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Research paper

Clock genes polymorphisms in male bipolar patients with comorbid alcohol abuse

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ABSTRACT

Background: Psychiatric comorbidity affects 24–65% patients with bipolar disorder (BD), 45% of which have alcohol abuse/dependence (AAD). Despite the fact that BD has an equal incidence in both genders, AAD more often occurs in men. We hypothesized that the presence of BD and AAD, reported as a secondary diagnosis, may result from a common genetic background. However, specific genetic factors predispose to gender differences.
Methods: Based on the relationship between circadian clock genes pathway and BD/AAD we decided to test the connection of four core clock genes with common genetic background of both diseases. We analyzed 436 patients with BD, among which 17% were diagnosed with AAD. The control group consisted of 417 healthy subjects. We analyzed 44 SNPs of the previously described core molecular clock genes: *CLOCK, ARNTL, TIMELESS* and *PER3*.
Result: We found association of *ARNTL* gene (rs11600996) and *PER3* gene (rs228642) polymorphisms with an increased risk of BD/AAD in a group of male patients. We also found that two other polymorphisms of *PER3* gene, rs228682 and rs2640909, were associated with both AAD and family history of affective disorders.
Limitations: Possible factors that could have influenced the results are: relatively small sample size, gender disproportion and unverifiable data form the patient interview.
Conclusions: Our study confirms the existence of a link between clock genes and increased risk of alcohol abuse/dependence in male patients and the accumulation of risk genes in patients with a positive family history.

1. Introduction

Statistics show that 65% of bipolar disorder (BD) patients have one psychiatric comorbidity, 42% have two and 24% - three or more (McElroy et al., 2001). What is more, 45% of BD patients have comorbid alcohol abuse/dependence (AAD) (Farren et al., 2012). Co-transmission of AAD in families has been reported for both disorders (BD and AAD) (Maier and Merikangas, 1996). Adoption studies revealed that substance abuse and BD was more common in biological relatives than in adoptive relatives, what confirms the genetic contribution (Ingraham and Wender, 1992).

Circadian clocks measure the length of day and night, and actively generate and maintain the routine variation in a range of functions of the organism (Partonen, 2015). At the molecular level the molecular clock consists of:

- CLOCK – clock circadian regulator
- ARNTL – aryl hydrocarbon receptor (also known as BMAL1)
- PER – period circadian regulator (include *per1, per2,* and *per3*)
- CRY – cryptochrome circadian regulator (include *cry1* and *cry2*)

CLOCK and ARNTL are transcription factors. In the nucleus, CLOCK forms a heterodimer with ARNTL. This complex activates transcriptions of *PER3* and Cry by binding to E-box elements in their promoters (Partonen, 2015; Udoh et al., 2015). Clock genes are expressed in the brain as well as in peripheral tissues and blood cells (Perreau-Lenz and Spanagel, 2015).

Data show that circadian clock genes pathway is involved in BD and

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AAD development (Hall et al., 1991; Partonen, 2015; Perreau-Lenz and Spanagel, 2015; Spanagel et al., 2005). The connection between clock genes and disrupted circadian rhythm in mood disorder patients was postulated according to observed symptoms like depressed/elevated mood, activity level and quality of sleep. What is more, worsening of depression symptoms in the morning is a characteristic trait of affective disorders (Hall et al., 1991).

In alcohol research, biological clocks are investigated as contributors to alcohol use disorders and sleep disorders (Udoh et al., 2015). Spanagel et al. (2005) showed that alcohol intake can specifically influence the functions of the circadian body clock (Spanagel et al., 2005).

In our study, we investigated the possible relationship among polymorphisms of *CLOCK, ARNTL, TIMELESS* and *PER3* genes (complex analysis of single polymorphisms and haplotypes – SNP interactions) and the comorbidity of AAD in BD patients. Based on the association of circadian genes with circadian rhythm disruption and the predisposition to BD (Oliveira et al., 2018), we decided to investigate whether the essential four clock gene polymorphisms are also connected with the increased risk of AAD among men with BD. We hypothesized that presence of BD and AAD, reported as a secondary diagnosis, may result from the common genetic background.

2. Materials and methods

2.1. Participants

The study was performed on 436 BD patients and 417 control group participants. The group of BD patients consisted of 237 females (aged 44 ± 15) and 199 males (aged 47 ± 14). They were recruited as consecutive patients among inpatients from Greater Poland region, treated at the Department of Psychiatry, University of Medical Sciences in Poznan. Patients agreed to enter the study after giving written informed consent. For each patient a consensus diagnosis was made according to DSM-IV criteria and by at least two psychiatrists, using structured clinical interview for DSM-IV Axis I disorders SCID-1 (First et al., 2007). Additionally, in the group of BD patients, we selected a subgroup consisting of 76 patients with AAD, 51 of them having a family history of affective disorder.

The control group consisted of 202 males (aged 45 ± 8) and 215 females (aged 46 ± 6) from Greater Poland region. We recruited healthy participants as a control group from among blood donors, students and other volunteers with no history of any psychiatric disorder, substance abuse or serious somatic illnesses. The Polish version of M.I.N.I screen (Mini International Neuropsychiatric Interview) was used to determine exclusion criteria such as the presence of any serious mental health problems (Sheehan et al., 1998) (Table 1).

The study was approved by the Ethics Committee of University of Medical Sciences in Poznan.

2.2. Influence of age and gender

Spearman's rank-order correlation showed significant ($p < 0.05$) negative correlation between sex and alcohol abuse (Spearman's rank correlation coefficient $r_s = -0.3$). This result supports that there is a higher prevalence of alcohol abuse in male than in female patients. Age, age of onset and duration of illness showed no significant influence on the analyzed variables. Correlation analysis let us identify other significant variables for further analysis. As the data provided were not significant enough, we did not carry out such an analysis in order not to minimize the tested groups.

2.3. Genotyping

DNA was extracted from 10 ml of EDTA anticoagulated whole blood samples using the salting method (Miller et al., 1988).

We analyzed polymorphisms of *CLOCK* (10), *ARNTL* (19), *TIMELESS* (6) and *PER3* (9) genes using TaqMan SNP Genotyping Assays and TaqMan SNP Genotyping Master Mix (*Applied Biosystems*). A detailed description of the protocol and analyzed SNPs has been published in Dmitrzak-Weglarz et al. (2015). The discrepancy in the size of the analyzed groups between "subjects" and the results presented in tables are due to missing data in genotyping.

Amplification for TaqMan SNP genotyping assay plates was done in ABI PRISM® 7900HT Sequence Detection System. Data acquisition and analysis were performed using the allelic discrimination analysis module in SDS v2.1 software (*Applied Biosystems*). Each reaction was prepared following the manufacturer's protocol.

2.4. Statistical analysis

Based on the recommendations of Ryckman, we performed the Hardy-Weinberg equilibrium (HWE) test in both patients and healthy participants as members of the same population (Ryckman et al., 2008). For computations, we used SNPassoc package.

We used classical case–control study calculation methodology – analysis of distribution was performed using Pearson's chi-square test for genotypes and Fisher's Exact Test for alleles (*VassarStats* package). The p value of less than 0.05 was considered statistically significant. The statistical analyses were performed with licensed statistical package STATISTICA v. 12.5.

The p-value less than 0.05 was considered statistically significant. The nominal p-values are presented the results section. Controlling for the number of investigated genes, the significant p-value after Bonferroni correction was set at $p < 0.01$ according to Pawlak et al. (2016). Additionally we calculated the false discovery rates and their q-values to better show which of the results are likely to be truly significant (supplementary results).

3. Results

Genotype distribution in our samples was in accordance to HWE except for rs4757142 (*ARNTL*). This polymorphism was eliminated from further analysis ($p < 0.001$) of all the studied polymorphisms (Dmitrzak-Weglarz et al., 2015).

We found significant differences in genotype distributions in the polymorphisms of *ARNTL*: rs11600996 CC ($p = 0.038$) and *PER3*: rs228642 TT ($p = 0.043$) genes that were associated with risk of BD.

Table 1
Sample characteristics.

	N	Age	Age of onset	Duration of illness (in years)	Patients with AAD	Patients with FH	Patients with AAD + FH
BD patients	436	45 ± 14	29.8 ± 11.2	16.7 ± 11.8	76	145	51
Female	237	44 ± 15	29.8 ± 10.7	17.6 ± 11.5	18	131	12
Male	199	47 ± 14	29.7 ± 11.8	15.4 ± 12.0	58	114	39
Controls	417	33 ± 13					
Female	215	46 ± 6					
Male	202	45 ± 8					

AAD – Alcohol abuse/dependence, FH – family history of affective disorders.