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1. Introduction

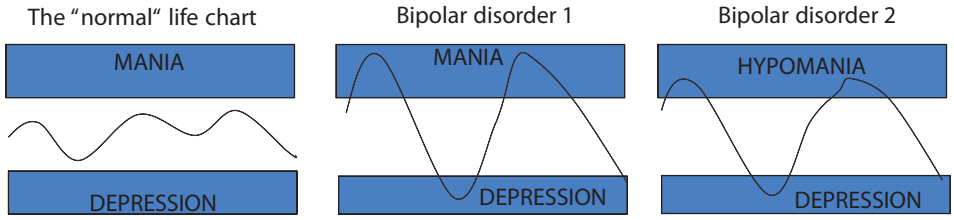
Bipolar disorder (BD) is a common mood disorder, with an estimated prevalence of 2.4 % in the Netherlands (1). According to the Trimbos institute and the most recent World Health Organization's report "The world health report 2001 - Mental Health: New Understanding, New Hope", in the Netherlands the burden of the disease is 30.000-40.000 Disability-Adjusted Life Years (DALY's), indicating a chronic disease disabling patients in normal functioning (2). Worldwide, BD is estimated to stay in the top ten causes of Years Lived with Disability (YLDs), accounting for 2.5% of total global YLDs (3). BD was once considered an episodic illness with a favorable outcome, however, nowadays seems to be a disease with a more chronic course with cognitive deficits in between episodes, residual mood symptoms and impaired functioning in daily life roles (4).

History

Bipolar disorder is characterized by mood episodes, with cycling patterns of both depression and mania or hypomania. Mania and hypomania are episodes of abnormal elevations of mood or irritability for at least 1 week (mania) or 4 days (hypomania), with raised activity level, inflated self-esteem, increased risk taking behavior, involvement in pleasant activities, and other features. Mania and hypomania differ with respect to severity of symptoms; when psychotic symptoms occur or admission is required, criteria for mania are met by definition. Furthermore, mania and hypomania can be distinguished in severity and duration of symptoms. Mania has a greater negative impact than hypomania in daily life with e.g. social and financial consequences. Depression is characterized by for at least 2 weeks of depressed mood or anhedonia for most of the time during the day. Several types of the disorder are distinguished according to the Diagnostic and Statistical Manual (DSM)-IV criteria: BD1 includes a history of at least one manic episode, though also depressive and hypo manic episodes are frequent, BD2 is marked by at least one depressive episode and one hypo manic episode. In figure 1, differences are shown. Furthermore, the DSM-IV includes cyclothymic disorder, consisting of a cycling pattern of hypomania and depressive symptoms, not fulfilling the criteria for full depressive episodes. Finally, BD not otherwise specified as rest category for patients with drastic mood changes regarded as mood disease, but not meeting enough criteria for hypomania and depressive episodes.

Manic and depressive episodes have already been described in ancient Greek Hippocrates. In his description of the Greek woman Thasos suffering from racing thoughts, sleeplessness, euphoria or irritable mood, and in severe cases hallucinations, he referred to this condition as a maniac state. The German doctor Emil Kraepelin (1856–1926), described manic depressive psychosis, as an illness with acute episodes, characterized

Figure 1: schematic example of "normal" mood chart and examples of mood charts in BD1 and BD2.



by depression or manic psychosis, with largely symptom-free intervals in between (5). Only until 1957 unipolar depressive and BD were distinguished into two different entities by K. Leonhard (6). In 1976, Goodwin and colleagues introduced hypomania and BD2 as a separate diagnosis. BD as such was officially introduced as diagnosis in the DSM-III in 1980. Goodwin and Redfield note that a complicating factor in research is the poor reliability of BD2 diagnosis resulting from the difficulties of assessing the history of hypo manic symptoms, especially by depressed patients (6). It is under current debate in what respect BD2 and recurrent unipolar depression differ with respect to cycling patterns and polarity. Long-term studies to differentiate between and validate diagnostic boundaries are highly needed.

Within this historical context, it is clear that the diagnosis and course of BD according to its modern criteria are only recently on their way to be further described and validated with respect to clinical course and treatment of the disease. Specifically validation of the "diagnostic" entity BD is subject to criticism (6), indicating the need for further research to the boundaries and criteria of this diagnosis.

In addition to the diagnostic difficulties, one must to realize the clinical course of the disease as highly variable and unpredictable. The DSM-IV criteria for BD predominantly focus on mood, with characteristics of mood episodes. Number of mood episodes, and recovery in between episodes, are thus key features in evaluating course of disease. However, in the past years evidence is increasing that impaired daily life functioning in patients with BD is caused by for example mood disturbance, especially residual depressive symptoms, as well as cognitive deficits.

Risk factors influencing clinical course

In identifying risk factors influencing clinical course, distal and proximal risk factors can be distinguished. Proximal factors are more immediately related to the actual episode of the disorder (preceding stress, interruptions daily rhythms, medication changes)

than distal factors which play a background role from conception and early childhood increasing imminent risk (maternal stress, childhood abuse). Several risk factors known to be associated with a worse long-term course of BD are already identified. In a landmark retrospective study of almost 1,000 BD patients in the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD) (7), the following was found: early onset was identified as risk factor, predicting higher rates of co morbid anxiety disorders, substance abuse, more recurrences, shorter periods of euthymia, greater likelihood of suicide attempts and violence. In longitudinal studies a relation was found between number of episodes in the index year and prior course of illness (history of rapid cycling), family with substance abuse, and poor occupational functioning (8, 9). In other studies, early childhood trauma was repeatedly associated with earlier disease onset and more severe course (10, 11). It has been thought that alcohol use can cause vicious circles leading to depression and vice versa, influencing the course of illness. Alcohol use as "self medication" strategy during (bipolar) depression occurs frequently (41% of bipolar patients) (12), increasing the risk on developing alcohol dependence. However, in a recent longitudinal study, no relation was found between alcohol use and longitudinal course of BD (13). Other environmental factors known to influence BD are life events and daily life stress. Life events are found to be of major impact in the year before the first depressive or manic episode (14). There are several, though somewhat contradictory, studies underlining the influence of life events on course of BD in longitudinal studies (15). Stressful life events may trigger depressive episodes in BD by inducing changes in social "Zeitgebers", circadian rhythms and the regulation of the stress response (15, 16). Thus, illness history, early childhood trauma, alcohol abuse and stressful life events influence course of the illness. In this study, we included social support, life events, medication use as proximal factors, and childhood trauma as distal factor. However, in this dissertation we will step back towards a preceding level, namely the underpinnings of the disease. In order to be able to further improve the understanding of the disease, we will first focus on two endophenotypes, namely stress hormone exposure and cognitive performance, in relation with clinical course of the disease.

Clinical course: endophenotypes as intermediate factors

Although clinical correlates associated with the course of the disease are known, the causes of the disease have still not been elucidated. Genetic risks seem to overlap with schizophrenia (17) in genome-wide association studies (GWAS), and currently, due to lack of convincing evidence, samples for a GWAS are now collected in 2,500 BD1 patients in the Dutch study named Bipolar Genetics. A major limitation of genetic association studies is that DNA nucleotide sequences themselves are not predictive for "in vivo"

gene expression, RNA transcription and proteome function. Research to protein function in relation with mental diseases is promising by detailing pathophysiological processes, which could lead to understanding of the biochemical overlap and differences between for example schizophrenia and BD (18). However, core pathophysiological underpinnings of BD have to be elucidated to be able to understand genetic, epigenetic and proteomic findings. One of the possible entrances in finding pathophysiological processes is the introduction of endophenotypes as simpler clues to find genetic underpinnings than complex phenotypic diseases. Endophenotypes are known as sub clinical quantitative traits that exist in affected and unaffected relatives independent of the disorder (19). The traits can differ in their presentation as they can be neurophysiological, biochemical, endocrine, neuroanatomical, or cognitive in nature (20).

Endophenotypes are generally less complex than their associated phenotype in a disorder as they have fewer genes, allowing easier linkage due to their higher signal-to-noise ratio. They are therefore useful indicators of processes that mediate between genotype and phenotype (21). The concept of endophenotypes was originally introduced by Gottesman and Shields in the early 1970s. A renewed interest has emerged due to the limited success of genetic linkage and association studies. For an endophenotype to be useful in genetically identifying a psychiatric disorder, it should meet a set of criteria as proposed by Gottesman and Gould (22): first, it must be associated with the disorder; second, it should be primarily independent of clinical state (i.e. it manifests whether or not illness is active); third, it should be heritable (i.e. more common in the non-affected relatives than in the general population) and fourth, it must be associated with a candidate gene or gene region. A valid endophenotype should hence have trait-like properties (21); in contrast to a state variable, an endophenotype it is not only affected by mood phase. Because having a stable and permanent presentation, endophenotypes may be more reliable than mood symptoms in predicting the long-term course of BD.

However, other concepts such as “biomarker” and “vulnerability trait” indicate the same need for better defined biological substrates for complex disease phenotypes. These terms refer to the search for biological underpinnings of diseases, while endophenotypes aim to serve as intermediate factors in finding genetic underpinnings of diseases. In BD, biological endophenotypes such as the status of the Hypothalamic- Pituitary- Adrenal (HPA-)-axis, could serve as endophenotype (23, 24). In addition, cognitive performance has been proposed to serve as a cognitive endophenotype (25-27) in BD, which also appears to be under influence of cortisol effects in the hippocampus (28, 29) and prefrontal cortex (30).

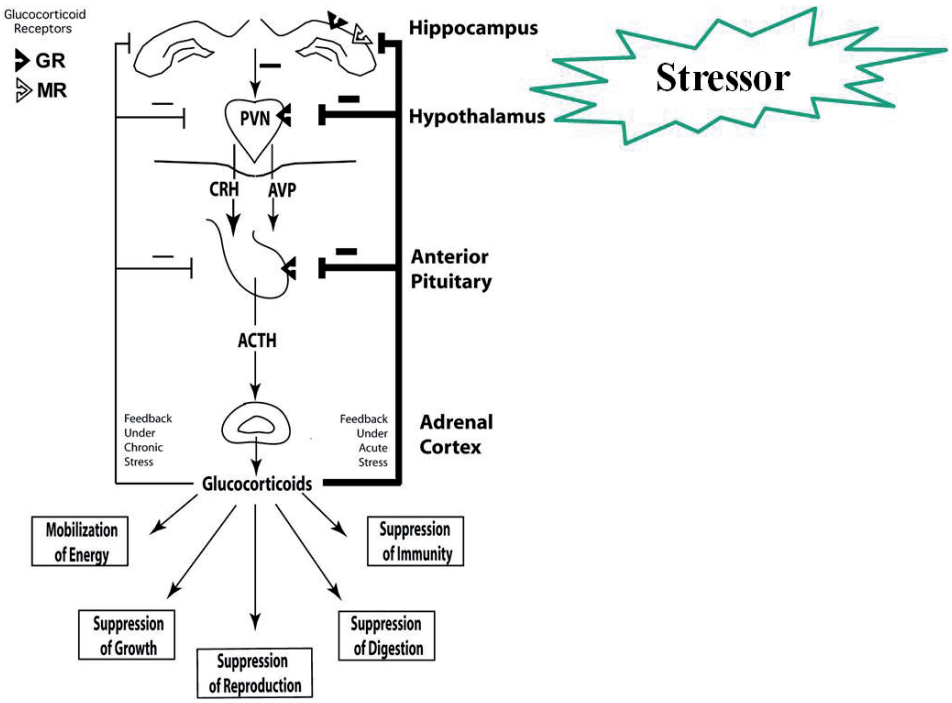
In the next paragraphs the HPA-axis as a biological endophenotype will be described in relation to BD. Furthermore, an overview will be provided with current assessments of

the HPA-axis, and new developments will be described. As a second endophenotype, cognition will be described in its relation both with the clinical course of BD and in its relation with the functioning of the HPA-axis. Although in this thesis the discussion of the role of endophenotypes will be limited, the introduction of this concept is needed to introduce the theoretical background and subsequently the choices made in our study design. The articles in this thesis focus mainly on the results of the cross sectional data.

Endophenotype 1: the HPA-axis

The biological stress response consists of a precisely regulated cascade of release of hormones and neurotransmitters. The direct route is stimulating the adrenergic pathways, inducing the sympathetic response; the slower hormonal route is regulating the HPA-axis. To maintain stability in a continuously changing environment, the human

Figure 2: Schematic overview of the HPA-axis, the major impacts on body processes, and the glucocorticoid (GC) feedback in the brain through Glucocorticoid Receptors (GRs) and Mineralocorticoid Receptors (MRs) (31) . GR and MR in the brain is included in this figure .Copied and adjusted with permission from Boonstra, 2004, in accordance with the Scientific Technical and Medical publishers permissions guidelines 2012.



body and mind are required to respond with flexibility, in which the HPA-axis plays an important role. The stress response is triggered by a stressor of any kind (psychological, physical). In figure 2 a schematic overview is provided.

Corticotropin Releasing Hormone (CRH) is a hormone that fulfils a central role in the stress response and is released by the hypothalamic paraventricular nucleus (PVN) under influence of a stressor. It is able to rapidly activate the sympathic response and plays an important role in orchestrating the so -called "fight, freeze or flight" reaction. In a second, slower way, CRH activates through ACTH- release in the pituitary, the release of cortisol in the adrenal glands. The combined system of CRH-ACTH-cortisol release is referred to as HPA-axis. Cortisol stimulates and regulates the mobilization of glucose in the liver, supporting the fight or flight reaction by stimulatory effects on the muscles, heart and blood pressure. Cortisol also affects cognitive processes such as memory of interpretation and decision-making in different situations. Furthermore, cortisol has suppressive effects on different organs and hormone systems not directly needed during

Table 1: Cortisol effects in and outside the brain

Cortisol	Central/ Brain effects	Systemic effects
Acute effects	Negative feedback of cortisol production by inhibition of CRH and ACTH production	Mobilization of glucose
		Increased blood pressure
	Suppression of growth hormone and sex hormone production	Suppression of bone re-calcification and immunity/inflammation.
	Inhibition of long-term potentiation neurons	
Chronic effects	Affective dysregulation, depression, euphoria	Hypertension, cardiovascular diseases
	Neurotoxic effects: cognitive deficits	Fatigue, myopathy
	Anxiety, eating problems, insomnia	Amenorrhea and impotency
		Impaired immune defenses, increased risk of regular infectious diseases
		Osteoporosis
		Diabetes

Based on PhD Thesis E. Van Rossum (2005) and "Stress, the brain and depression" – H.M. Van Praag, R. De Kloet and J.H. Van Os (2004).

the stress response, such as the gastrointestinal system, the immune system, the gonadal hormones, growth hormone and bone calcification (32). In sum, cortisol acts at multiple sites within the body to maintain homeostasis. Because of the damaging effects of chronic exposure to cortisol, the HPA axis is tightly regulated through negative feedback on glucocorticoid receptors: cortisol inhibits its own release and thereby finalizes the stress response. Chronic raised cortisol levels could be very damaging. Long-term effects of increased cortisol levels are: osteoporosis, myopathy, fatigue, abdominal obesity, diabetes and dyslipidemia leading to increased risk of cardiovascular disease, impaired immune defenses, affective symptoms and neurotoxicity, especially in the hippocampus area associated with cognitive symptoms such as memory deficits and problems with concentration and executive functioning. In table 1, cortisol effects are summarized.

Dysregulation of the HPA-axis in BD as endophenotype

Dysregulation of the HPA-axis is known to occur in several psychiatric disorders such as anxiety disorders like post traumatic stress disorder (33) and mood disorders including BD (34-40), in patients as well as in healthy offspring of bipolar parents (24, 37). Activation of the HPA-axis during depressive episodes exists in more than 80% of the patients (41), this association seems at least partly state independent in bipolar patients (39), indicating a trait phenomenon: remitted patients have higher overall cortisol levels, reduced cortisol reactivity to negative daily events, and flatter diurnal slopes associate all with more episodes (42), reflecting subtle but on the long-term clinically relevant influence. Daban (43) and Watson (39), reviewed the abnormalities in HPA-axis functioning in patients with BD. Both conclude that the Dex/CRH test, the most sensitive challenge test provoking a stress response and measure HPA axis abnormalities, is abnormal in remitted and non-remitted bipolar patients, indicating that this could serve as a trait marker in BD as well as in MDD (40). In chapter 1 and 2, this will be discussed in further detail. All mentioned arguments plead to the HPA-axis functioning as endophenotype in BD, following the aforementioned criteria according to Gottesman and Gould: it is associated with BD, it is at least partly independent of clinical state, it is found in healthy family members. In the next section we will focus on the final criteria, namely the genetic basis of the HPA axis regulation in relation with BD.

Dysregulation of the HPA-axis and the role of cortisol receptors: link to genetic underpinning.

There are two main cortisol receptors by which cortisol exerts its effect: the Mineralocorticoid Receptor (MR) and the Glucocorticoid Receptor (GR). In the brain, MR

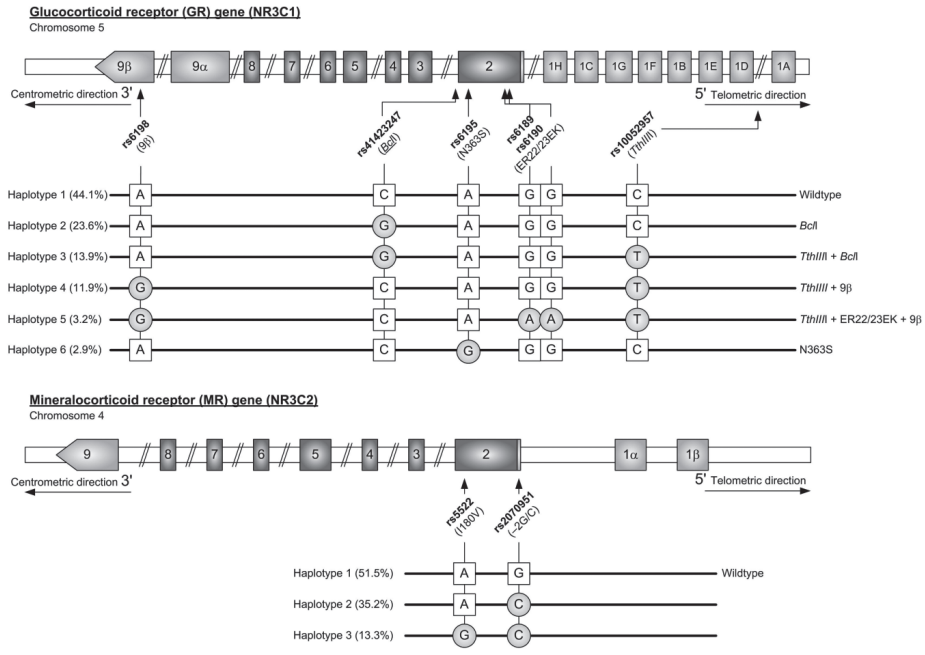
is predominantly localized in the hippocampus, the amygdala and the prefrontal cortex, and has a dominant function in controlling sensitivity of the stress response system, limiting disturbance of cellular homeostasis and for example acquisition and appraisal of information, and behavioral selection (prefrontal) (44).The GR is widespread throughout the brain, but has a high density in the hippocampus, the prefrontal cortex and the periventricular nucleus and is involved in facilitating recovery of cellular homeostasis, restraining stress induced responses and learning and memory processes promoting behavioral adaptation for future events (44). The affinity of the MR for cortisol is 10 times higher than the affinity of the GR for cortisol. In normal circumstances cortisol predominantly binds to the MR, during the stress response cortisol also binds to the GR, whereas the GR is important for the negative feedback thereby determining the regulation of the HPA-axis (45-47). During periods of stress and elevated plasma cortisol, there is increased occupation of GR.

The lipophilic cortisol is thought to enter the cell by passive diffusion through the cell membrane. In the cytoplasm it is binding with the GR or MR, after which the complex travels through the nuclear membrane into the nucleus. The MR and the GR function through several modes of action. An important route to affect gene transcription is the direct binding of GR or MR dimers to target genes. Metabolic effects and the feedback mechanism are exerted by stimulation of gene transcription, also transcription inhibition can occur through this mechanism. The second route is through to regulate gene expression by binding to gene regulating proteins like AP-1 (activating protein-1) and NF-κB (nuclear factor- κB) (48). These pathways influencing gene expression are time-consuming, as it is clear that changing protein production by altered gene expression is a slow process, applicable in regulatory but not acute processes. In animal studies, the MR is also thought to be active as membrane MR in regulating the HPA-axis in a fast way, by regulating the threshold for stress induced surge and neuronal excitability (49). The effects of GR and MR dimers are complex and divers, and can modulate the signal transduction of for example serotonin and adrenaline. However, these hypotheses are based on animal studies, in which the predominant glucocorticoid is corticosterone; in humans it is cortisol; it is unclear to what extend these findings remain the same in humans. The therapeutic applications of these receptors is currently under debate in trials with GR-antagonists, such as mifepristone in treatment of depression with psychotic features (50), which is continued in a placebo controlled trial in depressed patients with psychotic symptoms (ClinicalTrials.gov: NCT00637494), planned until the end of 2013. In bipolar depressed patients it has been found to improve mood and neurocognitive functioning (51). However, it was not effective for treatment of major depressive episodes in previous trials. In the current treatment of BD, lithium en valproate take a central place. These drugs seem to have an effect through GR-co-chaperone proteins,

that attenuates GR translocation in the nucleus, and indirectly reduce hypercortisolemic effects at tissue level (52).

Several different MR gene and GR gene variants (polymorphisms), appear to alter glucocorticoid sensitivity, which are described in figure 3. These polymorphisms are known to result in clinically relevant consequences (53), and discussed in detail in chapter 1.

Figure 3: Overview of the GR gene and MR gene, showing the single nucleotide polymorphisms (SNPs) and haplotypes



In sum, these haplotypes have been known to lead to altered HPA-axis regulation with clinical consequences:

GR gene haplotypes:

- Haplotype 2 (*Bcl*I) and haplotype 3 (*TthIII*I+ *Bcl*I): both involving a C to G single nucleotide polymorphism (rs6189/rs6190) at a polymorphic *Bcl*I restriction site, were associated with hypersensitivity to GCs (54). Several previous studies reported associations with unfavorable metabolic characteristics, such as increased body

mass index (BMI) (55), increased abdominal visceral fat(56, 57), hypertension(58), and lower amount of lean mass in the elderly (54).

- Haplotype 4 (*TthIII*I + 9β): The 9β polymorphism (rs6198) is a single nucleotide polymorphism (SNP) in exon 9β of the *GR* gene. An ATTTA sequence in the 3' UTR is changed into GTTTA. This polymorphism is associated with an increased stability of the mRNA of the inactive GR-β isoform and may lead to a relative glucocorticoid resistance by increased inhibition of the active GR-α isoform (59). This polymorphism results in reduced transrepressional effects (by which, for example, genes important in the immune system are regulated), whereas the transactivational effects appeared to be normal(60). The 9β polymorphism has also been reported to be associated with rheumatoid arthritis (59), and a 68% reduced risk of carriage of nasal Staphylococcus aureus in carriers of this polymorphism (61).

- Haplotype 5 (*TthIII*I + 9β + ER22/23EK): is also associated with a relative resistance to GCs, but in contrast to haplotype 4, this resistance is linked to the transactivational effects of the GR, by which most of the adverse effects of GCs are being mediated. The ER22/23EK polymorphism (rs6189/rs6190) is located in the transactivation domain of the GR gene, and involves two nucleotide changes in codons 22 and 23 (GAG AGG -> GAA AAG), which have been shown to be linked. This variant has been found to be associated with a healthy metabolic profile (lower C-reactive protein (CRP) levels, lower cholesterol levels and increased insulin sensitivity), longevity in the elderly and a beneficial body composition in young adults (48, 53).

- Haplotype 6 (N363S): this polymorphism (rs6195) is located in codon 363 of exon 2 of the *GR* gene. The nucleotide change (AAT -> AGT) results in an asparagine to serine amino acid. It is associated with lower cortisol levels and higher insulin levels after DST, suggesting increased GC sensitivity (62).

MR gene haplotypes:

- Haplotype 2 (-2G/C): the C allele of this polymorphism (rs2070951) is in vitro resulting in more MR protein. These carriers have a more sensitive suppression of cortisol after low dose (0.25 mg) Dexamethasone, with a highest decrease in women (63). The Cortisol Awakening Rise was not affected (63). During the Trier Social Stress Test, these persons showed highest saliva cortisol and heart rate, indicating that more MR protein leads to a higher acute stress response (64).

- Haplotype 3 (-2G/C and I180V): this haplotype is consisting of the C allele of the -2G/C polymorphism in combination with the A -> G allele of the I180V polymorphism

(rs5522). The G allele of the I180V polymorphism is associated with higher cortisol levels in men (63).

The genetic basis of HPA-axis functioning is fulfilling the final criteria to serve as endophenotype according to Gottesman and Gould. Recently, the GR polymorphisms *Bcl*I and ER22/23EK have been associated with unipolar depression in several studies (65, 66). In addition, the ER22/23EK polymorphism seems to be associated with a decreased risk of dementia in healthy individuals. MR haplotype 2 has been found to correlate with optimism and a lower risk of depression, both in women (66b).

In this thesis we investigated the association between GR and MR polymorphisms and clinical characteristics of BD. The future plan is to study the possible associations between these genetic variations and cognitive performance.

Analyzing the HPA-axis: how to assess a system in continuous change?

Until now, there are several methods to investigate the HPA-axis functioning in vivo. Multiple consecutive saliva and serum cortisol levels can provide insight in cortisol curves, total daily cortisol production and the cortisol awakening response (CAR). Circulatory cortisol levels are known to depend on chronobiological rhythms (ultradian, circadian as well as seasonal rhythms) and daily life stress.

Both the ultradian and circadian pacemakers reside in the hypothalamus (67), influencing hormonal release and hence cortisol level variability. First, ultradian rhythm is caused by oscillatory neurons in the hypothalamus, leading to CRH release and consequently, ACTH pulses and cortisol pulses, with a normal hourly rhythm (68). Contrary to GC binding to the MR (t1/2= 45 minutes), GR function is depending on this pulsatility, with a rapid dissociation of GCs after a rapid pulse (t1/2 = 5 min) (68). The timing of a stressor in a decreasing or increasing point of the pulse, has consequences for the magnitude of the stress response, which is absent in rats in the falling phase and vice versa in the increasing phase of GCs during the pulse. Second, circadian rhythms influence cortisol levels. Moreover, they are known to be disturbed during mood episodes, which in rats have been shown to be restored after fluoxetine (69). Third, seasonal variations influence HPA-axis activity, with higher cortisol levels in the morning and higher sensitivity of the stress response (increased feedback) during winter months, protecting against energy waste during winter sleeps in animals (70, 71). Therefore, it is crucial to be aware of the fact that the timing of an assessment during the day has limitations due to chronobiological rhythms in providing information about the HPA-axis functioning.

Table 2: Methods to evaluate HPA-axis functioning – pros and cons

Test	Method	Assessment	+	-
Cortisol day curve (73)	Saliva or serum sampling during the day	Assessment of circadian rhythm	Robust impression of day rhythm Mean cortisol level during the day	Low sensitivity for detecting psychopathology in psychiatric patients No evaluation of stress response Intensive for patients, serum sampling requires admission.
Urinary free cortisol (74)	Mean daily cortisol in 24h urine sampling	Reflection of total cortisol production as diagnostic tool for Cushings' syndrome	Comparison inter individual differences Information on total cortisol production	No information on stress response, no information on ultradian or circadian changes Increasingly unpopular due to low sensitivity as well as specificity in detecting Cushings' syndrome, however still broadly applied for diagnostic purposes
Scalp hair cortisol (73)	Cortisol levels in scalp hair strands	Long-term cortisol: mean cortisol level over months to years (depending on length of hair)	Easy, non invasive, information on long-term mean systemic cortisol levels. Not affected by daily fluctuations Possibility to assess cortisol in retrospect	New method: clinical relevance?
CAR (75)	First hour following awakening saliva or serum collection.	Assessing basal activity as well as response to awakening.	Associated with mood disorders Non invasive, outpatients, home and non-stress situations	Highly dependent on time of awakening Compliance
TSST (76)	Psychosocial stress test under laboratory conditions	Possibility to evaluate cortisol levels as well as other HPA-axis parameters pre, during and post acute stress	Induced "real" moderate to severe stress Responsiveness of endocrine and immunological parameters	Ethical considerations in patient populations Time consuming, expensive
ESM (77)	Saliva sampling after beeps (e.g. 10 beeps per day) in daily life, simultaneous report of daily life experiences	Insight in HPA-axis responsivity in daily life experiences	No recall bias Associated with reduced flexibility of stress response and higher overall cortisol levels in bipolar patients (42)	Depending on compliance Time consuming and demanding for participants

DST (41)	0.25 mg, 0.5 mg or 1.0 mg Dex in evening; morning awakening cortisol level	Testing negative feedback of the central GR	Easy to perform 1-mg DST is valuable in diagnosing Cushing's syndrome 0.25 mg DST is valuable in determining GR sensitivity in normal individuals.	Low sensitivity in psychiatric populations (<45%)
DEX-CRH test (34)	1.5 mg Dex at 11 pm followed by 100 µg CRH at 3 pm next day, blood sampling from 2 pm- 6 pm	Testing negative feedback of the central GR	High sensitivity in psychiatric inpatients (80-90%) Higher cortisol levels predictive for relapse depression (78)	Not suited for outpatients due to frequent sampling Expensive in large cohorts
Metapyrone test	Blocking cortisol production by inhibition of 11-deoxycortisol into cortisol	Diagnostic tool for testing the HPA-axis functioning	Widely used in endocrinology to diagnose secondary adrenal insufficiency Compensatory increasing ACTH levels indicate good functioning of HPA-axis on pituitary level	Not suited for subtle changes in HPA-axis regulation
Insulin tolerance test	By inducing hypoglycemia the HPA-axis is strongly activated	Diagnostic tool for testing the HPA-axis functioning	Diagnosing adrenal insufficiency	Risk for lethal hypoglycemia, should always be performed in clinical settings with closely monitoring of vital functions.
ACTH stimulation test, or, Synacthen test (79)	Administration of intravenous or intramuscular synthetic ACTH	Diagnostic tool for primary adrenal insufficiency	High sensitivity and specificity	Narrow indication, no information about feedback functioning.

Abbreviations: ESM: Experience Sampling Method; CAR: Cortisol Awakening Rise; DST: Dexamethasone Suppression Test; DEX/CRH test: Dexamethasone/ Cortisol Releasing Hormone test; TSST: Trier Social Stress Test

To get insight in set point, negative feedback sensitivity and functioning of cortisol receptors, challenge tests are needed to evaluate HPA-axis functioning during the acute stress response. Mean cortisol levels during the day, and as a new method, mean cortisol levels over months may provide information concerning the long-term cortisol levels as result of HPA axis sensitivity in relation to psychopathology. The most sensitive way to establish sensitivity of the GR is to perform the earlier mentioned Dex/CRH tests (72). Other methods include the Trier Social Stress Test and the Dexamethasone Suppression Test. Alternative ways to assess the functioning of the HPA-axis in basal and daily life conditions are by measuring cortisol levels in saliva, morning awakening responses, or by the Experience Sampling Method. In table 2 these different assessments are summarized.

Worldwide, we are the first to use scalp hairs to measure cortisol levels in patients with BD. This recently developed method is feasible to determine long-term cortisol levels and appears to yield a reliable estimate of long-term HPA-axis activity (80-82), with promising potential to inform about the consequences of increased long-term cortisol levels (83). A positive correlation has been found between cortisol levels in hair and waist circumference and Waist Hip Ratio (73), and higher cortisol levels have been found in patients with high endogenous cortisol levels (e.g. Cushings' syndrome). Since hair grows with an average rate of 1 cm per month, a hair segment of e.g. 3 cm would reflect mean cortisol levels over a period of approximately 3 months. This long-term cortisol measurement is therefore not influenced by the time of sample collection or acute stress due to daily circumstances or the research setting. Decreased cortisol levels are found in hair of patients with generalized anxiety disorder (GAD), but no differences in salivary cortisol levels between GAD patients and healthy controls (84). This suggests that hair cortisol levels may reflect the chronic cortisol secretion, whereas the results found with saliva or serum cortisol levels might also include acute responses to the measurement circumstances. Several other studies have shown that hair cortisol is indeed a marker of long-term cortisol exposure (80, 85-88). Previously, hair cortisol was used to provide a tool for doping control regarding chronic glucocorticoid use by top athletes (89). Van Uum et al used it to provide a technique to assess the cortisol levels due to chronic stress (88), which was confirmed one year later to be a reliable retrospective marker for cortisol levels for at least 6 months up to several years (90). This method proves promising in providing a retrospective calendar of cortisol production (73). Very recently, a relation has been found between higher cortisol levels over 6 months and depression (91), whereas lower hair cortisol levels relate to generalized anxiety disorder (92).

In order to explore the possibilities and relevance of the new available long-term cortisol assessment in hairs in patients with BD, we decided to use this technique to assess cortisol levels in scalp hair in our cohort. Previous studies did not report on hair cortisol levels in BD patients yet, the results of the patients in our cohort are described in chapter 5. Furthermore, we collected saliva samples at two consecutive evenings at 22.00h. Several studies have shown that saliva samples taken in the late evening on different days show the lowest variation in cortisol levels compared to diurnal cortisol measurements and the cortisol awakening response (93-95). This suggests that late evening cortisol levels are not as much influenced by acute stressors and daily influences than e.g. the cortisol awakening response or other daytime cortisol measurements. Therefore, it is most likely that *if* there is a correlation between hair and saliva cortisol measurements, it may have been with evening salivary cortisol measurements.

Endophenotype 2: Cognitive functioning

The word cognition refers to mental functions or processes including attention, memory, language skills, planning and supervising behavior. It is a widely used term with different meanings depending of the field it is used in. Here, we use cognition to refer to mental processes in the prefrontal cortex (executive functions), and hippocampus (memory). Additionally, attention is necessary to select information for further processing. This might be located in parietal lobes, where sensory information is selected to be transformed into "noticed" information. To avoid pollution of the meaning of these 3 terms, the content is described in Box 1. Cognitive functions like attention, memory and executive functions are not stringent different, but show overlap: without focused information selection there is nothing to remember. Without working memory it is not possible to plan and coordinate information into behavioral selection.

Box 1: Definitions of cognitive functions used in this thesis

Attention:

Attention is the spotlight on non selective sensory information, enabling persons to select and subsequently process selected information efficiently. This function is useful in preventing sensory overload.

Working memory:

Short term memory is useful for "holding" information (visual, phonological) in the present with direct past and direct future, with limited capacity. The information held is coordinated by executive skills like retrieving memories, selection and initiation of behaviour, etc. "This interaction between flexible executive functions and passive processing routines is an essential characteristic of working memory" .

Executive functions:

These functions act as the brain's chairmen, coordinating information, silencing input, activating and synchronizing other brain regions. Prefrontal located; not one specific domain, but using for example attention, working memory and language. It is distinguished from "automatic" selection of behaviour; executive functions are needed when automatic behaviour appears to be insufficient. Five situations are identified (Ward, 2006, p.286): 1) planning, decision making; 2)

error correction and troubleshooting; 3) not well learned or novel sequences of actions; 4) dangerous or difficult actions; 5) need to overcome habitual actions or resisting temptation.

Based on:"The Student's Guide to cognitive neuroscience" – Jamie Ward; 2006; Psychology Press, Hove, East Sussex.

Until recently, due to Kraepelin's influence, BD was regarded as an episodic illness with the assumption that patients resumed complete recovery, cognitive function included, between episodes (96). Modern clinical neuropsychological batteries, have found that in BD there is a clear impact on cognitive function between episodes (21), with a clinically relevant relation between for example processing speed and a poorer outcome with respect to social and global functional (97), and verbal memory as a significant factor in determining functioning at work. This is in line with the finding that light depression and impairments in memory and executive functioning are predicting long-term (5 year) functional (negative) outcome of the disease (98). Poor outcome is highly associated with cognitive impairments, particularly executive dysfunction, persisting during euthymic phases, leading to impairment of psychosocial and occupational functioning (99). These studies underline that in addition to the clinical mood episodes (especially sub-threshold depression) also cognitive impairments should be taken into account to establish a functional prognosis of the clinical course of the disease.

Several domains of cognitive function are recently recommended to focus on attention, processing speed, executive functioning, working memory and visual and verbal learning memory (21, 100-102). These appear to influence daily life functioning significantly (98, 99). In 2010 the International Society of Bipolar Disorders (ISBD) reached consensus about the preference of assessment of these cognitive functions in BD (102). The ISBD Battery for Assessment of Neurocognition (ISBD-BANC) is proposed as still preliminary, still needing empirical validation towards a further delineation of a core set of cognitive tests. In 2005 however, we decided to use the Test for Attentional Performance(www.psytest.net). This instrument is developed to assess attention performance, working memory and executive functions in adults and children with brain damage, and later applied in psychiatric patient populations. The development of these tests was based primarily on the needs of low complex, easy understandable neuropsychological diagnostics, which could be applied easily to patient groups and investigators (103).

Until now, cognitive domains which are found to be disturbed in BD differ somehow in different mood phases of the disease. During euthymia, mainly deficits in sustained attention, divided attention, verbal and working memory, processing speed, and impairment of executive functioning appear to be most consistently observed according to a recent meta-analysis (100). However, these deficits are not limited to euthymic phases and seem to exacerbate by depressive and/or manic symptoms as well. Furthermore, depressive mood states are characterized by lack of concentration, deficits in working memory, and executive functioning (104). (Hypo)manic states are accompanied by lack of sustained and selective attention, and long-term working memory. However, with the aforementioned criteria of Gottesman and Gould (22) in mind, cognitive deficits are associated with BD, and seem at least partly state independent. Genetic factors are probably most important in explaining both cognitive impairments (105). The final criteria, namely the prevalence in unaffected family members, is met in some other studies (26), arguing that cognitive functioning could serve as an endophenotype in future research. However, this is still under debate, as cognitive functioning in unaffected family members is not convincingly found to be disturbed (106) and is still in need for future studies.

While cognitive abnormalities are recognized as an important feature of BD (107), the nature and extent of these are less clear in terms of their existence before onset of affective symptoms, their etiology, their relationship to underlying neuroanatomical abnormalities and how they are affected as illness progresses. Here we discuss the influence of medication on cognition, the hypothesis of cognitive damage due to HPA axis dysregulation and the scar hypothesis.

Cognition and medication

However, besides being a disease trait, cognitive performance is known to be influenced by other factors as well. Well known influences of better cognitive performance are younger age, higher level of education and euthymia. Furthermore, the influence of treatment and specifically medication on cognitive performance are other important factors. Effective medication became available in the early fifties of the twentieth century. Together with the development of more standardized diagnostic procedures, psychopharmacological treatment options were developed firstly to treat acute episodes and later to prevent new episodes. Lithium was first used in 1949 by John Cade for treatment of mania (108), and approved by the American Food and Drug Administration in 1970. Since then, drug treatment became more and more common in

the treatment of acute mood episodes as well as after recovery. Maintenance treatment preventing new episodes is a relative new phenomenon, influencing the long-term outcome of the disease. However, results have been contradictory; for example, in 2008 it was convincingly argued that (long-term) antidepressant use is increasing the risk on developing a rapid cycling pattern of BD on the long-term (109). In a naturalistic setting, patients with a history of psychotic episodes including 31 bipolar patients, were followed during 15 years and compared off and on medication use. On the long-term, it appeared that a subgroup of these patients did very well on symptom scores and global functioning (110). The influence of medication use on the long-term course of BD has to be further entangled, with respect to clinical observable mood episodes as well as cognitive functioning and physical health. It is already known that cognitive performance can be influenced by psychotropic medication. Although genetic factors are considered as most important influences on cognition in BD, the role of residual mood symptoms and of medication use is also topic of discussion (105). The impact of Lithium use on cognition concerns mainly a slight slowing of processing speed and subjective impairment of cognitive functioning; no other strong significant influences on cognitive performance have been found (111-113). Antipsychotic use seems associated with level of memory and executive functioning in a group of 40 BD patients compared with 40 healthy controls (112). Despite all the research, little attention has been given to the consequences for cognition of using polypharmacy, which is common practice in the treatment of BD.

To be able to investigate cognitive performance as endophenotype in relation with clinical course and with cortisol exposure, the role of medication cannot be neglected. Therefore, in chapter 6 the association between medication and cognition is investigated.

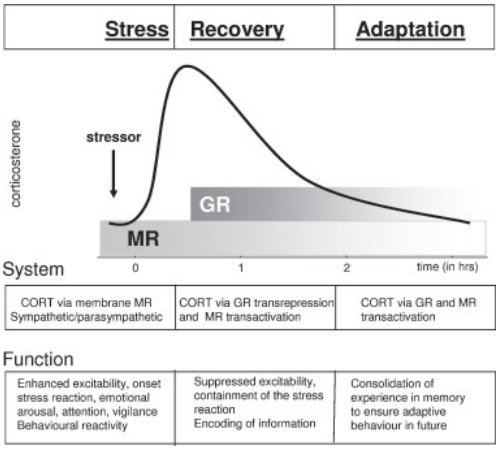
Cognition and impact of cortisol exposure: relation between two endophenotypes?

Recent studies suggest that abnormalities in the HPA-axis of BD patients may cause or exacerbate the cognitive impairments (114, 115). The negative influence of cortisol on neuroplasticity , which is disrupted in mood disorders, may well play a role in the development of cognitive deficits (116). In general, acute stress or the so called ‘fight or flight’ response improves cognitive functions such as attention and memory for the time the stressor is present. However chronic or excessive activation of the stress-system has disruptive effects on cognitive functioning (75). Patients with Cushings’ syndrome with excess of ACTH as well as cortisol production, are known to suffer from cognitive deficits. Furthermore, cortisol excess causes hippocampal atrophy in these patients (117, 118), which is reversible after successful treatment (119). The cognitive deficits found in BD

patients might therefore be related to the dysregulation of the HPA-axis. Recently, a relationship between higher cortisol levels and hippocampal related cognitive functions such as verbal memory, and executive functioning in unipolar depressed patients (120). The proposed relationship between cognition and HPA-axis functioning as described above, is giving ground to the choice of assessing both HPA-axis functioning as well as cognitive functioning in this study. However, in this thesis, the relationship will not be further described.

Having responsibility for the onset and termination of the stress response, the GR and MR also seem to be important in cognitive functioning. The MR as well as the GR are involved in learning and working memory (121-123). In figure 3, different functions of MR and GR are shown.

Figure 3: MR and GR- level of activity and cognitive function.



Based on (with permission): Brain development (in animals) under stress: Hypotheses of glucocorticoid actions revisited (30).

Van Rossum and colleagues reported an association between a GR polymorphism (ER22/23EK) and reduced risk for dementia in the general population (124) and less divided attention disturbances in depressed patients (65) indicating that this GR polymorphism may influence neuro-cognitive processes. It is hypothesized that one explanation could be a decreased direct effect of cortisol on brain tissue is mediated by a relatively insensitive GR (124). The MR is known to be involved in appraisal of critical situations and selection of behavioral adaptation (30). At a genetic level, MR haplotype 2 (the -2G/C SNP) is known to be associated with reduced depression, reduced hopelessness and more optimism (66b).

There are a number of indications that MR correlates to cognition; in patients with Addison's disease, a role of MR in encoding learning material has been found (125). The authors show the interaction between GR and MR, with optimal performance on working memory tests, when both activated. Further evidence for a role of the MR in cognitive performance has been found in blocking the MR with spironolactone in healthy individuals, which leads to impairment of attention, visuospatial memory and mental flexibility (126). Still, the connection of the MR with cognition has been mainly studied in depression: the MR polymorphism rs5522 (I180V) moderates reward processing and stress-induced reward learning deficits (127). However, the complex interaction of GR and MR in cognitive performance in health and disease needs to be further clarified. One hypothesis is, due to a shift in the MR:GR balance by chronic stress, MR-mediated behaviors are altered and consequently, GR mediates the consolidation of these altered behaviors (30).

The HPA-axis functioning and cognitive functioning: the scar hypothesis

A new approach to explaining brain damage in mood disorders is the scar theory (128), describing long lasting changes in the function of the brain (cognition, biological) following depression, increasing the risk for developing future depressive episodes. The pre-morbid regulated "set-point" of the HPA-axis is thought to be changed by epigenetic changes in DNA methylation of the GR caused by for example childhood abuse in suicide victims (129) or motherly depression during pregnancy (130). This can make an individual in advance more prone to be sensitized by stress. However, it is still questioned to what extend causes of depression lead to scarring, including sensitivity for stress (as measured by the ESM, TSST, cortisol assessments), and a changed set-point of the HPA-axis due to DNA methylation of the GR; on the other hand it is thought that depressive episodes themselves lead to further scarring of the functioning of the brain with respect to cognition and HPA-axis sensitivity. This scarring process is possibly a structural phenomenon developed during life, starting at early childhood and never ending, probably influenced by protective, as well as harmful circumstances.

The Bipolar Stress Study

In 2006 a study named The Bipolar Stress Study, was started at the outpatient department for Bipolar Disorders of PsyQ The Hague. The general topic of the Bipolar Stress Study is to identify risk factors, that have impact on the clinical course of BD and treatment of these patients. In the study approach three levels and their interactions with the environment (stressful life events and social support) were distinguished: 1.

clinical functioning (phenotype) , 2. genetic variations and vulnerability (genotype) and 3. cortisol exposure and cognitive functioning (endophenotypes).

In this dissertation, a part of the results is presented with the emphasis on the role of genotypes of the cortisol receptors and two endophenotypes, namely cortisol exposure and cognitive functioning. The results of the Bipolar Stress Study may help to develop clinically relevant interventions to improve the course of BD and subsequently the quality of life of patients with BD.

In general, there is a trend in psychiatric research towards early detection of disease and early identification of people at risk, also in research in the field of BD (131). It is expected that diagnostic processes will be more and more supported by genetic, biochemical, and neuropsychological assessments, to enable focused treatment strategies. As Hyman already pointed out that "defining a rational nosology for disorders of the brain, the body's most complex organ, is clearly one of the great challenges for modern medical science. Nonetheless, fundamental advances in our understanding of the genetic and environmental determinants of disease risk, and of the neural circuitry supporting normal and pathological mental processes promises to form the basis of improved classification in the coming decades" (132).

The Bipolar Stress Study is a project that consists of a cross sectional study followed by a 24 month longitudinal study, in part of the original patient group. The data analysis of the longitudinal study are ongoing.

For the cross sectional study the data of 366 patients were collected (genotype , phenotype and endophenotype). In the 24 month longitudinal study 189 patients participated.

The first patients were enrolled in 2006, data collection stopped at the end of 2011.

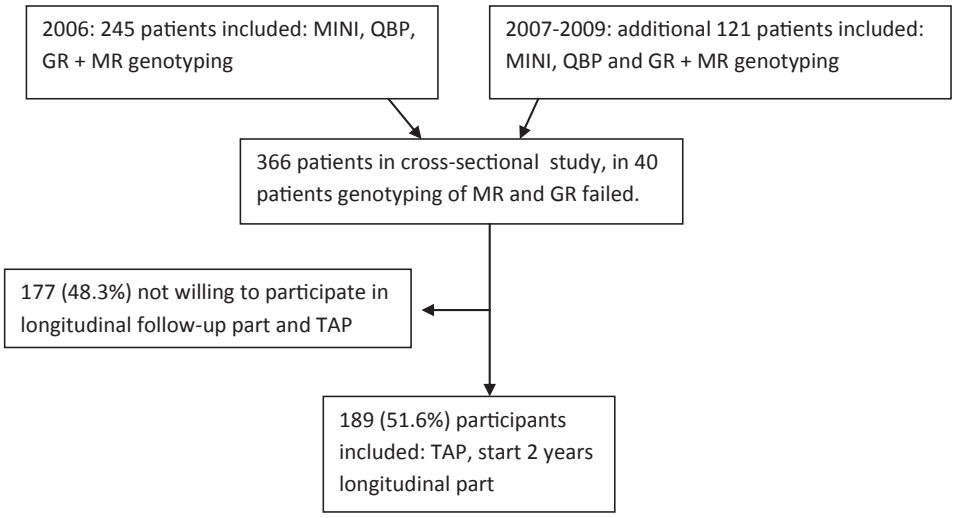
In figure 4, a flow chart is shown of our cohort.

Although in this thesis the cross sectional but not the longitudinal collected data are presented, a description of both the cross-sectional and longitudinal part is provided to clarify the study design and to be able to discuss the results within a consistent framework.

I. Cross-sectional part

First, in 2006, the first 245 patients were enrolled. They started with the assessment of the MINI, the QBP and GR and MR gene genotyping. In 2006 we extended the protocol, and recruited an additional 121 patients for baseline measurements. Furthermore

Figure 4: Flow chart of patient numbers and dropout rates in the Bipolar Stress Study.



we asked the first 245 patients to participate again in the extended study. In total we included 366 patients for the cross-sectional baseline measurements.

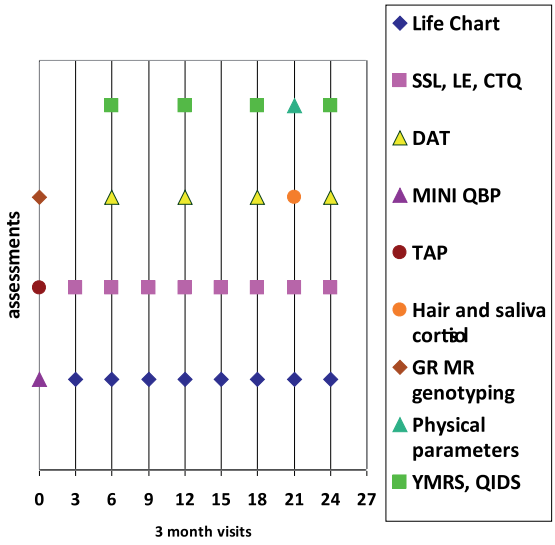
In 2007-2009, of these 366 patients, 189 participants were enrolled in the longitudinal study which also included the Test for Attentional Performance (TAP), which was not available for the complete 366 patients in the cross-sectional part. Please take note of the fact that the first visit includes the TAP and the MINI + QBP for most patients; however, the cross-sectional measurements with the patients of the cohort included in 2006 were separated in time with the MINI QBP and genotyping measured by entrance of the study, the TAP was assessed by continuing in the extended study the next year.

II. Longitudinal part

In the follow-up period of 24 months, all patients filled in a monthly Life Chart, and every three months the Social Support List and the Life Event List. Every 6 months they did a subtest of the TAP, namely the Divided Attention Test, together with measurement of current mood (the QIDS and the YMRS). Data on cortisol levels and physical health were collected in a subgroup of 100 patients in hair and saliva during the 7th visit at 21 months.

In figure 5, a timeline is provided with the various assessments during each visit in the longitudinal part of the study.

Figure 5: Timeline of longitudinal measurements



Abbreviations: MINI: Mini International Neuropsychiatric Interview; QBP-NL: Questionnaire for Bipolar Illnes- Dutch version; TAP: Test for Attentional Performance; YMRS: Young Mania Rating Scale; QIDS: Quick Inventory for Depressive Symptoms; LCM: Life Chart Method; SSL: Social Support List; LE: Life Events list; DAT: Divided Attention Test.

In this thesis, the following results will be described:

- The cross-sectional data of the first visit (t0) with emphasis on the genotype data and the role of GR and MR polymorphisms on the clinical course of BD;
- The cortisol levels in hair and saliva (21 month visit) in relation with clinical course of BD;
- The results of the divided attention test after 6, 12, 18 and 24 months, in relation to the QIDS and YMRS scores, as first analysis of the longitudinal data set.

In chapter 1 and 2 an overview of current studies is provided in two reviews, thereby giving the further theoretical underpinning of our research project. Chapter 1 focuses on the regulation of the HPA-axis in mood disorders, and especially on how to assess the HPA-axis. Furthermore, in this chapter findings of deregulation of the HPA-axis and mood will be discussed. Chapter 2 will discuss findings regarding genetic polymorphisms in the GR and MR gene in relation to mood and cognition.

In Chapter 3 and 4 we investigated the GR gene polymorphisms (chapter 3 and 4) and MR gene polymorphisms (chapter 4) in relation to cross-sectional retrospective collected data regarding previous illness course and clinical characteristics. These articles intend to identify the risk for the course of BD of these genetic variations influencing stress sensitivity.

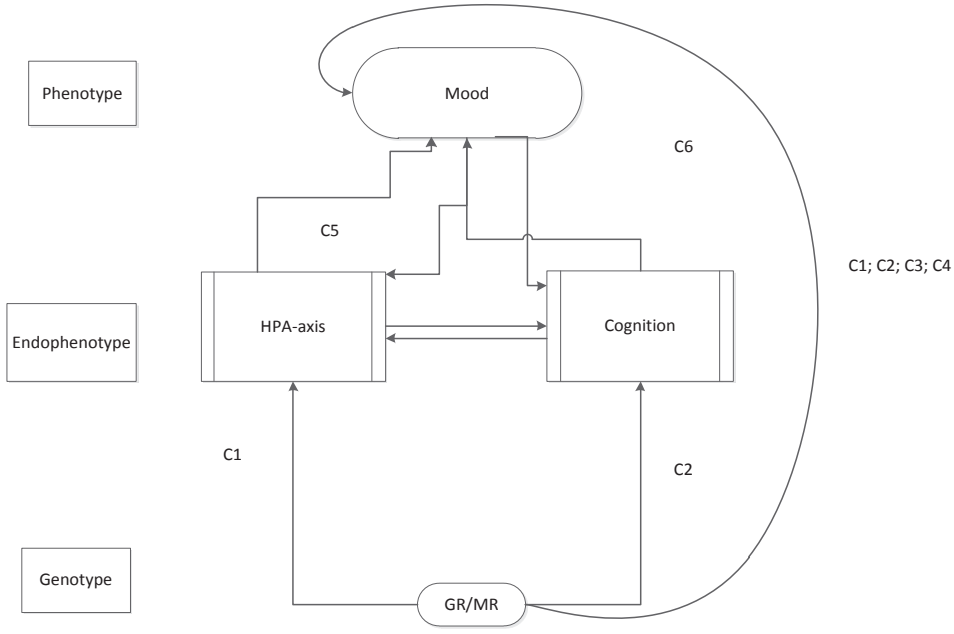
In Chapter 5 hair and saliva cortisol levels are described and related to clinical correlates of BD.

In Chapter 6 the cognitive functioning in relation to is described, in relation to clinical characteristics and medication use in BD patients is described.

Although the results presented in this dissertation are part of the larger ongoing Bipolar Stress Study, this thesis must be seen as a step forward in elucidating the role of cortisol exposure in relation to illness course and cognition. This may lead to new directions in future research and subsequent treatment. This will be discussed in more detail in the General Discussion.

In figure 6 an overview is provided from the content of this thesis.

Figure 6: Chapter contents (C1-C6) schematically shown



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