Psychiatry Research ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Brief report

Suicidal behavior in the context of disrupted rhythmicity in bipolar disorder—Data from an association study of suicide attempts with clock genes

Joanna Pawlak [a,*], Monika Dmitrzak-Weglarz [a], Malgorzata Maciukiewicz [a,d,e], Monika Wilkosc [c], Anna Leszczynska-Rodziewicz [a,b], Dorota Zaremba [a], Pawel Kapelski [a,b], Joanna Hauser [a]

[a] Psychiatric Genetics Unit, Department of Psychiatry, Poznan University of Medical Sciences, Poland
[b] Department of Adult Psychiatry, Poznan University of Medical Sciences, Poland
[c] Department of Individual Differences, Institute of Psychology, University of Bydgoszcz, Poland
[d] Pharmacogenetics Research Clinic, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Ontario, Canada
[e] Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada

ARTICLE INFO

Article history:
Received 1 October 2014
Received in revised form
28 December 2014
Accepted 12 January 2015

Keywords:
Clock genes
Suicide
Circadian rhythm

ABSTRACT

Suicidal behavior exhibits both circadian and annual rhythms. We were seeking an association between selected candidate clock genes and suicidal behavior in bipolar patients. The study included 441 bipolar patients and 422 controls and we genotyped 41 SNPs of the *CLOCK*, *ARNTL*, *TIMELESS*, *PER3* genes. The main positive findings built up associations between selected polymorphisms and:

- violent suicide attempts (*CLOCK*, *TIMELESS*);
- multiple suicide attempts (*TIMELESS*);
- a family history of suicide attempts (*TIMELESS*).

1. Introduction

Bipolar disorder is associated with both a high risk of suicidal behavior (SB) and with disrupted biological rhythms (McCarthy and Welsh, 2012). The disturbances first are those in the rhythms of activity and sleep. Melancholic depression is characterized by early morning waking and the occurrence of the worst mood in the morning and partial recovery during the evening. These symptoms interfere with cognitive functions, memory and attention.

In depression, patients experience many metabolic and hormonal pattern disruptions (nocturnal melatonin release (Koenigsberg et al., 2004); cortisol release rhythm, (Koenigsberg et al., 2004); body temperature (Posener et al., 2000), eating patterns (Wirz-Justice, 1995) and glucose metabolism (Buysse et al., 2004; Germain et al., 2007)).

Clock gene expression may also influence behavior indirectly by affecting cell function at the molecular level. The clock protein acts as a histone acetyl transferase enzyme and controls chromatin reorganization (Doi et al., 2006).

Moreover, studies show suicidal behavior to exhibit both circadian (van Houwelingen and Beersma, 2001; van Houwelingen et al., 2010) and seasonal rhythms (Germain and Kupfer, 2008). Both in attempted (Valtonen et al., 2006) and completed suicides (van Houwelingen and Beersma, 2001) time patterns were detected. The suicide rate varies between males and females (Preti et al., 2000). The fluctuation rate throughout the year was more pronounced among patients with mood disorders, in comparison to those with schizophrenia (Valtonen et al., 2006).

We hypothesized that suicidal behavior, as a rhythmic phenomenon, may be an element of a disrupted biological rhythm and be associated with polymorphisms of the clock genes. The primary aim was to seek an association between candidate clock genes and suicidal behavior, and type of suicide attempt, in bipolar patients. The secondary aim was to seek a linkage between clinical traits related to suicide and the genetic background, including G × G (gene × gene) interactions within clock genes.

* Corresponding author. Tel. +48 61 8491311; fax: +48 61 8480392.
E-mail address: joanna.pawlak@gmail.com (J. Pawlak).

http://dx.doi.org/10.1016/j.psychres.2015.01.010
0165-1781/

2. Material and method

The inclusion and exclusion criteria for bipolar patients employed here were previously described (Pawlak et al., 2013a, 2013b, 2014). A total of 441 patients (254 female and 187 male) and 422 controls from the region of Wielkopolska, Poland were included. The suicide attempt data used came from the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (section X-with the exception of eight patients) and from an additional interview. The subjects with the suicide attempt were divided into two groups: 61 persons who had taken violent or life-threatening attempts (hanging oneself, jumping from heights, throwing oneself under a vehicle, shooting, bleeding, drowning) (Beautrais, 2001) and 111 persons who did not apply these methods (Table 1).

The control group included blood donors and other volunteers without a history of any psychiatric disorder. The Ethics Committee of Poznan University of Medical Sciences approved the study. All the study participants were Caucasians of Polish origin and gave their written, informed consent.

Candidate polymorphisms were chosen according to a previously described protocol (Maciukiewicz et al., 2014). TaqMan SNP (single nucleotide polymorphism) genotyping was performed using an ABI Prism® 7900HT Sequence Detection System. We applied the allelic discrimination analysis module in SDS v2.1 software (Applied Biosystems, Foster City, CA).

The computational methods used were: the Kruskal–Wallis test, the Mann–Whitney *U* test with false discovery rate (FDR) adjustment for multiple testing, regression models for additive model, nonparametric evaluation of quantitative traits, multifactor dimensionality reduction (MDR). For the quantitative MDR 10,000 permutations were used as a correction method. For the power test the log additive mode of inheritance was employed. The presence of haplotype variants was detected using Haploview software (Barrett et al., 2005). We also used R environment (The R Development Core Team, 2013), Statistica 10, 64 bit Statsoft software and PLINK software (Purcell et al., 2007) (Details are available from the authors and in Supplementary tables).

3. Results

Polymorphisms violating the Hardy–Weinberg equilibrium were excluded from further analysis (Barrett et al., 2005; Lin et al., 2010). The power of association tests was estimated at the level of about 5%, which is characteristic for complex disorder analyses (Dmitrzak-Weglarz et al., 2013).

Neither allelic nor genotypic associations of polymorphisms of clock genes and suicide attempters vs nonattempters; suicide attempters vs controls and non-attempters vs controls were detected. After applying the Westfall–Young permutation test for multiple testing correction, no significant haplotype variants associated with suicidal risk were detected.

We found associations between selected gene variants and: 1) type of suicide attempt (violent vs not violent): rs3805148 ($p=0.035$; corrected=0.045 AC/AA), rs534654 ($p=0.0195$; corrected=0.04 AG/AA), rs11171856 ($p=0.0338$; corrected=0.032 CT/CC) and rs2291739 ($p=0.039$; corrected=0,045 AG/GG); 2) number of suicide attempts (multiple vs single): rs2291739 ($p=0.0169$; corrected=0.0257 GG/AG) (see Tables in Supplementary materials).

A family history of suicide attempts was linked with rs11171856 ($p=0.006$ corrected =0.0062 CT/TT) and rs2291739 ($p=0.018$ corrected=0.012 AG/GG). Other positive findings were associations between gene variants and age at first suicide attempt: rs7396943 ($p=0.00996$ corrected=0.0162 CG/CC) and rs11022778 ($p=0.0454$ corrected=0.047 GG/GT).

Two gene variants built up nominally significant relations: namely rs6850524 and rs3805148 for single/multiple attempts ($p=0.0221$) and for violent/non violent attempts ($p=0.0236$). rs6850524 was found to be significant by analysis of allele associations only.

We also looked for any epistasis interactions between circadian gene variants and suicide attempt characteristics. Gender, the familial burden of suicide attempts, a history of suicides in the family and the familial burden of affective disorders were all taken as covariates. High risk (HRR) genotypes were only analyzed to seek for factors which increase the risk.

When the type of suicide attempt was analyzed (violent versus non violent), interactions between rs11022779 and rs6849474 appeared. We analyzed age at the first suicide attempt, and a familial burden of suicide attempts, as dependent traits. However, the three methods used did detect rs3805148 as being associated with suicide attempt characteristics, at least on a nominal level. rs2291739 was found by both the Kruskal–Wallis test and qMDR. rs10462021 and rs3789327 produced a significant model when we searched a relation to suicidal attempts in the family. Also rs10462021was found to be significant when allele associations were computed (see Tables 2 and 3 in Supplementary materials).

The number of suicide attempts (multiple vs single suicide) was related to rs1982350 and rs12648271. The simple model appeared as the strongest ($p=0.0027$)

4. Limitations

A limitation of this study is the lack of comprehensive analysis of the numerous suicide risk factors that potentially influence suicide behavior simultaneously (comorbidity with substance abuse/dependence, personality disorder or anxiety disorder; treatment with lithium and its discontinuation; patient drug compliance or life events (Kovacsics et al., 2009; Mathews et al., 2013). The number of suicide attempters and SNPs included is a limitation too.

5. Discussion

The lack of positive findings in association studies and haplotype analyses between suicide attempters and controls, indicates that clock genes do not play the main role in the pathogenesis of

Table 1
Sample characteristics.

	Controls	BP
Number	422	441 (BPI $n=344$ 78.005%; BPII $n=97$ 21.995%)
Age (years)	18–68, mean=40.28, S.D.=5.31	18–84, mean=46.39, S.D.=13.75
Female	234 (55.450%),	254 (57.596%),
Male	188 (44.550%)	187 (42.404%)
Age at onset	–	10–63, mean=30.59, S.D.=11.42
Duration of the disease (years)	–	1–54, mean=15.18, S.D.=11.07
Family history of suicide attempt	0	7 (1.587%)
Family history of suicide committed	1 (0.237%)	34 (7.710%)
Suicide attempt(s) in lifetime history	–	180 (40.816%)
Violent suicide attempt(s) in lifetime history /nonviolent suicide attempt(s) in lifetime history	–	61 (33.889%) /111 (61.667%) $n=8$ data not available (4.444%)
Single/multiple suicide attempts	–	94 (52.222%) /78 (43.333%) $n=8$ data not available

Please cite this article as: Pawlak, J., et al., Suicidal behavior in the context of disrupted rhythmicity in bipolar disorder—Data from an association study of suicide attempts with clock genes. Psychiatry Research (2015), http://dx.doi.org/10.1016/j.psychres.2015.01.010