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Short communication

Evaluation of previous substance dependence genome-wide significant findings in a Spanish sample

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ABSTRACT

Background: Substance dependence is a chronic and relapsing disorder explained by genetic and environmental risk factors. The aim of our study is to replicate previous genome-wide significant (GWS) hits identified in substance dependence in general or in cocaine dependence in particular using an independent sample from Spain.

Methods: We evaluated, in a Spanish sample of 1711 subjects with substance dependence (1011 of them cocaine dependent) and 1719 control individuals, three SNPs identified as GWS in previous studies: rs1868152 and rs2952621 (located near *LINC02052* and *LINC01854,* respectively), associated with substance dependence, and rs2629540 (in the first intron of *FAM53B*), associated with cocaine dependence.

Results: We replicated the association between rs2952621 and substance dependence under the dominant model ($P = 0.020$), with the risk allele (T) being the same in our sample and in those two reported previously. We then performed a meta-analysis of the two samples used in the original study that reported the association of rs2952621 with substance dependence (Collaborative Studies on Genetics of Alcoholism (COGA) and Study of Addiction: Genetics and Environment (SAGE)) together with our Spanish sample. The meta-analysis of 3747 cases and 4043 controls confirmed the association (OR = 1.26, 95% CI = 1.15–1.39).

Conclusions: The rs2952621 variant, located downstream from the yet uncharacterized gene *LINC01854,* is associated with substance dependence in our Spanish sample. Further research is needed to understand its contribution to the susceptibility to substance dependence.

1. Introduction

Substance dependence is a complex psychiatric disorder characterized by loss of control in drug intake, craving, and withdrawal (Koob and Volkow, 2010). The heritability estimates of substance dependence range from 39% to 72% depending on the drug of abuse (Goldman et al., 2005),

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although other factors such as clinical assessment, ethnicity, or age may also have an impact on these scores. Despite the fact that the genetic contribution to the disorder is substantial, the specific underlying genetic risk factors remain largely unknown. Candidate gene association studies assessing susceptibility to dependence to drugs of abuse have focused mainly on genes involved in the neurotransmitter systems of the brain, but only a few consistent associations have been found. These include the genes *CNR1*, *CHRNA5* and *DRD2* (reviewed by Bühler et al., 2015). On the other hand, Genome-Wide Association Studies (GWAS) have highlighted new candidate genes such as *LINC01854*, *ARHGAP28* (Wetherill et al., 2014), or *KAT2 B* (Johnson et al., 2015) for substance dependence and *FAM53 B* (Gelernter et al., 2014) for cocaine dependence. However, most GWAS have been performed for alcohol dependence (Tawa et al., 2016). These GWAS have highlighted some risk genes that had already emerged through candidate gene studies, like some members of the *ADH* family and *ALDH2*, encoding alcohol and aldehyde dehydrogenases, respectively (Samochowiec et al., 2014). Others have emerged that were not *a priori* candidates for the disorder, pointing at new functions that may be related to its etiology.

Replication of previous GWS findings in independent samples is key to validating association signals, especially when they have been identified in studies with limited sample sizes, as is the case for most studies performed in substance dependence so far. The aim of the present study is to replicate, in a Spanish sample, several GWS findings reported in previous GWAS of substance dependence in general and dependence to cocaine performed in samples with European ancestry (Gelernter et al., 2014; McGue et al., 2013; Wetherill et al., 2014). Our analyses include meta-analytical approaches and functional annotation of replicated findings.

2. Materials and methods

2.1. Samples and DNA isolation

The case sample consists of 1711 substance dependent patients, including 1011 cocaine-dependent subjects (Supplementary Table S1 and Supplementary Fig. 1). All patients were diagnosed under DSM-IV-TR criteria (Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Text revision) and the SCID (Structured Clinical Interview) (First et al., 1997) at the Addiction and Dual Diagnosis Unit of the Psychiatry Department of Hospital Universitari Vall d'Hebron (Barcelona, Spain). They were included in the study if they met the criteria for dependence for at least one drug of abuse (Supplementary Table S1 and Supplementary Fig. 1). The control sample consisted of 1719 individuals (Supplementary Table S1) recruited at the Blood and Tissues Bank of Barcelona. All cases and controls were unrelated, Spanish, Caucasian, and sex-matched. The study was approved by the ethics committee of our institution according to the Helsinki Declaration. Population stratification of our sample was discarded in a previous study of our group (Fernàndez-Castillo et al., 2013) by genotyping 48 unlinked anonymous SNPs located at least 100 kb from any known gene and analyzing the results using the STRUCTURE software (Pritchard et al., 2000), the Fst coefficient (Goudet, 1995), and the Pritchard and Rosenberg method (Pritchard and Rosenberg, 1999).

Genomic DNA was isolated from peripheral blood lymphocytes using the salting-out method (Miller et al., 1988). DNA concentration of all samples was measured on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Termo Fisher Scientific Inc., Wilmington, DE, USA).

2.2. SNP selection and genotyping

We selected SNPs that showed a GWS association ($P < 5e-08$) with substance dependence (dependence to at least one drug of abuse) in samples with European ancestry (see Supplementary Table S2 for details). Two SNPs were identified under these criteria and were selected for genotyping: rs1868152, located 5′ from *LINC02052* (McGue et al., 2013), and rs2952621, located 3′ from *LINC01854* (Wetherill et al., 2014). Both were associated with substance dependence. We also considered a third SNP that was GWS for cocaine dependence in a meta-analysis of samples of African- and European-American individuals: rs2629540, within the first intron of the *FAM53B* gene (Gelernter et al., 2014). This previous study included two additional SNPs, rs2000085570 and rs2456778, showing GWS associations in some of the individual samples but not in the meta-analysis, and they were not considered in our replication study. Genotyping of all SNPs was performed using KASP technology (LGC genomics, UK). Replicates of some samples and negative controls were included.

2.3. Statistical analyses

The statistical power of our samples was calculated *post hoc* using the Genetic Power Calculator software (http://csg.sph.umich.edu/abecasis/cats/) (Purcell et al., 2003). We calculated the power under the additive, dominant, and recessive models using a level of significance of 0.05, odds ratio of 1.2, a disease prevalence of 0.026 (Compton et al., 2007), and the minor allele frequency (MAF) calculated in our control sample (MAF = 0.1 for rs1868152, MAF = 0.42 for rs2952621 and MAF = 0.27 for rs2629540).

Departure from Hardy-Weinberg equilibrium (HWE) ($P < 0.05$) was tested for all the SNPs separately in controls and cases using the SNPassoc package of the R library (González et al., 2007). The case-control association test was carried out using the same package under four different genetic models: additive, dominant, recessive and codominant. All results were adjusted by age, as significant differences in age were detected between cases and controls using the non-parametric test U Mann-Whitney with SPSS22 (SPSS Inc., Chicago, IL, USA). Bonferroni correction threshold for multiple testing was set at $P < 4.16e-03$ (0.05/(3 SNPs*4 genetic models)).

Finally, for rs2629540, we also performed a symptom count analysis similar to the one reported in the original study (Gelernter et al., 2014) by including the severity of the consumption of opioid, alcohol, and nicotine as covariates.

2.4. Meta-analysis

Meta-analysis for rs2952621 (3747 cases and 4043 controls) was performed with the meta R package (www.cran.r-project.org/web/packages/rmeta/index.html) for three different substance dependence samples: the Collaborative Studies on Genetics of Alcoholism (COGA) (genotype data from Wetherill et al., 2014), the Study of Addiction: Genetics and Environment (SAGE) (data obtained through dbGAP application, accessions phs000092.v1.p1.c1 and c2, under project 15342), and our Spanish sample. The samples gathered by COGA and SAGE have been widely used in substance dependence GWAS either as discovery or as replication samples. The COGA sample included 824 cases and 935 controls. For the SAGE sample, which consisted of 1224 cases and 1390 controls, we used European-American individuals and excluded related individuals and subjects also present in the COGA sample from the analysis. We considered the dominant model, which displayed the best *p*-value in the Spanish sample. The Mantel-Haenszel test (fixed effects model) was applied, as the samples did not show heterogeneity.

2.5. Functional annotation

We searched for other variants in linkage disequilibrium (LD) with rs2952621 using the SNAP tool (http://archive.broadinstitute.org/mpg/snap/ldsearch.php) (Johnson et al., 2008), considering $r^2 \geq 0.8$ and the genotype data from the Utah Residents (CEPH) with Northern and Western European Ancestry (CEU) population of 1000 Genomes Pilot 1. The predicted functional effect of all these variants (38 in total) was evaluated using the SNP Function Prediction software (https://

Table 1
Replication study of three genome-wide significant associations in a sample of Caucasian Spanish subjects with substance dependence and controls.

						Genotypes									
		N				Cases (%)			Controls (%)			p-value			
SNP	Phenotype	Cases	Controls	Alleles	MAF	11	12	22	11	12	22	Codominant	Dominant	Recessive	Additive
rs1868152	SD	1698	1707	C > T	0.1	79.6	19.3	1.1	79.6	19.4	1.0	0.8039	0.7338	0.6270	0.8401
rs2952621	SD	1699	1718	C > T	0.42	31.3	50.7	18.0	35.0	46.7	18.3	**0.0488**	**0.0196**	0.9675	0.1109
rs2629540	CD	1011	1144	C > G	0.27	53.7	38.7	7.6	53.9	38.4	7.7	0.6347	0.4154	0.8258	0.5837

Bold values: significant *p*-values; MAF: Minor Allele Frequency; SD: Substance Dependence; CD: Cocaine Dependence.

snpinfo.niehs.nih.gov/snpinfo/snpfunc.html) in the CEU population. These SNPs were tested as potential quantitative trait loci (eQTLs) using GTEx Analysis, Release V6p (GTEx Consortium et al., 2013). Histone modification data for H3K27ac, H3K9ac, H3K9me1 or H3K9me3 of 10 different brain regions (hippocampus middle, substantia nigra, anterior caudate, cingulate gyrus, inferior temporal lobe, angular gyrus, dorsolateral prefrontal cortex, germinal matrix, and male and female fetal brain) related to enhancer or promoter regions were explored using the Haploreg v4.1 tool (Ward and Kellis, 2016).

3. Results

We assessed three previous genome-wide significant findings identified in GWAS of substance dependence in general or cocaine dependence in particular (rs1868152, rs2952621, and rs2629540, see Supplementary Table S2) in a Spanish Caucasian sample of substance dependence (59% of the subjects being cocaine-dependent) and the corresponding controls (Supplementary Table S1 and Supplementary Fig. 1).

The statistical power of our sample, assuming an OR of 1.2, was 6–68% (rs1868152), 40–97% (rs2952621), and 12–79% (rs2629540), depending on the model of inheritance considered. All three SNPs were in Hardy-Weinberg Equilibrium (HWE) in controls and cases.

We identified an association between rs2952621 and substance dependence under the dominant model (best *p*-value, $P = 0.020$) and the codominant model ($P = 0.049$), neither of which overcame the Bonferroni correction for multiple testing (Table 1). These nominal associations showed the same direction as in the original study (Wetherill et al., 2014), with the T allele as the risk variant. As the association was described under the additive model in the original study, we inspected the genotypes of COGA (discovery) and SAGE (replication) samples and observed that the associations were also positive for the dominant model ($P = 3.3e\text{-}04$ and $P = 9.9e\text{-}03$, respectively) and the codominant model ($P = 1.0e\text{-}05$ and $P = 0.035$, respectively). Then, we performed the meta-analysis of the COGA, SAGE, and Spanish samples (3747 cases and 4043 controls in total) under the dominant model, which yielded the best association results in most samples. The association was confirmed in the meta-analysis (OR = 1.26, 95% CI = 1.15–1.39). All three datasets showed the same direction of the effect, and T was the risk allele (Fig. 1). We did not observe significant associations for rs1868152 or rs2629540 under any of the tested genetic models (Table 1).

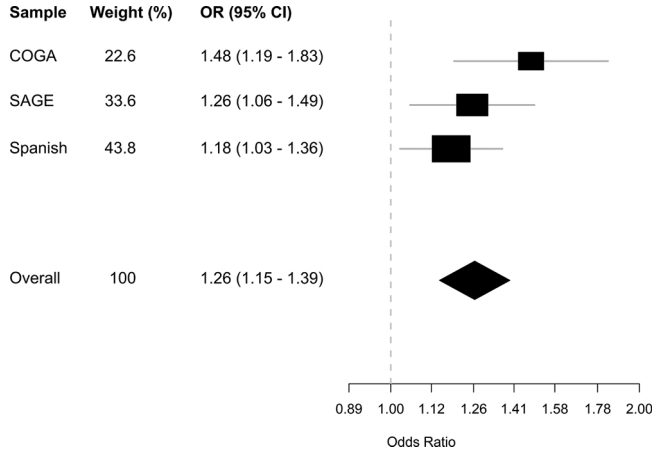

Fig. 1. Meta-analysis of rs2952621. Forest plot of the ORs (95% CI) of marker rs2952621 for our Spanish sample and the COGA and SAGE datasets.

We then assessed the possible functional effect of the rs2952621 variant using bioinformatics tools. This SNP is in high LD ($r^2 \geq 0.8$) with 37 SNPs (Supplementary Table S3). Our SNP of interest, and those located closest to it, overlap with multiple histone marks classically related with enhancer regions in different brain areas (Supplementary Table S3). On the other side, although rs2952621 is not an eQTL and is not predicted to have any functional effect, eight SNPs out of those 37 that are in LD with it are located in predicted binding sites for transcription factors, and two of them overlap with splice sites within the *LINC01854* gene. Finally, 11 out of these 37 SNPs are eQTLs for the pseudogene *FAR2P1* (located 790 kb distal from rs1251179) in the nerve-tibial tissue.

4. Discussion

The aim of this study is to follow-up previous GWAS results on substance dependence in general or on cocaine dependence through a replication study in a Spanish sample. One of the three GWS signals, the rs2952621 SNP associated with substance dependence (Wetherill et al., 2014), was replicated in our study. We did not replicate the associations reported for rs1868152 with substance dependence or rs2629540 with cocaine dependence.

The original study (Wetherill et al., 2014) identified an association between rs2952621 and substance dependence ($P = 1.77e\text{-}08$) in a discovery sample (COGA, Collaborative Study on the Genetics of Alcoholism) of around 800 cases and 900 controls. The authors replicated these findings in a second sample of 2647 individuals (SAGE, Study of Addiction: Genetics and Environment) with the T allele as the risk variant in both groups (Wetherill et al., 2014). Our study replicates this finding in the same direction in a Spanish Caucasian sample ($P = 0.020$, dominant model). Finally, the meta-analysis of the three samples showed an effect for the risk allele T with an OR = 1.26 (95% CI = 1.15–1.39) (Fig. 1).

The rs2952621 SNP is located 1.3 Kb downstream from a yet uncharacterized gene, *LINC01854*, identified in two previous studies (Ota et al., 2004; Strausberg et al., 2002). It is expressed in most tissues, including the brain. Unfortunately, no further information is available about the function of this gene to understand its possible role in substance dependence. Rs2952621 seems to be located in an enhancer region, but it is not described as an eQTL and does not have a predicted functional impact. Several SNPs are in high LD with it and were explored for potential functional involvement. Among them, rs1251179 seems to be the most interesting one: the degree of LD with rs2952621 is high ($r^2 = 0.97$ in the CEU population), it is predicted to be located in a transcription factor binding site, and it is described as an eQTL in the

nerve-tibial tissue for the pseudogene *AC018865.9* (located 790 kb distal from rs1251179). Further research is needed to clarify whether it could have a role in the susceptibility to substance dependence.

The lack of replication of the previously associated SNPs rs1868152 and rs2629540 could be explained by phenotypic heterogeneity of the substance dependence samples used in the different studies or by lack of statistical power due to limited sample size and/or modest effect size of the variants. In the case of rs2629540, the reported association (Gelernter et al., 2014) reached GWS in a *meta*-analysis of European-American and African-American individuals (P = 4.3e-08). However, when the two ethnic groups were analyzed separately, the association with rs2629540 was only nominal, with the African sample performing much better (P = 1.4e-06) than the European one (P = 2.6e-03) with comparable sample sizes. The lack of replication in our sample, which is also of European origin, would make sense in this context.

5. Conclusions

We replicated the reported association between substance dependence and the variant rs2952621, located close to the uncharacterized gene *LINC01854*. A meta-analysis of our sample and the discovery and replication samples of the original study confirmed the association and the direction of the effect. This risk variant for substance dependence (T allele) may contribute to the susceptibility to this chronic relapsing disorder, although further studies are required to understand the molecular basis of this contribution.

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Contributors

LP-C performed the association study and the statistical analysis; LP-C and JC-D performed the meta-analysis; LG-L, CR, ACA, NM-L, MR-M, JAR-Q and MC participated in the recruitment of patients and clinical assessment and coordinated the clinical research; LP-C, JC-D, CS-M and NF-C isolated genomic DNA from samples; LP-C, BC and NF-C designed the study; MR contributed to the genetic analysis. LP-C prepared the first draft of the manuscript and all figures and tables; BC and NF-C coordinated the study and supervised the manuscript preparation. All authors contributed to and approved the final version of the manuscript.

Conflict of interest

JAR-Q was on the speakers' bureau and/or acted as a consultant for Eli-Lilly, Janssen-Cilag, Novartis, Shire, Lundbeck, Almirall, Braingaze, Sincrolab, and Rubió in the last 5 years. He also received travel awards for taking part in psychiatric meetings from Janssen-Cilag, Rubió, Shire, and Eli- Lilly. The Department of Psychiatry chaired by him received unrestricted educational and research support from the following pharmaceutical companies in the last 5 years: Eli-Lilly, Lundbeck, Janssen-Cilag, Actelion, Shire, Ferrer, and Rubió. The rest of the authors declare no conflicts of interest or relevant financial interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.drugalcdep.2018.03.013.

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