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The association between *SYT1*-rs2251214 and cocaine use disorder further supports its role in psychiatry

Bruna S. da Silva[a,b,1], Renata B. Cupertino[a,b,1], Jaqueline B. Schuch[c,d], Djenifer B. Kappel[a,b], Breno Sanvicente-Vieira[e], Cibele E. Bandeira[a,b], Lisia von Diemen[d], Felix H.P. Kessler[d], Eugenio H. Grevet[b,f], Rodrigo Grassi-Oliveira[e], Claiton H.D. Bau[a,b], Diego L. Rovaris[b,f,*]

[a] Department of Genetics, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[b] ADHD Outpatient Program, Adult Division, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
[c] Laboratory of Immunosenescence, Graduate Program in Biomedical Gerontology, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil
[d] Center for Drug and Alcohol Research, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[e] Developmental Cognitive Neuroscience Lab, Biomedical Research Institute, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil,
[f] Department of Psychiatry, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

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ABSTRACT

Synaptotagmin-1 is an essential regulator of synaptic vesicle exocytosis, and its encoding gene (*SYT1*) is a genome and transcriptome-wide association hit in cognitive performance, personality and cocaine use disorder (CUD) studies. Additionally, in candidate gene studies the specific variant rs2251214 has been associated with attention-deficit/hyperactivity disorder (ADHD), antisocial personality disorder and other externalizing phenotypes in adults with ADHD, as well as with response to methylphenidate (MPH) treatment. In this context, we sought to evaluate, in an independent sample, the association of this variant with CUD, a phenotype that shares common biological underpinnings with the previously associated traits. We tested the association between *SYT1*-rs2251214 and CUD susceptibility and severity (addiction severity index) in a sample composed by 315 patients addicted to smoked cocaine and 769 non-addicted volunteers. *SYT1*-rs2251214 was significantly associated with susceptibility to CUD, where the G allele presented increased risk for the disorder in the genetic models tested ($P = 0.0021$, OR = 1.44, allelic; $P = 0.0012$, OR = 1.48, additive; $P = 0.0127$, OR = 1.41, dominant). This is the same allele that was associated with increased risk for ADHD and other externalizing behaviors, as well as poor response to MPH treatment in previous studies. These findings suggest that the neurotransmitter exocytosis pathway might play a critical role in the liability for psychiatric disorders, especially externalizing behaviors and CUD.

1. Introduction

The influence of exocytosis-related variants, including those on Synaptotagmin-1 (SytI) encoding gene (*SYT1*), has been investigated in psychiatric disorders since they play a critical role in neurotransmitter release (for review see Cupertino et al., 2016). *SYT1* gene has been linked to cocaine use disorder (CUD) in a candidate pathway association study (Fernàndez-Castillo et al., 2012), as well as in a recent genome and transcriptome-wide-association study, where it presented a nominal association with CUD in a gene-based analysis and a significant association in a differential expression analysis in postmortem brain tissue (Huggett and Stallings, 2019). A particular SNP on *SYT1* gene - rs2251214 - was associated with adulthood attention-deficit/hyperactivity disorder (ADHD) in two independent clinical samples (Cupertino et al., 2017; Sánchez-Mora et al., 2013), as well as with antisocial personality disorder (ASPD) and other externalizing phenotypes in patients with ADHD (Cupertino et al., 2017). This SNP was also associated with methylphenidate (MPH) treatment response in adults with ADHD, being involved with both symptom response and treatment persistence (da Silva et al., 2018). Therefore, this variant previously implicated in stimulant treatment response is a candidate to be associated with CUD.

SytI acts as a Ca^{2+} sensor to induce the fusion of presynaptic vesicles with the plasma membrane through soluble N-ethylmalemide-

* Corresponding author at: Faculdade de Medicina, Campus Saúde, Rua Ramiro Barcelos, 2400 2° andar, Porto Alegre, RS CEP: 90035003, Brazil.
E-mail address: drovaris@hcpa.edu.br (D.L. Rovaris).
[1] These authors contributed equally to this work.

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sensitive factor attachment protein receptors (SNARE) complex binding, being an important regulator of synaptic vesicle exocytosis (Südhof, 2013). This cascade of events culminates in the release of stored neurotransmitters into the synaptic cleft (Tucker and Chapman, 2002; Wu and Schulten, 2014), and the proper regulation of this process is crucial for the modulation of behavioral responses, including craving for cocaine (Adinoff, 2004; Nestler, 2005). Studies using *in vivo* and *in vitro* models suggest that synaptotagmins could exert a role in stimulants actions. The infusion of low doses of MPH was able to modulate *SYT1* mRNA levels in PC12 cells (Bartl et al., 2010) and treatment with cocaine led to increased *SYT4* mRNA levels in the striatum of rats (Courtin et al., 2006; Denovan-Wright et al., 1998). Moreover, intraperitoneal injection of 3,4-methylenedioxymethamphetamine in adult mice induced increases in SytI and SytIV protein levels in different brain regions (Peng et al., 2002). These alterations of exocytosis regulatory proteins expression induced by stimulant drugs could modulate the efficacy of synaptic transmission and consequently affect functions related to the reward system.

The main mechanism of action of both cocaine and MPH involves the inhibition of the reuptake of neurotransmitters into presynaptic neurons. These stimulants blockade dopamine, norepinephrine and serotonin transporters (DAT, NET and SERT, respectively), leading to increased levels of these neurotransmitters in the synaptic cleft (Han and Gu, 2006; Hannestad et al., 2010). An alternative mechanism has also been proposed to explain cocaine actions (see Heal et al., 2014). Cocaine would act as an inverse agonist of DAT, resulting in reversion of the dopamine transport into the synaptic cleft. This hypothesis is in accordance with the fact that cocaine and MPH are weak dopamine uptake inhibitors and seem to have dopamine releasing effects, differently from other competitive reuptake inhibitors (Heal et al., 2014).

Therefore, since cocaine seems to present a complementary exocytosis-related mechanism of action and can modulate expression of critical elements of neurotransmitter release, it is plausible that *SYT1*-rs2251214 might also influence the susceptibility and severity to CUD. Considering the overall evidence, including an intriguing set of findings involving *SYT1*-rs2251214 and externalizing behaviors (Cupertino et al., 2017; Sánchez-Mora et al., 2013) and MPH treatment response (da Silva et al., 2018), we sought in an independent sample to extend the association to CUD, a phenotype that shares common biological underpinnings with the previously associated traits (Gurriarán et al., 2018).

2. Material and methods

2.1. Sample

The sample was composed by crack cocaine addicted patients ($N = 315$) who voluntarily sought specialized treatment in hospital addiction units from the metropolitan region of Porto Alegre, Southern Brazil, and by healthy non-addicted volunteers ($N = 769$) recruited at a blood donation center (Hospital de Clínicas de Porto Alegre) and at a community from the same city. All individuals self-reported themselves as of European descent, a measure that is significantly correlated with genomic estimates of interethnic admixture in Latin populations (Ruiz-Linares et al., 2014). Moreover, in the specific region where the sample was collected, the population presents nearby 90% of European ancestry (Ruiz-Linares et al., 2014). This project was carried out in accordance with the Declaration of Helsinki. The procedures had been clarified to all subjects, who then signed an informed consent approved by the Research Ethics Committees of the participating institutions.

2.2. Diagnosis

Diagnosis of CUD followed the diagnostic and statistical manual of mental disorders, 4th edition (DSM-IV; APA, 1994) criteria, confirmed through the structured clinical interview for DSM-IV axis I disorders (SCID-I; First et al., 2002) or Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). Information on the potential problem areas (severity) within the sample of crack cocaine addicted patients was assessed through the sixth version of addiction severity index (ASI-6; Cacciola et al., 2011; Kessler et al., 2012; McLellan et al., 2006). ASI-6 is a semi-structured interview that besides gathering information about substance use problems, it provides composite scores for the severity of problems in other life domains that potentially contribute for negative outcomes (e.g., legal, education/employment, medical, etc.). The higher the scores computed, the greater the severity of the problems the interviewee has for that domain. The severity of general psychiatric symptoms and drug use were evaluated in this study. The calculation of the composite score for the Psychiatry domain combines information about different manifestations (e.g., suicidality, delusions, hallucinations), overall frequency of such symptoms, impairment caused by those symptoms, distress caused and need for treatment specifically for those symptoms. The calculation of composite scores for the Drugs domain is based on data related to drugs use, including information on frequency of use and time since last use, problems due to drug use, how often these problems occur, how much trouble these problems cause and how much treatment is needed. Healthy non-addicted participants screened negatively for crack and/or cocaine use through the SCID-I/P screening module (First et al., 2002) or the alcohol, smoking, and substance involvement screening test (ASSIST; Henrique et al., 2004; WHO ASSIST Working Group, 2003). The exclusion criteria for both cases and controls were presence of schizophrenia and other psychotic disorders and/or severe cognitive deficit that would impair the capacity of answering the instruments.

2.3. Polymorphism selection and genotyping

The *SYT1*-rs2251214 (Chr12:79430071) SNP was selected based on its previous association with ADHD, ASPD and other externalizing phenotypes within ADHD subjects, as well as with MPH treatment response (Cupertino et al., 2017; da Silva et al., 2018; Sánchez-Mora et al., 2013). It was genotyped using a Taqman allelic discrimination assay, according to the manufacturer's instructions (Step One Plus, Applied Biosystems, Foster City, CA, USA).

2.4. Statistical analysis

The influence of *SYT1*-rs2251214 on the susceptibility of CUD was evaluated by logistic regression analysis, while its effects on the quantitative measures of the problem areas of ASI-6 (i.e. drugs and psychiatric scales) were evaluated through linear regression. Different genetic models (allelic, additive, and dominant) were tested using PLINK software v1.07 (Purcell et al., 2007). Sex and age were included as covariates in all analyses, except for the allelic model that runs under the command –assoc at PLINK, and this is not compatible with adjustment for covariates. The significance level was set at 0.05.

2.5. Public databases analyses

HaploReg v4.1, RegulomeDB, and the SNP Function Prediction of the SNPinfo were used to evaluate the possible role of rs2251214 in regulatory mechanisms. HaploReg is a tool that examines annotations of the noncoding genome at disease-associated loci by genome-wide association studies (GWASes) (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php Ward and Kellis, 2011). RegulomeDB annotates SNPs with known and predicted regulatory elements in the intergenic regions of the human genome (http://www.regulomedb.org/ Boyle et al., 2012). The SNPinfo web server is a set of web-based tools to predict functional characteristics of both coding and noncoding SNPs (https://snpinfo.niehs.nih.gov/ Xu and Taylor, 2009).

Table 1
Sample characteristics and genotypic frequencies.

	Cases (n = 315)	n	Controls (*n* = 769)	n
	Mean (SD)		*Mean (SD)*	
Age (years)	30.9 (8.0)	315	29.2 (8.6)	769
Drugs[a]	53.3 (6.0)	283	–	
Psychiatric[a]	49.7 (8.5)	283	–	
Age of first cocaine use	18.5 (5.7)	259	–	
Years of regular cocaine use	5.8 (6.2)	259	–	
Age of first crack use	23.0 (8.3)	279	–	
Years of regular crack use	5.6 (5.0)	277	–	
	n (%)		*n (%)*	
Gender (male)	165 (52.4)	315	435 (56.6)	769
Tobacco use disorder[b]	241 (83.1)	290	69 (9.0)	763
Alcohol use disorder[c]	85 (31.3)	272	5 (0.7)	747
Major depressive disorder[c]	57 (20.8)	274	172 (22.9)	750
Bipolar disorder[c]	97 (35.8)	271	30 (4.0)	748
Anxiety disorders[c,d]	105 (38.2)	275	117 (15.6)	750
SYT1-rs2251214		315		769
GG	204 (64.7)		439 (57.1)	
AG	106 (33.7)		283 (36.8)	
AA	5 (1.6)		47 (6.1)	

[a] Subscales of the sixth version of addiction severity index (ASI-6).
[b] Current information.
[c] Lifetime information.
[d] Anxiety disorders include generalized anxiety disorder, obsessive-compulsive disorder, panic disorder and agoraphobia.

3. Results

Clinical characteristics of the sample and genotype frequencies are presented in Table 1. No deviation from the Hardy-Weinberg Equilibrium was found ($P = 0.483$, $\chi^2 = 0.492$).

3.1. Genetic association analyses

SYT1-rs2251214 was significantly associated with susceptibility to CUD in our sample, where the G allele presented increased risk for the disorder in all genetic models tested ($P = 0.0021$, OR = 1.44, allelic; $P = 0.0012$, OR = 1.48, additive; $P = 0.0127$, OR = 1.41, dominant). However, *SYT1*-rs2251214 does not seem to influence the severity of CUD, since no association was observed for ASI-6 severity subscales (drugs and psychiatric status) or for age of first use of either smoked or snorted cocaine (Table 2).

3.2. Public databases analyses

Additional analyses in public databases were performed to predict the potential functionality of *SYT1*-rs2251214 and refine our results. RegulomeDB showed minimal evidence of transcription factors binding on this SNP (score of 6). The SNP Function Prediction of the SNPinfo web server did not reveal any involvement of *SYT1*-rs2251214 in splicing regulatory mechanisms. Furthermore, HaploReg did not show differences in chromatin state nor the presence of histone modifications in the brain tissues evaluated, suggesting that this SNP is not involved in gene expression regulatory mechanisms. Besides, *SYT1*-rs2251214 was not in high LD (R^2 and D' > 0.8) with other variants in the European population.

4. Discussion

In this study, we observed that *SYT1*-rs2251214 G allele is associated with increased risk for CUD. This result extends preceding findings implicating this SNP on externalizing phenotypes and stimulant responses to a different albeit neurobiologically related phenotype in an independent sample. The previous studies reported that the same allele was associated with increased risk for ADHD, and its externalizing-related phenotypes (Cupertino et al., 2017; Sánchez-Mora et al., 2013), as well as for poor treatment response to MPH (da Silva et al., 2018). These results point towards an idea that although both MPH and cocaine target DAT, other mechanisms possibly involving the release of neurotransmitters are major factors to be considered in the actions of these stimulants.

In line with the hypothesis that exocytosis-related genes could be involved in the mechanisms underlying the susceptibility for CUD, a previous candidate pathway association study sought to evaluate SNPs covering 16 genes involved in the release of neurotransmitters (Fernàndez-Castillo et al., 2012). They found that polymorphisms in *SYT1* and *SYT2* genes were nominally associated to CUD severity and susceptibility (Fernàndez-Castillo et al., 2012). This study also reported a significant association for the *NSF* (N-ethylmaleimide sensitive fusion protein) gene, which encodes a protein involved in the recycling of SNARE complex. Such gene was also associated with CUD in a subsequent study (Cabana-Domínguez et al., 2016). In a recent genome and transcriptome-wide-association study, *SYT1* gene presented a nominal association with CUD in a gene-based analysis and a significant association in a differential expression analysis in the human hippocampus (Huggett and Stallings, 2019). Additionally, *SYT1* gene is a hit in genome-wide association studies (Supplementary Fig. 1) since it was associated in gene-based analyses with neuroticism (Luciano et al., 2018; Nagel et al., 2018a,b) and its domains of irritability (Nagel et al., 2018b) and fed-up feelings (Nagel et al., 2018a,b), educational attainment (Lee et al., 2018) and cognitive performance (Lee et al., 2018).

The fact that *SYT1*-rs2251214 was previously associated with different outcomes of MPH treatment (da Silva et al., 2018), and in the present study with CUD susceptibility, but not severity, suggests that in CUD these effects might not involve a genetic modulation of a pharmacodynamic effect, as observed for MPH (da Silva et al., 2018), but

Table 2
Association between *SYT1*-rs2251214 and cocaine use disorder.

	Allelic model			Additive model[a]			Dominant model[a]		
	OR	*CI95%*	*P-value*	*OR*	*CI95%*	*P-value*	*OR*	*CI95%*	*P-value*
Case control status	1.44	1.14–1.82	0.0021	1.48	1.17–1.88	0.0012	1.41	1.08–1.85	0.0127
	beta	*SE*	*P-value*	*beta*	*SE*	*P-value*	*beta*	*SE*	*P-value*
Drugs[b]	−0.03	0.08	0.6987	−0.03	0.08	0.6952	−0.03	0.08	0.6957
Psychiatric[b]	−0.09	0.08	0.2770	−0.09	0.08	0.2746	−0.12	0.09	0.1802
Age of first cocaine use	0.01	0.08	0.8904	0.01	0.07	0.8524	0.03	0.08	0.7001
Years of cocaine use	0.06	0.07	0.3755	0.07	0.07	0.3359	0.06	0.07	0.4170
Age of first crack use	0.03	0.08	0.7126	0.04	0.06	0.5263	0.02	0.06	0.7234
Years of crack use	0.09	0.08	0.2806	0.09	0.08	0.2478	0.09	0.08	0.2783

Effect allele = G.
OR = odds ratio. CI = confidence interval. SE = standard error.
[a] Adjusted for age and sex.
[b] Subscales of the sixth version of addiction severity index (ASI-6). *P*-value calculated using linear regression analysis.

rather an influence on susceptibility solely. It is important to mention that the interpretation of the findings in patients with CUD may reflect the fact that the chronic and heavy use of smoked cocaine, which is usual among addicted individuals, could potentially alter the functioning of neurotransmission systems (for example through the down-regulation of dopamine receptors) at a point that the subtler effects of genetic variability on CUD severity might become undetectable.

In addition, the overwhelming recent evidence towards the shared heritability in psychiatric disorders (Brainstorm Consortium et al., 2018; Gurriarán et al., 2018) indicates that *SYT1* may indeed represent a relevant biological factor underpinning all the previously associated phenotypes, including CUD. We suggest that *SYT1*-rs2251214 effects in CUD would emerge similarly to what was reported to a range of externalizing (Cupertino et al., 2017) and internalizing (Luciano et al., 2018; Nagel et al., 2018a) behaviors. Conversely, a mediating relationship between *SYT1*-rs2251214 and other phenotypes such as conduct disorders, ADHD, mood and anxiety disorders could be influencing CUD susceptibility indirectly, since patients with CUD present high rates of psychiatric comorbidities (Daigre et al., 2013; Narvaez et al., 2014; Saunders et al., 2015).

This hypothesis-driven study presents some limitations. The first is the fact that we focused on a single SNP previously associated with externalizing phenotypes and stimulant treatment response in our target population. However, other variants of *SYT1* gene, that are not in LD with rs2251214 (Supplementary Table 1 and Supplementary Fig. 2), recently achieved GWAS significance level in cognitive performance and personality studies in non-Latin American populations. Therefore, the inclusion of our sample in future GWAS will extend the analysis to additional variants and genes related to the neurotransmitter exocytosis pathway to confirm their involvement in CUD. This hypothesis is relevant considering the previous evidence from in vitro/in vivo models and association studies involving psychiatric phenotypes and genes related to this pathway. Second, it is not possible to infer putative mechanisms underlying the *SYT1*-rs2251214 association since this intronic SNP has no described functionality yet, and it might not have a functional effect by itself, but instead is in linkage disequilibrium with other not-studied functional SNP. Third, we should not discard the possibility that other psychiatric comorbidities might be playing a role in the association observed. Finally, the fourth limitation relates to the lack of genome-wide data to estimate principal components for more precise control for population stratification, especially considering the significant but moderate correlation between self-perception and genetically determined ancestry in Latin America. However, this possible bias is minimized by the fact that, among Latin-Americans, individuals that self-identified as of European descent presented a trend for higher European ancestry ($\approx$ 90%), as opposed to other groups (Ruiz-Linares et al., 2014).

5. Conclusion

Our results showing an association between *SYT1*-rs2251214 and CUD, combined with previous evidence of the influence of neurotransmitter exocytosis-related genes on psychiatric disorders and stimulants use/abuse, highlight the importance of such pathway in this context. Although these results still need replication, they indicate that additional studies should further explore the effects of genes involved in neurotransmitter exocytosis pathway in other psychiatric phenotypes.

Contributors

BSS and RBC prepared the first draft of the manuscript. BSS, RBC, JBS and DLR participated in the design of the study and in the statistical analysis. BSS, RBC, DBK and CEB worked on the laboratorial techniques, such as blood extraction and genotyping. BSV, LVD, FHP and RGO were involved with the recruitment of patients and the management of the database for analysis. EHG, RGO, CHDB and DLR reviewed all drafts of the manuscript and actively contributed to the writing of the final version. All authors have read and approved the final version of this article.

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Ethical statement

This project was carried out following the Declaration of Helsinki, and all subjects signed an informed consent form that was previously approved by the Research Ethics Committees of the participating institutions.

Declaration of interest

The author(s) declare the following potential conflict of interest with respect to the research, authorship and/or publication of the present article: Dr. EHG was on the speaker's bureau for Novartis and Shire for the last 3 years. He also received travel awards (air tickets and hotel accommodations) for participating in two psychiatric meetings from Shire and Novartis. All other authors report no financial interests or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2019.109642.

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