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Chronotype and sleep quality as a subphenotype in association studies of clock genes in mood disorders

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Genetic background and clinical picture of mood disorders (MD) are complex and may depend on many genes and their potential interactions as well as environmental factors. Therefore, clinical variations, or endophenotypes, were suggested for association studies. The aim of the study was to investigate association between the chronotype (CH) and quality of sleep characteristics with polymorphisms *CLOCK, ARNTL, TIMELESS* and *PER3* genes in MD. We included a total sample of 111 inpatients and 126 healthy controls. To assess CH we applied Morningness-Eveningness Questionnaire (MEQ). Additionally, we defined the quality and patterns of sleep using The Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS). We applied Kruskal-Wallis test to determine associations. The main positive findings refer to associations between selected polymorphisms and:

1) chronotype with the *ARNTL* gene (rs11824092 and rs1481892) and the *CLOCK* (rs1268271),

2) sleep duration with the *CLOCK* gene (rs3805148) and the *TIM* gene (rs2291739),

3) daytime dysfunction with the *PER3* gene (rs228727, rs228642, rs10864315),

4) subjective sleep quality with the *ARNTL* gene (rs11824092, rs1982350),

5) sleep disturbances with the *ARNTL* gene (rs11600996).

We also found the significant epistatic interactions between polymorphism of the *PER3* gene (rs2640909) & the *CLOCK* gene (rs11932595) and following sleep quality variables: sleep duration, habitual sleep efficiency and subjective sleep quality.

The present study suggests a putative role of the analyzed clock genes polymorphisms in chronotype in the control group and in sleep quality disturbances in the course of MD. The results indicate that PSQI variables can be used to refine phenotype in association studies of clock genes in MD.

Key words: chronotype, sleep quality varies, clock genes, mood disorders

INTRODUCTION

Objectives

Genetic background and clinical picture of mood disorders (MD) are complex and may depend on many genes and their potential interactions as well as environmental factors. Therefore, clinical variations, or endophenotypes, were suggested for association studies.

The human biological clock determines synchronization of light-dark rhythm with physiological and mental processes (Lemoine et al. 2013, Frank et al. 2008, Ohayon 2007a). Chronotype (CH) is an expression of individual circadian rhythm differences, defining the preference of morning (lark) or evening (owl) activity (Pracki et al. 2014). CH is considered as the combination of both biological (genetic factors, aging of the body) and psychosocial (hours of study or work, the rhythm of family life) processes (Allebrandt and Roenneberg 2008). CH is highly heritable (heritability ~50%) (Koskenvuo et al. 2007, von Schantz et al. 2015). Studies suggested relationships between CH and both differences in the activation level (extraversion/introversion, novelty seeking, mobility, strength of activation/inhibition of the nervous system) and severity of depression and anxiety (Frank et al. 2013, Hasler et al. 2013, Ottoni et al. 2012). As the result, CH may be considered

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as a potential phenotype in association studies of "clock genes" in MD.

Sleep disturbances are common in patients diagnosed with MD. The prevalence of insomnia or excessive sleepiness during course of MD suggested a pivotal role of the circadian system in the development of this disorder (Frank et al. 2008, Ohayon 2007b). If confirmed, treatment of sleep disorders should be parallel with pharmacotherapy as a part of new therapeutic approach (Bellivier et al. 2015). New approach may result in faster remission and reduce risk for suicide attempts (Franzen and Buysse 2008). The master biological clock is located in the suprachiasmatic nuclei of the anterior hypothalamus and is crucial for the organization of the circadian rhythms. The group of genes known as "clock genes" is responsible for the maintenance of circadian rhythm on the subcellular level (Tardito et al. 2010).

Several studies documented that seasonality of mood is connected with seasonal exposure to light (Geoffroy et al. 2015, Patten et al. 2016, Savides et al. 1986). Thus we expect that season of fulfilling self-assessment questionnaire may involve on giving answers.

The aim of current study is to investigate genetic associations between preferences of daily activity, characteristics of sleep quality and polymorphisms of pivotal "clock genes" in Polish, MD cohort.

METHODS

Participants

We included total number of 111 inpatients, who met DSM-IV criteria for MD in the course of bipolar (70 BPD) and unipolar disorder (41 UPD). The lifetime diagnosis was established using SCID-I (Structured Clinical Interview for Axis I clinical disorders for DSM-IV) (First et al. 2007). We applied the OPCRIT Checklist to determine lifetime perspective of MD and sleep disturbances symptoms. Our sample consisted of predominantly female (76 female, 35 male), adults aged 18–78 years (mean=42, SD±17). The age of onset (mean=30, SD±12) and duration of disease (mean=12, SD±12) were determined based on the first episode meeting the DSM-IV criteria evaluated by a psychiatrist. We recruited patients in the hospital by the Department of Psychiatry, University of Medical Sciences in Poznan.

The control group (CG) consisted of 126 healthy volunteers (66 female, 60 male), aged 22–83 years (mean=39 years, SD±12). Control volunteers subjects were assessed using the Polish version of M.I.N.I screen (Mini International Neuropsychiatric Interview). M.I.N.I screen is compatible with ICD-10 and DSM-IV criteria. It allows for exclusion 14 disorders on I axis such as: major depressive disorder, dysthymia, manic symptoms, panic disorder, agoraphobia, social phobia, obsessive-compulsive disorder, post-traumatic stress disorder, alcohol abuse/dependence, drugs abuse/dependence, psychotic symptoms, anorexia & bulimia nervosa and generalized anxiety disorder (Lecrubier et al. 1997). The exclusion criteria from the control group were as follows: sleep problems (difficulty in falling asleep, waking up in the middle of the night, waking up early or too long sleep) identified in M.I.N.I. screen as well as more than 14 points obtained in the Epworth Sleepiness Scale (ESS).

Patients fulfilled self-questionnaires during hospitalization after obtaining euthymic state evaluated by psychiatrist. To standardize the interval of retrospective studies, patients were asked to give answers describing the period of remission before the current/last episode. The study was approved by the Ethics Committee, University of Medical Sciences in Poznan. All study participants were Caucasians of Polish origin and gave the written, informed consent.

Questionnaires

Morningness-Evenigness Questionnaires (MEQ)

We assessed chrontype using Polish, validated adaptation of Morningness-Evenigness Questionnaires previously used by (Ciarkowska in unpublished results) Circadian Activity Rhythm Questionnaire – standardisation on Polish population (N=12080) MEQ (Morningness-Evenigness Questionnaires) (Horne and Ostberg 1976). The questionnaire consisted of 21 questions that cover basis of subjective personal observations, behavior, experiences, senses etc. Resulting data was used to assess individual chronotype (morning, moderate/neutral and evening) was assessed following suggestions by (Pracki et al. 2014).

The Pittsburgh Sleep Quality Index (PSQI)

We applied The Pittsburgh Sleep Quality Index (PSQI) to assess the quality and patterns of sleep in adults. The PSQI consists of 19 questions covering: subjective sleep quality, sleep latency (the time it takes to fall asleep), sleep duration, habitual sleep efficiency (the ratio of total sleep time to time in bed), sleep disturbances, the use of sleep-promoting medication (prescribed or over-the-counter), and daytime dysfunction. Each item is scored from minimum of 0 (better) to maximum of 3 (worse). The seven component scores may be summed to provide a global score (PSQI), with higher scores reflecting poorer sleep quality. A score of ≥5 on the global rating suggests moderate sleep problems in ≥3 areas, or more severe problems in at least two areas (Buysse et al. 1989).

Epworth Sleepiness Scale (ESS)

We assessed the likelihood of falling asleep due to excessive sleepiness using Epworth Sleepiness Scale (ESS), one of the most common scales in sleep medicine. The participant is asked to assess, on a scale of 0–3, the probability of falling asleep in eight typical situations from everyday life. Number of points below 10 indicates a lack of excessive sleepiness; score above 14 indicates pathological sleepiness (Johns 1991).

Clinical variables from OPCRIT

OPCRIT provides a convenient, reliable, rapid and valid approach to polydiagnostic assessment with consensus best-estimate lifetime diagnoses. It provides a synthesis of the SCID interview. In its basis we chose the demographic data (age, gender, age of onset); variables related to sleep problems (reduced need for sleep, initial insomnia, middle insomnia (broken sleep), early morning waking and excessive sleep) and course of disorder in lifetime, which can be omitted in the self-assessment questionnaires provided according to the last period of remission (Craddock et al. 1996).

Genotyping

Blood [10 ml collected in a vacuum tube with anti-coagulant ethylenediaminetetraacetic acid (EDTA)] samples were obtained from each participants. Genomic DNA was extracted from collected blood using the Miller's salting-out method (Miller et al. 1988). We select candidate genes according to review of literature. The SNP in genes were chosen according to tag SNP selection in Haploview v 4.2 (Barrett et al. 2005) using HapMap (International HapMap 2003, Zhang et al. 2015) database for Caucasian population and supplemented by SNPs previously reported associations for psychiatric disorders. Analyzed polymorphisms are as follows: 9 polymorphisms of *CLOCK* gene (rs1801260, rs3805148, rs6849474, rs11932595, rs12648271, rs6850524, rs12649507, rs4340844, rs534654), 18 polymorphisms of *ARNTL* gene (rs1481892, rs4146388, rs10766075, rs4757142, rs7396943, rs11824092, rs7947951, rs7937060, rs1562438, rs3816360, rs7126303, rs3789327, rs11022778, rs11600996, rs11022779, rs11022780, rs7107287, rs1982350), 6 polymorphisms of *TIMELESS* gene (rs2291739, rs2291738, rs7302060, rs10876890, rs11171856, rs2279665) and 9 polymorphisms of *PER3* gene (rs836755, rs228727, rs10864315, rs4908694, rs228682, rs228642, rs2172563, rs2640909, rs10462021). Detailed description of protocol and analyzed polymorphisms and are presented in previous references (Dmitrzak-Weglarz et al. 2015, Maciukiewicz et al. 2014). TaqMan SNP (single nucleotide polymorphism) genotyping was performed using an ABI Prisms 7900HT Sequence Detection System. We applied the allelic discrimination analysis module in SDS v 2.1 software (Applied Biosystems, Foster City, CA).

Statistics

Statistical analyzes were performed at the Department of Computer Science and Statistics and at the Department of Psychiatric Genetics of Poznan University of Medical Sciences.

P value of less than 0.05 was considered statistically significant. Categorical variables are expressed as percentages. Continuous data was checked for normal distribution by Shapiro-Wilk's test. Variables measured on an ordinal scale are presented as the medians with 25th and 75th percentiles. Univariate analysis was performed. Categorical variables were analyzed by the $\chi 2$ test or Fisher-Freeman-Halton test, when applicable. The Spearman's rank correlation test is used to evaluate the associations between the variables measured on an ordinal scale. Comparisons of continuous data, which are non-normally distributed, are performed by using the Mann-Whitney U test. Comparisons of ordinal variables are analyzed by the Mann-Whitney U test or the Kruskal-Wallis test with Dunn's multiple comparisons test. The regression models for additive model was performed to find association between genotypes and selected variables. The multifactor dimensionality reduction (MDR) to compute epistatic interaction of genes was used. 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. We also used R t, Statistica 10 (v 10, StatSoft, Krakow, Poland), and StatXact (v 8, Cytel, Cambridge, Massachusetts), 64 bit Statsoft software and PLINK software.

RESULTS

Chronotype

We noticed no significant differences between patients and controls in frequency of chronotype regardless of the type of disease ($p>0.05$) (Table I, Fig. 1).

Sleep variables

Table II presents results of comparisons between controls and selected group of patients. All characteristics were significantly worse in patients then controls ($p<0.05$) except duration of sleep with no significant differences

Table I. Frequency of chronotypes in patients & controls

	Evening chronotype	Intermediate chronotype	Morning chronotype	ch2 (df=2)	p value
Controls n=126	51 (40.48)	45 (35.71)	30 (23.81)		–
Patients n=111	54 (48.65)	41 (36.94)	16 (14.41)	3.598	0.1655
BPD patients n=70	30 (42.86)	30 (42.86)	10 (14.28)	2.662	0.2662
UPD patients n=41	24 (58.54)	11 (26.83)	6 (14.63)	4.183	0.1235

BPD *vs.* UPD p=0.1758; ch2 Pearson test; % in the brackets

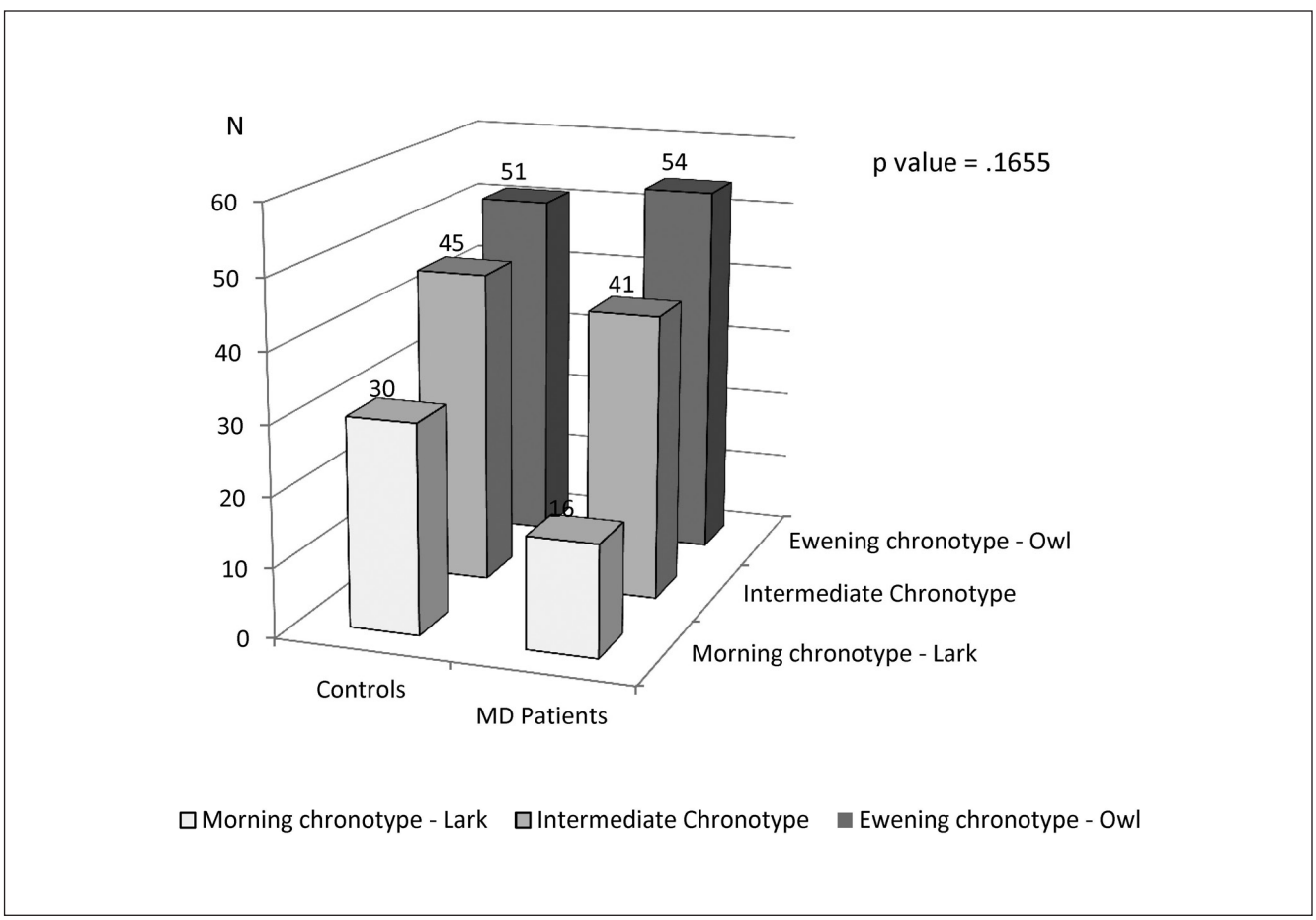

Fig. 1. Frequency of chronotypes in patients & controls. N – number of subjects, MD – mood disorder.

(p>0.05). We noticed difference in the sleep latency when compared BPD and UPD groups (p=0.0092), with higher values in UPD patients.

Sleep variables: chronotype and season

We were interested in whether sleep quality variables are dependent on chronotype or season of fulfilling the survey. For this purpose we have made an independent assessment in a control group and patients to exclude the impact of diagnosis on the results.

We detected association between chronotype and individual components of sleep quality in neither MD nor controls groups (data not shown). However, in both groups the deteriorating of total sleep quality with shift chronotype towards the evening preference of activity was observed (CG: K-W H=8.648, p=0.0132; MD: K-W H=6.325, p=0.0432) (Fig. 2). Thus we can indirectly infer that evening chronotype is associated with poorer quality of sleep.

In the control group, we observed relation between the sleep quality and the season of fulfilling the questionnaire (K-W H=10.2192, p=0.0168) (Fig. 3), with

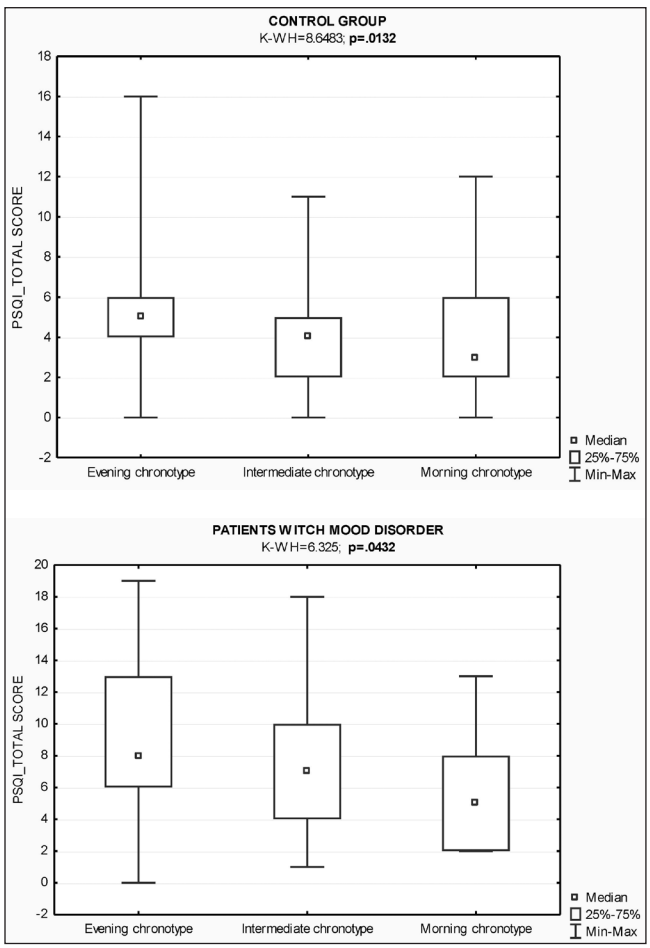

Fig. 2. Sleep quality difference between chronotypes in CG & MD.

worse quality of sleep in winter when compared to summer (Dunn's test p=0.0414). This may be a result of overall well-being during summer and relaxation connected with the holiday period. This dependency is not found in MD patients. This means that the quality of sleep is the same, irrespective of the season and indicates a direct relationship with the disease (K-W H=1.5387, p=0.6733).

Correlation with clinical variables

Several items of OPCRIT describe sleep problems during illness episode and may be correlated with chronotype (Table III). We detected suggestive correlations between reduced need for sleep, initial insomnia and early morning waking with chronotype or course of disorder with total score of PSQI and ESS. Higher results obtained in total score of PSQI (r_s=–0.2654) and reduced need for sleep (r_s=–0.2319) demonstrating the poorer quality of sleep were associated with evening chronotype. In contrast, initial insomnia (r_s=0.3485), early morning

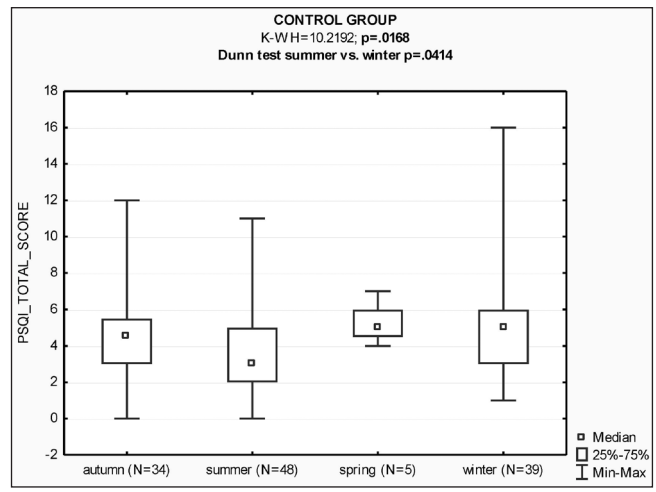

Fig. 3. Sleep quality difference between seasons in CG.

waking (r_s=0.2830) and increased sleepiness during the day (measured by ESS) (r_s=0.3800) were correlated witch morning chronotype.

All significant correlations (<0.05) ranged from 0.17 to 0.38 which is a poor correlation/weak relationship, thus should be treated as preliminary results and need confirmation in further studies.

Chronotype, sleep quality variables and genes

To determine the relationship of studied genes and their polymorphisms with diagnosis and the preference of daily activity or sleep variables, analyzes were performed independently for groups of patients and control subjects.

We conducted genetic association between genotypes and chronotype, sleep quality variables using Kruskal-Wallis test. Additionally, we used regression models applied in PLINK to look for association between genotypes and selected variables.

Significant, nominal associations were observed in the case of one polymorphisms of the *CLOCK* gene (rs1268271, p=0.0402) and two polymorphisms of *ARNTL* gene (rs11824092, p=0.0375, and rs1481892, p=0.0309) with chronotype only in control case (Table IV).

Significant nominal association in patients group was observed in cases as follows:

1) sleep duration with *CLOCK* (rs3805148, p=0.0118) and *TIM* (rs2291739, p=0.0184),

2) daytime dysfunction with *PER3* (rs228727, p=0.0465, rs228642, p=0.0280 and rs10864315, p=0.0161),

3) subjective sleep quality with *ARNTL* (rs11824092, p=0.0366, and rs1982350, p=0.0487),

4) sleep disturbances with *ARNTL* (rs11600996, p=0.0447) (Table V).

Table II. Sleep variables differences between controls and selected groups of patients

Compared groups	CG vs. MD	CG vs. BPD	CG vs. UPD	BPD vs. UPD
Sleep variables	p value	p value	p value	p value
Sleep duration	0.5232	0.7412	0.2079	0.2770
Sleep disturbances	**0.0002**	**0.0011**	**0.0071**	0.4819
Sleep latency	**0.00001**	**0.0002**	**0.000000**	**0.0092**
Daytime dysfunction	**0.00001**	**0.0011**	**0.000079**	0.0568
Habitual sleep efficiency	**0.0008**	**0.0318**	**0.0002**	0.4487
Subjective sleep quality	**0.0002**	**0.0472**	**0.00005**	0.0601
Medicine-Induced Sleep	**0.000034**	**0.000072**	**0.000056**	0.5953
PSQI_TOTAL	**0.000000***	**0.000000***	**0.000002***	0.1897*
ESS-sleepiness	**0.000000***	**0.000000***	**0.000000***	0.2790*

CG – control group, MD – mood disorders, BPD – bipolar disorder, UPD – unipolar disorder, PSQI_TOTAL – global score of sleep quality, ESS – Epworth Sleepiness Scale; ch2 Pearson test or Fisher-Freeman-Halton test in case <5, *Mann-Whitney test; significant p value – in bold

Table III. Correlation between chronotype, total sleep quality and sleepiness and selected variables from OPCRIT (R Spearman) – only in patients

	CHRONORTYPE	PSQI_TOTAL	ESS
CHRONORTYPE		**−0.2654**	**−0.1697**
PSQI_TOTAL	**−0.2654**		**0.3800**
ESS	**0.3800**	**−0.1697**	
Gender	0.3139	0.0455	0.2017
Age of onset	0.0569	−0.1368	−0.1786
Reduced need for sleep	**−0.2319**	0.1603	0.0955
Initial insomnia	**0.3485**	−0.0163	−0.0699
Middle insomnia (broken sleep)	0.2221	0.0916	−0.1066
Early morning waking	**0.2830**	0.0967	−0.0867
Excessive sleep	−0.0876	−0.1334	0.1184
Course of disorder (with or without remission)	−0.0487	**−0.2405**	0.0807

PSQI_TOTAL – global score of sleep quality, ESS – Epworth Sleepiness Scale; significant correlations with p value <0.05 – in bold

Epistatic interaction

During second stage of genetics analyzes, we employed Multifactor Dimensionality Reduction (MDR) for chronotype and sleep quality variables to compute pairwise epistatic interaction between circadian genes variants.

MDR analysis did not show significant epistatic interactions between circadian genes variants with chronotype in control group as well as MD patients (data not showed).

Nevertheless, significant epistatic interaction between polymorphism rs2640909 of *PER3* gene and rs11932595 of *CLOCK* gene were obtained in: sleep duration (p=0.0123), habitual sleep efficiency (p=0.0132) and subjective sleep quality (p=0.0163) in MD patients (Table VI). No significant interactions were observed in controls.

Table IV. Significant association between analyzed polymorphisms and chronotype in control group

	Gene	SNP ID	K-W H	p value		Genotypes, p value	
						CC	CG
	CLOCK	rs1268271	6.4277	**0.0402**	CG	0.5447	NA
					GG	0.8726	**0.0342**
						CC	CT
Chronotype	*ARNTL*	rs11824092	6.5688	**0.0375**	CT	0.6299	NA
					TT	0.0601	**0.0305**
						CC	CG
	ARNTL	rs1481892	6.9531	**0.0309**	CG	**0.0253**	NA
					GG	**0.0253**	0.9831

Kruskal-Wallis test, significant p value – in bold

Table V. Significant associations between analyzed polymorphisms and sleep quality variables in patient group

	Gene	SNP ID	K-W H	p value		Genotypes, p value	
						AA	AC
	CLOCK	rs3805148	8.8762	**0.0118**	AC	0.7567	NA
					CC	**0.0217**	**0.0124**
Sleep Duration						CC	CG
	TIM	rs2291739	7.9894	**0.0184**	CG	**0.0224**	NA
					GG	0.5760	**0.0224**
						CC	CT
		rs228727	6.1370	**0.0465**	CT	0.6952	NA
					TT	**0.3368**	**0.0363**
						AA	AT
Daytime Dysfunction	*PER3*	rs228642	7.1528	**0.0280**	AT	**0.0218**	NA
					TT	0.2095	0.6554
						CC	CT
		rs10864315	8.2581	**0.0161**	CT	**0.0186**	NA
					TT	0.1095	1.000
						AA	AG
		rs11824092	6.6149	**0.0366**	AG	0.1327	NA
					GG	0.0864	0.1327
Subjective sleep Quality	*ARNTL*					CC	CT
		rs1982350	6.0435	**0.0487**	CT	0.1027	NA
					TT	0.0477	0.3895
						CC	CT
Sleep Disturbances	*ARNTL*	rs11600996	6.2145	**0.0447**	CT	0.5445	NA
					TT	0.4111	**0.0434**

Kruskal-Wallis test, significant p value – in bold

Table VI. Significant results of MDR analyzis

PSQI	SNP1_PER3	SNP_CLOCK	beta H	WH	beta L	WL	W_{max}	Perm, P
Sleep duration	rs2640909	rs11932595	2.434	7.230	–1.955	9.470	9.470	**0.0123**
Habitual sleep efficiency	rs2640909	rs11932595	2.250	9.453	–2.250	9.453	9.453	**0.0132**
Subjective sleep quality	rs2640909	rs11932595	1.840	4.610	–1.352	4.932	4.932	**0.0163**

beta H – high risk category, WH – the Wald statistics for the High risk category, beta L – low risk category, WL – the Wald statistics for the Low risk category, W_{max} – the maximum between the two Wald statistics, Perm, P – the adjusted permutation p-value obtained from the permutation distribution of W_{max}, significant p value – in bold

DISCUSSION

Chronotype

Mood disorders are associated with disrupted biological rhythmes and as the result with sleep disturbances. Chronotype allows description of individual preferred activity pattern. The comparison of subjective and objective method of daily activity preferences in a group of healthy subjects, proved that the use of Polish adaptation of the MEQ is a valid method of the chronotype assessment (Pracki et al. 2014). However, we failed to observe any significant differences between patients and controls in the frequency of chronotype regardless of the type of disease, probably due to small sample size. Our results may be due to relatively not enough numerous groups, as well as with the restriction applied a statistical approach. On the other hand, we observed the deteriorating of total sleep quality with shift chronotype towards the evening preference of activity in CG and as MD groups. This is consistent with previous findings and suggests we selected appropriate tool and research direction.

Research provided by National FINRISK Study showed relations between preference for morning activity and lower depressive symptoms (Konttinen et al. 2014). In turn, subsample of outpatients with insomnia, those with morning chronotype showed the greatest reduction of depressive symptoms after a cognitive behavioral therapy (CBT) (Bei et al. 2015).

Research by Merikanto and others (2015) confirmed that the diurnal preference towards evening activity is accompanied by three or more lifetime mental disorders, more sleeping problems, more seasonal variation in mood and behaviour, and more burnout when compared with those with the diurnal preference towards morningness. All researches mentioned above suggest that evening preference may have distinct relationships with the deterioration of mood, while morning chronotype seems to be a protective factor in depression, as well as is associated with a better prognosis.

Sleep variables

Sleep disorders in the course of affective disorders for a long time have been considered as a result of circadian rhythm disturbances. Changes in a sleep pattern, e.g., changes of time or amount of sleep are associated with circadian rhythm. Many researchers have postulated that sleep disorders are the cause and/or trigger of MD. They also indicated that the process can proceed bidirectionally, which means that MD can result in disturbed sleep (Geoffroy et al. 2014, Frank et al. 2008). Therefore, we conducted a detailed analysis of quality sleep variables. We used a self-assessment questionnaire PSQI and ESS with proven utility in routine practice (Boudebesse et al. 2014).

All sleep characteristics were significantly worse in MD patients than controls. Only duration of sleep was comparable in all analyzed groups. Our results are consistent with those presented by Wang and others (2014) and Geoffroy and colegues (2014) for BPD patients, as well as with the results presented by Saunders and others (2015) for UPD patients (Geoffroy et al. 2014, Saunders et al. 2015, Wang et al. 2014).

We also provided a comparison of sleep quality variables between BPD and UPD patients. The sleep latency was the only variable that significantly differentiated the BPD and UPD groups. The waiting time for sleep was longer in patients with UPD. According to our knowledge, there is no such comparison in the literature.

Sleep variables: chronotype and season

We investigated if sleep quality variables are dependent on chronotype or season of fulfilling the questionnaire and discovered that regardless of diagnosis the evening chronotype is connected with the poorer quality of sleep. Similar conclusions were obtained by studying the Haregu and others (2015) who made circadian rhythm, sleep quality and daytime sleepiness characteristics among Thai college students. The General Health Questionnaire-12 (GHQ-12) was used to evaluate the presence of common psychiatric disorders (CPDs). Using logistic regression models, they found a significant association between CPDs and sleep characteristics, such as evening chronotype, poor sleep quality and daytime sleepiness (Haregu et al. 2015). To our knowledge, so far no one has checked whether sleep quality variables depend on the season in which the questionnaire has

been completed. As could be expected in control group sleep quality is better in the summer. Conversely, in patients poor quality of sleep is constant and independent of the season.

Correlation with clinical variables

Based on our previous studies, we selected OPCRIT items describing sleep problems during illness episodes such as: reduced need for sleep, initial insomnia, middle insomnia (broken sleep), early morning waking and excessive sleep (Maciukiewicz et al. 2014). We found no significant effect of gender or age of onset on chronotype or sleep quality. Correlations suggested significant relations between our chronotype (reduced need for sleep, initial insomnia, early morning waking) and sleep quality (course of the disease). We found significant correlations worthy of further analysis, but our results are limited by the size of the analyzed group. Maciukiewicz and others (2014) used sleep disturbances as dimension created form OPCRIT variables such as middle insomnia (broken sleep) and early morning walking. This dimension was used as a quantitative trait in computations. Although the approach used by Maciukiewicz was not possible in the present study (due to the size of the group), current results confirmed a direct link between the quality of sleep before a disease and sleep disorders in the course of the disease. We did not observed significant correlation between initial insomnia variable from OPRRIT check list and PSQI or EES variables. The reason for this result may be that the initial insomnia is a symptom heralding a relapse and does not relate to the period of remission described in self-assessment questionnaire.

Chronotype, sleep quality variables and genes

The present study is the first association study of chronotype with such a wide range of "clock genes" and their polymorphisms.

The significant nominal association was observed in the case of 2 polymorphisms of *ARNTL* gene (rs11824092 and rs1481892) and one polymorphism of the *CLOCK* gene (rs1268271) with chronotype in control case. No statistically significant association in MD patients was observed.

The present study suggests a putative role of the *CLOCK* and *ARNTL* polymorphisms in the determination of chronotype in control group. The lack of significant association with chronotype in MD patients may results from masking effect due to significant association of "clock gene" polymorphisms with MD demonstrated in earlier studies (Dmitrzak-Weglarz et al. 2015, Maciukiewicz et al. 2014). We noticed mainly effect of between variable number tandem-repeat (VNTR) in the *PERIOD3* gene (*PER3*, rs57875989) and chronotype. Previous studies suggested association between the longer *PER3*(5) allele and diurnal preference, while shorter *PER3*(4) allele is linked with preference for evening, respectively (Archer et al. 2003, Dijk and Archer 2010, Ebisawa et al. 2001, Nievergelt et al. 2006, Pereira et al. 2005). Other study conducted on Chinese and Norwegian populations indicated lack of association between the *PER3* clock gene and chronotype. It suggests, that the proposed role of *PER3* needs further clarification, especially in context of ethnic differences (An et al. 2014, Osland et al. 2011).

Very few association studies of clock gene polymorphisms and the sleep quality variables became a contribution to carrying out this study. We observed significant nominal association only in patients group in cases as follows: sleep quality with *ARNTL* (rs11824092, rs1982350); sleep duration with *CLOCK* (rs3805148) and *TIM* (rs2291739); daytime dysfunction with *PER3* (rs228727, rs228642, rs10864315); sleep disturbances with *ARNTL* (rs11600996).

The present study suggests that "clock genes" may be involved in disturbances in sleep, observed in the course of MD. Moreover, PSQI variables can be used to refine phenotype in association studies of clock genes in MD. Parsons and others (2014) found a significant association between guanine nucleotide binding protein beta 3 (*GNβ3*) (rs5443) and global sleep quality, & between a rare polymorphism in period homologue 2 (*PER2*) (rs2304672) and both sleep duration and quality measured by PSQI. The differences in our and mentioned above study may arise from:

1) different groups of subjects (MD patients in our study vs. healthy young adults aged 18–27),

2) different sets of tested genes and their polymorphisms (42 polymorphism from 4: *CLOCK*, *ARNTL*, *TIMELESS* and *PER3* genes *vs.* 10 polymorphisms from 8: *ARNTL2*, *CLOCK*, *DBP*, *PER1*, *PER2*, *PER3*, *FBXL3*, *GNβ3*),

3) the results obtained in different models (additive models of inheritance – justified in complex diseases vs. recessive model).

Despite significant methodological differences in both studies, common denominator is that findings suggest that genes with circadian expression may play a role in regulating both the circadian clock and sleep homeostasis. It also highlights the importance of further studies aimed at dissecting the specific roles that circadian genes play in these two interrelated but unique behaviors in control group, as well as in patients with MD.

Finally, we looked for any epistatic interactions between circadian gene variants and chronotype, as well as with sleep quality variables. Rs2640909 of *PER3* gene and rs11932595 of *CLOCK* gene produced a significant model when we searched a relation to sleep duration, habitual sleep efficiency and sleep quality in MD patients only. No significant interactions were observed in controls. The results again indicated the relationship of "clock gene" with the PSQI variables in MD patients with poor quality of sleep regardless of disease status.

Limitations

A fundamental limitation of the present study is the analyzed sample size. While number of fulfilled surveys is satisfactory in relation to publications mentioned above, it is still too low for association studies. Therefore, we failed to carry out more advanced and comprehensive calculation methods, e.g. data mining. The lack of using objective methods to assessing chronotype and quality of sleep, as well as the number of selected polymorphisms also may be the limitation.

CONCLUSIONS

The present study suggests a putative role of the *CLOCK* and *ARNTL* polymorphisms in chronotype determination in control group. The lack of significant association with chronotype in MD patients may results from masking effect due to significant association of "clock gene" polymorphisms with MD demonstrated in earlier studies.

Obtained results confirms role of the analyzed "clock genes" polymorphism in sleep quality disturbances in the course of MD. The results indicate that PSQI variables can be used to refine phenotype in association studies of clock genes in mood disorders.

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