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Short communication

Association of age-of-onset groups with GWAS significant schizophrenia and bipolar disorder loci in Romanian bipolar I patients

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ABSTRACT

We investigated the influence of the age-of-onset (AO) on the association of 45 loci conferring risk for bipolar disorder (BP) and schizophrenia with BP-type-I in a Romanian sample (461 patients, 436 controls). The AO-analysis implicated the *EGFR* gene, as well as loci in other genes, in the AO variation of BP-type-I and revealed for the first time the link between BP-type-I and risk variants considered specific to schizophrenia (polymorphisms in *MMP16*/*RIPK2* and *CNNM2* genes).

1. Introduction

Bipolar disorder (BP) and schizophrenia are severe heritable psychiatric disorders. Epidemiologic (Lichtenstein et al., 2009) and molecular studies suggest that BP and schizophrenia partly share their genetic liability. The Cross-Disorder Group of Psychiatric Genomics Consortium (PGC) (2013) analyzed genome-wide data obtained for five psychiatric disorders and demonstrated high SNP-based co-heritability between schizophrenia and BP (0.68). Schizophrenia Working Group of the PGC (2014) identified 108 loci significantly associated with schizophrenia at genome-wide level; some of these loci were involved not only in schizophrenia, but also in BP (Chen et al., 2013; Mühleisen et al., 2014).

Evidence is growing that the contribution of genome-wide significantly associated risk loci to the genetic liability to major psychoses in different populations might depend on disorder subphenotype, in particular on the subphenotype generated by the age-of-onset (AO) (Mathieu et al., 2010; Priebe et al., 2012; Jamain et al., 2014). Several clinical studies showed that early- and late-onset forms of BP and schizophrenia are accompanied by different morbid risk for major psychoses to first degree relatives of probands (Rice et al., 1987; Schürhoff et al., 2000; Byrne et al., 2002; Grigoroiu-Serbanescu et al., 2001, 2014), which might suggest a different expression of the genetic propensity. Therefore the *aim* of the present study was to investigate the hypothesis of a possible AO influence on the association of selected risk loci for BP and schizophrenia revealed by large-scale genome-wide association studies (GWAS) with BP-type-I (BP-I) in the Romanian population.

2. Methods

2.1. Patient and control samples

The study was approved by the grant committee of the Romanian Ministry for Education and Research. All participants provided written informed consent following a detailed explanation of the study aims and procedures.

All patients and controls were of Romanian descent. Genealogical information about parents and all four grandparents was obtained through direct interview of the subjects.

461 unrelated BP-I patients were recruited from consecutive hospital admissions. The diagnosis of BP-I was assigned according to DSM-IV criteria (APA, 1994) on the basis of both the Diagnostic Interview for Genetic Studies (DIGS) (NIMH, 1995) and medical records. Patients were included in the sample if they had at least two documented hospitalized illness episodes (one manic/mixed and one depressive or two manic episodes) and no residual mood

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incongruent psychotic symptoms during remissions. This information was confirmed by first degree relatives for 64% of the cases. Detailed clinical-demographic information about patients is presented in Table S_1.

AO was defined as the age at which the proband first met DSM-IV criteria for a manic, mixed, or major depressive episode.

Population-based controls were screened with DIGS for lifetime history of any major affective or schizoaffective disorders, schizophrenia and any other psychosis, obsessive-compulsive disorder, eating disorders, alcohol/drug addiction. 438 unaffected controls were included in the study.

There were no significant differences between cases and controls in terms of age and sex distribution (Table S_1).

2.2. SNP selection and genotyping

Fifty genome-wide significant SNPs in SCZ and BP studies with more than 10,000 subjects (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011; The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013), as well as SNPs considered relevant for these disorders in the review by Sullivan et al. (2012) were selected for genotyping in our samples. Primer molecules for the multiplex reaction were designed using the Assay Design Suite tool (www.mysequenom.com, Sequenom, San Diego, CA, USA). Genotyping was performed in 2013 at the Department of Genomics, Life & Brain Center (University of Bonn, Germany) using Sequenom's Mass Array System and iPlex Gold reagents in accordance with the manufacturer's instructions. A detailed description of the method can be found in Blondal et al. (2003).

2.3. Quality control

Quality control (QC) was performed using INTERSNPv.1.11 software (Herold et al., 2009). Only SNPs with call rate $\geq 90\%$ and no significant deviation from Hardy–Weinberg–Equilibrium at $p=0.05$ in controls and patients were included in the analysis. Individuals were removed from the analysis if their DNA call rate was $<90\%$ or there was discrepancy between genotyped and phenotypic sex. After quality control, six patients, nine controls and five genotyped SNPs were excluded from the downstream analysis. The list of SNPs that passed the QC is shown in Table S_2.

The final samples consisted of 455 cases (267 females and 188 males) and 429 controls (256 females; 173 males).

2.4. Statistical analysis

The association between disease status and SNP-genotypes, as well as the AO-influence on this association were analyzed using both logistic regression and linear regression as implemented in INTERSNP software (Herold et al., 2009).

Two AO-groups (early-onset, late-onset) were defined for the logistic regression using commingling (admixture) analysis performed with the SEGREG program of S.A.G.E.v6.1 software (S.A.G.E., 2012). The model best fitting the data was chosen based on the smallest AIC-value.

Since there was no significant difference between cases and controls in terms of age and sex distribution, two variables that might have influenced the regression results, they were not included as covariates in the analysis.

The Bonferroni correction for multiple testing (45 SNPs) was applied to the nominal *p*-values. This resulted in a corrected *p*-value of 0.001 for determining significance of results.

The sample power to detect an allelic association at alpha = 0.05 (uncorrected for multiple testing) was 45% for a minor allele frequency (MAF) of 0.20 (average MAF-value in our samples) and 36% for a MAF of 0.10 (lowest MAF-value in our samples). The expected effect size was 1.3. The computation was performed with the Genetic Power Calculator (http://pngu.mgh.harvard.edu/purcell/gpc).

3. Results

Following the results of commingling/admixture analysis of AO in our sample (Fig. S_1) we chose the age 25 as cut-off point for the early- and late-onset (for details see Grigoroiu-Serbanescu et al. (2014)). 220 patients had an early-onset ($AO < 25$ years) and 235 patients had a late-onset ($AO \geq 25$ years).

None of the 45 SNPs passing the QC was significantly associated with BP-I in our total patient sample after Bonferroni correction of the *p*-values. But two SNPs reached nominal significance in the total sample (Table 1-panel A) replicating the discovery studies: rs11764590 (intronic, *MAD1L1* gene) and rs9834970 downstream of *TRANK1/LBA* genes. Two other SNPs reached nominal significance in the total sample (rs4939921, *MYO5B* gene, $p=0.026$ and rs3818253, *TRPC4AP* gene, $p=0.025$), but the opposite alleles were the risk alleles compared to the discovery studies.

The logistic regression (Table 1-panel A) showed that two SNPs reached nominal significance in the *early-onset group* [rs7004633 (intergenic, MMP16/RIPK2), rs9834970 (*TRANK1/LBA*)], while in the *late-onset* group seven SNPs showed nominally significant associations with BP-I: rs11191454 *(AS3MT)*, rs11764590 *(NT5C2)*, rs17172438 *(EGFR)*, rs729969*(EGFR)*, rs11764590 *(MAD1L1)*, rs7914558 *(CNNM2)*, and rs1064395 *(NCAN)*. The SNPs in the genes *AS3MT, CNNM2, NT5C2* are in LD. An additional SNP, rs10503253, located in *CSMD1,* showed a trend towards association ($p=0.074$).

The linear regression of the AO in the total sample replicated the involvement of most of these SNPs in the AO variability of BP-I (Table 1-panel B); a positive *Beta* indicates an association of the risk allele with a later AO and a negative *Beta* supports an association with an earlier AO. The SNP rs17172438 (*EGFR* gene) withstood the Bonferroni correction and a new SNP *(ANK3* gene) displayed nominally significant association with AO.

4. Discussion

Our study is the first one to examine the possible association between SNPs significant in large-scale GWAS of schizophrenia and BP and AO in BP-I, the most severe form of BP disorder. It provided suggestive evidence that AO might contribute to the specificity of the molecular overlap between schizophrenia and BP-I and that two new loci (rs7004633 in *MMP16/RIPK2* and rs7914558 in *CNNM2*), previously linked only to schizophrenia, might be involved in BP-I too.

The linear regression of the investigated SNPs on the disease AO evidenced a SNP in the *EGFR* gene that withstood the Bonferroni correction and contributed to the variability of the AO in our total sample, being mainly associated with later onset. Our study is the first one that replicates the association between *EGFR* (epidermal growth factor receptor) and BP, first described by Sklar et al. (2008). The *EGFR* is involved in neural stem cell proliferation and in circadian rhythm alterations through increasing phosphorylation activity of GSK3b (Kramer et al., 2001; Foltenyi et al., 2007). This is consistent with the effects of mood stabilizers that target the inhibition of GSK3b activity in BP-I patients. Weber et al. (2011) considered *EGFR* and its regulators candidate molecules for psychiatric disorders.

Several SNPs, nominally significant in the AO analysis in our sample *(AS3MT*, *NT5C2*, *CNNM2*, *MAD1L1*, *TRANK1*, *NCAN*, *CSMD1*,