both AA and EA due to relatively small sample size. In an attempt to address this limitation, we have carried out a combined analysis that shows an association of rs1342043 with CA in AA. We find no association with CA in our EA population, possibly due to the underpowered sample size but also possibly due to limited SNP coverage in the EA population of only 12%. Since the coverage in the AA population is only 42%, further genotyping is warranted to fully characterize the association of common genetic variation in *NCS-1* with CA.

5. Conclusion

In conclusion, the present study shows that genetic polymorphisms (rs1342043, rs7849345) in *NCS-1* are associated with CA in AA individuals. The current study also highlights gender-specific effects of *NCS-1* polymorphisms and susceptibility to drug dependence. Future studies are necessary to further evaluate the role of *NCS-1* in cocaine dependence.

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References

1. Ballon N, Leroy S, Roy C, Bourdel MC, Olie JP, Charles-Nicolas A, Krebs MO, Poirier MF. Polymorphisms TaqI A of the DRD2, BalI of the DRD3, exon III repeat of the DRD4, and 3'UTR VNTR of the DAT: association with childhood ADHD in male African-Caribbean cocaine dependents? American Journal of Medical Genetics. Part B Neuropsychiatric Genetics. 2007; 144:1034–1041.
2. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

3. Bergson C, Levenson R, Goldman-Rakic PS, Lidow MS. Dopamine receptor-interacting proteins: the Ca(2+) connection in dopamine signaling. *Trends in Pharmacological Sciences*. 2003; 24:486–492. [PubMed: 12967774]
4. Bermak JC, Li M, Bullock C, Zhou QY. Regulation of transport of the dopamine D1 receptor by a new membrane-associated ER protein. *Nature Cell Biology*. 2001; 3:492–498.
5. Berrettini WH, Persico AM. Dopamine D2 receptor gene polymorphisms and vulnerability to substance abuse in African Americans. *Biological Psychiatry*. 1996; 40:144–147. [PubMed: 8793046]
6. Bloch PJ, Nall AH, Weller AE, Ferraro TN, Berrettini WH, Kampman KM, Pettinati HM, Dackis CA, O'Brien CP, Oslin DW, Lohoff FW. Association analysis between polymorphisms in the dopamine D3 receptor (DRD3) gene and cocaine dependence. *Psychiatric Genetics*. 2009; 19:275–276. [PubMed: 19503018]
7. Burgoyne RD. Neuronal calcium sensor proteins: generating diversity in neuronal Ca²⁺ signalling. *Nature Reviews Neuroscience*. 2007; 8:182–193.
8. Cox JA, Durussel I, Comte M, Nef S, Nef P, Lenz SE, Gundelfinger ED. Cation binding and conformational changes in VILIP and NCS-1, two neuron-specific calcium-binding proteins. *The Journal of Biological Chemistry*. 1994; 269:32807–32813. [PubMed: 7806504]
9. Dahl JP, Jepsen C, Levenson R, Wileyto EP, Patterson F, Berrettini WH, Lerman C. Interaction between variation in the D2 dopamine receptor (DRD2) and the neuronal calcium sensor-1 (FREQ) genes in predicting response to nicotine replacement therapy for tobacco dependence. *The Pharmacogenomics Journal*. 2006; 6:194–199. [PubMed: 16402081]
10. Haile CN, Kosten TR, Kosten TA. Genetics of dopamine and its contribution to cocaine addiction. *Behavior Genetics*. 2007; 37:119–145. [PubMed: 17063402]
11. Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. *Annual Review of Neuroscience*. 2006; 29:565–598.
12. Kabbani N, Negyessy L, Lin R, Goldman-Rakic P, Levenson R. Interaction with neuronal calcium sensor NCS-1 mediates desensitization of the D2 dopamine receptor. *Journal of Neuroscience*. 2002; 22:8476–8486. [PubMed: 12351722]
13. Kendler KS, Karkowski LM, Neale MC, Prescott CA. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. *Archives of General Psychiatry*. 2000; 57:261–269. [PubMed: 10711912]
14. Kendler KS, Prescott CA. Cocaine use, abuse and dependence in a population-based sample of female twins. *British Journal of Psychiatry*. 1998; 173:345–350. [PubMed: 9926041]
15. Kim OJ, Ariano MA, Lazzarini RA, Levine MS, Sibley DR. Neurofilament-M interacts with the D1 dopamine receptor to regulate cell surface expression and desensitization. *Journal of Neuroscience*. 2002; 22:5920–5930. [PubMed: 12122054]
16. Koob GF. The neurobiology of addiction: a neuroadaptational view relevant for diagnosis. *Addiction*. 2006; 101(Suppl 1):23–30. [PubMed: 16930158]
17. Li M, Bermak JC, Wang ZW, Zhou QY. Modulation of dopamine D(2) receptor signaling by actin-binding protein (ABP-280). *Molecular Pharmacology*. 2000; 57:446–452. [PubMed: 10692483]
18. Lohoff FW, Weller AE, Bloch PJ, Nall AH, Ferraro TN, Kampman KM, Pettinati HM, Oslin DW, Dackis CA, O'Brien CP, Berrettini WH. Association between the catechol-O-methyltransferase Val158Met polymorphism and cocaine dependence. *Neuropsychopharmacology*. 2008; 33:3078–3084. [PubMed: 18704099]
19. Martone ME, Edelman VM, Ellisman MH, Nef P. Cellular and subcellular distribution of the calcium-binding protein NCS-1 in the central nervous system of the rat. *Cell and Tissue Research*. 1999; 295:395–407. [PubMed: 10022960]
20. Messas G, Meira-Lima I, Turchi M, Franco O, Guindalini C, Castelo A, Laranjeira R, Vallada H. Association study of dopamine D2 and D3 receptor gene polymorphisms with cocaine dependence. *Psychiatric Genetics*. 2005; 15:171–174. [PubMed: 16094250]
21. Nestler EJ, Terwilliger RZ, Walker JR, Sevarino KA, Duman RS. Chronic cocaine treatment decreases levels of the G protein subunits Gi alpha and Go alpha in discrete regions of rat brain. *Journal of Neurochemistry*. 1990; 55:1079–1082. [PubMed: 2117048]

22. Persico AM, Vandenbergh DJ, Smith SS, Uhl GR. Dopamine transporter gene polymorphisms are not associated with polysubstance abuse. *Biological Psychiatry*. 1993; 34:265–267. [PubMed: 8399824]
23. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*. 2007; 81:559–575. [PubMed: 17701901]
24. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science (New York NY)*. 1987; 237:1219–1223.
25. Storey JD. A direct approach to false discovery rates. *Journal of the Royal Statistical Society Series B-Statistical Methodology*. 2002; 64:479–498.
26. Storey JD, Taylor JE, Siegmund D. Strong control, conservative point estimation and simultaneous conservative consistency of false discovery rates: a unified approach. *Journal of the Royal Statistical Society Series B-Statistical Methodology*. 2004; 66:187–205.
27. Storey JD, Tibshirani R. Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:9440–9445. [PubMed: 12883005]
28. Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Gifford A, Hitzemann R, Ding YS, Pappas N. Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. *The American Journal of Psychiatry*. 1999; 156:1440–1443. [PubMed: 10484959]

HIGHLIGHTS

- The neuronal Ca^{2+} sensor-1 (NCS-1) gene encodes a dopamine receptor-interacting protein.
- Dopamine receptor-interacting proteins (DRIPs) represent novel targets for cocaine addiction.
- rs1342043 was associated with cocaine addiction in African Americans.
- Sex specific analysis of cases and controls shows an association of rs1342043 in males and rs7849345 in females.
- Future studies are necessary to elucidate the role of NCS-1 in addiction.

Fig. 1.

(A) Gene structure of *NCS-1* and location of the genotyped SNPs in reference to the *NCS-1* gene (<http://genome.usc.edu>) February 2009 (GRCh37/hg19) assembly. (B) Linkage disequilibrium (LD) between 8 SNPs genotyped in African Americans. (C) LD between 3 SNPs genotyped in European Americans. The shading represents $r^2 \geq 0.8$ and numbers inside the boxes refer to 'D'.

Demographic information of study subjects. African American (AAs) and European American (EAs) ethnic groups were studied for genetic association between variants in the *NCS-1* gene and cocaine addiction.

Table 1

Ethnicity	Cases	Controls	Male cases	Male controls	Female cases	Female controls
AA-TRC (discovery sample)	796	416	591	159	205	257
AA-Rutgers (replication sample)	335	336	161	138	174	198
Total AA	1131	752	752	297	379	455
EA (replication sample)	336	656	169	333	167	323

Table 2

(A) Genotypic and allelic distributions for NCS-1 in cocaine-AA addicted and control samples (discovery sample/phase 1 genotyping). (B) Sex specific analysis of genotypic and allelic distributions for NCS-1 in cocaine-AA addicted and control samples (phase 1 genotyping). Bold highlighted *p*-values remain significant ($p < 0.05$) after correction for multiple testing.

(A)					
Phase 1 SNP genotyping	Genotypic frequency	Genotypic <i>p</i> -values		Allelic <i>p</i> -values	
rs1342043					
Cases (<i>n</i> = 781)	0.10 (CC, <i>n</i> = 84)	0.47 (CT, <i>n</i> = 374)	0.41 (TT, <i>n</i> = 323)	0.0005	0.34 (C)
Controls (<i>n</i> = 408)	0.18 (CC, <i>n</i> = 77)	0.43 (CT, <i>n</i> = 176)	0.38 (TT, <i>n</i> = 155)		0.40 (C)
rs4837479					
Cases (<i>n</i> = 740)	0.14 (CC, <i>n</i> = 106)	0.47 (CT, <i>n</i> = 350)	0.38 (TT, <i>n</i> = 284)	0.035	0.38 (C)
Controls (<i>n</i> = 393)	0.17 (CC, <i>n</i> = 69)	0.40 (CT, <i>n</i> = 155)	0.43 (TT, <i>n</i> = 169)		0.37 (C)
rs7849345					
Cases (<i>n</i> = 804)	0.17 (TT, <i>n</i> = 139)	0.51 (TC, <i>n</i> = 415)	0.31 (CC, <i>n</i> = 250)	0.08	0.43 (T)
Controls (<i>n</i> = 410)	0.21 (TT, <i>n</i> = 87)	0.52 (TC, <i>n</i> = 217)	0.25 (CC, <i>n</i> = 106)		0.47 (T)
rs3824544					
Cases (<i>n</i> = 743)	0.24 (TT, <i>n</i> = 183)	0.50 (TC, <i>n</i> = 375)	0.24 (CC, <i>n</i> = 185)	0.31	0.43 (T)
Controls (<i>n</i> = 357)	0.24 (TT, <i>n</i> = 89)	0.46 (TC, <i>n</i> = 165)	0.28 (CC, <i>n</i> = 103)		0.47 (T)
rs10819611					
Cases (<i>n</i> = 805)	0.08 (TT, <i>n</i> = 65)	0.42 (TC, <i>n</i> = 346)	0.48 (CC, <i>n</i> = 394)	0.09	0.29 (T)
Controls (<i>n</i> = 405)	0.11 (TT, <i>n</i> = 47)	0.38 (TC, <i>n</i> = 157)	0.49 (CC, <i>n</i> = 201)		0.31 (T)
rs947513					
Cases (<i>n</i> = 710)	0.26 (GG, <i>n</i> = 188)	0.36 (GA, <i>n</i> = 262)	0.36 (AA, <i>n</i> = 260)	0.28	0.45 (T)
Controls (<i>n</i> = 390)	0.30 (GG, <i>n</i> = 117)	0.32 (GA, <i>n</i> = 127)	0.37 (AA, <i>n</i> = 146)		0.46 (T)
rs2277200					
Cases (<i>n</i> = 827)	0.19 (GG, <i>n</i> = 162)	0.49 (GT, <i>n</i> = 412)	0.30 (TT, <i>n</i> = 253)	0.58	0.44 (G)
Controls (<i>n</i> = 412)	0.22 (GG, <i>n</i> = 91)	0.48 (GT, <i>n</i> = 200)	0.29 (TT, <i>n</i> = 121)		0.46 (G)
rs7873936					
Cases (<i>n</i> = 651)	0.10 (AA, <i>n</i> = 70)	0.39 (AG, <i>n</i> = 256)	0.49 (GG, <i>n</i> = 325)	0.97	0.31 (A)
Controls (<i>n</i> = 372)	0.11 (AA, <i>n</i> = 41)	0.39 (AG, <i>n</i> = 39)	0.49 (GG, <i>n</i> = 49)		0.32 (A)

(B)

Phase 1 SNP genotyping	Genotypic frequency	Genotypic <i>p</i> -values	MAF	Allelic <i>p</i> -values		
rs1342043						
Cases (males <i>n</i> = 529)	0.11 (CC, <i>n</i> = 59)	0.48 (CT, <i>n</i> = 258)	0.40 (TT, <i>n</i> = 212)	0.005	0.35 (C)	0.16
Controls (males <i>n</i> = 157)	0.20 (CC, <i>n</i> = 32)	0.38 (CT, <i>n</i> = 61)	0.40 (TT, <i>n</i> = 64)		0.40 (C)	
rs7849345						
Cases (females <i>n</i> = 188)	0.14 (CC, <i>n</i> = 28)	0.46 (CT, <i>n</i> = 89)	0.37 (TT, <i>n</i> = 71)	0.005	0.38 (T)	0.001
Controls (females <i>n</i> = 253)	0.22 (CC, <i>n</i> = 58)	0.52 (CT, <i>n</i> = 133)	0.24 (TT, <i>n</i> = 62)		0.5 (T)	

MAF = minor allele frequency.

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Table 3

(A) Genotypic and allelic distributions for *NCS-1* in cocaine-AA addicted and control samples (replication sample/phase 2 genotyping). (B) Genotypic and allelic distributions for *NCS-1* in cocaine-EA addicted and control samples from the Rutgers repository (replication sample/phase 2 genotyping). (C) Combined analysis of genotypic and allelic distributions for *NCS-1* in cocaine-AA addicted and control samples (phase 1 plus phase 2 genotyping). Bold highlighted *p*-values remain significant ($p < 0.05$) after correction for multiple testing.

(A)		(B)		(C)	
Phase 2 SNP genotyping in AAs		Genotypic frequency		Genotypic <i>p</i> -values	
rs1342043		Genotypic frequency		MAF	
Cases (<i>n</i> = 332)	0.15 (CC, <i>n</i> = 50)	0.46 (CT, <i>n</i> = 154)	0.38 (TT, <i>n</i> = 128)	0.29	0.38 (C)
Controls (<i>n</i> = 288)	0.20 (CC, <i>n</i> = 57)	0.43 (CT, <i>n</i> = 124)	0.37 (TT, <i>n</i> = 1107)		0.41 (C)
rs7849345		Genotypic frequency		MAF	
Cases (<i>n</i> = 332)	0.18 (TT, <i>n</i> = 61)	0.52 (TC, <i>n</i> = 173)	0.29 (CC, <i>n</i> = 98)	0.96	0.44 (T)
Controls (<i>n</i> = 333)	0.19 (TT, <i>n</i> = 64)	0.51 (TC, <i>n</i> = 172)	0.29 (CC, <i>n</i> = 97)		0.45 (T)
rs10819611		Genotypic frequency		MAF	
Cases (<i>n</i> = 232)	0.14 (TT, <i>n</i> = 34)	0.62 (TC, <i>n</i> = 146)	0.22 (CC, <i>n</i> = 52)	0.95	0.32 (T)
Controls (<i>n</i> = 236)	0.13 (TT, <i>n</i> = 32)	0.20 (TC, <i>n</i> = 49)	0.65 (CC, <i>n</i> = 155)		0.31 (T)
(B)		(C)		(D)	
Phase 2 SNP genotyping in E/A		Genotypic frequency		Genotypic <i>p</i> -values	
rs1342043		Genotypic frequency		MAF	
Cases (<i>n</i> = 284)	0.0 (CC, <i>n</i> = 0)	0.31 (CT, <i>n</i> = 83)	0.70 (TT, <i>n</i> = 201)	0.01	0.14 (C)
Controls (<i>n</i> = 630)	0.02 (CC, <i>n</i> = 16)	0.29 (CT, <i>n</i> = 187)	0.67 (TT, <i>n</i> = 427)		0.17 (C)
rs7849345		Genotypic frequency		MAF	
Cases (<i>n</i> = 329)	0.23 (TT, <i>n</i> = 78)	0.46 (TC, <i>n</i> = 152)	0.30 (CC, <i>n</i> = 99)	0.96	0.46 (T)
Controls (<i>n</i> = 653)	0.24 (TT, <i>n</i> = 159)	0.46 (TC, <i>n</i> = 302)	0.29 (CC, <i>n</i> = 192)		0.47 (T)
rs10819611		Genotypic frequency		MAF	
Cases (<i>n</i> = 329)	0.08 (TT, <i>n</i> = 26)	0.47 (TC, <i>n</i> = 156)	0.44 (CC, <i>n</i> = 147)	0.33	0.31 (T)
Controls (<i>n</i> = 650)	0.1 (TT, <i>n</i> = 64)	0.42 (TC, <i>n</i> = 279)	0.47 (CC, <i>n</i> = 307)		0.31 (T)
(C)		(D)		(E)	
Phase 1 and phase 2 SNP genotyping in AAs		Genotypic frequency		Genotypic <i>p</i> -values	
rs1342043		Genotypic frequency		MAF	

(C)

Phase 1 and phase 2 SNP genotyping in AAs	Genotypic frequency		Genotypic <i>p</i> -values		MAF	Allelic <i>p</i> -values
Cases (<i>n</i> = 1111)	0.12 (CC, <i>n</i> = 134)	0.47 (CT, <i>n</i> = 528)	0.40 (TT, <i>n</i> = 449)	0.0001	0.35 (C)	0.002
Controls (<i>n</i> = 696)	0.19 (CC, <i>n</i> = 134)	0.43 (CT, <i>n</i> = 300)	0.37 (TT, <i>n</i> = 262)		0.40 (C)	