Schizophrenia Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

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

Altered circadian clock gene expression in patients with schizophrenia

Anne-Sofie Johansson [a,1], Björn Owe-Larsson [b,1], Jerker Hetta [b], Gabriella B. Lundkvist [a,*]

[a] Department of Neuroscience, Karolinska Institutet, Retzius väg 8, 17177 Stockholm, Sweden
[b] Department of Clinical Neuroscience, Karolinska Institutet, Section of Psychiatry at Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 7 October 2015
Received in revised form 17 April 2016
Accepted 18 April 2016
Available online xxxx

Keywords:
Biological clock
Human
Psychiatric disorder
Schizophrenia
Circadian rhythm
Sleep
Molecular clock

ABSTRACT

Impaired circadian rhythmicity has been reported in several psychiatric disorders. Schizophrenia is commonly associated with aberrant sleep-wake cycles and insomnia. It is not known if schizophrenia is associated with disturbances in molecular rhythmicity. We cultured fibroblasts from skin samples obtained from patients with chronic schizophrenia and from healthy controls, respectively, and analyzed the circadian expression during 48 h of the clock genes *CLOCK*, *BMAL1*, *PER1*, *PER2*, *CRY1*, *CRY2*, *REV-ERBα* and *DBP*. In fibroblasts obtained from patients with chronic schizophrenia, we found a loss of rhythmic expression of *CRY1* and *PER2* compared to cells from healthy controls. We also estimated the sleep quality in these patients and found that most of them suffered from poor sleep in comparison with the healthy controls.
In another patient sample, we analyzed mononuclear blood cells from patients with schizophrenia experiencing their first episode of psychosis, and found decreased expression of *CLOCK*, *PER2* and *CRY1* compared to blood cells from healthy controls.
These novel findings show disturbances in the molecular clock in schizophrenia and have important implications in our understanding of the aberrant rhythms reported in this disease.

1. Introduction

In psychiatric disorders such as depression, bipolar disorder and schizophrenia, dysregulations of circadian rhythmicity have been reported, manifested as alterations in the sleep-wake cycle, body temperature cycle and rhythmic hormonal profiles. Disturbances in the circadian timing system may not only be a symptom of these diseases, but have also been proposed to trigger these severe conditions (Karatsoreos, 2014). Schizophrenia is commonly associated with aberrant sleep-wake cycles and insomnia (reviewed in Monti et al., 2013), presented as poor sleep initiation, poor sleep consolidation, reduced slow wave sleep and shortened REM sleep latency with frequent sleep onset REM periods (Afonso et al., 2011a; Bromundt et al., 2011; Chouinard et al., 2004; Wirz-Justice et al., 2001; Wulff et al., 2012). These alterations occur also in non-medicated patients (Chouinard et al., 2004). Altered melatonin and cortisol profiles, a common marker of endogenous circadian rhythmicity, have also been shown in schizophrenia. Although the reports differ in results regarding the phase of the melatonin rhythm (advanced versus delayed compared to sleep onset), they all suggest a misalignment, or an altered phase angle, between the sleep-wake cycle and the melatonin profile in patients suffering from schizophrenia (Afonso et al., 2011b; Bromundt et al., 2011; Wirz-Justice et al., 1997; Wulff et al., 2012). Phase advances of the body temperature rhythm, serum tryptophan and prolactin have also been reported (Morgan and Cheadle, 1976; Rao et al., 1994). Taken together, these numerous studies strongly suggest a perturbed circadian regulation in schizophrenic patients (Lamont et al., 2010). Circadian molecular rhythms have been demonstrated in a number of tissues and are generated by complex autoregulatory transcription and translational feedback loops of so called "clock genes" and their protein products, which are found in most cells in the body and are expressed with a circa 24 h periodicity. Among the most important are two transcription factors Circadian Locomotor Output Cycles Kaput (CLOCK) and brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1 (*BMAL1*), which together activate transcription of *period* (*PER1*, *PER2* and *PER3*), *cryptochrome* (*CRY1* and *CRY2*), *retinoic acid receptor-related orphan receptor alpha* (*RORα*) and *the orphan nuclear receptor from the reverse erythroblastosis virus* (*REV-ERB*) genes. PER and CRY proteins inhibit their own transcription whereas RORα and REVERBα rhythmically activate and repress transcription, respectively, by acting on *BMAL1* promotor region (Buhr and Takahashi, 2013). The master clock is localized to a well-defined structure in the brain's hypothalamus, the suprachiasmatic nuclei (SCN), which coordinate clock rhythms in other brain regions and peripheral organs and thus acts as a "pacemaker" of daily rhythms (Buhr and Takahashi, 2013). One of the clearest examples of SCN activity is the sleep-wake cycle, but also endocrine, metabolic and immunological activities are governed by the SCN. It was reported in 1998 that fibroblasts have the same molecular clock machinery as SCN and can be used as a bona fide in vitro model of SCN cells (Balsalobre et al., 1998; Yagita et al., 2001).

* Corresponding author.
E-mail address: gabriella.lundkvist@ki.se (G.B. Lundkvist).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.schres.2016.04.029
0920-9964/

Furthermore, fibroblasts contain endogenous, self-sustained clocks. Brown and coworkers showed that molecular clock properties differ in skin fibroblasts from human individuals with different chronotypes; individuals with preference for evening activity had a longer circadian molecular day than individuals with morning preference (Brown et al., 2008). This finding, although questioned (Hasan et al., 2012), suggests that behavioral circadian activity can be traced in fibroblasts, and the molecular clock function is reflected in skin cells. It is therefore possible that cellular functional disturbances, such as circadian rhythmicity, associated with psychiatric disorders also can be expressed and detected in fibroblasts. Indeed, Yang et al have reported attenuated expression of specific clock genes in bio-bank fibroblasts originally obtained from bipolar patients (Yang et al., 2009).

Studies reporting clock gene polymorphisms or altered clock gene regulation in schizophrenia are scarce (Aston et al., 2004; Kishi et al., 2011; Mansour et al., 2009; Takao et al., 2007). Interestingly, Sun et al (2016) recently reported altered expressions of *PER1*, *PER2*, *PER3* and *NPAS2* in white blood cells in schizophrenic patients. We sampled skin biopsies and cultured fibroblasts from patients diagnosed with schizophrenia and studied the circadian expression of 8 clock genes in the fibroblast cultures. In another patient group, we analyzed clock gene expression in mononuclear cells (MNC) from blood sampled from patients with first onset psychosis who later developed schizophrenia.

We report, to our knowledge, for the first time that circadian clock gene expression is disturbed in fibroblasts from schizophrenic patients.

2. Materials and methods

2.1. Patients with chronic schizophrenia: studies of fibroblasts and sleep patterns

2.1.1. Study objects (for skin biopsy and sleep measurements)

All patients and control individuals were recruited at Karolinska University Hospital Huddinge. Subjects were provided with detailed oral and written information about the research project and gave written consent to participate. The local Ethics Committee approved the study (no. 04-273/1, supplements 2006/637-32 and 2009-06-12). Patients and control individuals were evaluated using Structured Clinical Interview for DSM-IV-Axis I Disorders (SCID-I). All subjects were of Caucasian origin.

Twenty-two individuals were enrolled in the study. Eleven patients with chronic schizophrenia were recruited at an inward unit. Females represented 54.5% and average patient's age was 44.4 ± 11.9 years (age span 25–59 years). All patients were under neuroleptic medication, as specified in Supplementary material.

Out of eleven age and sex matched, healthy control individuals, six were females (54.5%), and the average age of subjects was 41.2 ± 12.8 years (age span 22–59 years).

2.1.2. Sleep measurement in study objects

Subjects were asked to complete the questionnaire USI (Uppsala Sleep Inventory), which includes the Minimal Insomnia Symptom Scale (MISS), a 3-item scale including the cardinal symptoms of insomnia, i.e., difficulties falling asleep, night awakenings and not becoming rested by sleep, respectively (Broman et al., 2008).

2.1.3. Preparation of fibroblast cultures for qPCR

To establish fibroblast cultures, a small piece of the skin (approximately 2 × 4 mm) was cut longitudinally with a scalpel on the medial part of the upper arm at the site of *Musculus biceps brachii* of the individuals. Tissues were prepared and fibroblasts were cultured and serum-shocked in order to re-set the molecular clock in all cells (detailed description in Supplementary material). The cells were sampled at time points 0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48 h after serum shock, which occurred at time point 0 h.

2.1.4. Real-time PCR and data analysis

Total RNA was extracted from the cells and cDNA templates were run in triplicates using Platinum SYBR Green qPCR Supermix UDG (Invitrogen). *CLOCK*, *BMAL1*, *PER1*, *PER2*, *CRY1*, *CRY2*, *REV-ERBα* and *DBP* were analyzed, with *glyceraldehyd-3-phosphate dehydrogenase* (*GAPDH*) as internal control. Fold-differences in the levels of transcripts between groups were calculated according to the formula $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). For a more detailed description of the procedure, see Supplementary material.

2.1.5. Western blot

Fibroblast cells were serum shocked and harvested 24, 28, 32 and 36 h after serum shock. Cells were lysed, and proteins were separated and stained with antibodies against CRY1, CRY2 and VINCULIN. For details, see Supplementary material.

2.1.6. Data analysis and statistics

Student's *t*-test was used to compare overall levels of clock gene and protein expression, as well as symptoms of insomnia, between the healthy and schizophrenic group. Circadian rhythmicity in individual gene expression profiles of *CLOCK*, *BMAL1*, *PER1*, *PER2*, *CRY1*, *CRY2*, *REV-ERBα* and *DBP* was detected using a harmonic regression analysis (CircWave 1.4 software; courtesy of Dr. Roelof Hut; www.euclock.org). The program produces a Fourier-curve that describes the data by adding as many harmonics to the wave fit as the data allow. The program uses F-testing for each added harmonic like a step-wise multiple regression. For details of the analysis, see Supplementary material.

The significance level (alpha) for the F-test was set to 0.05. Number of significantly rhythmic individual gene expression patterns was calculated for each clock gene and compared with Chi-square analysis.

Table 1
Minimal Insomnia Symptom Scale (MISS): individual answers given by study objects, from whom a skin biopsy was taken for fibroblast studies. Each item is classified with the following response categories: none (0); small (1); moderate (2); severe (3); very severe (4). In addition, rhythm in *CRY1* and *PER2* is indicated in each individual.

MISS items (value 0–4 in each case)	Difficulties falling asleep	Night awakenings	Not being rested by sleep	Total score MISS items	*CRY1* rhythm (x) or not (−)	*PER2* rhythm (x) or not (−)
Patient						
S1	1	2	2	5	–	–
S2	2	2	3	7	–	–
S3	4	4	3	11	–	–
S4	1	1	1	3	–	–
S5	0	0	1	1	x	x
S6	0	0	1	1	–	–
S7	1	2	2	5	–	–
S8	0	4	3	7	x	–
S9	0	3	3	6	x	x
S10	0	0	0	0	–	–
S11	4	4	4	12	–	–
Control						
C1	0	0	0	0	x	x
C2	0	0	0	0	x	x
C3	0	0	1	1	x	x
C4	0	0	0	0	x	x
C5	0	0	0	0	x	x
C6	0	0	0	0	x	x
C7	2	0	0	2	x	–
C8	0	0	0	0	x	x
C9	0	0	0	0	x	–
C10	0	1	2	3	x	–
C11	0	0	0	0	x	x

Please cite this article as: Johansson, A.-S., et al., Altered circadian clock gene expression in patients with schizophrenia, Schizophr. Res. (2016), http://dx.doi.org/10.1016/j.schres.2016.04.029