Cortisol exposure, cognition and clinical course of bipolar disorder

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8. Discussion

In 2005, we started a study named The Bipolar Stress Study, at the outpatient department for Bipolar Disorders of PsyQ The Hague. The general topic of the Bipolar Stress Study was and is to identify risk factors that have impact on the clinical course and treatment of BD. In the study three levels and their interactions with the environment (stressful life events and social support) were distinguished: clinical functioning (phenotype), genetic variations and vulnerability (genotype) and two endophenotypes, namely cortisol exposure and cognitive functioning. For the cross-sectional study the data of 366 patients were collected (genotype and phenotype); 189 patients participated in the extended part of the study including the cognitive test at first visit and the 24 month longitudinal study.

In this dissertation, a part of the results is presented with the emphasis on the role of two endophenotypes, namely cortisol exposure and cognitive functioning. Here, the main findings will be critically presented, including the strengths and limitations.

Subsequently will be discussed:

1 GR /MR polymorphisms and clinical course of BD;

2 Cortisol levels in saliva and hair, the relation with clinical course of BD;

3 Associations of cognitive functioning with medication use in BD.

1. GR /MR polymorphisms and clinical course

Of the 366 patients enrolled in the cross-sectional part of the study, 326 patients were included in the genotypic assessments. Due to organizational reasons, the last 40 patients could not be genotyped. We found that several GR gene polymorphisms altering cortisol sensitivity associate with seasonal patterns of mood episodes, especially hypomania. In particular the 9β (rs6198), and, to a lesser extent, both the ER22/23EK (rs6189/rs6190) and the *Bcl*I (rs41423247) polymorphisms seem to associate with clinical characteristics of BD.

Even though this cohort is a large group with bipolar patients, for genetic association studies it is rather small. For example, only 8% of a healthy population (n=350; age is between 13-36 years old; not screened for psychiatric diagnoses), is carrier of the ER22/23EK on at least one allele. More frequent polymorphisms in healthy populations are the *Bcl*I (63% is carrier in the healthy population) and the 9β (31%) (1). However, contrary to Genome Wide Association Studies needing thousands of patients to find genetic associations with disease, this study is not designed to detect causes of BD. It is theory driven, expecting to unravel associations between several clinically relevant

genetic polymorphisms involved in regulation of cortisol sensitivity at the cellular level. The associations we found with clinical course of BD shed some light on the influence of changes in HPA-axis regulation on the disease. For example, the activity of the HPA-axis is known to be regulated by seasonal changes, with mild hypersensitivity for glucocorticoids during the winter. BD patients who also have a genetic vulnerability for dysregulation in the HPA-axis by GR polymorphisms may be more likely to develop seasonal mood episodes. However, several limitations have to be noticed. First, in this part of the study, the approach was cross-sectional with clinical data gathered retrospectively, leading to potential recall bias. This might have affected the reliability of the retrospectively collected data such as the reported age of onset of symptoms. On the other hand, we expect that due to the impact of first hypomania and mania these might be remembered quite well. Second, patients were questioned about their first illness symptoms, which hamper a sharp differentiation between symptoms and episodes. Third, results should be taken with caution, since we cannot rule out false positive genetic findings. Future studies are needed to replicate our findings.

Our findings of a relationship between GR polymorphisms and the course and characteristics of BD and the effect of treatment, can be added to a growing number of studies, in which the regulation and functioning of the GR has been shown to be related to clinically relevant aspects of mood disorders. One study showed a lower frequency of the G –allele of the *Bcl*I polymorphism in patients with excellent Lithium response (2). Other haplotypes are also more frequent in patients with partial or no Lithium response, further underlining the influence of differences in stress hormone (cortisol) sensitivity on medication response defining course of the disease(2). In another study, three GR gene polymorphisms in exon 9 (among which the 9β variant) were found to be associated with response to Lamotrigine but not to Olanzapine/Fluoxetine treatment (3). This is evidence in the body of literature supporting the relevance of GR polymorphisms in effect of antidepressant and mood stabilizing treatment. In addition to genetic variations in the GR, also changes of the GR function through gene activity and expression appears to be important (4-7). However, the exact mechanism is not elucidated yet. Direct evidence of involvement of the GR in BD has been shown by effects of GR antagonist mifepristone in bipolar depression, improving cognitive function and mood after one week treatment compared with placebo (8, 9, 9b).

In more detail it is interesting to notice the differences in direction of influence between polymorphisms. Specifically the 9β and the ER22/23EK polymorphisms are known to lead to mild glucocorticoid resistance, whereas other GR gene polymorphisms in this study (most important is the *Bcl*I polymorphism) are leading to mild glucocorticoid hypersensitivity (10-12). In trying to grasp this paradox, some issues have to be noted.

First, higher susceptibility to develop depressive episodes is found for both mild glucocorticoid hypersensitivity due to the *Bcl*I polymorphism (13), as well as mild glucocorticoid resistance due to the ER22/23EK polymorphism (13). This might indicate that the HPA-axis functioning knows an optimal set point with a U-shaped curve. Polymorphisms leading to relative resistance as well as SNPs leading to relative hypersensitivity in response to GCs, can both be involved in development of mood disorders. In this thesis we found that the ER22/23EK polymorphism is associated with an almost 8 years younger age of onset of BD (14). With the influence of GR polymorphisms, specifically the 9β or *Bcl*I polymorphism, on HPA-axis regulation, other influences, like seasonal induced changes in cortisol sensitivity, can further deregulate the system, leading to proneness for seasonal episodes as shown in chapter 4 (14).

Second, polymorphisms differentially affect intracellular signaling pathways of the GR, which might explain why for example both the ER22/23EK and 9β polymorphisms lead to resistance for GCs, but seem to differ in susceptibility for mood episodes. The 9β polymorphism has been shown to increase mRNA stability of the GR-β splice variant. This may result in more of the GR-β at the protein level, which dominantly inhibits the active GR-α isoform. Carriers of the 9β polymorphism have a higher susceptibility to develop rheumatoid arthritis (15, 16). Additionally, homozygous carriers of this polymorphism have been shown to be 70% less likely to be carrier of nasal S. Aureus (17). This indicates that the increased level of the GR-β splice variant leads to activation of inflammatory activity and resistance for GCs. The ER22/23EK polymorphism on the other hand is inducing decreased transcriptional activity because of an increase in a less transcriptional active translation isoform of the GR (the GR-A) (18), leading to diminished effects of GCs. This might be one of mechanisms involved in differences for susceptibility for developing mood episodes. However, further research is needed to shed light on exact consequences of GR signaling pathways in relation to mood.

Third, the already mentioned effects on the immune system should be taken into account to understand the effects of the polymorphism in relation to mood. In the afore mentioned study of Van Oosten et al. (15), in addition to the 9β polymorphism carriers, also the ER22/23EK carriers show a higher risk on developing rheumatoid arthritis, indicating a possible indirect effect on inflammation and the immune system. This is in line with the effect of the other polymorphisms, the *Bcl*I and N363S (rs6195), known to induce hypersensitivity for glucocorticoids, which lead to a lower risk on developing rheumatoid arthritis. Both may reflect a reduced (ER22/23EK and 9β) or increased (N363S and *Bcl*I) GC induced immunosuppression. Hypothetically, this may result in a more pro-inflammatory state of the immune system, which is also known to be the case during mood episodes and in BD in general (19, 20). The intertwining of the immune system and the HPA-axis is a complex and not fully understood field. However, it is important for

future research to study both systems in relation to each other. This may help to identify new pathways for treatment.

To conclude, further understanding is needed about the relation between functional polymorphisms of the GR gene, like *Bcl*I, 9β and the less frequent but influential ER22/23EK and clinical phenotypes in BD (and other mood disorders) like seasonal patterns, age of onset of disease and clinical course as well as therapy response. The role of MR gene polymorphisms in BD is not related to these factors in BD. However, future analyses will focus on elucidating the role of GR and MR polymorphisms in cognitive impairments in BD.

Furthermore, a complicating factor in understanding the role of GR and MR polymorphisms is that serum cortisol seems to fail in providing information about the potential mediating effects of cortisol on the relationship between GR and MR polymorphisms and clinical characteristics in bipolar patients. This might be due to the circadian rhythm of cortisol levels and the pulsatile way cortisol is secreted. Also, large daily variations due to e.g. acute stress or infection are also an important limitation of measuring serum cortisol levels. Using information about genetic variations known to be associated with altered cortisol sensitivity, results in a more adequately assessment of the lifelong true impact of cortisol at the tissue level. Taking GR genotypes into account may contribute to better understanding the relationship between glucocorticoids and BD. In the next paragraph this relation will be further discussed.

2. Cortisol levels in saliva and hair, the relation with clinical course of BD

In the introduction, an overview with different methods to evaluate HPA-axis functioning is provided. Previous studies used salivary or serum cortisol levels to evaluate HPA-axis functioning. With these assessment methods, insight in total cortisol levels, the Cortisol Awakening Response and changes in cortisol levels during the day can be obtained. However, these assessments do not provide insight in long-term functioning of the HPA-axis, due to the frequent fluctuations and influenceable cortisol levels. In BD studies, as well as in other studies with patients categorized according DSM-IV diagnoses, there were conflicting results. In addition to the changing levels of cortisol during the day, these results could reflect the limitations of the DSM-IV categories. Recently, clusters of symptoms in patients with depression and/or anxiety have been shown to correlate nonlinearly with the cortisol awakening rise (21), indicating the added value of analyzing symptom clusters above DSM-IV categories in depression and anxiety. In studies including bipolar patients, results vary from no differences in basal salivary or serum cortisol levels

in patients with BD versus healthy controls (22-24) to elevated cortisol levels in other studies(25, 26). Vreeburg et al, found that smoking has been found to associate with resistance for GCs, other factors influencing different HPA-axis measurements included sampling factors, health factors, sex and age. In order to minimize the influence of all factors involved in this variability, it is necessary to adjust analyses for confounders (27).

In order to obtain insight in long-term functioning of the HPA-axis, we used a newly developed assay with which cortisol can be measured in scalp hair. This method offers the opportunity to determine mean long-term cortisol levels and appears to yield a reliable estimate of long-term HPA-axis activity (28, 29). Furthermore, it is unique in its ability to retrospectively assess cortisol levels, an approach which can help evaluating the consequences of life events, or therapy interventions (28). Recent evidence shows that in veterans higher hair cortisol relates with PTSD and number of traumatic events (30). Additionally, long-term cortisol was higher in patients admitted for acute myocardial infarction compared to patients with other indications for admission on internal wards (31): cortisol levels were raised 3 months prior to the event. Shift work is also associated with higher long-term cortisol levels in hair, as well as with higher BMI in shift workers and healthy persons (32). This method seems promising for the future in following HPA-axis functioning over prolonged periods of time.

However, several issues regarding cortisol assessment in scalp hair have to be addressed:

First, it is not clear what is measured in hair: free or total cortisol. In the circulation approximately 75% of cortisol is bound to cortisol binding globulin (CBG), which is thought to be the biologically inactive state, circa 20% is bound to serum albumin, and around 4% is free cortisol. It is already convincingly found that total cortisol levels, vary significantly within and between individuals, thereby affecting the interpretation of HPA-axis test results (33). Still under debate but with support of recent evidence is the hypothesis that hair cortisol is reflecting free circulating cortisol levels. Evidence to support this is found in estrogen users. Oral contraceptives (OAC) stimulate CBG levels, but in OAC users, cortisol levels in scalp hair are similar to non users. This suggests that hair cortisol reflects free (biologically active) cortisol levels, and not total cortisol levels (28).

Second, long-term hair cortisol and short term saliva cortisol do not relate (34), which is also found in our study (chapter 5). The retrospective calendar provided by long-term hair cortisol could provide information about mean cortisol exposure and reflect long-term (weeks to months) gradual changes (28, 35-38), in contrast to saliva samples reflecting levels of acute stress response (minutes to hours). This indicates that these two methods could be nicely applied together, serving different purposes in evaluating the HPA-axis. The hair cortisol levels provide insight in long-term consequences for cortisol levels due

to life events, chronic stress, cardiovascular diseases, and treatments, e.g. treatment of Cushing's Syndrome (28). Saliva cortisol provides information of daily cortisol changes, with the CAR as awakening stress response, responsivity during the day by Experience Sampling Method and the cortisol day curve as information about the daily changes and diurnal rhythm. Challenge tests on the other hand, such as the Dexamethasone Suppression Test (DST) or the more sensitive Dex/CRH-Test, provide information about the magnitude and variability of the sensitivity of the negative feedback at pituitary level on cortisol production.

Third, the clinical relevance of this novel method of hair cortisol measurement has to be further investigated. Currently, evidence is swiftly mounting in a growing number of patient populations. For example, in generalized anxiety disorder (GAD), decreased cortisol levels in hairs have been found, but no differences in salivary cortisol levels between GAD patients and healthy controls (39). This is in line with our findings of lower hair cortisol levels in BD patients with co-morbid panic disorder and suggests also that hair cortisol levels may reflect the long-term cortisol secretion, whereas the results found with saliva or serum cortisol levels might include acute responses to the time and circumstances of assessment. Depressed medicated patients were found to have higher cortisol levels during 6 months before cutting hair strands, compared with healthy controls (40). In the future it would be very interesting to analyze hair cortisol levels in relation to clinical parameters such as medication effects, life events, remission and recurrence of mood episodes.

Fourth, it is not yet clear to what hair length retrospective cortisol levels can be measured reliably. The maximum length of hair strands differs between laboratories, differing between 6 cm (=6 months) (41) to several years depending on the length of the hair (28). This is probably due to differences in processing of the hair samples which slightly differs between laboratories.

Fifth, it seems that over time cortisol levels in scalp hairs remain stable. In over 2000 years old mummies changes in hair cortisol levels were assessed, with possibly one case of pathological cortisol raise (42), indicating the reliability over time of found cortisol levels. However, this needs further replication. Currently, a growing number of studies is validating this method and define the clinical relevance.

As already stated in the introduction of this thesis, in addition to medication use, the chronic course of the disease is also defined by another endophenotype, namely cognition. This endophenotype will be discussed below, but in this paragraph we will discuss the relation between cognition and long-term hair cortisol. In our study cognitive performance was measured at baseline, while the hair strands were in some cases cut almost 2 years later, at the end of the study period, which is leading to difficulties in

interpreting the results. Hair cortisol reflects long-term cortisol levels, and cognitive deficits are thought to be related with high cortisol levels on the long-term as well (43). It is known that patients with Cushing's Syndrome defined by chronic excessive cortisol levels suffer from early aging in cognitive performance and general cerebral loss of volume (44-46), specifically hippocampal atrophy.

In our preliminary analyses a negative relationship was found between executive functioning and hair cortisol levels. Patients with an above average score showed lower cortisol levels (p=.02; after adjustment for age, gender, and hair treatment, the use of hair products and frequency of hair wash, p=0.015). It has to be noted that these analyses need further replication and comparison with healthy controls. Although in healthy controls hair cortisol levels are stable over time, the fact that the cognitive tests in BD patients have been performed at baseline of the study and the hair analyses includes the period between the 18th and 21th month of the study complicates the interpretation of the results. Taken together, these findings underline the preliminary and explorative character of this new technique, which is in need for further validation and interpretation in a clinical relevant way.

To conclude, we and others found associations of hair cortisol with psychopathology (mood and anxiety), which are promising for future research. Hair cortisol measurements have a promise to serve as an endophenotype in identifying the role of regulation of the HPA-axis in the long-term course of BD. As we did not find any difference between healthy controls and BD as disease entity, we would recommend to study long-term hair cortisol levels in relation to cognitive performance over time, and also focus on symptom level like for example mood and anxiety over time. This is in line with recent studies focusing on symptoms clusters or dimensions in relation with for example HPA-axis functioning and metabolic syndrome (21, 47).

3. Associations of cognitive functioning with medication use in BD

In our study, cognitive performance was assessed by means of the Test for Attentional Performance ('Testbatterie zur Aufmerksamkeitsprüfung' (TAP), version 2.1, http://www.psytest.net/ ; Zimmermann & Fimm, 2002). The TAP is a widely used computer based standardized test battery and easy to use in clinical practice (48-50). However, when we started our study, no international consensus was reached about the use of instruments. Recently, the International Society for Bipolar Disorders (ISBD) proposed a more complete neurocognitive battery to asses global cognitive impairment and improvement, the ISBD Battery for Assessment of Neurocognition (ISBD-BANC)(51). This ISBD battery is composed of the neuropsychological tests that show the largest

patient-control effect size differences across the literature. Cognitive domains tested with this battery, include attention/ vigilance, processing speed, verbal learning and memory, executive functioning and working memory, visual learning and memory. Of these domains, the TAP considered attention, executive functioning and working memory. However, using a common set of standardized procedures will probably lead to more comparable results across different research groups spanning the international community.

As stated in the introduction, cognitive deficits are known to have “trait” as well as “state” features.

Cognitive deficits, as “trait” feature, are thought to be mainly caused by genetic factors and thus are an endophenotype of BD (52). This is in line with findings in unaffected siblings of BD patients performing worse on memory and executive functioning tasks compared with healthy controls (53), arguing that cognitive functioning could serve as an endophenotype in future research. This is also concluded in an earlier meta-analysis of Bora et al., finding that impairments of executive functioning, sustained attention and verbal memory were common both for patients as well as for relatives with larger effect sizes for the patient group (54).

However, in this meta-analysis deficits in processing speed and visual memory were only observed in patient groups, not in relative groups. Possibly, these functions reflect the influence of patient specific factors such as medication use (associated with psychomotor slowing) and earlier age of onset (associated with psychomotor slowing and verbal memory impairment). Thus, other, more “state-like” factors influencing cognitive abilities, seem important as well. This means that, in addition to the “trait” part of cognitive impairments in BD, it is clear that also “state” dependency like current mood, as well as other factors like medication use, are worsening cognitive performance (52). In this dissertation, before using cognition as endophenotype, we decided to first investigate the influences on the “state” of cognitive performance.

In chapter 6, we showed an association between use of number of medication types and worsening of executive functioning; antipsychotics and antidepressants associate respectively with poorer executive functioning and attention. Lithium showed no association with cognitive functioning. Regarding the literature about the relation between medication and cognition in BD, our results seem in line with previous findings: 1) Lithium induces mainly a slight slowing of processing speed and subjective impairment of cognitive functioning; no strong other significant influences on cognitive performance have been found (48, 55, 56). 2) Antipsychotic use was associated with level of memory and executive functioning in a group of 40 BD patients compared with 40 healthy controls (48). 3) Despite the large body of research, little attention has been

given to the long-term consequences for cognition of polypharmacy, a common practice in the treatment of BD. Moreover, long-term studies are scarce. One 15 year longitudinal study showed in patients with non-schizophrenic psychosis a more than twice better functioning and social adjustment in non medicated patients compared with patients on any psychiatric medication (46).

To conclude, the results in this thesis underline the importance of thoughtful prescription of medication, and especially caution in prescribing antipsychotics as well as in the use of 2 or more different types of medications. However, an important limitation of our study was the cross-sectional study design, which made it impossible to make statements about causality. For example, it could well be that patients with a more severe course of illness use more types of medication as well as suffer from more severe cognitive deficits. To be able to elucidate the direction of causality, we adjusted our analyses for all variables regarding severity of the disease, which did not change any finding. This supports our view that polypharmacy and especially antipsychotics, seem to negatively influence executive functioning.

In thinking about causes of cognitive impairments in BD, the “trait” as well as the “state” need attention. Moreover, as the scar hypothesis (57) suggests, cognitive performance can evolve during life under influence of for example number of mood episodes. This, together with medication use, current mood, and the genetically vulnerable profile (possibly caused by genetic vulnerability for BD) of cognitive performance could result in a detrimental evolution of cognition during the disease course. However, this topic should be considered more in detail, with attention for different domains of cognitive performance.

Strengths and limitations

In general, this thesis presents the first results of a cross-sectional and longitudinal study, based on a relatively large and well phenotyped cohort. Proper phenotyping is known to be crucial for this type of epidemiological genetic association studies (58). In addition to clinical information, data were also thoroughly collected with respect to endophenotypic (biological data, cognitive performance) and genotypic information, to explore the influence of cortisol exposure on BD. Selection of genetic polymorphisms was based on functionality of polymorphisms and was driven by the hypothesis that HPA-regulation (mediated by GR and MR) is influencing the parameters chosen on endophenotypic as well as phenotypic level. The functionality of the GR and MR polymorphisms has been convincingly shown, which is quite unique, for all polymorphisms in in vivo studies (with the DST, and somatic consequences of increased or decreased cortisol levels), and also

in in vitro bio-assays (GR: ER22/23EK, 9β, N363S; MR: I180V and -2G/C (59)) and even on molecular level (GR: ER22/23EK (18) and 9β(16)).

In the analyses of the cross-sectional data, several limitations have to be noticed:

First, data regarding illness characteristics were gathered by interviewing the patients with questionnaires, with a risk of recall bias.

Second, it is not possible yet at this point of the study to already claim clear causative conclusions, but only associative findings. However, our findings give direction to our future analyses of the longitudinal data. The longitudinal data hopefully will provide insight in the long-term influence of medication on cognition; furthermore, the influence of cortisol exposure on the longitudinal outcome will be analyzed.

Third, the clinical relevance of our genetic analyses of the MR and GR is yet limited. For a genetic association study, our cohort is relatively small. This is foremost of importance for less frequent polymorphisms like the ER22/23EK and the N363S SNP. Statistical power was appropriate for the more frequent polymorphisms (GR-9β, *Bcl*I). Our genetic association study suffers, like all such studies, several other possible fallacies. One of them is the risk on finding false positive or false negative results. In genetic studies, depending on SNP frequency in healthy populations, high numbers of participants are needed (58). To decrease the risk of having such false positive findings, we corrected all analyses for multiple testing. Nevertheless, it remains uncertain whether the finding in chapter 3, regarding lower number of hypomanic and manic episodes in carriers of the 9β polymorphism in patients with BD, is a false positive finding. After including more patients in the cohort, the strength of the association diminished to sub threshold significance level. Replication is needed in another independent cohort. Furthermore, besides false positive findings, and the importance of reliable phenotyping; other fallacies in genetic associations include influence of racial heterogeneity, and differences in gender and age (58). In our study, the cohort consisted of 95 % Caucasian people from Dutch ascend which could be regarded an asset of this study. Furthermore, patients were matched on age and gender with blood bank donors in this study (1). Notwithstanding these limitations these genetic studies merit continuation, as studies focusing on medication targeting the HPA-axis are promising and underline the relevance of the GR and MR function in mood disorders and cognition.

Fourth, the assay for cortisol assessment in scalp hair has only recently been developed. Although in the past years clinical data are rapidly accumulating that this is a reliable marker of long-term mean systemic cortisol levels, in this stage application in psychiatry as well as in other specialisms, is new. There are several issues that need to be addressed regarding this technique (60) : 1) the exact mechanism of how (through blood, sweat,

or sebum; probably most important through blood) and where (hair cortex or medulla) cortisol is incorporated in hair shafts, need to be further clarified; however, for our findings this is of no direct relevance, but in interpreting cortisol levels in relation with other HPA-axis assessments, this would be of relevance; 2) the grow speed of hairs is important in creating timelines of cortisol levels and therefore more research on possible interracial differences is important.

Fifth, hair cortisol analysis needs further embedding in clinical practice of mood disorders. Cortisol in hair can be regarded as a trait feature; however, severe stress, caused for instance by mood disorders, may lead to increased mean cortisol levels. Thus, the relation between life events and mood, but also between therapeutic success and mood, can be focus of research in the future. In our sample, numbers of patients with severe mood episodes were too low to be useful for analysis in this study. Our findings warrant further study of the use of hair cortisol in relation to BD.

Conclusive remarks

In this thesis several potential candidates are identified as risk factors influencing clinical course of BD. At the genotypic level, several GR gene polymorphisms changing cortisol exposure by sensitivity, associate with seasonal patterns of mood episodes, especially hypomania. In particular, the 9β polymorphism seems to associate with clinical characteristics of BD. At the endophenotypic level, higher cortisol exposure assessed by hair analysis is associated with more psychiatric co-morbidity and an older age of onset of the disease. However, due to our finding that no difference is found between the total group of patients with the broad phenotype BD and healthy controls, we would recommend to focus in future analyses with long-term cortisol on more defined and unambiguous long-term parameters like mood episodes in preceding months, anxiety symptoms, life events, and cognition, all in preceding months.

Cognitive function, as second endophenotype, is related to the number and types of medication used. It should be noted that while lithium had no effect on cognition, antipsychotics significantly did. In the analyses performed in this thesis regarding cognition, the main conclusion is that the rate impact of medication use on cognitive performance is still under debate. With these results in mind, the next phase in the analysis of this study is to investigate cognition as an endophenotype, in relation to e.g. GR and MR polymorphisms, cortisol in hair, and clinical course of BD.

Summarizing, cortisol exposure as determined by cortisol sensitivity at the genetic level and by measuring long-term cortisol levels using hair extracts, is associated with several clinically relevant phenomena defining the course of bipolar disorders. Furthermore,

cognitive functioning as second endophenotype, appears to relate to (number of different types of) medication.

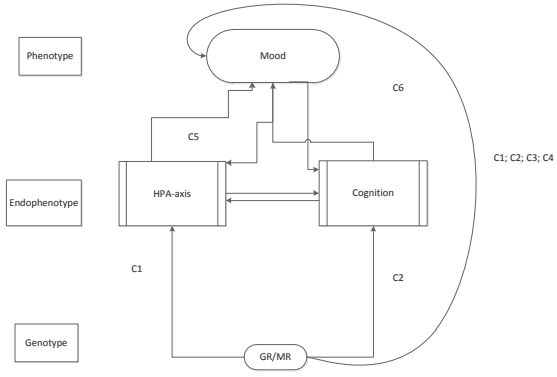
Future perspectives

First, as already noted, it would be very interesting to relate our data to the longitudinal course of BD. In box 1, the current findings are summarized in the light of the Bipolar Stress Study.

Box 1: findings and future directions with this thesis as part of the Bipolar Stress Study;

C1-C6 = Chapter 1 – Chapter 6.

Aims Bipolar Stress Study: *Identifying risk factors influencing clinical course of BD following figure 6 in the introduction:*



Current status:

In this figure knowledge is added on

1) *the relation of GR and MR polymorphisms to clinical characteristics of BD;*
2) *on the relation of endophenotype of the HPA axis to clinical characteristics of BD;*
3) *on influences like medication use on cognition to establish confounders in using this as endophenotype.*

Future directions: *The longitudinal part of the study will be investigated (life chart data):*

a. *What is the influence of genetic variations of the cortisol receptor on mood as measured by life chart? Is indeed the GR 9β and MRI180V protective? Can we confirm the relation to vulnerability for seasonal patterns?*
b. *How is the relationship between cortisol in hairs, environmental factors and course of mood, for example with sub analyses to patients with and without co-morbid panic disorder?*
c. *What is the influence of current mood as "state-factor" on cognitive performance?*
d. *What is the relation of cognitive performance to prospective course of the disease?*
e. *What is the influence of childhood trauma on endophenotypic as well as phenotypic level?*

Second, it would be very interesting to study immunological and inflammatory parameters in relation to the HPA-axis in BD and subsequent phenotypic data. The intertwining nature of both systems requires an integral vision to interpret the data in a meaningful way. Chronic stress is usually accompanied with raised pro-inflammatory cytokines (61), leading to higher risk of daily life infections and prolonged wound healing (62). In BD inflammatory changes are shown by a higher prevalence of organ auto-immunity (20) and pro-inflammatory activity in monocytes and anti-inflammatory activity in T cells (63), showing an imbalance in interleukin levels, which is normalized with Lithium treatment (19). An altered gene expression of inflammatory genes, found as a mRNA gene signature, was found in patients with BD (64), with a clear pro-inflammatory state of genes in monocytes (65). However, replication and meaning of those findings need further research. Possibly, these genes are also influenced by factors like altered GR sensitivity or medication use.

Third, a range of medical problems has been associated with BD (66). The most frequently described conditions are cardiovascular diseases, diabetes, and obesity (66). These are all related to what is referred to as the Metabolic Syndrome (MetS) (67), defined by the criteria from the National Cholesterol Education Program (NCEP) Adult Treatment Program III (ATP III)(68) these risk factors comprise three or more of the following five criteria: 1) an increased abdominal circumference (> 102 cm for men and > 88 cm for women), 2) hypertriglyceridemia (> 150 mg/dl), 3) a low level of high-density lipoprotein cholesterol (< 40 mg/dl for men and < 50 mg/dl for women), 4) hypertension (> 130/80 mmHg), and 5) a high fasting glucose level (> 100 mg/dl). Although several definitions of the MetS have been developed (69-71) the NCEP/ATPIII criteria are the most widely used in studies investigating the MetS. Taken into account the recent insights in metabolic changes due to for example atypical antipsychotics (commonly used in treatment of BD), it is urgently needed to understand shared mechanisms in psychopathology and hence be able to assess individual risks**.** The metabolic syndrome is highly prevalent in the general population (around 25% in the US (72) and around 15% in a Dutch population aged 28-59(73) to almost 20% in the healthy control cohort of the Netherlands Study to Anxiety and Depression (NESDA) (74). A relation with severity of depressive symptoms was predominantly found with abdominal obesity and dyslipidemia. MetS seems even more prevalent in bipolar patients (30% in the US study of Fagiolini (72) and 25.3% in an Italian sample (75)). In our sample almost 33% fulfilled the criteria of metabolic syndrome (unpublished analyses). The metabolic syndrome is repeatedly associated with increased sensitivity for glucocorticoids and with a pro-inflammatory state (76). Thus, physical health is important to evaluate in studies with attention for possible shared biological pathways.

And fourth, as a consequence of the above, the influence of medication has to be studied separately in longitudinal studies with respect to biological changes. It would be very relevant to study the consequences of different types of medication use on endophenotypes such as cortisol sensitivity and cognitive performance, but also on clinical phenotype with respect to number and severity of mood episodes. Iatrogenic damage due to antipsychotics or anti-epileptics in high risk patients should be avoided or minimalized.

As a final conclusive remark, the influence of the HPA-axis in relation with other biological systems, should be noticed to understand the clinical course of BD. Mood disorders are not limited to the brain, but involves wide bodily processes through hormones, cytokines and neural regulation.

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