THE SAHLGRENSKA ACADEMY

Connection between seasonality in hospitalizations and sleeping traits in bipolar disorder

Degree project in Medicine

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Program in Medicine

Gothenburg, Sweden 2021

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ABBREVIATIONS

BD: Bipolar Disorder

GWAS: Genome Wide Association Study

NPR: National Patient Register

SNP: Single Nucleotide Polymorphism

PRS: Polygenic Risk Score

OR: Odds Ratio

SCN: Suprachiasmatic Nucleus

SWEBIC-1: Swedish Bipolar Cohort Collection 1

SBP: St. Göran Bipolar Project

NPÖ: Nationell patientöversikt

CI: Confidence interval

CV: Coefficient of variation

BipoläR: Swedish National Quality Registry for Bipolar Disorder

ABSTRACT

Title: Connection between seasonality in hospitalizations and sleeping traits in bipolar disorder.

Master thesis, Program in Medicine, Sahlgrenska Academy at the University of Gothenburg, Sweden, 2021.

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Background: Bipolar disorder (=BD) is a relatively common mood disorder which is characterized by episodes of depression and mania/hypomania. These episodes are not evenly distributed along the year but show a seasonal trend: there are more manic episodes during spring and summer and more depressive episodes during autumn and winter. This phenomenon is called seasonality. Leading theory explaining this phenomenon is so called light hypothesis; amount of sunlight through the year affect mood of BD patients and therefore acts a episode provoking factor. Additionally genetic predisposition to BD is considered high (73-93%) but exact genes and mechanism is widely unknown. It has been previously pointed out that sleep traits differ between normal population and those with BD. For example ”night owl” -chronotype, hypersomnia and insomnia are more common with those with BD. Whether this has to do with lifestyle or has a connection to genetic factors is unknown. It has been hypothesized that society living on 8-16 schedule is harmful to the ”night owl” chronotype and therefore could be BD provoking factor, on the other hand there is a speculation that same genes that determinate chronotype and other sleeping traits also play a role with predisposition to BD. Additionally previous studies have pointed out that one mechanism for developing BD is malfunction of clock genes that control our circadian rhythm, both on daily- and seasonal level. Most important of these genes are PER- and CRY-gene families. Furthermore, one of the most potent drug against BD is lithium that affects expression of the exact same clock genes. This leads to hypothesis that there could be same genetic factors that contribute to both seasonality and sleeping traits.

Aims: Purpose of this master thesis work is to further clarify the role of chronotype and other sleeping traits in BD. More specific our aim was to study connection between genetic predisposition to following sleeping traits: chronotype, daytime sleepiness, sleep duration and insomnia and their connection to seasonality in BD. Furthermore this thesis focuses on trying to understand genetic predisposition to BD itself and it most distinguish subtypes BD-I and BD-II.

Methods: Genetic patient data for this study has been provided by Swedish Bipolar Collection (SWEBIC 2009-2013). SWEBIC consists of data of over 6000 Swedish BD patients. Data includes both information of patient's hospitalization history and genetic information in form of GWAS (genome wide association study). We used statistical programming software R to generate phenotype using clinical data from NPR (National Patient Register) to represent seasonality in hospitalizations among BD patients. As a result we got a specific value that describes seasonality for every individual in our database that fulfills inclusion criteria of our study. Our geneticist created PRS's (polygenic risk score) for every individual in our database that describes individual's genetic predisposition to sleeping traits we are examining: chronotype, sleep duration, daytime sleepiness and insomnia. Lastly, we calculated OR (Odds Ratio) comparing correlation between our phenotype and all of our genotypes separately using linear regression analysis.

Results: Our preliminary findings suggest that there is slight, yet statistic significant connection between seasonality in hospitalizations and both daytime sleepiness and sleep duration genotypes. On the other hand, we could not find statistic relevant connection between seasonality of hospitalizations and two other genotypes, chronotype and insomnia.

Conclusions: Our results suggest that among BD patients chronotype and other sleeping traits do play a role in the disease and its role should be further investigated.

Keywords: bipolar disease, circadian rhythm, sleep, seasonality, chronotype, daytime sleepiness, sleep duration, insomnia.

BACKGROUND

What is bipolar disorder, and why is it important?

Bipolar disorder (=BD) is a relatively common chronic mood disorder which is characterized by cyclic periods of depression and mania/hypomania. Mean onset age for BD is approximately 20 years. Lifetime prevalence of BD in population is estimated to be around 1-2% globally (1). This estimation includes BD subtypes I and II, whereas other prevalence studies that report lifetime prevalence as high as 5% usually also include so called bipolar subthresholds. Two most distinguish subtypes of BD are subtypes I and II. Difference between subtypes is that type I is consistent with manic- and depressive episodes whereas type II consists of hypomanic- and depressive episodes. Other BD related affective diseases are for example cyclothymia and schizoaffective disease.

Manic episodes are characterized by elevated/irritable mood, increased energy and psychomotoric activity, decreased need of sleep, impulsive behavior, fast and abundant speech and numerous goal-oriented ideas. In severe manic episodes even psychotic symptoms may arise, grandiosity being common characteristic. If untreated, manic episodes usually last between two weeks to five months. Hypomania can be seen as a milder version of mania; patient's capacity of everyday activities remains manageable although decreased compared to baseline levels, no psychotic symptoms arises and these episodes rarely demand hospitalizations.

Depressive episodes are characterized by decreased mood, persistent sadness, lack of interest in activities that previously were experienced as interesting, sleep disturbation, feelings of self-worthlessness or even -loathing, thoughts about death/suicide and decreased will to live. Depressive episodes usually last about six months, rarely longer than a year.

Ratio between manic- and depressive episodes is considered to be close to 1:1 but because of the considerably longer duration of depressive episodes patients usually spend much more time being depressive compared to manic (2). Between manic- and episodes patients are symptom free which is also called euthymia.

Moreover, there are mixed affective episodes in which patient experiences both manic- and depressive symptoms simultaneously.

Bipolar disorder has a significant negative impact on patient's life. Patients suffer of several comorbidities, both somatic and psychiatric. In fact WHO has listed BD as one of ten most disabling diseases globally counted on DALY-scale (3). Interesting observation is that according to WHO, BD is even more disabling than schizophrenia. Somatic comorbidities consist of for example metabolic syndrome, obesity and diabetes type II. When it comes to psychiatric comorbidities, for example anxiety disorder, personality disorder, substance abuse and higher suicide rate are these more frequent with bipolar patients compared to normal population (4, 5).

As a big picture bipolar disorder causes considerable suffering as a both somatic and psychiatric comorbidities both also as social factors such as lower income, higher level of unemployment and higher involvement in criminal activities. On top of that cost of the disease are rather high to society in a form of absent work effort and health care costs.

What are the mechanisms behind the disease?

Pathophysiology behind BD has been a mystery to psychiatrists and neuroscientists for a long time. Recent decades have brought us information on various alterations in BD patients compared to normal population, yet exact pathophysiology beyond disorder is not exactly clear to us. Leading theory beyond pathophysiology in BD, proposed by Strakowski et. al, is dysfunction of visceromotor network in the brain consisting of anterior cingulate cortex, orbitofrontal cortex, prefrontal cortex, hippocampus, amygdala, hypothalamus, striatum and thalamus (6).

Neuroimaging has consistently shown that BD patients have abnormalities in both grey- and white matter brain areas compared to normal population. When grey matter is concerned; abnormalities usually appear later on in the course of disease and seem to correlate with duration of illness and level of cognitive deterioration (7). When it comes to white matter abnormalities, lesser volume of white matter seems to be linked even to the first BD episodes, thus suggesting this could have something to do with onset of the disease (8). Another observation when white matter is considered is the fact that BD patients have increased hyperintensities of white matter which could play a role in reduced connectivity between brain regions (8, 9). Most significant brain areas having these white matter

abnormalities are cingulum, corpus callosum, frontal areas, parahippocampal areas and uncinate fasciculus and fornix which are tracts connecting the limbic system (10). These observations would make logically sense, since above mentioned brain areas are important in emotional processing. Furthermore, observations of increased size of amygdala and increased activity in the HPA-axel promote significance of limbic system in BD (11).

Alterations in the function of immunological responses have been consistently observed in active episodes (i.e. manic- or depressive episode) of BD. Especially pro-inflammatory cytokines, for example IL-6, TNF-α, IFN-γ and CRP seem to be upregulated during both manic and depressive episodes (12) and to normalize or significantly decrease again in euthymic state. This suggests connection between BD and activation of immune system.

Genetic predisposition and role of environmental factors?

Genetic predisposition to BD is considered high (73-93%) but exact genes and mechanism are widely unknown. Furthermore concordance rate for identical twins is significantly higher (39-43%) compared to heterozygotic twins (5-6%) (13). It has been well studied that BD in family significantly increases risk for disorder. Swedish family study showed that risk of BD was 7,7-, 3,3 and 1,6-times higher for those with first-, second- respectively third-degree relatives with BD (14). Moreover familial BD increases also risk for schizophrenia and major depression and vice versa (15) which implies common genetic predisposition to these diseases.

Genome Wide Array Studies (GWAS) have observed several Single Nucleotide Polymorphism (SNP) of which most important are CACNA1C, ODZ4 and TRANK1 (16). Consensus around BD's genetic predisposition is that it is not one single gene that causes the disease but many genes that together cause risk for onset of the disease. In combined GWAS analyses there are 30 different loci, mostly associated with ion-channels, neurotransmission and neural development, with significantly associated with BD.

Circadian rhythm and BD

Previous clinical studies have revealed that BD patients suffer from disruptions in seasonal- and circadian rhythms even between active episodes and that intensity of sunlight would be influential factor to BD (17). Circadian rhythm is a natural internal

process that synchronizes us to both 24h cycle and seasonal cycle throughout the year. Purpose of circadian rhythm is to regulate our 24h sleep-wake cycle by controlling for example, body temperature, alertness and hormone production. Circadian rhythm is mainly controlled endogenously, but is also affected by external stimuli, so called zeitgebers. Primary circadian clock is in humans located in suprachiasmatic nucleus (SCN). Correct function of our circadian clock is ensured by so called clock genes which are expressed in SCN. Of these clock genes the most important gene families are: PER-, CRY- and Clock gene families. Our genetic predisposition for these clock genes determines for example our chronotype. Furthermore, misfunction in these clock genes can cause problems in maintaining correct circadian rhythm, for example in familial advanced sleep phase syndrome which is associated to a nonsense mutation in PER2.

When it comes to disturbances of circadian rhythm in BD one of the most consistent observations is that melatonin production significantly decreases not only in active episodes but euthymia as well (17). There are observed abnormalities even in cortisone response and levels. Importance of circadian rhythm's role in BD is furthermore assured by the fact that one of the most common drug used in treating BD, lithium, has influence on the expression of clock genes that regulate circadian rhythm and ability to normalize circadian rhythm abnormalities with lithium-responding patients (18). Despite these notable associations between BD and circadian rhythm, GWAS studies have not been able to find association between clock genes and BD.

Seasonality and BD

Even more evidence of disrupted seasonal rhythm in BD is provided by the fact that hospitalizations of BD patients are unevenly distributed through year. In general, trend is that manic episodes peak during spring and early summer months whereas depressive episodes peak during autumn and winter months (19). In systematic reviews females show slightly higher tendency for seasonality in their seasonality in both manic and depressive episodes compared to males. There is a significant connection between amount of sunlight and peaks of affective episodes. Is has been hypothesized that increased amount of sunlight would be mania provoking factor in BD whereas decreasing amount depression provoking factor. This hypothesis is further backed up by the fact that amount of increase in sunlight is not even between days but has its peak at spring equinox (between 19-21.3 yearly), respectively most rapid decreasing around autumn equinox (between 22-23.9

yearly), which is right before highest peaks of hospitalization for mania respectively depression. Naturally change in sunlight exposure varies depending on latitude, as changes in northern latitudes are much stronger compared to those near equator. Furthermore evidence that fluctuation of sunlight has an impact to BD is provided by previous studies that have founded that the larger the increase in amount of sunlight the younger the age of BD onset will be (20). The difference in BD onset between sites nearest the equator and the sites furthest away from it ie. fluctuation in sunlight was according to the study almost 5 years. This observation also suggest that circadian rhythm plays a role in BD's pathophysiology.

What is chronotype's and other sleeping traits role in BD

Obvious example of sleep's role in BD comes from decreased need of sleep during manic episodes and even as a prodromal symptom of them. Even depressive episodes are often coupled with either insomnia or hypersomnia and often with daytime sleepiness (21). Above mentioned three sleeping traits are even more common in BD patients compared to normal population between episodes (22). As mentioned before, circadian rhythm is a major factor in controlling our sleep. Therefore, there has been a lot of research focusing on sleep of BD patients.

Definition of chronotype is: preference of the individual in which to carry out activities rather than resting or sleeping. i.e. in which period of the day individual prefers to sleep. Chronotype is usually divided in three main categories: individuals who prefer morning activities and wake up early, also referred as "morning lark" chronotype, individuals who prefer evening activities and therefore go to sleep later, also referred as "night owl" chronotype and individuals who have no preference and whose sleep pattern is something in between of the two others. It has been previously pointed out that people of "night owl" -chronotype suffer more often of BD compared to "morning lark" chronotype (23). Whether this has to do with lifestyle of connection genetic factors is unknown. It has been hypothesized that society living on 8-16 schedule is harmful to the "night owl" chronotype and therefore could be BD provoking factor, on the other hand there is a speculation that same genes that determinate chronotype also play a role with BD.

There has been previous GWAS investigating if BD patients have genetic predisposition to above mentioned sleep traits (24). Study was conducted in the same SWEBIC cohort this master thesis is using and found correlation between BD and PRS for night owl-

chronotype, daytime sleepiness and sleep duration. Same study did stratify between BD-I and -II and found significant differences between the two, therefore supporting hypothesis of heterogenicity between these two subtypes.

AIM OF THE STUDY

Specific aims of this thesis

The primary aim of this study is to find out if there is a connection between seasonality of hospitalization in BD episodes i.e. our phenotype and genetic predisposition to four following sleep traits: chronotype, insomnia, sleep duration and daytime sleepiness i.e. our genotype.
Secondary aim is to find out if there are any distinguish subgroups, such as BD subtype I and II, age and sex, that would have greater connection between phenotype and genotype. The purpose is to further investigate connection between BD, circadian rhythm and sleep and therefore help us to understand heterogenic nature of BD better. Such knowledge may help us to offer more individual treating methods for BD patients in the future.

MATERIAL AND METHODS

Study design and participants

This study is a retrospective cohort study. Cohort that was used retrieve the patient data was SWEBIC-1.

Swebic-1

Swedish Bipolar Cohort Collection 1 (SWEBIC-1) is a national cohort consisting of 12680 individuals, of which 5899 individuals with diagnosed BD and 6781 controls, conducted in Sweden between 2009 and 2013. Most of the patients were recruited out of Swedish National Quality Registry for Bipolar Disorder (BipoläR)(25). Registry contains following ICD-10 diagnosis: F25.0 (schizoaffective disorder, manic type), F30.1 – F 30.2

(mania with or without psychotic symptoms), F30.8 – F30.9 (other manic episodes and unspecified manic episodes), F31.0 – F31.9 (any type of bipolar affective disorder) and F34.0 (cyclothymia). In addition, 702 individuals, of which 514 with diagnosed BD and 188 controls, were recruited out of St. Göran Bipolar Project (SBP). SBP is a previous longitudinal study consisting of patients with diagnosed BD from Stockholm and Gothenburg areas. Rest of the 7185 individuals, of which 592 with BD and 6593 controls, were recruited from the National Patient Registry (NPR) or Statistics in Sweden. Individuals who fulfilled above mentioned criteria received a letter followed by a letter asking their wish to join SWEBIC cohort. Those who wished to participate signed a written informed consent. Afterwards participants participated in interviews and gave blood samples for later analysis.

Above mentioned patient data was linked to NPR using Swedish national registration numbers (personnummer). NPR data consist of all inpatient admission between 1973 and 2016 in Sweden, providing admission length, date, primary diagnosis (either ICD-8, -9 or -10) and secondary diagnoses.

Seasonality of hospitalizations, definition of our phenotype

To generate a phenotype to describe seasonality of hospitalizations we had to define season first. We decided to use so called Rosenthal's criteria to define season, i.e.. date of admission to hospital decided season of affective episode. Rosenthal's criteria divides year in to four quarters by months (26). Season in this study is therefore defined as follows: winter (from beginning of December to end of February), spring (from beginning of March to end of May), summer (from beginning of June to end of August) and autumn (from beginning of September to end of November). Rosenthal's criteria is also consequent with DSM-IV criteria of seasonality. Notable with this definition is the fact that it is not completely synchronized with amount of sunlight and changes in amount of sunlight. For example, darkest time of the year is approximately between 6-7.11 and 5-6.2 which makes a difference of 24-25 days between our definition and photoperiodic year. Reasoning for this comes from a fact that sunlight's effect on affect is not instant, but takes time to develop. Therefore Rosenthal's criteria is to estimated as a applicable measure of seasonality since both development of a affective episode and delay between onset and admission to hospital balances above mentioned difference.

As a measure of affective episode, we used hospitalization to general adult psychiatric ward. Psychiatric ward was in our data defined as hospitalization to general psychiatric health care unit for adults, which in NPR corresponds to Nationell patientöversikt (NPÖ) code 901. Notable with this choice of measure is that it does not take account the primary diagnosis of hospitalization. However, hospitalization in other psychiatric health care units such as addiction psychiatry was not included in our measure.

To avoid inclusion of other reasons than affective episodes in our study data we excluded all hospitalizations that were shorter than two days, i.e. hospitalizations with admission and discharge during the same day were excluded from this study.

To able to trustworthy evaluate seasonality of hospitalizations we decided to only include individuals with at least 3 hospitalizations in psychiatric ward.

Lastly, to avoid same affective episode to be counted as two or several hospitalizations we considered rehospitalization within eight weeks from discharge as a relapse of a previous affective episode, which is the time previous research suggest as a appropriate estimation in similar studies (27). Therefore we included hospitalizations with above mentioned criteria a one hospitalization.

Therefore our inclusion criteria is as follows: at least 3 hospitalizations to a psychiatric ward longer than 1 day, relapse hospitalizations excluded. Out of 12680 individuals in SWEBIC-1, 3104 fulfilled our inclusion criteria (Figure 1). Based on this patient data we generated an individual measurement to describe seasonality for every individual who fulfilled our inclusion criteria. This measure i.e. our phenotype was calculated as a coefficient of variation (CV) of season in hospitalizations that fulfilled inclusion criteria. The lower the CV of season, the higher individual's seasonality in hospitalizations and vice versa. Therefore individuals with higher CV of season show less seasonality in their hospitalizations.

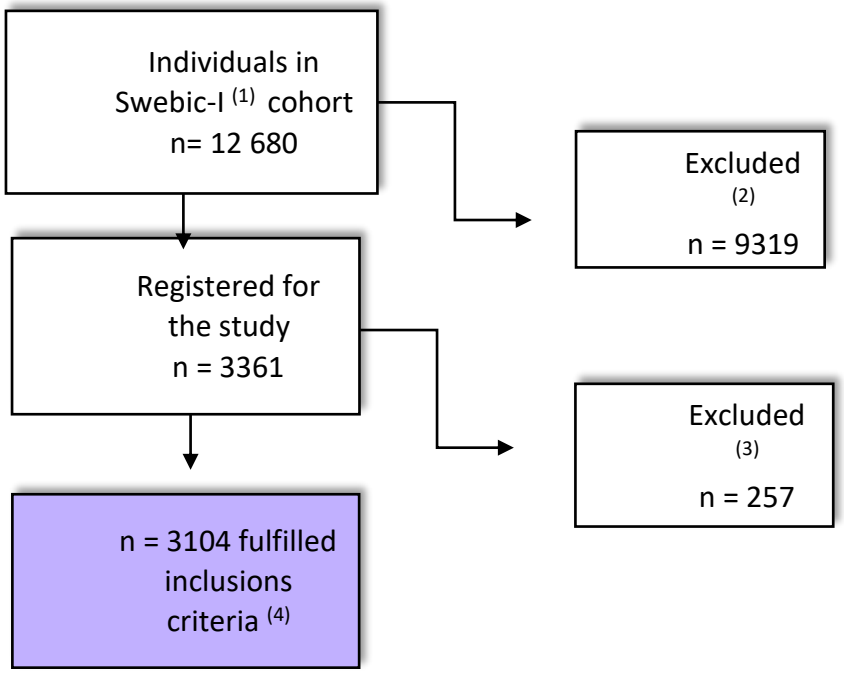

Figure 1. Flow chart of recruitment, excluded and included patients

(1) Swebic I = The Swedish Bipolar Collection I, The database of Swedish patients with bipolar disorder.
(2) 9319 patients excluded because of patients with <3 psychiatric hospitalizations and <1 day hospitalization
(3) Furthermore 257 patients excluded because of time between psychiatric hospitalizations ≤56 days decreased their number of hospitalizations below 3.
(4) 3104 patients fulfilled the inclusions criteria:
- ≥3 psychiatric hospitalizations and lasting at least 1 day
- Time between psychiatric hospitalizations > 56 days

PRS for sleeping traits, our genotype

Genetic data was collected as a part of SWEBIC-1. Blood samples from participants were taken at the Karolinska Institutet Biobank in Stockholm. Genotyping of genetic data was conducted in three waves at the Broad Institute of Harvard and MIT using Affymetric 6.0, Illumina OmniExpress chips and the Infinium PsychArray-24. Quality control of both samples and analysis was performed as appropriate (28). Polygenic risk scores (PRS) were calculated by our geneticist using previous discovery GWAS for our sleeping traits of interest (29-32): morningness, sleep duration, daytime sleepiness and insomnia . As a result, we received individual PRS for each of our study individual representing each of our genotypes containing ten different thresholds for SNP's (Single Nucleotide Polymorphism). Threshold means statistical limit when a certain SNP is included in the PRS. Each SNP has a certain effect on PRS. The higher threshold was, the less SNP's

included in PRS and vice versa. Therefore PRS with highest threshold has less SNP's compared with lower threshold and higher statistical certainty that they have effect on desired genotype.
In this study we are using morningness as a proxy for chronotype. Morningness is defined as a trait to prefer activities early in the 24 hour rhythm and early wake-up times corresponding to morning lark chronotype. Sleep duration is used as a proxy to hypersomnia since longer sleep periods than eight to nine hours a day can be seen pathological and are uncommon in normal population.

Statistical analysis

All statistical analysis was performed using custom made code in R version 4.1.1 (R foundation for statistical computing). Each study participant was assigned with a number (LöpNr) which allowed us to combine out phenotype with our genotype i.e. coefficient of variation of season in hospitalizations with individual PRS for each sleeping trait.
We used linear regression analysis to calculate odds ratio (OR) for every genotype using all 10 different thresholds for each PRS. We used phenotype as a dependent variable in the equation. Genotype was used as an explanatory variable. All analyses were adjusted by age, sex, amount of hospitalizations, platform (type of array chip that was used in genotyping analysis) and 10 population principal components. P-values lower than 0.05 or confidence interval (CI) not including 1.00 was regarded as statistically significant.

Ethics

Individuals Regional Ethical Review Board of Stockholm (2008/2009-31/2) approved SWEBIC-1 cohort. All participants were provided written informed consent. Data for each participant has been masked in a way that access code is needed to identify any specific participant.

RESULTS

Descriptive statistics

In total 3104 individuals fulfilled our inclusion criteria. The mean age for all participants was 33,38 years (range from 14 to 76 years). The mean age is counted as the age patient had their first admission to general adult psychiatric ward. The sex distribution among all participants was 62,7% females (1946/3104) and 37,3% males (1158/3104). The mean amount of hospitalizations to general adult psychiatric ward for participants was 8,62 (range from 3-76). Mean coefficient of variation was 0,446 (range from 0 to 0,866).

Table 1. Baseline characteristics of the study participants.

Basic characteristics	**Total** (n=3104)
Age (years), mean	33.38 (4.32)
Range (years)min; max	14-76
Sex	
Female	1946 (62.7%)
Male	1158 (37.3%)
Hospitalizations	
N, mean	8,62 (94.7%)
Range	3-76 (1.6%)
CV (season)[(1)]	18 (3.7%)
Coefficient of variation (season)	
Mean	1,271
Range	0-0.866
BD subtype	21 (4.3%)
Type I (n, %)	645 (20.8%)
Type II (n, %)	436 (14.07%)
NA (n, %)	1747(56.3%)

[(1)] Coefficient of variation of season of individuals based on an admission date to general adult psychiatric ward i.e. measure of seasonality in our study.

According to our data 20,8% (645/3104) of cohort had diagnosed BD-I, 14,0% (436/3104) had BD-II and with 56,3% (1747/3104) participants diagnosis was unknown (Table 1). Notable with these diagnoses are that our data was incomplete regarding them.

Total amount of hospitalization with individuals that upfilled out inclusion criteria was 35380 (Figure 2). As seen in the Figure 2, these hospitalizations follow previously observed trend with peaks of admissions in spring months (March, April and May) respectively autumn months (September and October). Note also that common observation i.e. systematic bias in December's admissions caused by holiday season is present with our data as well.

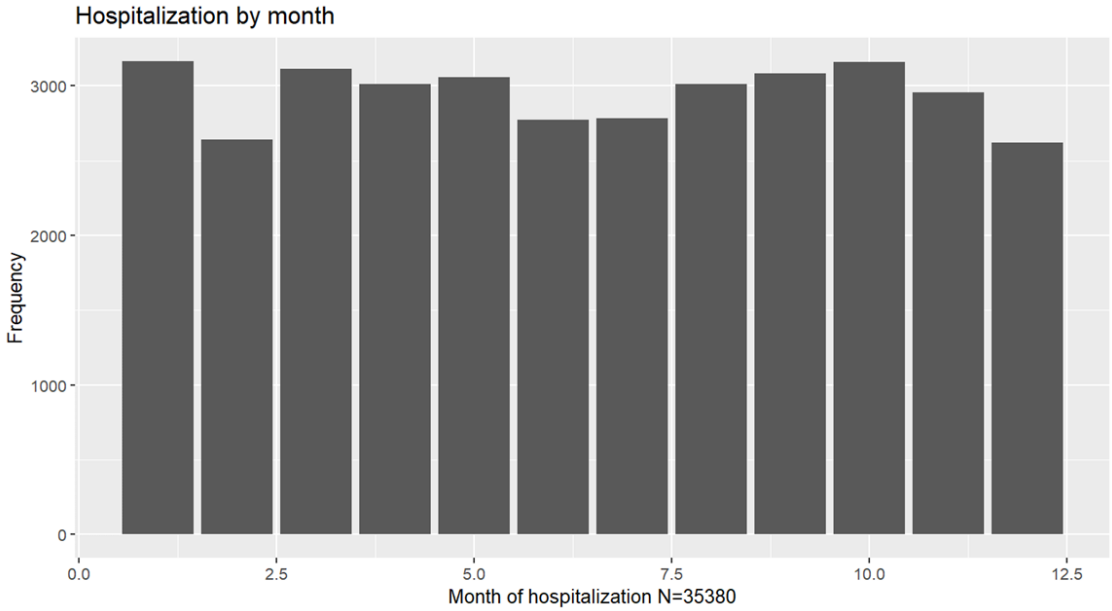

Figure 2. A bar plot showing all hospitalizations included in this by season. Frequency per given month on y-axis and month from January to December on x-axis.

Correlations between phenotype and genotype

Linear regression comparing individuals that showed low seasonality in their hospitalization respectively high seasonality revealed us that there statistically significant correlation only between seasonality and daytime sleepiness with two distinct thresholds. (OR 0,991 95% CI 0,985-0,987 with PRS pT < 5e8) and (OR 0,994 95% CI 0,988-0.999

with PRS pT <1e6). In this case OR below one suggest that individuals that show more seasonality in their hospitalizations have increased genetic predisposition to genotype in question. Other genotypes showed no statistically significant correlations. Following results are summarized by using thresholds giving most significant results and commenting overall trend of the data. All OR's are visible on Figure 3 and are reported on Table 2, -3, -4 respectively -5.

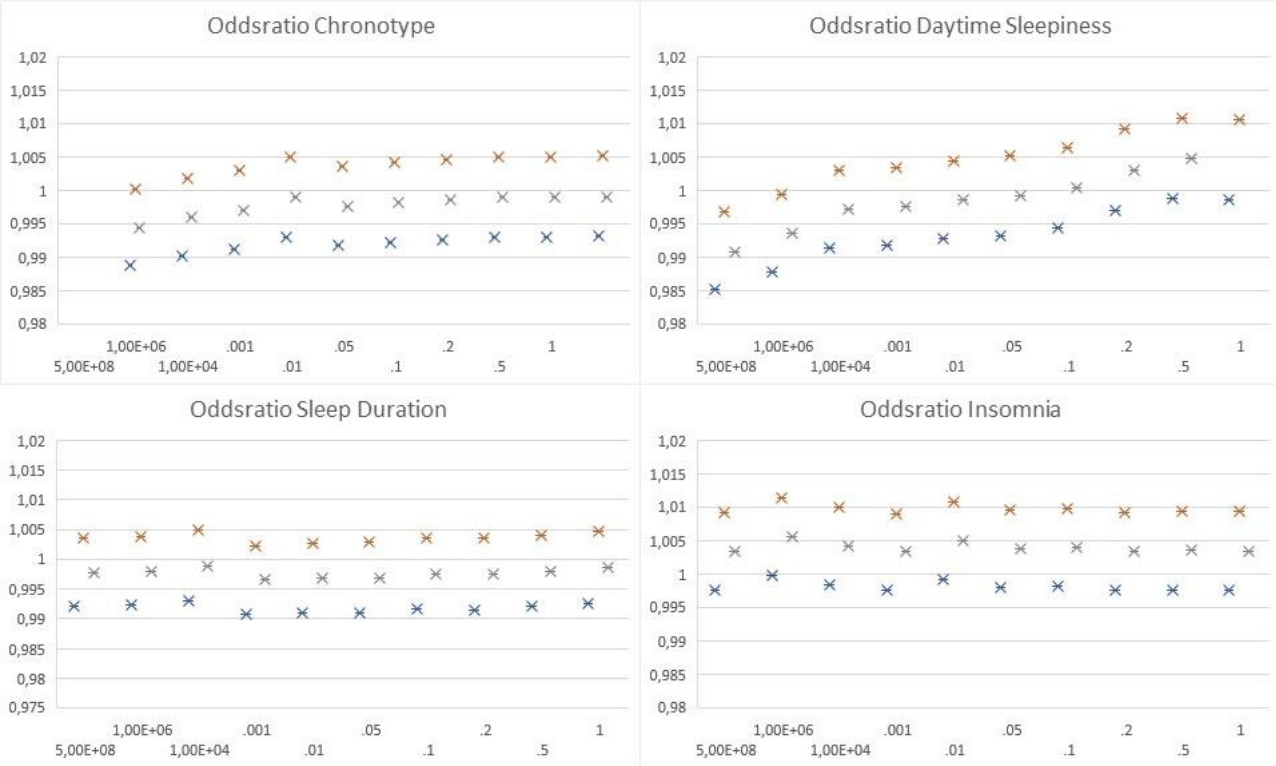

Figure 3. Odds ratio for regressions between polygenic risk scores for sleeping traits and study participants with low seasonality compared to high seasonality in their hospitalizations. Each separate figure has OR an y-axis and PRS threshold from lowest to highest on x-axis. Red crosses represent higher value of 95% CI whereas blue crosses lower value. Grey crosses represent actual OR.

Chronotype PRS and seasonality

No statistically significant correlation between chronotype and seasonality was found in this study. As seen in Table 2, closest threshold to significancy was one the one with highest threshold 5e8 (OR 0,994 95% CI 0,989-1,000 p-value 0,057). Since PRS was calculated as genetic predisposition to morningness, OR below one here suggests that individuals that show more seasonality have increased genetic predisposition to morningness i.e. are more inclined to morning lark chronotype. Also as seen in figure 3 and Table 2, OR shows an increasing trend as a threshold increases which could indicate

that the SNP's that have the highest effect on genotype considered have also strongest effect on seasonality.

Table 2. Regressions between polygenic risk scores for morningness and study participants with low seasonality compared to high seasonality in their hospitalizations

PRS P_T	OR	95% CI	P-value
p ≤ 1	0.999	0.993-1.005	0.769
p ≤ .5	0.999	0.993-1.005	0.745
p ≤ .2	0.999	0.993-1.005	0.730
p ≤ .1	0.999	0.993-1.005	0.661
p ≤ .05	0.998	0.992-1.004	0.540
p ≤ .01	0.998	0.992-1.004	0.423
p ≤ .001	0.999	0.993-1.005	0.728
p ≤ 1e4	0.997	0.991-1.003	0.321
p ≤ 1e6	0.996	0.990-1.002	0.185
p ≤ 5e8	0.994	0.989-1.000	0.057

Analyses controlling for age, sex, amount of hospitalizations, platform (type of array chip that was used in genotyping analysis) and 10 principal components. PRS pT = *P*-value threshold applied to discovery genome-wide association study in order to construct polygenic risk scores, OR = odds ratio, 95% CI = 95% Confidence Interval.

Daytime sleepiness PRS and seasonality

Statistically significant correlation was found between seasonality and daytime sleepiness with two distinct thresholds. (OR 0,991 95% CI 0,985-0,987 p-value 0,002 with PRS P_T < 5e8) and (OR 0,994 95% CI 0,988-0.999 p-value 0,031 with PRS P_T <1e6). In other words, those with higher tendency to seasonality have increased genetic predisposition to daytime sleepiness. Here as well these two thresholds were the ones with highest p-values meaning that SNP's that are responsible for strongest effect on considered genotype also have strongest correlation to seasonality. As seen in Figure 3 and Table 3 OR shows an increasing trend when threshold increases which supports above mentioned observation.

Table 3. Regressions between polygenic risk scores for daytime sleepiness and study participants with low seasonality compared to high seasonality in their hospitalizations

PRS P_T	OR	95% CI	P-value
p ≤ 1	1.005	0.999-1.011	0.134
p ≤ .5	1.005	0.999-1.011	0.116
p ≤ .2	1.003	0.997-1.009	0.318
p ≤ .1	1.000	0.994-1.006	0.902
p ≤ .05	0.999	0.993-1.005	0.798
p ≤ .01	0.999	0.993-1.004	0.627
p ≤ .001	0.998	0.992-1.003	0.419
p ≤ 1e4	0.997	0.991-1.003	0.352
p ≤ 1e6	0.994	0.988-0.999	0.031
p ≤ 5e8	0.991	0.985-0.997	0.002

Analyses controlling for age, sex, amount of hospitalizations, platform (type of array chip that was used in genotyping analysis) and 10 principal components. PRS pT = *P*-value threshold applied to discovery genome-wide association study in order to construct polygenic risk scores, OR = odds ratio, 95% CI = 95% Confidence Interval.

Sleep duration PRS and seasonality

No statistically significant correlation between sleep duration and seasonality was found in this study. As seen in Table 4, closest threshold to significancy was threshold .001 (OR 0,997 95% CI 0,991-1,002 p-value 0,240). No clear trend is observable between thresholds.

Table 4. Regressions between polygenic risk scores for sleep duration and study participants with low seasonality compared to high seasonality in their hospitalizations

PRS P_T	OR	95% CI	P-value
p ≤ 1	0.999	0.993-1.005	0.643
p ≤ .5	0.998	0.992-1.004	0.513
p ≤ .2	0.997	0.992-1.003	0.408
p ≤ .1	0.998	0.992-1.004	0.421
p ≤ .05	0.997	0.991-1.003	0.308
p ≤ .01	0.997	0.991-1.003	0.281
p ≤ .001	0.997	0.991-1.002	0.240
p ≤ 1e4	0.999	0.993-1.005	0.716
p ≤ 1e6	0.998	0.992-1.004	0.495
p ≤ 5e8	0.998	0.992-1.004	0.448

Analyses controlling for age, sex, amount of hospitalizations, platform (type of array chip that was used in genotyping analysis) and 10 principal components. PRS pT = *P*-value threshold applied to discovery genome-wide association study in order to construct polygenic risk scores, OR = odds ratio, 95% CI = 95% Confidence Interval.

Insomnia PRS and seasonality

No statistically significant correlation between insomnia and seasonality was found in this study. As seen in Table 5, closest thresholds to significancy were ones with thresholds 1e6 (OR 1,006 95% CI 1,000-1,006 p-value 0,060 with PRS P_T 1e6) respectively .01 (OR 1,005 95% CI 0,999-1,011 p-value 0,092 with PRS P_T .01). No clear trend is observable between the thresholds.

Table 5. Regressions between polygenic risk scores for insomnia and student participants low low seasonality compared to high seasonality in their hospitalizations

PRS P_T	OR	95% CI	P-value
p ≤ 1	1.003	0.998-1.009	0.250
p ≤ .5	1.004	0.998-1.009	0.243
p ≤ .2	1.003	0.998-1.009	0.255
p ≤ .1	1.004	0.998-1.010	0.183
p ≤ .05	1.004	0.998-1.010	0.202
p ≤ .01	1.005	0.999-1.011	0.092
p ≤ .001	1.003	0.998-1.009	0.260
p ≤ 1e4	1.004	0.998-1.010	0.164
p ≤ 1e6	1.006	1.000-1.011	0.060
p ≤ 5e8	1.003	0.998-1.009	0.261

Analyses controlling for age, sex, amount of hospitalizations, platform (type of array chip that was used in genotyping analysis) and 10 principal components. PRS pT = *P*-value threshold applied to discovery genome-wide association study in order to construct polygenic risk scores, OR = odds ratio, 95% CI = 95% Confidence Interval.

Subgroup analysis

We found no significant difference between male and female regarding their correlation between seasonality and above discussed sleeping traits. We analyzed BD subgroups -I and -II separately but because of low coverage in subtype data analysis missed statistical power and could therefore not provide statistically significant results.

DISCUSSION

Interpretation of findings

This master thesis is a retrospective cohort study examining connection between seasonality in hospitalizations and genetic sleeping traits most often coupled with BD patients. We found that BD patients that show seasonality in their psychiatric hospitalizations have genetic predisposition to daytime sleepiness compared to those who show less seasonality. Correlation is small, yet statistically significant. In contrast our study could not find statistically significant correlation between seasonality and three other sleeping traits: chronotype, insomnia. Although trend towards correlation between seasonality and chronotype and sub-significant correlation between seasonality and insomnia.

Connection between seasonality of hospitalizations and sleeping traits

This Bipolar disorder is in previous studies linked strongly to impaired sleep and BD patients do suffer more often of negative sleeping traits such as night owl chronotype, daytime sleepiness, hypersomnia and insomnia (21-23). Since BD is also strongly associated with impaired circadian rhythm which is also responsible our sleep regulation and -patterns (17), we hypothesized that the same genes that are responsible for seasonality could be responsible for sleep impairment and therefore would show a correlation in our analysis. In our knowledge, this was the first study to research this correlation.

Strengths and limitations

This retrospective cohort study was based on a data from SWEBIC-1 and Swedish NPR has many strenghts. SWEBIC-1 is considered to be trustworthy and genotyping was done with high standard quality control (28). Moreover it is one of world's biggest genetic database of BD patients. Swedish NPR is considered to be splendidly comprehensive and trustworthy patient registry. Level of missing data is relatively low and considering on a global level high standard psychiatric care and congruent diagnosis criteria within Sweden, systematic bias within data is considered to be low. Furthermore the long

duration that Swedish NPR has been collecting patient data (from 1973), enables analysis of a life long disease such as BD and seasonality within it. Another strength is rather big amount of individuals that fulfilled our inclusion criteria (n=3104) that enables statistical power. Lastly, heterogenic study population, both culturally and genetically is considered as a strength.

Moreover the GWAS of which this study used to generate PRS is considered to be trustworthy and excellently performed. Amount of individuals to evaluate significant SNP's were enormous (n=+450000) and study population both culturally and genetically heterogenic (29-32). Therefore significant SNP's we used to calculate our PRS can be considered as trustworthy.

When limitations are considered our measure of seasonality should be considered. First of all, using Rosenthal's criteria may neglect portion of seasonality. Using strict limits to season makes our model interpret for example admissions on 30.11 and 1.12 as a separate season even though they would be a day apart from each other, thus lowering seasonality in our measure. A better alternative to represent seasonality in such measure as ours would have been to use same 90-day periods i.e. admissions should be within ±45 days of each other seasonally to be considered occurring during same season. This definition would also be congruent with ICD-10 definition of seasonality (33). As seen in Figure 4, distribution of CV of season is close to normal distribution as it would be if that feature was generated by pure random. This could suggest that our measure did not manage to describe seasonality since there is rather good evidence of seasonal nature of BD.

It should also be considered how well do admissions to general adult psychiatric ward measure affective episode in BD. This definition does not take account to depressive- and hypomanic episodes that do not require inpatient care. Another problem with our definition is that it includes all admissions to general adult psychiatric ward, not only affective ones. This could alternate the results for example with patients with psychiatric comorbidities such as addiction, emotionally unstable personality and schizoaffective disorder thus possible compromising results from actual seasonal affective episodes. Another aspect that this definition does not take in account is the possibility that manic- and depressive episodes would have differed tendency to seasonality. We suggest that in future studies they would be studied separately.

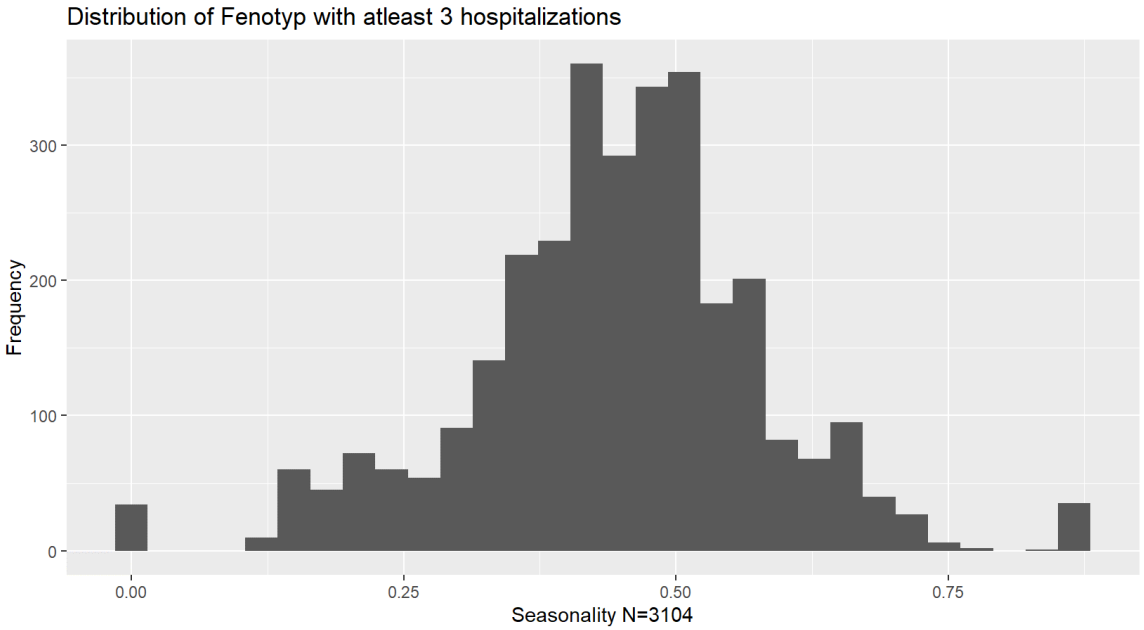

Figure 4. A bar plot showing distribution of coefficient of variation of season i.e. this study's phenotype. Frequency per given portion of CV of season on y-axis and portion of CV of season from 0 to 0.9 on x-axis. One bar/portion on x-axis equals approximately 0.03 units on CV of season's distribution.

Moreover, our measure of seasonality, coefficient of variation of hospitalizations, would seem to be greatly affected by the amount of hospitalizations given patient had thus being a confounder that would distort results. As seen in Figure 5, there seems to be rather clear connection that as an individual has more hospitalizations, CV of season will be closer to mean value.

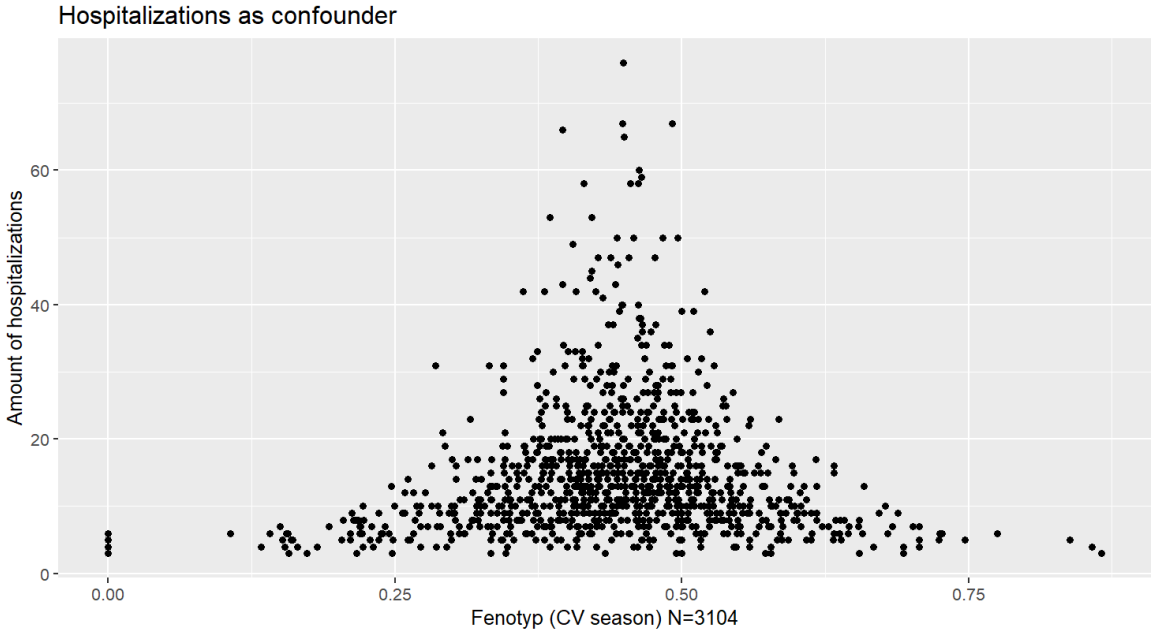

Figure 5. A scatter plot showing distribution of coefficient of variation (CV) of season i.e. this study's phenotype in relationship to amount of hospitalizations. Amount of hospitalizations on y-axis and CV of season from on x-axis.

The heterogenic nature of genetic predisposition to BD suggest that BD subtypes could have different predisposition to sleeping traits as well. Therefore our study that studied BD population as a whole can be seen as a limitation. Future study where distinguish subgroups such as BD-I and -II would be studied separately should be conducted.

Conclusions

In conclusion, our results suggest that among BD patients that show more seasonality in their hospitalizations have slightly increased genetic predisposition daytime sleepiness and possible to insomnia. Results were not as significant as we expected but as discussed in strength and limitations this could be due to study methods. In any case our results give an incentive to research connection between seasonality and sleep in future. Further knowledge is needed to provide better understanding of genetic factors and genetic heterogenicity behind BD and thus to enable more personalized treating methods. To identify BD patients suffering from circadian rhythm malfunction could be in future used for example to provide personalized chronobiological therapy such as light therapy and sleep phase advance (34) thus helping BD patients to cope with their disorder in a better manner.

Acknowledgements

I would like to thank my supervisor Erik Smedler for all the help and support with the writing of this thesis and enabling to combine my two subjects of interest into one study. I would also like to thank Lina Jonsson for providing PRS for sleeping traits.

Populärvetenskaplig sammanfattning

Utvärdering av samband mellan säsongsbundna inläggningar till stutenvård och sömnegenskaper vid bipolär sjukdom

Bipolär sjukdom är en skovvis affektiv psykiatrisk sjukdom som orsakar episoder med förhöjd sinnesläge, dvs. manier respektive med nedsatt sinnesläge, dvs. depressioner. Sjukdomen är relativt vanligt och drabbas ungefär 1-2% Sveriges befolkning. Manier karakteriseras av onormalt förhöjd humör, minskat sömnbehov, impulsivitet, tankeflykt och irritabilitet. Depressioner karakteriseras av depressiv humör, minskat intresse, förändrad matlust och sömnvaror samt möjligen skuldkänslor och även självmordstankar. Bipolär sjukdom delas i subtyper varav mest viktiga är subtyp I och -II. Skillnaden mellan subtyper är att subtyp I lider av manier som kräver psykiatrisk slutenvård medan typ II lider av hypomanier som kan betraktas som lindriga version av manier.

I tidigare studier har man konstaterat att individer som lider av bipolär sjukdom lider oftare sömnförstörning jämfört med normal befolkningen. Sömnförstöring definieras som insomnia dvs. svårigheter att upphålla sömn, dagtrötthet och hypersomnia dvs. onormal mycket sömn. Dessutom har man märkt att individer som har bipolär sjukdom är oftare ”kvällsmänniskor” jämfört med normalbefolkningen.

Man har konstaterat att genetiska faktorer förklarar 73-93% av risk att man drabbas av bipolär sjukdom. Man har dessutom konstaterat att bipolär sjukdom är kopplat till förstörd circadiska rytm dvs. rytm som sköter vår dygnsrytm och sömnmönster. Circadiska rytmen motsvarar också av vår anpassning till olika säsonger dvs. årstider.

Den här studien försökte svara följande frågan: finns det någon genetisk samband mellan sömnegenskap och säsongsbundna psykiatriska inläggningar till slutenvård hos bipolära patienter dvs. har de individer som har mer säsongsbundna inläggningar till sluten psykiatrisk vård ökad genetisk sårbarhet för sömnstörning.

Den här studien skapade utifrån svenska patient registret ett mått som avspeglade enskilda patients tendens att visa säsongsbundna inläggningar och jämförde detta med polygenic risk score för följande fyra sömnegenskaper: kronotyp (dvs. om man är morgon- eller kvällsmänniska), dagtrötthet, insomnia och sleep duration. Polygenic risk score skapades för varje individ som deltog i studien och blev kopplat med samma individens tendens för att visa säsongsbundna inläggningar.

Resultatet av studien var att individer som har tendens för säsongsbundhet i inläggningar visade sig att ha lite ökad risk att ha genetisk sårbarhet för dagtrötthet men inte för de 3 andra sömnegenskaper.

Slutsatsen från den här studien är att det finns inte någon tydlig samband mellan säsongsbundhet i inläggningar och sömnegenskaper, förutom liten samband med dagtrötthet. Flera studier krävs för att identifiera de individer som drabbas av säsongsbundhet för att kunna erbjuda mer individualiserade behandlingsmetoder i framtid.

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