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Genetic basis of psychopathological dimensions shared between schizophrenia and bipolar disorder

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ABSTRACT

Shared genetic vulnerability between schizophrenia (SCZ) and bipolar disorder (BP) was demonstrated, but the genetic underpinnings of specific symptom domains are unclear. This study investigated which genes and gene sets may modulate specific psychopathological domains and if genome-wide significant loci previously associated with SCZ or BP may play a role.

Genome-wide data were available in patients with SCZ (n = 226) or BP (n = 228). Phenotypes under investigation were depressive and positive symptoms severity, suicidal ideation, onset age and substance use disorder comorbidity. Genome-wide analyses were performed at gene and gene set level, while 148 genome-wide significant loci previously associated with SCZ and/or BP were investigated. Each sample was analyzed separately then a meta-analysis was performed.

SH3GL2 and CLVS1 genes were associated with suicidal ideation in SCZ (p = 5.62e-08 and 0.01, respectively), the former also in the meta-analysis (p = .01). SHC4 gene was associated with depressive symptoms severity in BP (p = .003). A gene set involved in cellular differentiation (GO:0048661) was associated with substance disorder comorbidity in the meta-analysis (p = .03). Individual loci previously associated with SCZ or BP did not modulate the phenotypes of interest.

This study provided confirmatory and new findings. SH3GL2 (endophilin A1) showed a role in suicidal ideation that may be due to its relevance to the glutamate system. SHC4 regulates BDNF-induced MAPK activation and was previously associated with depression. CLVS1 is involved in lysosome maturation and was for the first time associated with a psychiatric trait. GO:0048661 may mediate the risk of substance disorder through an effect on neurodevelopment/neuroplasticity.

1. Introduction

Schizophrenia (SCZ) and bipolar disorder (BP) are major psychiatric diseases associated with substantial morbidity and mortality as well as personal and societal costs (Chong et al., 2016; Ferrari et al., 2016). The heritability of both these disorders is very high: 81% for SCZ (Sullivan et al., 2003) and 85% for BP (McGuffin et al., 2003). Multiple lines of evidence indicate shared neurobiological alterations and genetic

vulnerability across SCZ spectrum and psychotic BP (Cardno and Owen, 2014; Clementz et al., 2016; Forstner et al., 2017; Ivleva et al., 2008; Schulze et al., 2014). The Psychiatric Genomics Consortium (PGC) es- timated a 68% genetic correlation between BP and SCZ using genome-wide SNPs (single nucleotide polymorphisms). However, the genes in- volved in this etiological overlap remain largely unknown. These findings have thus blurred the boundaries of classic nosography and established the importance of investigating psychopathological

dimensions that cut across different psychiatric diseases (Lee et al., 2013).

Despite the genetic variants shared between SCZ and BP are largely unknown, PGC identified 108 and 30 independent loci showing genome-wide association with SCZ and BP, respectively, thanks to large case-control samples (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Stahl et al., 2017). These loci were asso- ciated with broad diagnosis, but the specific psychopathological di- mensions they may modulate are unknown. PGC also found 10 in- dependent loci underlying both diseases at a genome-wide significance level, but few information is available on the specific loci or genes in- volved (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Ruderfer et al., 2014). Symptom overlap between SCZ and BP occurs in several areas: positive, negative, manic and depressive symptoms (Pearlson, 2015; Peitl et al., 2017). Few studies examined the shared genetic factors that may be involved, suggesting a significant overlap between a BP polygenic risk score and the clinical dimension of mania in SCZ (Ruderfer et al., 2014) and a higher burden of SCZ risk alleles in psychotic BP (Allardyce et al., 2018; Leonenko et al., 2018). Other clinical features are relevant because they are associated with poorer outcome, though not specific of SCZ and BP solely, and they include suicidal ideation (Chesney et al., 2014), substance use disorder comorbidity (Messer et al., 2017; Thoma and Daum, 2013; Vandaele and Janak, 2017) and age at disease onset (Immonen et al., 2017; Joslyn et al., 2016).

Single loci identified by adequately powered genome-wide asso- ciation studies (GWAS) are of undoubted value, but SNPs do no act as single units, they interact among each other, within the same gene and across different genes. Gene and gene-set analyses are statistical methods for analyzing multiple genetic markers simultaneously to de- termine their joint effect. These methods provide higher power than single-variant analysis, because they study the aggregated effect of variants in genes or pathways and they reduce the number of performed tests (~20,000 genes are known in the human genome, while tens of million SNPs) (de Leeuw et al., 2015). Gene-set analysis can also pro- vide insights into the functional and biological mechanisms underlying the pathogenesis of a trait.

Given the still largely unknown genetic factors involved, the present study aimed to investigate the genetic basis of psychopathological dimensions shared between SCZ and BP. We analyzed single variants using a candidate approach that included genome-wide significant loci associated with SCZ and/or BP by the PGC (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Ruderfer et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Stahl et al., 2017) since the strong evidence supporting a role of these loci but their unclear contribution in modulating specific psychopathological domains. In addition, we studied the role of genes and gene-sets (pathways and functional categories) with a genome-wide approach, but particular attention was directed toward genes and gene-sets including the cited genome-wide significant loci.

2. Materials and methods

2.1. Samples

For all samples ethical approval was obtained from local research ethics committees. The clinical-demographic characteristics of the samples are described in Table 1.

2.1.1. SCZ Sample

Patients were recruited at two sites in Italy: 111 patients in Bologna ("Maggiore" Hospital, SCZ sample I) and 115 patients in Rome ("San Filippo Neri" Hospital or psychiatric inpatient facility (RSA) "San Raffaele Villa dei Fiori", SCZ sample II), for a total of 226 patients.

For the former cohort, patients were enrolled at the moment of admittance to the Psychiatric inpatient Unit. Inclusion criteria were age

from 18 to 75 and a diagnosis of schizophrenia according to the DSM-IV-TR criteria confirmed using the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). Patients were included if they needed to start or to change antipsychotic treatment because of an acute psychotic relapse. Exclusion criteria were severe/unstable medical conditions, cognitive impairment that would interfere with the ability to participate in the study, pregnancy or breast-feeding. Clinical and demographic characteristics of patients were assessed at inclusion in the study, psychotic symptoms and depressive symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS) (Peralta and Cuesta, 1994) and the Hamilton Depression Rating Scale 21 items (HAMD-21) (Hamilton, 1980), respectively.

For the second cohort, included subjects were inpatients with a diagnosis of schizophrenia (DSM-IV-TR criteria) who were recruited between 2011 and 2012. All patients ranged between 45 and 55 years of age and were of Italian origin. Subject were included if they gave informed consent, had sufficient Italian language skills to complete the study measures and were not considered at risk of injurious behaviors toward themselves and others. Clinical-demographic characteristics were collected at inclusion in the study, psychotic symptoms were assessed using the PANSS (Peralta and Cuesta, 1994).

2.1.2. BP Sample

Patients were recruited at one site in Italy and one site in Spain: 79 patients in Bologna ("Maggiore" Hospital, BP sample I) and 149 patients in Barcelona (Hospital Clinic of Barcelona, BP sample II), for a total of 228 patients.

For the former cohort, the same inclusion and exclusion criteria described for the SCZ

Bologna sample were applied, except for diag- nosis (patients had a diagnosis of BP type I or II according to DSM-IV-TR criteria and confirmed using the MINI) (Sheehan et al., 1998) and treatment (patients needed to start or change mood stabilizing treat- ment because of an acute phase of disease). The PANSS and HAMD-21 scales were used to assess psychotic and depressive symptoms, re- spectively.

For the Spanish cohort, out-patients were enrolled in a naturalistic cohort study, consecutively admitted to the out-patient Bipolar Disorders Unit. Inclusion criteria were a diagnosis of Bipolar Disorder (type I or II) according to DSM-IV-TR criteria and age of 18 years or older. The current and lifetime diagnoses of psychiatric disorders were formulated by independent senior psychiatrists (diagnostic con- cordance: Kappa = 0.80) according to DSM-IV-TR clinical criteria and confirmed through the semi-structured interviews for Axis I disorders according to DSM-IV-TR criteria (SCID I) (First et al., 2002). Clinical- demographic characteristics were collected at inclusion and depressive symptoms were measured using the HAMD-17 scale.

2.2. Phenotypes

Five psychopathological dimensions or disease severity indicators were considered (Table 1): depressive and psychotic positive symptoms, suicidal ideation, age at disease onset and substance use disorder.

2.2.1. Depressive symptoms severity

In the SCZ sample and BP Barcelona sample, severity of depressive symptoms was evaluated using the following items of the HAMD-17 scale (Hamilton, 1980): 'depressed mood' (HAMD-1), 'feelings of guilt' (HAMD-2), 'work and interest' (HAMD-7), 'retardation' (HAMD-8), 'anxiety-psychic' (HAMD-10) and 'somatic symptoms-general' (HAMD-13) items. These constitute a selection of scale items with the highest internal, interrater and retest reliability, measuring the core set of de- pressive symptoms (Bagby et al., 2004; Bech et al., 1975). In the Italian BP sample a comparable phenotype was calculated using the sum of the following PANSS items (Peralta and Cuesta, 1994): 'anxiety' (G2), 'de- pression' (G6), 'motor retardation' (G7) items and three independent items collected in the sample – 'excessive self-reproach', 'loss of pleasure', 'loss of energy/tiredness', each recorded as continuous vari- ables (0–3 points). The resulting measures were standardized to make them comparable among samples.

2.2.2. Positive symptoms severity

In the SCZ sample and the Italian BP sample the severity of positive symptoms was evaluated using the following PANSS (Peralta and Cuesta, 1994) items: 'delusions' (P1) and 'hallucinatory behaviour' (P3). For the Barcelona BP sample a comparable phenotype was derived using two equivalent independent items – 'delusions' and 'hallucina- tions', both recorded as continuous variables (0–2 points). The resulting measures were standardized to make them comparable among samples.

2.2.3. Suicidal ideation

In the Italian SCZ and BP samples, patients were considered having suicidal ideation if scoring

at least 3 on HAMD-17 (Hamilton, 1980) 'suicide' (HAMD-3) item. In the other samples suicidal ideation was recorded as binary variable.

2.2.4. *Onset age*

Patients were differentiated between either early-onset or not-early- onset, depending on whether disease onset occurred at/before age 20 or later. Early disease onset is a predictor of poorer outcome and the early- onset group has typically been found to have an upper-bound of 18–22 years. We set the cut-off at 20 years consistently with the ma- jority of prior research (Immonen et al., 2017; Joslyn et al., 2016).

2.2.5. *Substance use disorder comorbidity*

Substance use disorder comorbidity was investigated through medical records and in clinical interviews for alcohol, cannabis, hal- lucinogens, opioids and/or stimulants. The phenotype was recorded as binary.

2.3. *Genotyping and imputation*

Patients were genotyped using the Illumina Infinium PsychArray 24 BeadChip (Illumina, Inc., San Diego). Genotypes were imputed using the Haplotype Reference Consortium (HRC version r1.1 2016) panel as reference and Minimac3 (Das et al., 2016).

Pre-imputation quality control was carried out according to the following criteria: 1) variants with missing rate $\geq 5\%$; 2) monomorphic variants; 3) subjects with genotyping rate $<$ 97%; 4) subjects with gender discrepancies; 5) subjects with abnormal heterozygosity; 6) re- lated subjects (identity by descent [IBD]) > 0.1875 (Anderson et al., 2010). Hardy–Weinberg equilibrium (HWE) was not used as an

exclusion criterion, as departures from HWE are expected in a case-only study. However, since violation of HWE may reflect technical artifacts, HWE was tested for a pool of relevant genes. Variants within such genes had HWE $p > .001$, supporting good quality genotyping (Wittke-Thompson et al., 2005).

Post-imputation quality control was performed according to the following criteria: 1) poor imputation quality ($R2 < 0.30$ (Li et al., 2010; Pistis et al., 2015)) and 2) minor allele frequency (MAF) < 0.05.

2.4. *Statistical analysis*

Association analyses were independently conducted in SCZ and BP samples and then a fixed-effects meta-analysis was performed at SNP, gene and gene-set level. All phenotypes were adjusted for age, gender and the first 10 population principal components to correct for popu- lation stratification (Patterson et al., 2006). Age and gender were chosen as covariates in line with the previous literature (Becker and Hu, 2008; Freeman et al., 2017; Grossman et al., 2006; Immonen et al., 2017; Koechl et al., 2012). Age and gender were included as covariates also because they showed an effect on the most part of the investigated phenotypes in our samples (Supplementary Table 1). The distribution of the first 10 principal components revealed good population homo- geneity. In fact, no subject from the pooled samples lied beyond six standard deviations from the mean for each of the first ten principal components (Price et al., 2006; Wang et al., 2009).

2.4.1. *Gene and gene-set analysis*

These analyses were performed in SCZ and BP samples and then a meta-analysis was carried out using MAGMA (de Leeuw et al., 2015).

Gene-sets (pathways and functional categories) were downloaded from the GSEA Broad Institute database version 6 (Liberzon et al., 2015). MAGMA performs both a self-contained and a competitive gene-set analysis, the latter is more conservative since it reflects if genes in a gene set are more associated with the outcome than genes outside that gene set, thus the competitive method was used. The Bonferroni correction was applied in gene analysis and 10,000 permutations were run to calculate empirical *p* values for gene sets.

2.4.2. *Analysis of genome-wide significant loci reported by the PGC schizophrenia, bipolar and cross-disorder working groups*

The variants associated with either or both SCZ and BP (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Stahl et al., 2017; (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Ruderfer et al., 2014) were investigated for association with the phenotypes of interest using linear

or logistic regression models in SCZ and BP samples and then through a fixed-effects meta-analysis (Plink version 1.9) (Purcell et al., 2007). The Bonferroni correction was used to account for multiple testing on the basis of the number of tested SNPs.

Given the interest in the identification of possible genes and gene-sets mediating the effect of these loci on the phenotypes of interest, genes and gene-sets harboring them were extracted from the results of the analysis described in the previous paragraph and reported separately.

3. Results

5,484,300 SNPs / 226 patients in the SCZ sample and 5,475,874 SNPs / 228 patients in the BP sample were available after quality control. The clinical-demographic characteristics of the included patients included in the analyses are reported in Table 1.

3.1. *Gene analysis*

18,761 and 18,818 genes, respectively, were included in the analysis in the SCZ and BP samples. Genes showing nominal $p < .0001$ are reported in Supplementary Table 2, while results that survived multiple-testing correction are summarized in Table 2. Manhattan plots for gene-based not corrected *p* values are available in Supplementary Fig. 1.

In the SCZ sample, SH3GL2 (SH3 Domain Containing GRB2 Like 2) (corrected $p = 5.62e\text{-}08$) and CLVS1 (Clavesin 1) (corrected $p = .01$) were associated with suicidal ideation. No other association survived multiple-testing correction.

In the BP sample, SHC4 (SHC Adaptor Protein 4) (corrected $p = .003$) was associated with depressive symptoms severity. No other association survived Bonferroni correction.

In the meta-analysis of the two samples SH3GL2 was still associated with suicidal ideation (corrected $p = .01$), while no other finding was significant after multiple-testing correction.

3.2. *Gene-set analysis*

17,783 gene sets were examined for association with the phenotypes under investigation. In the meta-analysis of SCZ and BP samples, substance use disorder comorbidity was associated with the Gene Ontology (GO) term GO:0048661 that is involved in positive regulation of cell proliferation (permuted comparative $p = .03$). The gene set "Roversi glioma copy number up", member of the functional group chemical and genetic perturbations, was close to the significance threshold for association with the same phenotype (permuted comparative $p = .09$). These results are summarized in Table 2. Other gene sets showed corrected $p > .20$. Results with nominal $p < .0005$ are reported in Supplementary Table 3.

3.3. *Analysis of genome-wide significant loci reported by the PGC schizophrenia, bipolar and cross-disorder working groups*

The list and characteristics of the available SNPs are reported in Supplementary Table 4.

No SNP showed association with the phenotypes of interest in the SCZ, BP sample or their meta-analysis. The top findings were rs11191454 (AS3MT gene) in the SCZ sample (nominal $p = .003$) and rs2799573 (CACNB2 gene) in the BP sample (nominal $p = .003$) for association with substance use disorder comorbidity. SNPs with nominal $p < .1$ are reported in Supplementary Table 5.

The available SNPs were mapped to the corresponding 71 genes in SCZ and BP samples. The significant genes reported in paragraph 3.1 did not include any of these genes. The top genes were ITIH3 in the SCZ sample, PACS1 in the BP sample and meta-analysis for association with suicidal ideation (nominal $p = .004$, 0.004 and 0.002, respectively). Gene-based results with $p < .05$ are reported in Supplementary Table 6.

The significant gene set GO:0048661 did not include any of the candidate genes of interest. However, the Roversi glioma copy number up included GALNT10 and DGKI candidate genes and was close to the significance threshold for association with substance use disorder comorbidity (permuted comparative $p = .09$).

4. Discussion

This study investigated the genetic factors associated with specific symptom domains and clinical features shared between SCZ and BP. The analysis was carried out at SNP, gene and gene-set level. The possible effect of loci showing genome-wide association with SCZ and BP in previous GWAS was also considered (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Ruderfer et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Stahl et al., 2017).

At gene level, SH3GL2 and CLVS1 were associated with suicidal ideation in the SCZ sample, and the former result was confirmed in the meta-analysis with BP sample, while the SHC4 gene was associated with depressive symptoms in the BP sample but not in the meta-analysis. SH3GL2 codes for Endophilin A1, a protein implicated in synaptic vesicle endocytosis that modulates intracellular signaling, calcium homeostasis and neurotransmitter release (Martins-De-Sousa et al., 2009). Specifically, Endophilin A1 regulates glutamate release in neurons expressing the vesicular glutamate transporter (Weston et al., 2011). Previous studies suggested that SH3GL2 is differentially expressed in the gray matter of prefrontal cortex in patients with psychosis compared to controls (Martins-De-Sousa et al., 2009; Prabakaran et al., 2004) and variants of this

gene were associated with cognitive functions in psychotic disorders (Lencer et al., 2017). To the best of our knowledge, it was the first time SH3GL2 gene was reported in con- nection to suicidal ideation. This link may be interpreted in the

perspective of SH3GL2 role in glutamatergic neurotransmission that is relevant to suicide. Indeed ketamine, a glutamate *N*-methyl-D-aspartate (NMDA) receptor antagonist, is associated with a rapid reduction of suicidal ideation (Murrough et al., 2015). Additionally, variants within GRIN2B, which encodes a subunit of the NMDA receptor ion channel, were reported in connection to changes in suicidal behavior-related neuropsychological measures (Sokolowski et al., 2013). The CLVS1 gene codes for clavesin 1, which, along with clavesin 2, is expressed exclusively in neurons and supposedly provides a unique neuron-spe- cific regulation of late endosome/lysosome morphology. Since neurons are particularly sensitive to lysosomal dysfunction and alterations in lysosomal function are the underlying cause of numerous neurode- generative diseases, clavesins may possibly have a role in that respect (Katoh et al., 2009). The association between the SHC4 gene and de- pressive symptoms severity was in line with the previous literature. SHC4 is expressed in neurons and regulates BDNF-induced MAPK ac- tivation (You et al., 2010), which has been shown to be a key factor in major depression pathophysiology (Duric et al., 2010). Two GWAS identified a variant (rs8023445) on chromosome 15 located within the SHC4 gene showing association with major depression (Aragam et al., 2011; Sullivan et al., 2009). A recent genome-wide haplotype-based association analysis of major depressive disorder found an haplotype approaching genome-wide significance located within SHC4 region (Howard et al., 2017).

At gene-set level, substance use disorder comorbidity was associated with GO:0048661, involved in up-regulation of smooth muscle pro- liferation. There is no overlap between genes in this gene-set and most prominent genes described in connection to substance abuse disorder (Jones and Comer, 2015; Li and Burmeister, 2009). Many members of GO:0048661, however, take part in cellular development and differ- entiation, not only in smooth muscle tissue; addiction-related genes were shown to be highly enriched in neurodevelopment-related pro- cesses (Sun and Zhao, 2010). It is therefore possible that this gene-set may be relevant to substance use disorders affecting neurodevelop- ment. Further, GO:0048661 includes CAMK2D which is part of a larger family of type 2 Ca2+/calmodulin dependent protein kinase genes that are the common link between five proposed addiction-related genetic pathways (Li et al., 2008). Specifically, CAMK2 kinases have been found to have an important role in mediating stimulant-induced do- pamine release (Fog et al., 2006), conditioned place preference (Sakurai et al., 2007) and behavioral sensitization (Licata et al., 2004) and to participate in processes leading to the development of opioid tolerance and addiction (Tang et al., 2006).

No significant cross-phenotype genetic associations were detected. Considering suggestive signals from gene and gene-set analysis (Supplementary Tables 2 and 3), GO:0044849 was the only result with suggestive cross-trait association (depressive and positive symptoms severity in SCZ sample, depressive symptoms in the meta-analysis). This gene set takes part into ovulation cycle regulation, and this may be linked to the well characterized estrogens' influence on depressive (Ryan and Ancelin, 2012) and positive symptoms (Seeman, 2012; Zhu et al., 2018). OXTR (oxytocin receptor), is also a member of this gene set. The oxytocin system is relevant in the modulation of social beha- vior and it is dysregulated across various psychiatric diseases and symptom domains (Cochran et al., 2013). The cross-trait suggestive effect of GO:0044849 is

consistent with the current view that disruption in networks, such as the estrogens and oxytocin systems, may account for shared vulnerability across psychiatric traits (Doherty and Owen, 2014).

None of the genome-wide significant loci or corresponding genes or gene-sets previously associated with SCZ and/or BP was associated with the phenotypes of interest (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Ruderfer et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Stahl et al., 2017). Regarding gene-sets, the Roversi glioma copy number up, which includes the candidate genes GALNT10 and DGKI harboring

variants previously associated with SCZ (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), was close to the sig- nificance threshold for association with substance use disorder co- morbidity. Similarly to GO:0048661, this gene-set includes genes overseeing cellular development and differentiation, thus its connection on substance use disorder may be interpreted under the same light (Sun and Zhao, 2010). None of the individual loci analyzed was associated with the phenotypes of interest. The top findings were rs11191454 (AS3MT gene) in SCZ sample and rs2799573 (CACNB2) in BP sample for association with substance use disorder.

These results should be interpreted in consideration of some lim- itations. Firstly, the relatively small sample sizes, which limit the pos- sibility of assessing the impact of candidate loci on the examined phenotypes. For individual variants, our meta-analysis provided a power of 0.07 to detect risk alleles with odds ratios (ORs) ~ 1.1, i.e. the mean OR of significant variants reported by the PGC, setting the alpha value to 0.05 (two-tailed) and considering MAF = 0.30, in line with that of significant variants reported by the PGC. Indeed, the attainment of a power of 0.80 would require a much large sample size, over 16.400 subjects, and, applying such a power threshold to our study population, an OR of 1.8 could be detected for individual variants. However, spe- cific symptom domains were considered in our analysis not broad di- agnostic categories, thus expected effect sizes do not necessarily cor- respond to those reported by the PGC, which by the way includes very heterogenous samples, despite the fact that we could not provide direct evidence supporting this hypothesis and our findings should be con- sidered as suggestive only. Secondly, there was only partial compar- ability between the samples in terms of the phenotype construction, particularly for depressive and positive symptoms severity, and other sample characteristics, such as treatment setting.

In conclusion, our results support and detail previous findings and suggest some new associations. A role of SH3GL2, previously char- acterized as relevant to psychosis (Åberg et al., 2012; Lencer et al., 2017; Martins-De-Sousa et al., 2009; Prabakaran et al., 2004), was hypothesized in suicidal ideation. CLVS was also associated with sui- cidal ideation for the first time, this gene is neuron specific but still poorly characterized (Katoh et al., 2009). Our results confirmed the effect of SHC4 on depressive symptoms (Aragam et al., 2011; Duric et al., 2010; Howard et al., 2017; Siddiqui et al., 2009; Sullivan et al., 2003). Lastly, GO:0048661, involved in up-regulation of cell pro- liferation, may play a role in the risk of substance abuse disorder co- morbidity, possibly through a modulating effect on neurodevelopment and Ca2+/calmodulin dependent protein kinases (Sun and Zhao, 2010; Fog et al., 2006; Licata et al., 2004; Sakurai et al., 2007; Tang et al., 2006).

Supplementary data to this article can be found online at https:// doi.org/10.1016/j.pnpbp.2018.08.023.

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Ethical statement

Approval was obtained by local ethics committees for all the studies included. All study procedures were in accordance with the Declaration of Helsinki.

Conflict of interest

Alessandro Serretti is or has been consultant/speaker for: Abbott, Abbvie, Angelini, Astra Zeneca, Clinical Data, Boheringer, Bristol Myers Squibb, Eli Lilly, GlaxoSmithKline, Innovapharma, Italfarmaco, Janssen, Lundbeck, Naurex, Pfizer, Polifarma, Sanofi, Servier.

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The other authors declare no potential conflict of interest.

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Table 1

Clinical-demographic characteristics of the included samples. For each continuous and categorical variables mean ± standard deviation and distributions (per- centage) were reported, respectively. BDI=Bipolar disorder type I, BDII=Bipolar disorder type II, BDNos=Bipolar disorder not otherwise specified, NA=not available.

Variable	SCZ sample I (n=111)	SCZ sample II (n=115)	SCZ sample total (n=226)	BD sample I (n=79)	BD sample II (n=149)	BD sample total (n=228)
Age	43.03±13.38	48.93±14.27	46.03±14.12	47.30±12.83	48.62±14.03	48.16±13.63
Gender N (%)	65 M (58.56%), 46 (41.44%)	60 M (52.17%), 55 (47.83%)	125 M (55.31%), F (44.69%)	38 M (48.1%), F (51.9%)	81 M (54.36%), 68 (45.64%)	119 M (52.19%), F (47.81%)
Ethnicity caucasian/other	107/4	112/3	219/7	75/4	140/9	215/13
Age at onset	24.73±7.63 (36 NA)	22.83±6.66 (15 NA)	23.65±7.14 (51 NA)	27.99 ± 11.90 NA)	27.49±10.8 (18 NA)	27.66±11.19 (28 NA)
Diagnosis	/	/	/	58 BDI, 11 BDII, 10 BDNos	116 BDI, 21 BDII, BDNos	174 BDI, 32 BDII, 22 BDNos
Depressive symptoms severity (before standardization)	7.70 ± 3.78 (55	10.57±4 (27 NA)	Different scales used	7.83±4.83 (19 NA)	1.86 ± 2.69 (41	3.99±4.6 (60 NA)
Positive symptoms severity standardization)	7.70 ± 2.77 (14	6.67 ± 2.82 (27	7.21 ± 2.79 (41	4.61±2.50 (61 NA)	0.96 ± 0.67 (23	Different scales were used
Suicidal ideation yes/no	14/42 (55 NA)	14/96 (5 NA)	28/138 (60 NA)	22/38 (19 NA)	73/56 (20 NA)	95/94 (39 NA)
Early onset yes/no	26/49 (36 NA)	45/55 (15 NA)	71/104 (51 NA)	26/43 (10 NA)	48/83 (18 NA)	74/126 (28 NA)
Substance use disorder yes/no	47/28 (36 NA)	22/74 (19 NA)	69/102 (55 NA)	40/32 (7 NA)	74/55 (20 NA)	114/87 (27 NA)

Table 2

Summary of significant results of gene (a) and gene set analysis (b). CHR=Chromosome; NSNPS=number of SNPs annotated to that gene; ZSTAT=the Z-value for each gene; NGENES=the number of genes in the gene set.

Gene	CHR	Phenotype	Sample	NSNPS	ZSTAT	P	Corrected p
(a) SH3GL2							
	9	Suicidal ideation	SCZ	1005	6.88	3.01E-12	5.62E-08
CLVS1	8	Suicidal ideation	SCZ	410	4.87	5.56E-07	1.04E-02
SHC4	15	Depressive symptoms severity	BP	339	5.10	1.66E-07	3.13E-03
SH3GL2	9	Suicidal ideation	Meta-analysis	998	4.85	6.25E-07	1.18E-02

SET	Phenotype	Sample	NGENES	BETA	BETA STD	SE	P	Corrected p
(b) GO:0048661								
	Substance use disorder comorbidity	Meta-analysis	60	0.51	0.03	0.11	1.57E-06	3.12E-02