INTRODUCTION
1.0 INTRODUCTION

Bipolar disorder (BD) or manic depressive illness is a chronic heritable neuropsychiatric disorder with complex origins in gene-environment interactions. The characteristic features of BD are extreme shifts in mood, energy and functioning. It is called bipolar because the mood swings occur between two poles of emotion, mania - high and depression - low. The shifts in mood are not merely related to life events. Genetic, physiological, psychological and environmental factors contribute to the illness. The United States has the highest lifetime rate of BD at 4.4%, and India the lowest, with 0.1%. (Merkingas et al., 2011).

1.1 Mania or Hypomania

The high periods of BD are referred to as mania or hypomania. Symptoms of mania or hypomania include high energy level; minimal sleep; extreme irritability; racing thoughts/flight of ideas; increased self-esteem or grandiosity; impulsive behaviour with poor judgement; spending sprees; elevated mood; pressured speech; more talkative or social; distractibility; increased alcohol and drug use; increased sexual activity; risky and bizarre behaviours and increase in goal-directed activity.

1.2 Depression

The low periods of BD are referred to as depression. Symptoms of depression include decreased energy; irritability; feelings of sadness, anxiety and despair; changes in appetite and sleep; loss of interest or pleasure in activities or friends; suicidal thoughts; feelings of guilt or worthlessness; loss of self-esteem; indecision; isolation; spend most of the time in bed; slowed thinking; diminished activity; poor concentration; impaired memory and craving for sweets.
1.3 Mixed state

Mixed state refers to a condition when one is depressed and manic at the same time. Dr. Emil Kraepelin solidified the modern concept of BD (Kraepelin, 1921). He emphasized that most mixed states were temporary phenomena in the course of the disease and were seen in the transition periods between the two principal forms of the disease and labelled the various possible combinations of the mixed states as: anxious mania; mania with poverty of thought; inhibited mania; manic stupor; excited depression and depression with flight of ideas.

1.4 Causes of BD

1.4.1 Genetic factors

BD is a polygenic disorder (McGuffin and Katz, 1989; Gershon, 1990). Several family, twin and adoption studies have provided strong evidence for involvement of different genetic components in BD (Craddock and Jones, 1999). A family history of BD is one of the strongest and most consistent risk factor for BD. There is an average 10-fold increased risk among adult relatives of individuals with BD. Magnitude of risk increases with degree of kinship. The heritability of BD is estimated at about 85%, (Sullivan et al., 2000; McGuffin et al., 2003; Bienvenu et al., 2011) which makes BD one of the most heritable multi factorial medical conditions. Up to 10% of the human genome is believed to be involved in mood regulation (Patel et al., 2010). Genetic factors for BD are composed of many common alleles, each with a small effect on risk while there are many rare alleles with larger effects on risk. Despite this complex picture, there is considerable progress in identification of individual risk alleles by linkage and candidate gene association studies and genome-wide association studies (GWAS).
1.4.2 Physiological factors

Mood is regulated by an interplay of several chemical circuits in the brain which are disrupted in depression and mania. Neurotransmitter dysfunction, immune dysfunction, disturbances in body rhythms and endocrine dysfunction are associated with BD.

1.4.3 Psychological factors

Individuals with BD experience delusions and hallucinations. Delusion is a false belief based on an incorrect inference about external reality that is firmly sustained despite what everyone else believes. Hallucinations are false or distorted sensory experiences generated by mind rather than external stimuli and may be seen, heard, felt and even smelled or tasted.

1.4.4 Environmental factors

BD is caused by adverse environmental conditions. Stress plays a key role in precipitating BD. Figure 1.1 depicts a model which shows an individual’s vulnerability to stress. A relatively minor stressor such as a change in working hours is sufficient enough to elicit mood symptoms if an individual is born with a great deal of genetic or biological vulnerability. Less genetic vulnerability means BD will be triggered only by a relatively severe stressor like the death of a parent. Separated, divorced, or widowed individuals have higher rates of BD.

Figure 1.1 A vulnerability-stress model for understanding periods of illness and wellness

Courtesy: Zubin & Spring, 1977
1.5 Diagnostic procedures

The procedures which are used for diagnosing individuals with BD are the following:

- **Magnetic Resonance Imaging (MRI) of the Brain**
  
  MRI uses a strong magnetic field and radio waves to demonstrate structural and chemical changes in brain.

- **Neuropsychological testing**
  
  It evaluates one’s ability to concentrate, remember, abstract and solve problems.

- **Clinical interview**
  
  The interview will involve a series of questions and answers about one’s life, personal involvement, information about family and childhood.

- **Neurological examination**
  
  It evaluates how the brain and nerves are working by testing one’s mental, sensory, motor and reflex functioning.

- **Eye tracking**
  
  The subject is seated in a chair and asked to follow a target with the eyes while the eye movements are recorded.

- **Evoked Potential Electro Encephalogram (EEG)**
  
  EEG involves recording of the brain waves by attaching small disks to different places on the scalp. The responses of the brain to different flashes of light and noises are recorded.
• **Laboratory tests**

  Blood and urine samples are collected to test for common medical conditions and to screen for drug and alcohol use. The blood levels of lithium and valproate are monitored in BD patients who are taking these drugs. At present, there is no blood test for BD but is expected in the near future. The tests are likely to be based on arrays that examine multiple gene variants and biological pathways in a single test.

1.6 **Criteria for diagnosis of BD**

  Two different criteria are used by researchers worldwide for diagnosing individuals with BD. They include:

1.6.1 **International Classification of Diseases, tenth edition (ICD – 10)**

  ICD-10 is the World Health Organization’s statistical classification of diseases and health related problems. ICD criteria are used in Europe and other regions.

1.6.2 **Diagnostic and Statistical Manual of Mental disorders, fifth edition (DSM – 5)**

  DSM-5, published in May 2013 is the updated version of the American Psychiatric Association’s Diagnostic and Statistical manual of mental disorders used by clinicians and researchers to diagnose and classify mental disorders. DSM criteria are used in USA and other regions as well as prevailing in research studies.
1.7 The DSM subtypes of BD

1.7.1 BD I

BD I is characterised by full-blown manic attacks and deep paralyzing depressions. A schematic representation of the moods in BD I is shown in figure 1.2.

![Figure 1.2 Mood changes in BD I](image1)

1.7.2 BD II

BD II is characterised by fully developed depressive episodes and episodes of hypomania. A schematic representation of the moods in BD II is shown in figure 1.3.

![Figure 1.3 Mood changes in BD II](image2)
1.7.3 Cyclothymic Disorder

Cyclothymic disorder is characterised by frequent short periods (days to weeks) of depressive symptoms and hypomania separated by short periods of fairly normal mood. A schematic representation of the moods in cyclothymia is shown in figure 1.4.

![Figure 1.4 Mood changes in Cyclothymia](image)

1.7.4 Other subtypes of BD

Other subtypes of BD include:

- Substance/Medication-induced bipolar and related disorder
- Bipolar and related disorder due to another medical condition
- Other specified bipolar and related disorder
- Unspecified bipolar and related disorder

1.7.4.1 Seasonal Affective Disorder

Seasonal affective disorder (SAD) is a term coined by Dr. Rosenthal (1984) is a recurrent condition in which the mood episodes occur seasonally. Two types of SAD are present. The most common type is Winter form, in which patients diagnosed with SAD experience recurrent depressive episodes in Fall/Winter and remission the following Spring. In the Summer form, SAD patients experience
depression in the Spring/Summer months and recover outside of these seasons. SAD, a variant of BD (Magnusson & Partonen, 2005), is tied to the amount of daylight, which is a primary regulator of circadian rhythms and clock gene expression.

1.8 Suicide Risk

The lifetime risk of suicide in individuals with BD is estimated to be at least 15 times that of the general population. Risk of suicide in BD was found to be the highest of all psychiatric illnesses for males and second highest for females (Nordentoft et al., 2011). About 50% of adults with BD attempt suicide at least once in their lifetime (Hawton et al., 2005).

1.9 BD and Creativity

There are numerous reports that BD individuals possess a high degree of creativity as the popular saying “No great genius has ever existed without some touch of madness” (Aristotle, 1993). The following are artists, writers and composers with BD (Mondimore, 1999).

**Artists:** Paul Gauguin, Vincent van Gogh*, Mark Rothko*


**Composers:** Hector Berlioz, George Frederick Handel, Robert Schumann*, Hugo Wolf*

*Attempted or Committed suicide.
1.10 Treatment of BD

BD is generally treated with a range of drugs in combination with Counselling and Psychotherapy. Electroconvulsive Therapy (ECT) is also done. The treatment regime for BD includes:

- Mood stabilizers - lithium carbonate, divalproex sodium or lamotrigine.
- Anticonvulsant drugs like carbamazepine, valproate and gamapentin have been shown to exhibit efficacy as mood stabilizers.
- Atypical antipsychotics - quetiapine, risperidone or aripiprazole.
- Antianxiety agents - clonazepam or lorazepam.
- Antidepressants - sertraline, paroxetine, bupropion or citalopram.

1.10.1 Lithium

Lithium, an alkali metal is one of the first drugs used to treat BD. The therapeutic effects of lithium was discovered in 1949 by Dr. John Cade who wanted to test the toxicity of uric acid and urea, the by-products of protein metabolism obtained from the urine of manic patients by injecting small amounts into guinea pigs. To make uric acid and urea soluble in water, he conjugated them to lithium and injected lithium-urate and later lithium-carbonate to the guinea pigs and discovered that “after a latent period of about two hours the animals, although fully conscious, became extremely lethargic and unresponsive to stimuli for one to two hours before once again becoming normally active” which suggested to him that lithium component of the compound might have certain protective effect against urate toxicity. He tested this efficacy in humans in 1948 and 1949 by administering lithium to manic patients, most of whom experienced a dramatic recovery (Cade JF, 1949). Dr. Cade’s discovery as a breakthrough was reported by
(Schou et al., 1954). Lithium is used in salt form such as lithium carbonate and lithium citrate in treatment of BD since 1970’s.

For patients taking lithium, a lithium level in blood between 0.8 and 1.0 milli equivalents per litre (meq/L) (Gelenberg et al., 1989) and 0.5 to 0.8 meq/L (Schou M, 1997) is recommended. Due to its narrow therapeutic index lithium must be carefully monitored to avoid toxicity. Side-effects of lithium range from tremors, fasiculations, polydipsia and polyuria to symptoms of moderate toxicity (dizziness, muscle weakness, vomiting, headache and slurred speech) to signs of severe intoxication (ataxia, stupor, cardiac arrhythmias and seizures). Long-term therapeutic use of lithium is also associated with decreases in thyroid function (Gyulai et al., 2003), acne, psoriasis and kidney toxicity.

1.10.2 Valproate

Valproate is also used in the treatment of BD. The therapeutic effect of valproate in blood is 45 µg/ml and side effects become more problematic at levels greater than 125 µg/ml (Suppes et al., 2002). Valproate is broken down by the liver and it develops an elevation in liver enzymes, which in rare instances can lead to liver inflammation. Valproate can also affect the production of platelets. Hence liver enzyme tests and blood platelet counts should be conducted at regular intervals.

The therapeutic efficacy of mood stabilisers and antidepressants used to treat BD can be partially explained by their action on molecules regulating circadian rhythms.
1.10.3 Photoperiod

The word photoperiod refers to the length of daylight hours in the twenty-four-hour day. Photoperiod has a profound effect on living beings. Dr. Rosenthal found that Winter depression could be treated by artificially lengthening the photoperiod (Rosenthal NE, 1993) where he wrote “Phototherapy had been born”.

1.10.4 Chronotherapeutics

Non-pharmacological techniques such as bright light therapy and total sleep deprivation are used to treat BD by resetting the circadian clock (Wirz-Justice et al., 2009). Bright light is used as a therapeutic intervention in BD (Eastman et al., 1998; Lewy et al., 1998a; Terman et al., 1998). In BD, sleep loss or bright light can trigger mania, whereas extended darkness can reduce mania (Wehr et al., 1998). Exposure to dark diminishes manic symptoms similar to as antipsychotic drugs (Barbini et al., 2005). Sleep deprivation is used to treat severe depression which can lead to precipitation of manic episodes in BD patients (Wirz-Justice et al., 2004). Sleep deprivation (Boivin, 2000), and shifts of sleep timing (Wehr et al., 1979; Sack et al., 1985) are used as antidepressants. BD patients benefit by a strict sleep-wake cycle which regulates circadian rhythms (Leibenluft & Suppes, 1999; Wirz-Justice et al., 2005).

Stabilization of social zeitgeber factors may reduce the risk of relapse of BD (Leibenluft et al., 1996; Malkoff-Schwartz et al., 1998; Ashman et al., 1999; Frank et al., 2000). Interpersonal and social rhythm therapy (IPSRT) is effective in treating BD when practiced in combination with pharmacotherapy (Frank et al., 2005; Miklowitz et al., 2007). The neurobiological mechanisms of action of chronotherapeutic techniques involve the same targets of pharmacologic
antidepressants and antimanic substances, thus suggesting shared mechanisms among all effective treatment for BD (Benedetti & Smeraldi, 2009).

**Figure 1.5 Comprehensive model for BD pathophysiology.** This model is consistent with mood being a function of trophicity (Niculescu, 2005) through energy metabolism (Quiroz et al., 2008) as well as cellular growth and proliferation (Le-Niculescu et al., 2008). The organism reacts to a favourable environment by activity and expansion and to an unfavourable environment by inactivity and retraction (Niculescu, 2005).

### 1.11 Biological Clocks

Chronobiology (from Khronos, the Greek word for “Time”) is the science of bodily rhythms and biological clocks. Dr. Curt P. Richter conducted investigations in laboratory rats and found that they had biological clocks which
kept to a 24 hour cycle (Richter, 1965). When the rats were surgically blinded, their internal clocks kept to a twenty-four-hour cycle for a while, after which their activity schedule started shifting by strikingly constant intervals (Richter, 1965). The blinded rat’s internal clock no longer kept to a 24 hour schedule because it had lost its zeitgeber (a German word which means “time giver”) and could no longer be entrained by its external “setting” signal namely light.

The importance of circadian rhythm was first established during a series of experiments in the basement of a Munich hospital. A group of volunteers were placed in a windowless room, isolated from all external clues as to the time of day or the day of the week. They were allowed to establish and follow their own schedules and sleeping. This study revealed that when the clock runs at its own pace, the human body operates on a cycle of approximately 24 hours. The internal clock is entrained by environmental cues, primarily light, to a 24 hour cycle.

The internal clock can shift only about one or two hours per day. When we reset our watches for daylight-saving time or travel to an adjacent time zone, we hardly notice the change. But when we travel across several time zones, it takes several days for our internal clock to become entrained to the new time. The farther we have travelled, the longer it takes, about one day for each hour of time change. When the human volunteers were forced to live on a thirty-hour cycle, their internal clocks could never catch up with their sleep/wake cycle. The internal clock went out of synchronization with the artificially prolonged sleep/wake cycle resulting in a negative effect on mood (Mondimore FM, 1999). All the work on chronobiology and biological clocks indicates that there are important links between bodily rhythms and mood. BD is associated with disrupted circadian rhythms.
1.12 Circadian rhythms

The term “circadian” which derives from the Latin phrase “circa diem” meaning “about a day” refers to the biological processes that display rhythms during a period close to 24 hours. Circadian rhythms are one of the most critical biorhythms that are conserved among various species (Pittendrigh, 1993). The primary function of circadian rhythms is to allow living beings to adapt to their periodically varying environment, through entrainment of rhythms. Entrainment refers to the process where the circadian pacemaker resets itself in response to light to maintain synchrony of the clock to the 24 hour day. A pacemaker is a functional entity capable of self-sustaining oscillations that synchronizes other rhythms. Circadian clocks are capable of functioning autonomously without any external output or time cue, although environmental signals like day/night cycles can reset or entrain them (Cermakian & Sassone-Corsi, 2002). The light-dark cycle of the solar day has the largest impact on circadian regulation (Halberg et al., 1959). The circadian clock is evolutionarily conserved and dates back to the time when plants diverged from the common lineage with animals and fungi (Dunlap, 1999; Lowrey and Takahashi, 2004; von Schantz, 2008). The three major circadian pacemakers in mammals are the suprachiasmatic nuclei (SCN) of the anterior hypothalamus, the retina and the pineal gland. The SCN is the dominant circadian pacemaker (Welsh et al., 2010) which synchronizes to the environment by light input from melanopsin present in the ganglion cells of the retina (Ecker et al., 2010) through the retinohypothalamic tract (Moore & Lenn, 1972). Clocks are also present in many other brain regions (Reick et al., 2001; Abe et al., 2002; Granados-Fuentes et al., 2004; Lamont et al., 2005; Guilding & Piggins, 2007) and peripheral tissues (e.g., liver, kidneys, heart, muscle) (Yamazaki et al., 2000; Balsalobre, 2002; Yoo et al.,
Although the peripheral clocks are capable of generating oscillations independently, they are synchronized by the SCN clock (Kramer et al., 2001; Lowrey & Takahashi, 2004). In mammals, the central pacemaker in the SCN regulates diurnal rhythms of various physiological functions such as behaviour, feeding, blood pressure, and hormonal secretion, whereas peripheral clocks synchronize various cellular activities, including metabolism and cell cycles, in a tissue-specific manner (Reppert & Weaver, 2002). Cells throughout the body also display 24-hour rhythms (Reppert & Weaver, 2001; Welsch et al., 2004; Yoo et al., 2004; Nagoshi et al., 2005). Genes involved in oxidative phosphorylation, protein synthesis, and vesicle transport have also been identified as key cycling genes in the suprachiasmatic nucleus (Panda et al., 2002). Circadian rhythms regulate behaviour and a variety of physiological functions like sleep-wake cycles, hormonal secretion, body temperature and metabolism (Cameron et al., 2008; Green et al., 2008; Eckel-Mahan and Storm 2009). Circadian rhythmicity is cell-autonomous, in both SCN neurons (Welsh et al., 1995) and non-SCN cells (Welsh et al., 2004). A hallmark of circadian clocks is the display of rhythms in many messenger RNAs and proteins, including those encoded by so-called clock genes (Reppert & Weaver, 2002; Cermakian & Boivin, 2003). These clock genes are necessary for sustaining circadian rhythms under constant conditions. Mice with mutant clock genes display abnormal rhythms, and even arrhythmicity in some cases (Cermakian & Boivin, 2003; Ko & Takahashi, 2006). Genetic variants in clock and clock-related genes display abnormal circadian rhythms (Piggins 2002; Florez & Takahashi, 1995; Barnard & Nolan 2008; Menet & Robash, 2011). Circadian rhythmicity in SCN and peripheral tissues is tightly regulated by the cellular clock system which consists of transcription factors and their modulators.
1.12.1 Cellular circadian clock network

The cellular circadian clock comprises of clock genes which regulate and are themselves regulated by transcription-translation feedback loops to adjust rhythms to an approximate 24-hour cycle (Dardente & Cermakian, 2007; Takahashi, 2008). The positive loop of the mammalian clock system comprises of circadian locomotor output cycles kaput (CLOCK) or Neuronal period- aryl hydrocarbon receptor nuclear translocator – singleminded (PAS) domain protein 2 (NPAS2) and aryl hydrocarbon receptor nuclear translocator-like (ARNTL/ARNTL2) proteins which are members of basic helix–loop–helix (bHLH) Period-Arnt-Single-minded (PAS) domain, named for the first three proteins identified with this motif: the drosophila Period (PER), human ARNT, and drosophila single-minded (SIM) (Nambu et al., 1991). The sequences of the PAS domain of this family contain two copies of an approximately 50-amino-acid repeat, referred to as the PAS-A and PAS-B repeats separated by 100 amino acids of non-conserved sequence. PAS domain takes part in extensive transcriptional regulation, regulate biological responses to light and can mediate a number of biochemical functions needed for developmental and physiological events (Huang et al., 1993). The PAS domains appear to be important for protein-protein interactions (Lindebro et al., 1995). PAS domains have been found to bind ligands and to act as sensors for light and oxygen. ARNTL and CLOCK also contain a basic helix loop helix (bHLH) domain which mediates their DNA binding and heterodimerization (Crews ST, 1998). The basic region of ARNTL contains an E-R-X-R motif that is highly conserved among basic helix transcription factors that binds to E-box transcription element and is thought to constitute a structure required for recognition of this DNA sequence (van der Schalie et al., 2007). The
bHLH-PAS proteins comprise a prominent class of transcriptional regulators that control a variety of developmental and physiological events. Although some bHLH-PAS proteins can form homodimers, the most common functional unit is comprised of heterodimers (Crews ST, 1998). The bHLH region is located near the amino terminus. The basic region binds DNA and the HLH domain promotes dimerization. The carboxy-terminal residues contain transcriptional activation domains (Franks & Crews, 1994; Jain et al., 1994). Two of the basic biological processes that bHLH-PAS proteins participate in are biological rhythms and response to oxygen levels. The identification of related PAS proteins implicated in rhythms between insects and mammals (King et al., 1997; Sun et al., 1997; Tei et al., 1997) indicates that the mechanism of circadian regulation is evolutionarily well conserved. The fungal PAS proteins mediate light-controlled rhythmic behaviour (Linden and Macino 1997), suggesting an even stronger association between the PAS domain and regulation of rhythms. The bHLH domain and PAS elements are required for the association of CLOCK with ARNTL (Rutter et al., 2001; Kondratov et al., 2003).

1.12.2 Circadian rhythm cycle
The circadian rhythm cycle begins when the transcription activator CLOCK dimerizes with ARNTL to initiate the cellular circadian oscillation. CLOCK and ARNTL heterodimerize and bind to DNA elements called E-boxes (CACGTG), E1-boxes (CACGTT) (Hao et al., 1997; Gekakis et al., 1998; Hogenesch et al., 1998; Travnickova-Bendova et al., 2002; Etchegary et al., 2003; Ueda et al., 2005; Yoo et al., 2005), Noncanonical E-box (CATGTG) (Kiohara et al., 2008) and EL-box (CACGAG) (Ueshima et al., 2012) in the promoter of their target genes such as the clock genes PERIOD (isoforms PER1, PER2, and PER3) and
CRYPTOCHROME (isoforms CRY1 and CRY2) to activate their transcription during the daytime (Figure 1.6). Their protein products PER and CRY form a dimer in the cytoplasm and translocate into the nucleus at night, where they interact directly with CLOCK-ARNTL to repress their own transcription (Dunlap, 1999; Griffin et al., 1999; Kume et al., 1999; Young & Kay, 2001; Albrecht, 2002; Panda et al., 2002; Hastings & Herzog, 2004; Ye et al., 2011). CRY1 and CRY2 Proteins interact with NPAS2-ARNTL complex and repress their transcription (Kondratov et al., 2006). PER2 inhibits the transcription of CLOCK-ARNTL2 complex (Sasaki et al., 2009). In contrast, PER2 activates the transcription of NPAS2-ARNTL complex (Kaasik & Lee, 2004). PER also associates with TIMELESS (TIM) to turn off the CLOCK-ARNTL and NPAS2-ARNTL mediated transcription of the PER gene (Darlington et al., 1998; Sangoram et al., 1998). Consequently, PER and CRY levels fall as the PER-CRY repressor complex is targeted for degradation by specific E3 ubiquitin ligase complexes (Shirogane et al., 2005; Busino et al., 2007; Reischl et al., 2007; Siepka et al., 2007) and the negative repression is relieved resulting in CLOCK-ARNTL activating a new round of transcription to begin the circadian cycle anew. This cell autonomous, auto regulatory transcriptional feedback loop takes about 24 hours to complete and forms the core mechanism of the circadian clock in mammals (Lowrey & Takahashi, 2011).

1.12.3 Circadian rhythm signalling
The most powerful environmental cues for entrainment are the light-dark cycles. Two structurally related neuropeptides, pituitary adenylate cyclase-activating polypeptide (ADCYAP1 or PACAP) and vasoactive intestinal peptide (VIP), as well as their main receptor VIPR2 in SCN appear to play important roles in the
photoentrainment process and control of mammalian circadian rhythms by activating intracellular signaling pathways (Harmar 2003). The clock-controlled genes (ccgs) are also regulated by the clockwork (Duffield, 2003). Some of these genes are directly regulated by CLOCK-ARNTL (Jin et al., 1999). The transcriptional activity of CLOCK-ARNTL varies in a circadian way and hence the mRNAs from these ccgs display circadian rhythms. ARNTL displays circadian regulation of its steady state mRNA levels (Shearman et al., 2000; Lee et al., 2001). The core molecular mechanism of the circadian clock is similar in brain and peripheral cells (Yagita et al., 2001). Clock genes operate as a well characterised circuit. Figure 1.6 depicts the circadian rhythm signalling described as above.

The primary loop of circadian cycle is accompanied by two adjunct feedback loops. The first adjoining loop involves NR1D1/2 and RORα/β genes. NR1D1 and NR1D2 genes play major roles in shaping the circadian oscillation of ARNTL mRNA (Preitner et al., 2002; Ueda et al., 2002). NR1D1/2 protein binds to retinoic acid-related orphan receptor element (RRE) within the promoter region of ARNTL gene and represses its transcription whereas ROR proteins compete for the same site and activate its transcription (Giguere, 1999; Guillaumond et al., 2005). NR1D1 gene inhibits transcription of ARNTL gene by inhibitory binding at ARNTL gene at RORE promoter sites (Liu et al., 2008). PER2 interacts with peroxisome proliferator-activated receptor alpha and NR1D1 to modulate ARNTL gene (Schmutz et al., 2010). Transcription of NR1D1/2 and RORα/β genes is activated by CLOCK-ARNTL heterodimers acting through E-box enhancers in their promoters.
Figure 1.6 Circadian rhythm signalling

Courtesy: Soria et al., 2010
RORα gene expression oscillates rhythmically in some tissues (Yang et al., 2006). RORα gene binds to the promoter of ARNTL gene and enhances its transcription (Nakajima et al., 2004; Sato et al., 2004), thereby regulating its circadian oscillation (Akashi & Takumi, 2005). The transcription of NR1D1 gene is also repressed by the inhibitory action of CRY-PER complexes on CLOCK-ARNTL (Preitner et al., 2002; Guillaumond et al., 2005). The appropriately timed accumulation of CLOCK-ARNTL in nucleus is mainly ARNTL-dependent (Kwon et al., 2006). The second adjoining loop of circadian cycle involves the proline and acidic amino acid-rich domain basic leucine zipper (PAR bZip) transcription factors like, D-site of albumin promoter binding protein (DBP), thyrotroph embryonic factor (TEF), hepatic leukemia factor (HLF), the bZip protein, E4BP4 (NFIL3), bHLH proteins, DEC1 and DEC2 (BHLHE40, BHLHE41), all of which
are transcriptional targets of CLOCK-BMAL1 (Lowrey & Takahashi, 2004; Gachon, 2007; Takahashi et al., 2008) which bind to the D-box element of circadian clock genes and regulate their transcription (Lopez-Molina et al., 1997). The genes in the D-box loops are not required for the functioning of the clock although they may be involved in making the core oscillations more robust and add precision to the period (Preitner et al., 2002; Liu et al., 2008). The three binding elements namely E-box in the morning, D-box in the day, and RRE elements in the evening together provide the necessary delay to cycle at near 24 hr (Ukai-Tadenuma et al., 2011; Minami et al., 2013).

A model has been proposed which relates sunlight, lithium and circadian clock genes to MAOA, dopamine and mania (Kripke et al., 2009) (Figure 1.8). NR1D1 gene inhibits transcription of ARNTL gene by inhibitory binding at ARNTL gene RORE promoter sites (Liu et al., 2008). Peroxisome Proliferator-Activated Receptor-Gamma Coactivator 1 Alpha (PPARGC1A) gene is a regulator of ARNTL gene and additionally functions through regulation of NR1D1 and NR1D2 effects on ARNTL (Liu et al., 2007). PPARGC1A possibly binds to RORE sites both on NR1D1 and on ARNTL, and PPARGC1B may act similarly, perhaps providing a partial explanation for effects on both mania and depression, seeming opposites which are both aspects of BD. Bright light which promotes mania, tends to promote transcription of the PER1 and PER2 genes (Albrecht et al., 2001) which may then inhibit the action of the ARNTL-NPAS2 heterodimer in stimulating MAOA.
Figure 1.8 Model relating sunlight, lithium, and circadian genes to monoamine oxidase A (MAOA), dopamine and mania. This model relates sunlight and lithium to components of the circadian gene system, to monoamine oxidase A (MAOA), dopamine, and resultant stimulation of mania. Green solid arrows represent interactions which promote the function of the affected component. Red striped arrows represent inhibition of the function of the affected component. Components in white boxes hypothetically promote mania. Components in black boxes hypothetically inhibit mania. The red-green striped box for Peroxisome Proliferator-Activated Receptor - Gamma Coactivator 1 Beta gene (PPARGC1B) suggests its opposing roles in possibly stimulating both ARNTL and NR1D1, whereas NR1D1 then inhibits ARNTL protein.
1.13 Post-translational modifications of clock proteins

In addition to the core circadian clock mechanism, circadian rhythms are regulated by posttranslational modifications of clock proteins.

1.13.1 Phosphorylation

Phosphorylation of clock proteins by Casein kinases-1 δ/ε (CSNKδ/ε) and glycogen synthase kinase 3 beta (GSK3β) proteins are necessary to maintain the stability of clock proteins (Reischl and Kramer, 2011). GSK3β phosphorylates TIM gene (Martinek et al., 2001; Harms et al., 2003), CRY2 gene (Harada et al., 2005; Kurabayashi et al., 2006), PER2 gene (Iitaka et al., 2005) and NR1D1 gene (Yin et al., 2006).

Phosphorylation of PER and CRY proteins by CSNKδ/ε and GSK3β leads to its ubiquitination and proteasomal degradation (Akashi et al., 2002; Eide et al., 2005; Harada et al., 2005; Gallego et al., 2006; Partch et al., 2006; Vanselow et al., 2006). CKIε phosphorylates PER and CRYs in vitro, but apparently only after they form a complex with CKIε and the PERs (Eide et al., 2002). Degradation of the negative limb proteins PER and CRY is required to terminate the repression phase and restart a new cycle of transcription. Hence, stability/degradation rate of the PER and CRY proteins is crucial in determining the period of the clock.

Phosphorylation of CLOCK and ARNTL also regulate circadian rhythms. GSK3β phosphorylates ARNTL which controls the stability of the protein and the amplitude of circadian oscillation (Sahar et al., 2010). ARNTL was shown to be a substrate for CSNKε (Eide et al., 2002) and for mitogen-activated protein kinases (Sanada et al., 2002). Dimerization of CLOCK-ARNTL through the PAS domains is required for these phosphorylation events and for subsequent transactivation (Dardente & Cermakian, 2007).
Phosphorylation by the same kinase has opposite effects for different clock substrates (For example, phosphorylation by CSNK δ/ε leads to degradation of PER and stabilization of ARNTL). CSNKε phosphorylates several circadian proteins including ARNTL and PER (Knippschild et al., 2005; Gallego et al., 2007). ARNTL phosphorylation could enhance transactivation at E-box sites (Eide et al., 2002). This model is also supported by the fact that phosphorylated forms of ARNTL are predominantly found in the nucleus at the time of maximal transcriptional activity of CLOCK-ARNTL (Lee et al., 2001; Kondratov et al., 2003; Cardone et al., 2005; Akashi et al., 2006; Ripperger & Schibler, 2006). CRY interferes with the transactivation of CLOCK/ARNTL by reducing the phosphorylation of ARNTL and shifting the ratio of phosphorylated/unphosphorylated forms of ARNTL towards a predominance of unphosphorylated (transcriptionally inactive) form. CLOCK-ARNTL complex activity is repressed by CRY to maintain the circadian rhythmicity indicating that transcriptional feedback is required for mammalian clock function (Sato et al., 2006). Mutations in CSNKδ/ε results in altered kinase activities and cause shorter circadian periods in mammals (Ralph & Menaker, 1988; Lowrey et al., 2000; Xu et al., 2005; Gallego et al., 2006).

1.13.2 SUMOylation

The process of tagging small ubiquitin-related modifier protein (SUMO) to lysine residues of ARNTL called as SUMOylation is a reversible posttranslational modification controlled by an enzymatic pathway and is an important event in maintaining clock rhythmicity (Muller, 2001; Gill G 2004; Cardone et al., 2005). SUMOylation of ARNTL requires and is induced by CLOCK. SUMOylation plays an important role in ARNTL circadian expression and CLOCK rhythmicity.
SUMOylation of ARNTL constitutes another level of control within the core circadian clock.

1.13.3 Acetylation

Acetylation of proteins is another essential phenomenon in regulating the clock (Sterner and Berger, 2000). CLOCK acetylates ARNTL at a highly conserved Lys 537 residue. ARNTL undergoes rhythmic acetylation in mouse liver, with a timing that parallels the downregulation of circadian transcription of clock-controlled genes. ARNTL acetylation facilitates CRY1 interaction with the CLOCK-ARNTL complex. CLOCK induces ARNTL acetylation whereas CLOCK and PER1 do not undergo acetylation. CLOCK-ARNTL dimerization is essential for CLOCK-dependent acetylation of ARNTL (Hirayama et al., 2007). The chromatin remodeling necessary for cyclic transcriptional activity exerted by CLOCK-ARNTL is achieved by rhythmic acetylation/deacetylation of histones (H3 and H4) at multiple clock target genes (Gau et al., 2002; Travnickova-Bendova et al., 2002; Naruse et al., 2004). Histone acetyltransferases (HATs) proteins acetylate histones to enable the chromatin to open up. Histone deacetylases (HDACs) deacetylate histones, locking the chromatin such that it is not accessible to the transcriptional machinery. HAT activity of the CLOCK and chromatin remodelling are essential for the core clock mechanism (Doi et al., 2006). CLOCK plays a role as a HAT (Grimaldi et al., 2009). ARNTL enhances HAT function. PER1 recruits SIN3-HDAC complex and leads to the deacetylation of DNA bound by CLOCK-ARNTL transcriptional regulator (Duong et al., 2011). JARID1C and JARID1A (Jumonji ARID domain-containing protein) coactivate CLOCK-ARNTL mediated transcription of PER genes. JARID1A increases histone acetylation by inhibiting HDAC 1 function and has a nonredundant role in circadian oscillator...
function (DiTacchio et al., 2011). Rhythmic deacetylation of histone H3 at the promoters of circadian genes is also regulated by the deacetylase SIRT1, which is sensitive to NAD$^+$ levels (Asher et al., 2008; Nakahata et al., 2008). NAD$^+$ to NADH ratio has been shown to regulate the ability of CLOCK-ARNTL to bind DNA in vitro (Rutter et al., 2001). Thus, cellular metabolism may prove to play an important role in regulating the circadian clock (Marcheva et al., 2013).

1.13.4 Methylation

Methylation could be another histone modification of importance for clock function (Brown et al., 2005; Etchegaray et al., 2006; Ripperger & Schibler, 2006). The CLOCK-ARNTL complex recruits the methyl transferase MLL1 to cyclically methylated histone H3 and HDAC inhibitor JARID1A to facilitate transcriptional activation (Katada and Sassone-Corsi 2010; DiTacchio et al., 2011).

CLOCK gene (Vitaterna et al., 1994), CRY gene (Vanderhorst et al., 1999), ARNTL (Bunger et al., 2000) and PER2 gene (Akashi & Nishida, 2000; Zheng et al., 2001) are vital elements of clock.

The bHLH, PAS-A and PAS-B domains of CLOCK and ARNTL are tightly intertwined and variants in the dimer interfaces affect the stability and activity of the CLOCK-ARNTL complex as well as the periodicity of the circadian oscillator (Huang et al., 2012).

CLOCK or ARNTL by themselves had no activity on the E or EL box mediated transcription. CLOCK-ARNTL or NPAS2-ARNTL dimers are required to mediate this function. The link between CLOCK-ARNTL complex and the circadian cis-regulatory element E-box dates back to before insects and vertebrates diverged (Paquet et al., 2008). A direct repeat of E-box-like elements is required for cell autonomous circadian rhythm of clock genes (Nakahata et al., 2008). PER2
is a transcriptional repressor of the E-box (Ueda et al., 2005) and sets the pace for circadian oscillations (Akashi et al., 2006). Impairment of E-box or prime E-box regulation may therefore greatly affect the circadian system.

**ARNTL** gene is upstream of **DBP** gene in the circadian clock intracellular molecular machinery, driving the transcription of **DBP** gene (Ripperger & Schibler, 2006; van der Veen et al., 2006). **ARNTL, NPAS2** and **PER2** form a key functional unit at the core of the circadian clock (Kaasik & Lee, 2004). **PER2** expression is dependent on light and it resets the circadian clock (Pando et al., 2001). Activation of **PER1** and **PER2** genes occur in the morning whereas **CRY1** and **CRY2** genes occur in the evening (Lincoln et al., 2003a). CRY protein lacks a PAS domain. CRY2 inhibition of **ARNTL** is dose dependent and both CRY1 and CRY2 repress all four combinations of **ARNTL** (ARNTL2) - **CLOCK** (NPAS2) protein heterodimers (Dardente et al., 2007).

**NPAS2** can replace the function of **CLOCK** (Debruyne et al., 2006). **JARID1C** and **JARID1A** coactivates **CLOCK-ARNTL** mediated transcription of **PER** genes (DiTacchio et al., 2011). A domain in the extreme C- terminus of **ARNTL** plays an essential role in determining the balance between circadian transcriptional activation and suppression (Kiohara et al., 2006). The basic region of **ARNTL** contains an E-R-X-R motif that is highly conserved among bHLH transcription factors that bind to the E-box transcription element. Substitution of alanine for arginine which is within the basic region of **ARNTL** forms a heterodimer with **CLOCK** but is unable to bind to the E-box transcription element (Hosoda et al., 2004). Signaling mediated by the dopamine D2 receptor potentiates circadian regulation by **CLOCK**: **ARNTL** (Yujnovsky et al., 2006).
In Drosophila, *CLOCK* gene oscillates in a circadian manner, whereas *CYCLE* gene (*ARNTL* gene homolog) expression is constant (Bae *et al.*, 1998; Glossop *et al.*, 1999; Bae *et al.*, 2000). This is exactly opposite to the mouse mammalian system in which *ARNTL* gene cycles while *CLOCK* gene is constant (Oishi *et al.*, 1998; Shearman *et al.*, 2000). In the Zebrafish both *CLOCK* and *ARNTL* genes oscillate (Whitmore *et al.*, 1998; Cermakian *et al.*, 2000).

*ARNTL* reaches its peak level at the time of dark-light transition, maintaining its highest levels in the subjective night while *CLOCK* shows minimal variation during the 24 hour cycle (Albrecht *et al.*, 1997; Honma *et al.*, 1998; Oishi *et al.*, 1998; Takumi *et al.*, 1998).

### 1.14 Circadian clock genes in mammals

The genes involved in the circadian clocks in mammals are the following:

- ADCYAP1
- ARNTL
- AANAT
- ATXN2
- BHLHE40
- BHLHE41
- BTRC
- CSNK2A1
- CSNK2A2
- CHEK2
- CXCL12
- CLOCK
- CRY1
- CRY2
- CNGA3
- CCNA1
- CCNB1
- CDK1
- DBP
- ALAS1
- DRD2
- EP300
- EGR3
- EGFR
- FBXL3
- FMR1
- GJA1
- GCCR
- GSK3B
- GNAT13
- HLF
- HDAC
- HSD3B2
- HSD3B1
- IKBKG
- KDM5A
- MIR132
- MIRN219-1
- NPAS2
- NONO
- NFIL3
- NCO1
- NR1D1
- NR1D2
- NR2F6
- PER1
- PER2
- PER3
- PRDX2
- PPARGC1A
- PPARGC1B
- PHLP7P
- PCK1
- PLCB4
- PRNP
- PROK2
- PRKCA
- PRKAA1
- RORA
- RORB
- RORC
- RARA::RAI1
- RXRA
- SIRT1
- SFPQ
- THBD
- TEF
- TIM
- TMEM165
- VIP
- VIPR2
- WDR5
- WEE1

The “core” circadian clock consists of 18 genes namely *ARNTL1/2, CLOCK, NPAS2, PER1/2/3, CRY1/2, NR1D1/2, RORA/B/C, DECI1/2, CSNK1D/E* (Ueda *et al.*, 2005). 343 genes are reported to modulate circadian rhythms (Zhang *et al.*, 2009). Thousands of clock controlled genes oscillate rhythmically in some
tissues (Yan et al., 2008). The organization of the extended clock network is given in figure 1.9.

![Diagram of the extended clock network](image)

**Figure 1.9 Organization of the extended clock network.** The core clock oscillator comprises of 18 genes that encode for transcriptional regulators (middle). These proteins are organized in complex feedback loops with positive (green) and negative (red) limbs that generate approximately 24 hour rhythms in gene expression responsible for maintaining circadian rhythms. Upstream clock modulators influence the period and/or amplitude of rhythms by altering protein stability, cellular distribution, or phosphorylation of proteins within the core clock (top). Core clock transcriptional regulators generate expression rhythms in numerous downstream clock controlled genes that are not the “gears of the clock” involved in generating rhythms, but may be important effectors or “hands of the clock” (bottom).

### 1.15 Circadian rhythms and BD

The role of circadian system in BD is substantiated by several studies. Disruption in circadian rhythms leads to increased incidence of many diseases, such as cancer and mental illness (Reppert & Weaver, 2001; Gachon et al., 2004). Disrupted circadian rhythms could contribute directly to the pathophysiology of BD (Nikitopoulou & Crammer, 1976; Wehr et al., 1983; Wirz-Justice & van den Hoofdakker, 1999; Mitterauer, 2000; Jones, 2001; Lenox et al., 2002; Mansour et al., 2005a; Ghaemi SN, 2007; McClung, 2007; McCarthy & Welsch, 2012). BD patients exhibit cyclicity of mood and sleep disturbances (Frank et al., 2000;
Riemann et al., 2002; Jackson et al., 2003) suggesting the possibility of clock dysfunction. There are abnormalities in circadian alignments in BD patients (Lamont et al., 2007). Mutations in circadian clock genes alter circadian rhythms, rest-activity cycles and sleep patterns (Zheng et al., 1999; Bunger et al., 2000; Bunney & Bunney, 2000; Bae et al., 2001; Dudley et al., 2003; Franken et al., 2006; Xu et al., 2007). Circadian rhythm abnormalities in the sleep wake-cycle (excessive sleep in the depressive phase and reduced need for sleep in the manic phase) (Bauer et al., 2006) have been established in BD. The sleep-wake cycle is altered by variants in clock genes PER1 (Carpen et al., 2006); PER2 (Jones et al., 1999; Toh et al., 2001; Carpen et al., 2005; Xu et al., 2005); PER3 (Ebisawa et al., 2001; Archer et al., 2003; Carpen et al., 2005; Pereira et al., 2005); TIM (Utge et al., 2010) and CSNK1ε (Takano et al., 2004). Circadian rhythmicity of clock genes regulate energy metabolism and sleep (Franken & Dijk, 2009). Mutant mouse models of clock genes such as ARNTL, CLOCK, NPAS2, CRY1 and CRY2 also have alterations in sleep duration and homeostasis (Naylor et al., 2000; Wisor et al., 2002; Laposky et al., 2005; Franken et al., 2007; Franken & Dijk, 2009).

Circadian functions like variation in mood, body temperature and secretion of hormones like cortisol, norepinephrine, thyroid stimulating hormone and melatonin are disrupted in BD subjects (Atkinson et al., 1975; Sachar, 1975; Kripke et al., 1978; Mendlewicz, 1982; Souetre et al., 1989; Nurnberger et al., 1990; Linkowski et al., 1994; Leibenluft et al., 1996; Schreiner et al., 2001). Melatonin regulates sleep and other cyclical bodily functions and its synthesis is inhibited by light (Pandi-Perumal et al., 2006). Melatonin levels were significantly lowered in BD patients compared with healthy individuals (Beck-Friis et al., 1985; Kennedy et al., 1996; Nurnberger et al., 2000). A phase advance of melatonin levels was found in
manic patients (Lewy et al., 1979; Kennedy et al., 1989) and a delayed peak melatonin time was reported in euthymic bipolar patients (Nurnberger et al., 2000). Bright light and melatonin are used for treating circadian rhythm disorders (Lewy et al., 1998b; Bunney et al., 2005) and melatonin is the only option to treat blind people (Sack et al., 2000; Hack et al., 2003).

BD with seasonal pattern (mania during Spring and Summer, depression during Fall and Winter) referred to as seasonal affective disorder (SAD, Rosenthal et al., 1984) is associated with disrupted circadian rhythms. Patients with SAD generate a biological signal of change of season that is absent in healthy volunteers. The duration of nocturnal period of active melatonin secretion was shorter in Summer than in Winter (Wehr et al., 2001). In most photoperiodic mammals, long days (16 hours light: 8 hours darkness) activate prolactin release with a Summer physiology while short days (8 hours light: 16 hours darkness) suppress prolactin and produce a Winter condition (Lincoln et al., 2003b). The clock gene variants hinder the ability of BD subjects to appropriately adapt their circadian rhythms to their environment and subject them to sleep disturbances (Harvey, 2008). Life stress affects sleep-wake and social rhythms, leading to circadian clock disruption and subsequent mood episodes (Frank et al., 2000; Grandin et al., 2006; Shen et al., 2008). The social zeitgeber theory states that stress in life results in mood episodes which disrupts the social routines and thereby the biological rhythms (Ehlers et al., 1988). Figure 1.10 depicts the social zeitgeber theory.
1.16 Evidences for association of circadian clock genes in BD and SAD

Convergent Functional Genomics (GFG) of GWAS for BD is emerging as a potential tool to identify the potential candidate genes associated with BD. CFG is an approach that integrates genetics and functional genomic data from human studies and animal models (Figure 1.11).

DBP was identified as potential gene candidate for BD using CFG (Niculescu et al., 2000). CFG approach in a mouse pharmacogenomic model for BD identified ARNTL gene as potential bipolar candidate gene (Ogden et al., 2004). CFG analysis integrating genetics and functional genomics data identified ARNTL, GSK3β and RORα/β genes to be associated with BD (Le-Niculescu, 2008). A single
Figure 1.11 CFG: Multiple independent lines of evidence for Bayesian cross-validation of GWAS data. The maximal possible score from GWAS data (6pts) is equally weighed with the maximal possible score from other human and animal model gene expression and genetic data (6pts).

nucleotide polymorphism (SNP) in CLOCK gene (T3111C; rs1801260) is associated with greater insomnia and decreased need for sleep in bipolar patients (Serretti et al., 2003, 2005). CLOCK gene variant is associated with human diurnal preference (Katzenberg et al., 1998). NPAS2 gene deficient mice (Dudley et al., 2003) and CLOCK gene mutant mice display a behavioral profile that is similar to human mania (Roybal et al., 2007). The CLOCK gene mutation in mice led to a lengthening of the circadian period (Vitaterna et al., 1994). A knock-out of ARNTL gene reduced activity but was largely restored by replacing ARNTL function in muscle (McDearmon et al., 2006). DBP gene was identified as potential candidate for BD in gene expression studies (Niculescu et al., 2000). DBP gene knock-out mice display a bipolar-like phenotype (Le-Niculescu et al., 2008). A decreased expression of ARNTL, DBP and NR1D1 genes has been reported in fibroblasts from
bipolar subjects (Yang et al., 2009). An increase in ARNTL gene expression was reported in postmortem brains from bipolar subjects (Nakatani et al., 2006). PER2 gene variation was reported to be associated with depression (Lavebratt et al., 2010a). CRY2 gene variation was found to be associated with Winter depression and lowered CRY2 gene levels is associated with depression in BD (Lavebratt et al., 2010b). Evidence for the association of TIM gene variation with depression and sleep disturbance has also been reported (Utge et al., 2010).

SNP association studies (Benedetti et al., 2003; Johannson et al., 2003; Mansour et al., 2005b, 2006; Nievergelt et al., 2006; Szczepankiewicz et al., 2006; Benedetti et al., 2008; Kishi et al., 2008; Shi et al., 2008; Kripke et al., 2009; Lee et al., 2010; Severino et al., 2009; Mansour et al., 2009; Sjoholm et al., 2010a; Sjoholm et al., 2010b; Soria et al., 2010; Dallaspezia et al., 2011; McCarthy et al., 2012) and GWAS with evidences from gene expression and genetic data from human and animal model studies (Patel et al., 2010) have implicated variants in several clock genes in BD (Table 1.1). SNP association studies (Johannson et al., 2003; Partonen et al., 2007; Lavebratt et al., 2010b) have implicated variants in clock genes in SAD (Table 1.1).

1.17 Effects of treatment of BD on circadian clock

BD treatment studies provide indirect evidences for the involvement of clock genes in BD. Mood stabilizers and antidepressants used to treat BD exert their action through molecules associated with the regulation of circadian rhythms (Abe et al., 2000; Manji et al., 2001; Lamont et al., 2007; McClung, 2007). Mood stabilisers modulate circadian function (Atkinson et al., 1975; Johnsson et al., 1983; Bosch et al., 1986; Klemfuss H, 1992; Hafen & Wolnik, 1994; Klemfuss & Kripke, 1995; Dokucu et al., 2005; Roybal et al., 2007).
### Table 1.1 Studies with evidences for clock gene variants significantly associated with BD and SAD

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<th>SAD</th>
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</table>
Transgenic mice carrying a mutation in the *CLOCK* gene displaying a human mania-like behavioural profile, has reverted to nearly normal levels after chronic administration with lithium. Lithium phosphorylates and stabilizes the *NR1D1* gene (Kripke *et al.*, 1978). Lithium treatment in cells leads to rapid proteosomal degradation of *NR1D1* gene and activation of clock gene *ARNTL* (Yin *et al.*, 2006). The mood stabilizer lithium inhibited GSK3β (Klein and Melton, 1996; Kaladchibachi *et al.*, 2007; O’Brein & Klein, 2009) and regulated circadian rhythms of BD patients and model organisms (Kripke *et al.*, 1978; Hitzemann *et al.*, 1988; Campbell *et al.*, 1989; Abe *et al.*, 2000; Basturk *et al.*, 2001; Manji *et al.*, 2001). Specific GSK3β inhibition shortens period in mammalian cells (Hirota *et al.*, 2008; Vougogiannopoulou *et al.*, 2008). Lithium increased the amplitude of *PER2* and *CRY1* genes and reduced the amplitude of *PER3, CRY2, ARNTL, EABP4* and *NR1D1* genes and altered the period of *PER2* gene in serum shocked cultured murine fibroblasts (Osland *et al.*, 2011). Lithium regulated the circadian system by phosphorylating the clock components *CRY2* (Harada *et al.*, 2005), *PER2* (Iitaka *et al.*, 2005) and *NR1D1* (Yin *et al.*, 2006) and *ARNTL* (Sahar *et al.*, 2010) and by delaying the transcription of *PER2* gene (Li *et al.*, 2012). Lithium salts reduced melatonin suppression by light in BD patients (Nurnberger *et al.*, 2000). Lithium (Hallam *et al.*, 2005a) and Valproate (Hallam *et al.*, 2005b) reduced melatonin suppression by light in healthy controls. Lithium altered clock gene expression (MCQuillin *et al.*, 2007) and delayed circadian rhythms in rodents, monkeys and humans (Kripke *et al.*, 1978; Kripke *et al.*, 1979; Kripke *et al.*, 1980; Welsch & Moore-Ede, 1990). *GSK3β* gene has been implicated in both lithium’s effect on circadian rhythms (Iitaka *et al.*, 2005; Gould *et al.*, 2006; Yin *et al.*, 2006; Hirota *et al.*, 2008) and its therapeutic action in BD (McCarthy *et al.*, 2010).
Phosphorylation of clock proteins by GSK3β played a vital role in mood stabilization (Gould & Manji, 2005). The mood stabiliser valproate also regulated circadian rhythms by acting on GSK3β (Li et al., 2002).

The CFG approach identified ARNTL gene as the top candidate implicated for BD (Le-Niculescu et al., 2009, Patel et al., 2010) (Figure 1.11).

Figure 1.11 Top BD candidate genes. The lines of evidence (CFG scoring) is depicted on the right side of the pyramid

Legend:
Human Postmorem brain evidence
Human genetic evidence
Human blood evidence
*Mouse genetic evidence

Courtesy: Patel et al., 2010
Figure 1.12 Structure of ARNTL, Structure of CLOCK-ARNTL heterodimer and domain organization of ARNTL. (A) Crystal structure of ARNTL; (B) Ribbon diagram of CLOCK-ARNTL heterodimer. The CLOCK subunit is green and ARNTL subunit is blue. (C) Domain organization of ARNTL. Crystals were obtained from the truncated proteins (indicated by the amino acid residue number) encompassing the bHLH-PAS-AB domains.

Courtesy: Huang et al., 2012
1.18 Role of *ARNTL* gene in functions other than circadian regulation

ARNTL was identified by Ikeda and Nomura in 1997. It is called ARNTL in mouse and human and Cycle in drosophila. The circadian clock is interconnected with many aspects of cellular function (Zhang et al., 2009). CLOCK-ARNTL regulates the circadian expression of nicotinamide phosphoribosyltransferase (NAMPT), an enzyme that provides a rate-limiting step in the NAD\(^+\) salvage pathway. NAMPT is required to modulate circadian gene expression (Nakahata et al., 2009) implicating that cellular metabolism and circadian rhythms are linked. Metabolic and Circadian systems are interconnected at the transcriptional level (Balsalobre et al., 1998; Panda et al., 2002; Yang et al., 2006 McCarthy et al., 2007). *ARNTL* gene knock-out mice exhibit several physiological defects and a shortened life span (Rudic et al., 2004; Bunger et al., 2005; Laposky et al., 2005; Shimba et al., 2005; Turek et al., 2005; McDearmon et al., 2006; Curtis et al., 2007). ARNTL plays an important role in the regulation of adipose differentiation and lipogenesis in mature adipocytes (Shimba et al., 2005). The B-cell development is significantly impaired in *ARNTL* gene knock-out mice (Sun et al., 2006).

*ARNTL* gene polymorphisms result in susceptibility to hypertension and diabetes (Woon et al., 2007). Disruption of CLOCK and *ARNTL* genes leads to hypoinsulinaemia and diabetes (Marcheva et al., 2010). The dorsomedial hypothalamus contains an *ARNTL* gene-based oscillator that drives the food entrainment of circadian rhythms (Fuller et al., 2008). ARNTL modulates the expression of stem cell regulatory genes in an oscillatory manner. Disruption of this clock equilibrium through deletion of *ARNTL* or PER1/2 gene resulted in progressive accumulation or depletion of dormant stem cells and predisposition to
tumorigenesis (Janich et al., 2011). Deletion of ARNTL gene causes circadian arrhythmicity without the need to perturb any other core circadian member (Dibner et al., 2010) and its ubiquitous deletion causes premature aging, including defects in adult skin morphogenesis (Bunger et al., 2000; Kondratov et al., 2006; Lin et al., 2009). Conditional deletion of ARNTL gene in liver, retina and pancreas results in profound defects in tissue function (Storch et al., 2007; Lamia et al., 2008; Marcheva et al., 2010) and hematopoietic stem cells show a ARNTL-CLOCK dependent circadian release to the periphery (Mendez-Ferrer et al., 2008).

ARNTL is expressed at high level in some regions of the brain (Honma et al., 1998; Namihira et al., 1999), and synchronized peripheral oscillation is observed in muscle, lung, kidney, liver, and heart (Oishi et al., 1998). Mice homozygous for ARNTL gene deletion exhibit an immediate and complete loss of circadian rhythmicity in constant darkness (Bunger et al., 2000). Inactivation of ARNTL gene in mice is associated with a wide range of phenotypes, including decreased body weight, shortened life span, increased sleep time and sleep fragmentation, and altered regulation of blood pressure, glucose homeostasis, energy balance, lipid metabolism and adipogenesis (Bunger et al., 2000; Rudic et al., 2004; Shimba et al., 2005; Turek et al., 2005; Curtis et al., 2007). ARNTL gene double knock-out mice also display phenotypes including infertility (Kennaway 2005), idiopathic calcification and ossification of hind limb joints (Bunger et al., 2005), and increased sensitivity to chemotherapy and radiation (Gorbacheva et al., 2005) and have reduced life spans and show early aging and age-related pathologies (Kondratov et al., 2006). Hence ARNTL gene has an important role in a variety of functions other than regulating circadian rhythm, and its role depends on the tissue type in which it is expressed.
### Table 1.2 Description of *ARNTL* gene

<table>
<thead>
<tr>
<th>Official Full Name</th>
<th>Aryl Hydrocarbon Receptor Nuclear Translocator-Like</th>
</tr>
</thead>
<tbody>
<tr>
<td>Official Gene Symbol</td>
<td><em>ARNTL</em></td>
</tr>
<tr>
<td>Alternate Symbols for <em>ARNTL</em></td>
<td>BMAL1; BMAL1C; MOP3; PASD3; TIC; bHLHe5; MGC47515</td>
</tr>
<tr>
<td>Molecular Class</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Molecular Function</td>
<td>Transcription factor activity</td>
</tr>
<tr>
<td>Biological Process</td>
<td>Regulation of circadian rhythm</td>
</tr>
<tr>
<td>Molecular Weight</td>
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</tr>
<tr>
<td>Gene Map Locus</td>
<td>11p15.2</td>
</tr>
<tr>
<td>Gene ID</td>
<td>406</td>
</tr>
<tr>
<td>Human Protein Reference Database ID</td>
<td>03973</td>
</tr>
<tr>
<td>MIM</td>
<td>602550</td>
</tr>
<tr>
<td>Gene Type</td>
<td>Protein coding</td>
</tr>
<tr>
<td>Localisation</td>
<td>Primary – Nucleus; Alternate – Cytoplasm</td>
</tr>
<tr>
<td>Size</td>
<td>109,463 bases; 626 amino acids</td>
</tr>
<tr>
<td>Domains</td>
<td>PAS 146-213; PAS 327-393; PAC 400-443</td>
</tr>
<tr>
<td>Motif</td>
<td>HLH 78-131</td>
</tr>
<tr>
<td>Site of Expression</td>
<td>Amygdala, Brain, Caudate Nucleus, Corpus Callosum, Heart, Hippocampus, Skeletal Muscle, Substantia nigra, Subthalamic Nucleus</td>
</tr>
</tbody>
</table>
The gene encoding ARNTL maps to human chromosome 11p15.2. In rat, it is located in a region of chromosome 1q34 harbouring quantitative trait loci (QTL) for blood pressure, type 2 diabetes mellitus, body weight, cardiac mass, and kidney mass.

1.19 Why **ARNTL** gene was chosen for the study?

There is growing evidence that polymorphisms in circadian clock genes are associated with the pathophysiology of BD. The circadian clock gene **ARNTL** maps to 11p15 region which has been reported as the region of genetic linkage to BD initially by Egeland et al., (1987) and supportive evidences were reported by various other researchers (Kelsoe et al., 1989; Gurling et al., 1995; Smyth et al., 1997) (Source: Nurnberger & Berrettini, 1998).

The CFG approach identified **ARNTL** gene as the top candidate implicated for BD (Le-Niculescu et al., 2009, Patel et al., 2010). **ARNTL** gene knockout mice showed complete arrhythmicity when they were housed in constant darkness (Bunger et al., 2000). In mice, most of the core circadian clock genes exist as paralog pairs (**PER1** and **PER2**, **CRY1** and **CRY2**, **CLOCK** and **NPAS2**) and both the genes in the pair must be knocked out to confer arrhythmicity. The only exception to this pattern is **ARNTL**, the single knockout of which confers arrhythmicity, despite the presence of its paralog, **ARNTL2** (Takahashi, 2004; Dardente, 2008). Deletion of **ARNTL** gene causes circadian arrhythmicity without the need to perturb any other core circadian member (Dibner et al., 2010). Several polymorphisms in **ARNTL** gene have been reported to be associated with BD in different ethnic groups (Mansour et al., 2005b; Mansour et al., 2006; Nievergelt et al., 2006; Partonen et al., 2007; Mansour et al., 2009; Patel et al., 2010; Soria et al., 2010; McCarthy et al., 2012).
In Indians, although several individuals are diagnosed with BD, till date there are no reports available which relates them to *ARNTL* gene polymorphisms. Hence the present study has been undertaken to detect if the five SNPs across the *ARNTL* gene namely rs2279287 (A/G), rs1982350 (C/T), rs7126303 (C/T), rs969485 (A/G), rs2290035 (A/T) are present in the 30 members of close knit family with BD I of South Indian origin.