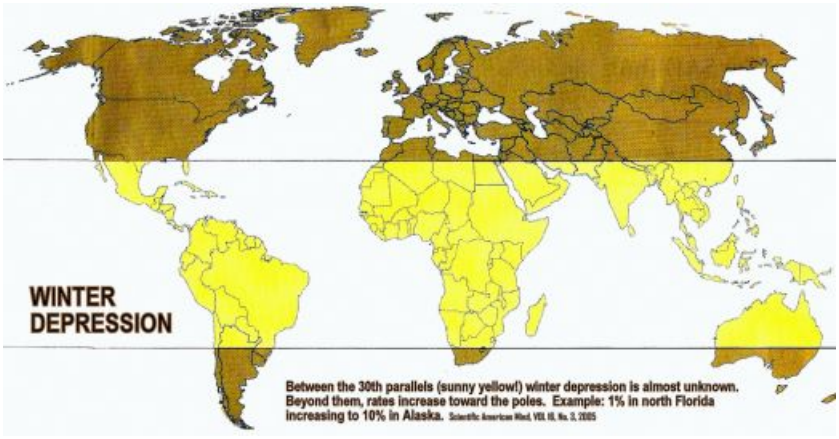
The influence of BMAL1 polymorphisms on the sensitivity to winter depression.



"People with seasonal depression experience something like constant jet lag."
Lighten Up by Ulrich Kraft, Scientific American Mind,Vol. 16, No. 3

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Date: 10 maart 2012

Summary

Some people experience a serious mood change when the seasons change. Seasonal Affective Disorder (SAD) is a mood disorder. The term 'winter depression' describes a form of SAD that occurs in the winter months: people who have normal mental health throughout most of the year experience depressive symptoms in the winter year after year. One of the major theories for explaining SAD involves the circadian rhythms of the body. Brain and muscle Arnt-like protein-1 BMAL1 is a transcription factor playing a central role in the regulation of circadian rhythms. Mutations in or deletions of a single circadian clock gene cause alterations to the circadian rhythms. Polymorphisms of circadian clock genes Per2, BMAL1, and Npas2 contribute to winter depression. Several polymorphisms of BMAL1 can be selected to determine their influence on winter depression sensitivity. Single-nucleotide polymorphism rs2290035 in the BMAL1 gene may regulate the circadian activities of BMAL1. Because of these alterations in the circadian rhythms, it has a direct influence on patients mood and can influence the person's sensitivity to winter depression.

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Introduction

There is a problem

The U.S. National Library of Medicine notes that "some people experience a serious mood change when the seasons change. They may sleep too much, have little energy, and may also feel depressed. Though symptoms can be severe, they usually clear up." What happens to those people? Why does human mood change when the seasons change? What are the reasons and what will be the consequences of thosechanges? Why is one person more sensitive to seasonal changes than another one? Where in the world dopeople suffer from seasonal mood changes? Are there only psychological answers or should we look deeper into the human body? What role do the circadian rhythms play in the sensitivity to winter depression? Which of the mechanisms of circadian rhythms influence the sensitivity and what happens to sensitivity if that mechanism changes/mutates? All these questions are very important and of great interest. We shall try to answer them in this essay.

The purpose of this essay is to show that winter depression is regulated by many factors in our body and understand the contribution of the transcription factor known to regulate circadian rhythm – BMAL1 to sensitivity of the human body to winter depression.

What is winter depression

Time and features

What are the mood disorders, that "some people experience … when the seasons change". There is a common term for these cases: Seasonal Affective Disorder (SAD). Winter depressionis a mood disorder in which people who have normal mental health throughout most of the year experience depressive symptoms in the winter year after year (Ivry, 2002). Symptoms of winter depression may consist of difficulty waking up in the morning, morning sickness, tendency to oversleep and over eat, especially a craving for carbohydrates, which leads to weight gain. Other symptoms of winter depression are: a lack of energy, difficulty concentrating on or completing tasks, and withdrawal from friends, family, and social activities. All of this can also lead to major depression, pessimistic feelings of hopelessness, and lack of pleasure which characterize a person suffering from this disorder. In the Diagnostic and Statistical Manual of Mental Disorders it is stated that winter depression is not a unique mood disorder, but is "a specifier of major depression". People who experience winter depression show sometimes symptoms of classic depression including insomnia, anxiety, irritability, decreased appetite, weight loss, social withdrawal and suicide. Furthermore susceptible individuals who work in buildings without windows may experience winter depression type symptoms at any time of year (Watkins, 2002). Some, but not all of these atypical individuals also had a seasonal pattern. Some people with winter depression also have mild or occasionally severe manic mood swings in the spring and summer. If these episodes are severe, the individual might be diagnosed with Bipolar Disorder (Lurie et al., 2006).

Why winter depression?

Patients with seasonal affective disorder have episodes of major depression that tend to recur during specific times of the year, usually in winter. In many species, activity is diminished during the winter months in response to the reduction in available food and the difficulties of surviving in cold weather. Hibernation is an extreme example, but even species that do not hibernate often exhibit changes in behavior during the winter. It has been argued that SAD is an evolved adaptation in humans that is a variant or remnant of a hibernation response in some remote ancestor

(Magnusson and Partonen, 2005). Presumably, food was scarce during most of human prehistory, and a tendency toward low mood during the winter months would have been adaptive by reducing the need for calorie intake. Some emerging evidence suggests that seasonal affective disorder may be associated with alcoholism and attention-deficit/hyperactivity disorder. The preponderance of women with SAD suggests that the response may also somehow influence reproduction. (Lurie et al, 2006). Patients with SAD are more likely to have family members with SAD, although this may be subject to reporting bias. Some studies have found that there may be a genetic component to susceptibility (Nillny et al., 2009). Patients with SAD visit their personal doctors more often in the winter than other patients, but rates between the groups are similar the rest of the year. Patients with SAD have more office visits, more diagnostic testing and more prescriptions throughout the year compared with age- and sex-matched controls (Lurie et al., 2006).

From problem to numbers

To understand the problem of winter depression better, we have to take a look at the world's population and compare the numbers of people that suffer from winter depression in different countries. In order to analyze winter depression rates worldwide in regard to the effects of latitude, genetic distance and cultural influences, data was extracted from a number of studies in which winter depression rates in percentage of population were obtained. The overall lifetime prevalence of SAD ranges from 0 to 9.7 percent. This estimate depends on the specific population studied, as well as whether SAD is diagnosed by a screening questionnaire or a more rigorous clinical interview. Prevalence may be higher at northern latitudes, and it may vary within ethnic groups at the same latitude.

Author/s of Study	Location	Latitude in Degrees	SAD Rate in %
Morrissey et al., 1992	Australia	19 S	1.7
Parslow et al., 2004	Australia	35.5 S	5.35
Axelsson et al., 2002	Canada	50 N	9.1
Han et al., 2000	China	35.4 N	2.4
Dam et al., 1998	Denmark	55 N	12.4
Hagfors et al., 1995	Finland	60 N	7.1
Magnusson and Axelsson, 1993	Iceland	64.5 N	3.6
Muscoletta et al., 1995	Italy	41.5 N	4.4
Imai et al., 2003	Japan	39.75 N	.89
Imai et al., 2003	Japan	33.35 N	.48
Ozaki et al.,1995	Japan	35 N	.86
Mersch et al., 1995	Netherlands	53 N	3.0
Konradson, 1996	Norway	64 N	9.65
Ito et al., 1992	Philippines	15 N	0.0
Booker et al., 1991	Russia	64 N	16.2
Eagles et al., 2002	Scotland	57 N	9.9
Hagfors et al., 1995	Sweden	60 N	3.9
Broman et al., 1998	Sweden	61.1 N	3.5
Wirz-Justice et al., 1992	Switzerland	47.5 N	2.2
Elbi et al., 2002	Turkey	39 N	4.8
Booker et al., 1991	USA	55 N	6.6
Booker et al., 1992	USA	65 N	9.2
Hedge et al., 1996	USA	30 N	3.7
Levine, 1995	USA	65 N	9.35
Low et al., 1998	USA	44.53 N	13.2
Rohan and Sigmon, 2000	USA	44.53	7.8
Rosen et al., 1990	USA	27 N	1.4
Rosen et al., 1990	USA	39 N	6.3
Rosen et al., 1990	USA	40 N	4.7
Rosen et al., 1990	USA	42.5	9.7

Table 1: Summary of cases that show the winter depression rate in percent of the population of the state and the author/authors of the study. Data from Whitehead 2004

In his review, Whitehead (2004) summarized a total of 30 studies that comprise the sample used in the different SAD analyses. Only those studies using the Seasonal Pattern Assessment Questionnaire (SPAQ) were used (see SPAQ, attachments). This was done out of necessity since studies using other diagnostic methods were minimal in number and did not use a common method (Magnusson and Partonen, 2000). This data and sources are summarized in Table 1 "Summary of Cases." It lists the country in which the study was conducted, the winter depression rate in percent and the author/authors of the study (Whitehead, 2004).

As we can see the rate of winter depression has a substantial variability across different countries. The incidence of SAD increases with increasing latitude up to a point, but does not continue increasing all the way to the poles. There seems to be interplay between an individual's innate vulnerability and her degree of light exposure (Whitehead, 2004). These are the results of several investigations that took place at the end of 90ties. According to Whitehead, winter depression is a common slump in the mood of some inhabitants of most of the Nordic countries and its rate has changed in the last ten years. According to the Central Statistics Office of the Netherlands, Dutch people are not an exception nowadays. An estimated 9% of the population in the Netherlands suffer from SAD (CBS Stat Line, 2011). If we take a look at the USA, in Alaska it has been established that there is a SAD rate of 8.9%.

Epidemiology

The survey shows women are more likely to be affected by SAD than men. About 70-80% of patients with SAD are women. The ratio of women to men was lower in patients suffering from bipolar affective disorder (1,5:1) than for unipolar depressives (5:1), a finding which has already been observed by Weissman et al., 1984 in a group of non-seasonal depressed patients. The prevalence in children and adolescents ranges from 3.3% to 4.2%, with the incidence increasing among girls during puberty (Nillny et al, 2009). Other studies have noted that parental ratings of depression are more severe among 16 to 18 year-olds than among 6 to 15 year-olds when assessed during the fall and winter months (Blazer et al, 1998). The most common age of onset is in one's thirties, but cases of childhood SAD have been reported and successfully treated. For every individual with full blown SAD, there are many more with milder "Winter Blues" (Magnusson and Partonen, 2005).

Circadian rhythms regulate sensitivity to winter depression

One of the major theories for explaining SAD involves the circadian rhythms of the body. What are circadian rhythms, what mechanisms control them and what role do circadian rhythms play in the modulation of winter depression?

Nature of circadian rhythms

A circadian rhythm is an endogenously driven roughly 24-hour cycle in biochemical, physiological, or behavioral processes. Circadian rhythms are also referred to by the term body clock. By carrying its own circadian clock, an organism can anticipate the regular daily changes in its environment and it can organize appropriate action in advance. One might think that the circadian clock would itself be a complex multicellular device with different groups of cells responsible for different parts of the oscillation mechanism. Bruce Alberts, in his book, The molecular biology of the cell, says that the circadian clock, in almost all organisms, including humans, is regulated by individual cells. According to Alberts (2002), understanding the working of the circadian clock is a fundamental problem in cell biology. To be called circadian, a biological rhythm must meet several general

criteria. The first one is, that the rhythms must repeat once a day, to have a 24-hour period. In order to keep track of the time of day, a clock must be at the same point at the same time each day. The rhythms have to be endogenous: they have to persist in the absence of external cues. A rhythm cannot be said to be endogenous unless it has been tested in conditions without external periodic input. The rhythms have to be able to match the local time. The rhythm can be reset by exposure to external stimuli such as light and heat. The rhythms have to maintain circadian periodicity over a range of physiological temperatures. Many organisms live at a broad range of temperatures, and thermal energy will affect the kinetics of all molecular processes in their cell(s). In order to keep track of time, the organism's circadian clock must maintain a roughly 24-hour periodicity (Shekhar, 2011).

Mechanisms that regulate circadian rhythms

Explanation of the molecular basis of circadian rhythms has progressed drastically over the past decade. It is known that in mammals these biological rhythms are regulated at the molecular level by core clock gene elements. These core clock gene elements are engaged in auto-regulatory feedback loops involving transcription and post translational modification. To understand that mechanism better we shall describe most of the processes using animal tests.
Mice as well as humans maintain a large number of physiological variables under continuous control of an internal clock. Humans and mice keep under circadian control the regulation of various processes such as the sleep-wake cycle, locomotor activity, temperature regulation, metabolism, water/food intake and levels of circulating hormones. Furthermore, disturbances of these circadian parameters have been associated with a number of psychiatric and neurological disorders in humans including seasonal affective disorder, bipolar disorder and neurodegenerative disorders (Hrabé et al, 2006). To understand the molecular basis of circadian rhythms, many molecular components remain to be identified in order to explain how the internal clock, residing within the suprachiasmatic nucleus of the hypothalamus, interacts with the environment and how it conveys its rhythm to oscillators in other tissues and brain regions (Harwell, 2011). Circadian rhythmicity is a consequence of intracellular molecular mechanisms. It also involves so-called clock genes. The products of some of these clock genes regulate their own expression. The outcome of the feedback loop is an oscillation in the levels of mRNAs and proteins. These mRNA and protein

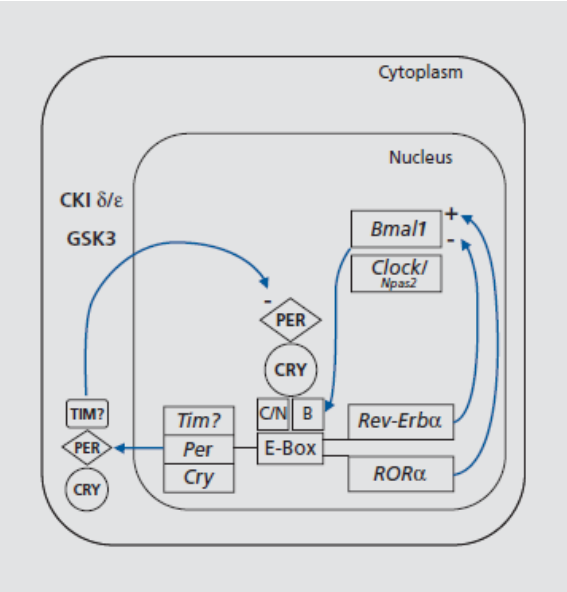

Figure 1: Simplified schematic diagram of the molecular mechanisms of the circadian clock in mammals. See the main text for details. Positive and negative feedbacks are indicated by arrows with a + and a - sign, respectively. Genes and mRNA are indicated by italics, proteins are in bold caps.
C = CLOCK protein; N = NPAS2 protein; B = BMAL1 protein
(Lamont et al, 2007)

rhythms are observed in the suprachiasmatic nucleus (SCN) of the hypothalamus, the master clock, as well as in other brain regions and peripheral tissues (King et al, 1997).
According to Lamont et al (2007), within the clock, other factors control the phosphorylation, stability, and localization of clock proteins, thereby regulating the oscillation, particularly the period. In mammals, Clock and Bmal1 encode transcription factors CLOCK and BMAL1 (brain and muscle ARNT-like protein 1). BMAL1 is also known as ARNTL or MOP3. These transcription factors form heterodimers that activate the transcription of Per1, Per2 and Per3(three Period genes) and two Cryptochrome genes Cry1 and Cry2 (Lamont et al, 2007). For a simplified schematic diagram of the molecular mechanisms of the circadian clock in mammals see Figure 1. We shall describe BMAL1, its functions and interaction with CLOCK in more detail later.

After activation of three Period genes and two Cryptochrome genes, PER and CRY proteins form complexes that are translocated back into the nucleus and inhibit their own expression. RORα and REV-ERBα act on BMAL1 to activate and repress transcription respectively. NPAS2 is an alternate dimerization partner for BMAL1 that may also regulate circadian rhythmicity in the fore brain, but it has not been consistently found in the SCN. Clock proteins are targeted for degradation by components of ubiquitin ligase complexes like FBXL3 and β-TRCP1, which together regulate the period of circadian oscillation by controlling the rate of accumulation, association and translocation of Per and Cry (Lamont et al, 2007). All these genes, protein products, and enzymes work together to control clock functioning.

Circadian rhythms and winter depression

So in what case do circadian rhythms refer to winter depression? Traits and symptoms of affective disorders appear to interweave with circadian clock work and rhythms in seasonal forms of depression (Bunney and Bunney, 2000). The term "circadian rhythm" describes the fact that bodily functions seem to follow a set pattern throughout the day as if the body is working to a set schedule or internal clock (Shekhar, 2011).
Manipulations of the sleep–wake cycle and circadian phase have proven beneficial for some patients; for example, sleep deprivation can give a temporary remission from a depressive episode (Wirz-Justice and Van den Hoofdakker, 1999) and morning bright light therapy is currently the treatment of choice for recurrent winter depression, or seasonal affective disorder (SAD) (Rosenthal et al, 1984). It is commonly thought, that in SAD this internal clock is disrupted. It leads to various biochemical abnormalities and symptoms associated with these abnormalities. SAD patients frequently have sleep complaints, particularly hypersomnia, with longer polysomnographically-recorded non-REM sleep and more slow-wave activity per minute of non-REM sleep (Lamont et al, 2007). Patients with SAD seem to generate a melatonin-dependent signal that is absent in healthy volunteers and that is similar to the signal that mammals use to regulate seasonal changes in their behavior (Partonen et al, 2007). This is demonstrated by the fact that an animal can interpret the same duration of melatonin secretion as an indicator of either winter or summer depending on whether the animal was previously exposed to shorter or longer periods of secretion, respectively (Wehr et al, 2001). The phase shift hypothesis postulates that SAD patients become depressed in winter because there is a season-specific shift in their endogenous circadian system with respect to their sleep-wake cycle (Lamont et al, 2007). There is an opinion, that circadian clock-related polymorphisms could be associated with an increased risk for SAD. Recent studies suggest that polymorphisms of PERIOD2, NPAS2, and BMAL1 can be interesting for further study. In this case we shall specifically discuss only BMAL1 and its influence on the sensitivity to SAD.

BMAL1 polymorphisms and winter depression

BMAL1

The molecular clock is composed of transcriptional feedback loops in organisms ranging from cyanobacteria to humans. As we already mentioned, BMAL1 is a transcription factor playing central roles in the regulation of circadian rhythms. BMAL1 is the only component of the mammalian circadian clock whose sole deletion in a mouse model generates arrhythmicity. In addition to defects in the clock, these BMAL1 null-mice also have reproductive problems, are small in stature, age quickly, and have progressive arthropathy that results in having less overall locomotor activity than wild type mice. Recent phenotyping data suggests that this gene and its partner Clock also play a role in regulation of glucose homeostasis and metabolism. Finally, BMAL1, Npas2, and Per2 have been associated with SAD in humans.

BMAL1 in healthy people

BMAL1 forms heterodimers with another basic helix–loop–helix/PAS protein, CLOCK, which drives transcription from E-box elements found in the promoter of circadian responsive genes, including Per1 and Cry. After translation of PER and CRY proteins, the PERCRY complex translocates to the nucleus, where it inhibits gene expression driven by BMAL1CLOCK (Lamont et al, 2007). In addition to these roles in the control of circadian rhythms, the contribution of BMAL1 to the regulation of sensitivity to winter depression has been suggested for several reasons. So far, genetic variations in circadian clock genes have been associated with sleep, mood, and metabolic disorders. Concerning these disorders, CLOCK gene variants have been linked to diurnal preference, delayed sleep phase syndrome, metabolic syndrome and obesity, ARNTL gene variants to bipolar disorder, type 2 diabetes and hypertension, and NPAS2 gene variants to diurnal preference and seasonal affective disorder. However, in some cases the aforementioned associations are conflicting, and the established links between circadian clock gene polymorphisms and disease susceptibility therefore remain incomplete (Kovanen et al, 2010).

BMAL1 polymorphisms contribute to winter depression

Mutations in or deletions of a single circadian clock gene cause alterations to the circadian rhythms, rest-activity cycles and sleep patterns, including the Per2 , BMAL1 and Npas2 genes. Many genes contribute to the phenotype of SAD, but since the circadian clock genes in particular are relevant , we focused on one of them BMAL1 (Partonen et al, 2007). Several studies have been done, to examine BMAL1 and its polymorphisms and their contribution to winter depression.

Pedrazzoli et al (2010) show BMAL1 interaction with other circadian clock genes. They also show the possible BMAL1 polymorphisms, their interactions and consequences of those interactions. From an initial screening of 1500 volunteers, a total of 98 volunteers of extreme types were selected to participate based on the Horne-Östberg (HO) questionnaire score (1976). A group composed of extreme morning subjects (n = 47) with a mean HO score of 68.1, 82% of them where Caucasian, 71.3% female, mean age 21.9 was compared with a group of extreme evening subjects (n = 51), mean HO score of 28.8, 80% of them where Caucasian, 71.6% female, mean age 22.9 (Pedrazzoli et al, 2010). Blood samples were taken from all participants and DNA was extracted from white blood cells. To determine the polymorphisms of BMAL1, they used a denaturing high performance liquid chromatography (DHPLC system). A semi-denaturing temperature of 59.7 °C

was used for the BMAL1 promoter region A-1420G polymorphism (rs4757138). It was the only polymorphism in this study not previously reported to be associated with any phenotype (Pedrazzoli et al, 2010). The Pedrazzoli group selected also polymorphisms for three other circadian clock genes. The polymorphisms of another three genes play also an important role in mood disorders, but we will not discuss them in this case in detail. One thing, that is important to mention, is, that the scientists have found 31 different four polymorphism combinations (polymorphisms present in each of four genes) in the sample out of the 81 that where mathematically possible. See Table 2 for details. The combinations involving low frequency alleles where much less likely to be found.

GC #	*Per3*	*Bmal1*	*Per2*	*Clock*	N	%
1	4/5	G/g	C/C	T/T	12	12.24
2	4/4	G/g	C/C	C/T	6	6.12
3	4/4	A/g	C/C	C/T	10	9.8
4	4/4	G/g	C/C	C/C	2	2.04
5	4/4	A/A	C/C	T/T	1	1.02
6	4/4	G/g	C/C	T/T	5	5.10
7	4/4	A/g	C/C	T/T	10	10.20
8	4/5	A/A	G/C	C/T	1	1.02
9	4/5	A/g	C/C	T/T	10	10.20
10	4/5	A/g	C/C	C/T	6	6.12
11	4/4	A/g	C/C	C/C	1	1.02
12	4/4	A/g	G/C	T/T	1	1.02
13	4/5	G/g	C/C	C/T	6	6.12
14	4/4	A/A	C/C	C/C	1	1.02
15	4/5	A/A	C/C	C/T	3	3.06
16	4/5	A/A	C/C	T/T	1	1.02
17	4/5	A/g	G/C	C/T	1	1.02
18	5/5	A/g	G/C	T/T	1	1.02
19	4/4	G/g	G/g	T/T	1	1.02
20	4/4	A/g	G/C	C/T	3	3.06
21	4/4	G/g	G/C	T/T	1	1.02
22	4/4	A/A	C/C	C/T	2	2.04
23	4/4	G/g	G/C	C/T	2	2.04
24	5/5	A/g	C/C	T/T	3	3.06
25	5/5	A/g	C/C	C/C	1	1.02
26	4/4	A/A	G/C	T/T	1	1.02
27	4/5	G/g	G/C	C/T	1	1.02
28	5/5	G/g	C/C	T/T	1	1.02
29	4/5	G/g	C/C	C/C	1	1.02
30	5/5	G/g	C/C	C/T	2	2.04
31	4/5	G/g	G/C	T/T	1	1.02
Total					98	100.00

GC#, Genotype combination number.

After different statistical analyses on the combination of four polymorphisms it became evident that there were statistically significant differences in the frequency distribution between combination #1, the morning sample, and combination #9, the evening sample (Pedrazzoli et al, 2010). Statistical analysis demonstrated that there could be an interaction among the gene polymorphisms, which would give a tendency/propensity to the by Pedrazzoli group determined chronotypes. This hypothesis was supported by the fact that the only difference between combinations #1 and #9 was the BMAL1 polymorphism, but this polymorphism alone did not provide any evidence of association. What was also interesting is that, combinations #1 #9 only differed in their BMAL1 genotypes, which where homozygous G and heterozygous A/G, respectively (Pedrazzoli et al, 2010). It was concluded that specific combinations of polymorphisms among different clock genes are stronger markers to chronotypes than single polymorphisms. Combinations of polymorphisms in these genes may influence phenotype, and that a combined analysis of the effects of different clock genes may be more accurate and more informative than single gene analysis.

Table 2: Genotype combinations of polymorphisms in the *Per3*, *Per2*, *Clock* and *Bmal1* genes. GC#, Genotype combination number. Statistically significant differences in the frequency distribution between combination #1, the morning sample, and combination #9, the evening sample. Data from Pedrazzoli et al, 2010

A research group under mentorship of Johansson in 2003 described circadian clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. They

introduced the results only for four circadian clock genes: CLOCK, PER2, PER3 and NPAS2. Genomic DNA was prepared from blood lymphocytes. Scientists have found a significant difference between SAD patients and controls for the NPAS2 471 Leu/Ser polymorphism, suggesting a recessive effect of the leucine allele. This study could be interesting for our case because the circadian clock genes interact with each other and polymorphisms of all circadian clock genes in combination and separately can influence the sensitivity to mood disorders (Johansson et al,2003).

Another research group under mentorship of Partonen, 2007, showed that three circadian clock genes Per2, BMAL1, and Npas2 contribute to winter depression. They hypothesized that, because Per2, BMAL1, and Npas2 genes form a key functional unit at the core of the circadian clock, variations in these three genes together could be associated with SAD. A total of 74 patients and 46 controls of Swedish or Finnish origin completed the SPAQ and Morningness Eveningness Questionnaire (MEQ) to assess the daily pattern of activities.

BMAL1 was analyzed for potentially functional genetic variations. To identify gene effects, the potentially functional single nucleotidepolymorphisms (SNPs) in BMAL1 were selected (see Figure 2).
In the BMAL1 gene, intronic allelic differences in transcription factor binding sites were found for rs3789327 and rs2290035. Linkage disequilibrium information led to the inclusion of rs2279287 and rs969485 (Partonen et al, 2007). The minor allele frequencies and genotypes among the patients and controls are presented in Table 3 and 4.

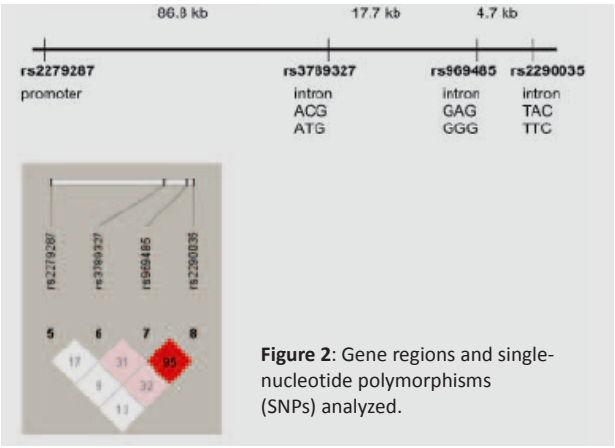

Figure 2: Gene regions and single-nucleotide polymorphisms (SNPs) analyzed.

Chromosome number	Gene name	Single-nucleotide polymorphism (SNP)			Alleles[a]	Minor allele frequency	
		Name	Id	Position	Name	Controls	Patients
2	PER2	10870	10870	238967505	G (A)	0.11	0.16
2	PER2	10562	rs2304674	238963904	C (T)	0.31	0.34
2	PER2	10563	rs13033501	238963860	A (G)	0.12	0.1
2	PER2	10565	rs10201361	238955905	G (C)	0.01	0.02
2	NPAS2	Y353Y	rs1562313	101045973	A (G)	0.15	0.18
2	NPAS2	A394T	rs2305160	101049822	A (G)	0.38	0.33
2	NPAS2	S471L	S471L	101052708	A (G)	0.2	0.2
2	NPAS2	A640A	rs1053091	101065328	T (C)	0.04	0.05
11	ARNTL		rs2279287	13255061	A (G)	0.3	0.28
11	ARNTL		rs3789327	13341892	G (A)	0.42	0.48
11	ARNTL		rs969485	13359619	G (A)	0.25	0.24
11	ARNTL		rs2290035	13364347	A (T)	0.43	0.42

[a] Minor allele (major allele in parentheses).

Table 3: Minor allele frequencies. Data from Partonen et al, 2007

The scientists assessed the individual contribution of the genetic variations of BMAL1 to the phenotype, using a step-wise regression analysis with forward selection. This gene-specific analysis identified the BMAL1 SNP rs2290035 as the only genetic variation having a significant association with SAD. See Table 5 for details.

Single-nucleotide polymorphism (SNP)	Controls			Patients		
	Alleles			Alleles		
	11 *n* (%)	12 *n* (%)	22 *n* (%)	11 *n* (%)	12 *n* (%)	22 *n* (%)
PER2:						
10870	143 (79.44)	36 (20.00)	1 (0.56)	128 (69.57)	52 (28.26)	4 (2.17)
rs2304674	82 (46.33)	81 (45.76)	14 (7.91)	81 (44.51)	79 (43.41)	22 (12.09)
rs13033501	139 (78.09)	35 (19.66)	4 (2.25)	148 (80.87)	33 (18.03)	2 (1.09)
rs10201361	176 (97.24)	5 (2.76)	0	180 (96.77)	6 (3.23)	0
ARNTL:						
rs2279287	80 (51.95)	56 (36.36)	18 (11.69)	85 (53.46)	59 (37.11)	15 (9.43)
rs3789327	67 (35.83)	84 (44.92)	36 (19.25)	53 (29.28)	84 (46.41)	44 (24.31)
rs969485	101 (56.42)	65 (36.31)	13 (7.26)	95 (55.23)	71 (41.28)	6 (3.49)
rs2290035	65 (34.39)	84 (44.44)	40 (21.16)	56 (29.63)	108 (57.14)	25 (13.23)
NPAS2:						
rs1562313	114 (71.70)	41 (25.79)	4 (2.52)	105 (66.04)	50 (31.45)	4 (2.52)
rs2305160	57 (35.85)	84 (52.83)	18 (11.32)	75 (47.17)	63 (39.62)	21 (13.21)
S471L	95 (59.75)	63 (39.62)	1 (0.63)	105 (66.04)	45 (28.30)	9 (5.66)
rs1053091	146 (91.82)	12 (7.55)	1 (0.63)	144 (90.57)	13 (8.18)	2 (1.26)

Table 4:genotypes among the patients and controls. Data from Partonen et al, 2007

Gene	Patients	Controls	Significance (model)	SNP	Significance (effect)	Odds ratio	
	n	*n*	*P*-value		*P*-value	OR	95% CI
PER2	177	173	0.03	10870	0.03	1.66	1.06–2.60
ARNTL	137	136	0.01	rs2290035	0.02	–	–
				A/A vs. T/T	0.19	0.62	0.31–1.27
				T/A vs. T/T	0.09	1.60	0.93–2.74
NPAS2	159	159	0.004	S471L	0.02	–	–
				A/A vs. G/G	0.05	8.14	1.01–65.48
				G/A vs. G/G	0.07	0.65	0.40–1.04

Table 5:logistic regression analysis of the single-nucleotide polymorphisms. Data from Partonen et al, 2007

BMAL1 as a partner of heterodimers with either NPAS2 or CLOCK is responsible for transcription from elements in promoters of the responsive genes included in the circadian pacemaker system. The SNP rs2290035 in the BMAL1 gene is located in the last intron. It alters a SP1 binding site and may thereby regulate the circadian activities of BMAL1. Because alterations to the circadian rhythms have a direct influence on patients mood and in SAD this internal clock is disrupted, we can conclude that one single-nucleotide polymorphism separately or in combination with other polymorphisms of the circadian clock genes can possibly influence the sensitivity to SAD .

Conclusion

The biology that underlies the association between circadian rhythms and mood disorders is still unknown, but may come from the influence of the molecular clock on certain neurotransmitters and their receptors. The interaction between the clock components is necessary for molecular clock function. The analysis of three key circadian clock genes points at the fact that the circadian clock is involved in the pathogenesis of winter depression. Because approximately 10% of all mood disorders follow a seasonal pattern , winter depression can be seen as a model for the molecular mechanisms in depressive disorders. It is important to mention, that the combination of three genes, that form a key functional unit at the core of the circadian clock, and their polymorphisms can possibly have more influence on sensitivity to SAD than one separate gene (Lamont et al, 2007). The knowledge accumulated up to date shows that the proteins coded by different clock genes interact physically with each other and act as transcription factors. Changes in protein

structure could alter the dimerization rates of proteins, thus causing alterations in the circadian regulation and leading to slightly different phenotypes, as seen with the different chronotypes. Several studies used gene-wise logistic regression analysis and showed that SAD was associated with variations in each of the three genes. Moreover, in an analysis of the combined effect of the three genes, additive effects were demonstrated and a genetic risk profile for the disorder was identified. Moreover different groups of scientistists presented a hypothetical mechanism of action for the observed effect for one of these genes, BMAL1. As mentioned, the SNP rs2290035 in the BMAL1 gene is located in the last intron. It changes a SP1 binding site and may thereby regulate the circadian activities of BMAL1. Expression of the BMAL1 gene is activated directly by RORA, a retinoic acid receptor-related orphan receptor, which is a key component of the circadian clock. The pineal gland hormone melatonin is a ligand for RORA, and therefore a signal of day length may have an additional effect on mood, as routinely seen in patients with SAD in specific. Availability of the ligands for RORA and subsequent drive for BMAL1 expression might thereby link to the interaction of depressive and seasonal components in patients with SAD, providing a further point of view on the dual vulnerability or two-trait hypothesis (Partonen et al, 2007). In summary, Partonen et al have found that SAD was associated with a potentially functional polymorphism in each of the three circadian clock genes. The proteins encoded by these genes are known to form a functional unit within the circadian clock, and there is a substantial circadian component in SAD. Their findings point at the possibility that these circadian clock genes are a key to the pathogenesis of SAD.

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Attachments

SEASONAL PATTERN ASSESSMENT QUESTIONNAIRE

1. Name _____ 2. Age _____

3. Place of birth - City / Province (State) / Country _____

4. Today's date _____ _____ _____
Month Day Year

5. Current weight (in lbs.) _____

6. Years of education
- Less than four years of high school 1
- High school only 2
- 1-3 years post high school 3
- 4 or more years post high school 4

INSTRUCTIONS

* Please circle the number beside your choice.

Example:
Sex Male 1 Female 2

7. Sex - Male 1 Female 2

8. Marital Status -
- Single 1
- Married 2
- Sep./Divorced 3
- Widowed 4

9. Occupation _____

10. How many years have you lived in this climatic area? _____

The purpose of this form is to find out how your mood and behaviour change over time. Please fill in all the relevant circles. Note: We are interested in your experience; not others you may have observed.

11. To what degree do the following change with the seasons?

	No Change	Slight Change	Moderate Change	Marked Change	Extremely Marked Change
A. Sleep length	0	1	2	3	4
B. Social activity	0	1	2	3	4
C. Mood (overall feeling of well being)	0	1	2	3	4
D. Weight	0	1	2	3	4
E. Appetite	0	1	2	3	4
F. Energy level	0	1	2	3	4

12. In the following questions, fill in circles for all applicable months. This may be a single month O, a cluster of months, e.g. O O O , or any other grouping.

At what time of year do you....

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	OR	No particular month(s) stand out as extreme on a regular basis
A. Feel best	O	O	O	O	O	O	O	O	O	O	O	O		O
B. Gain most weight	O	O	O	O	O	O	O	O	O	O	O	O		O
C. Socialize most	O	O	O	O	O	O	O	O	O	O	O	O		O
D. Sleep least	O	O	O	O	O	O	O	O	O	O	O	O		O
E. Eat most	O	O	O	O	O	O	O	O	O	O	O	O	OR	O
F. Lose most weight	O	O	O	O	O	O	O	O	O	O	O	O		O
G. Socialize least	O	O	O	O	O	O	O	O	O	O	O	O		O
H. Feel worst	O	O	O	O	O	O	O	O	O	O	O	O		O
I. Eat least	O	O	O	O	O	O	O	O	O	O	O	O		O
J. Sleep most	O	O	O	O	O	O	O	O	O	O	O	O		O

14. How much does your weight fluctuate during the course of the year?

0-3 lbs	1	12-15 lbs	4
4-7 lbs	2	16-20 lbs	5
8-11 lbs	3	Over 20 lbs	6

15. Approximately how many hours of each 24-hour day do you sleep during each season? (Include naps)

Winter	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Over18
Spring	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Over18
Summer	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Over18
Fall	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Over18

16. Do you notice a change in food preference during the different seasons?

No 1 Yes 2 If yes, please specify :

17. If you experience changes with the seasons, do you feel that these are a problems for you?

No	1	Yes	2	If yes, is this problem -	mild 1
					moderate 2
					marked 3
					severe 4
					disabling 5

Thank you for completing this questionnaire.

* Raymond W. Lam 1998 (modified from Rosenthal, Bradt and Wehr 1987).