Time after time: biological factors in the course of recurrent depression
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General Introduction

1.1 Introduction
1.1.1 Introduction and outline of thesis

Major Depressive Disorder (MDD) is a heterogeneous disorder with a highly variable and recurrent course, an inconsistent response to treatment, and is thus far without an established aetiology. This thesis presents some biological approaches associated with and/or predicting the course of recurrent depression.

The structure of this introductory chapter is as follows: first an introduction will be given on the recurrent type of MDD. This is followed by its relationship to cardiovascular risk. Some pathophysiological mechanisms, which possibly influence the course of MDD, will then be introduced. Thereafter, an overview of the DELTA-study, which forms the basis of most of the data used in this thesis, will be presented in association with a timeline figure. Lastly, the general aims and an outline of the succeeding chapters of the thesis will be given.

Recurrent depression

MDD is one of the most common forms of mental illness and affects approximately one in six men and one in four women over a lifetime. More recently, Bromet et al. reported that the occurrence of depressive episodes over a 12-month period was found to be 5.9% in low-middle income countries compared to 5.5% in high-income countries. MDD is also highly recurrent, as at least 50% of those who recover from an initial episode of depression will suffer from experiencing one or more additional episodes in their lifetime. Indicatively, approximately 80% of those with a history of two episodes will have further recurrence. Once a first episode occurs, recurrent episodes will usually begin within five years. In general, individuals with a history of depression will have five to nine separate major depressive episodes (MDEs) over their lifetime.

MDD is an illness with a well-established negative impact and is predicted to become the second-leading contributor to global burden of disease by the year 2020. This critical impact is mainly due to the recurrent nature of MDD, which results in a severe burden to patients, families, public health, and society.

*The current thesis uses the definitions of remission, recovery, relapse and recurrence. Recurrence, on the other hand, occurs when an individual experiences a new depressive episode after a full recovery had been achieved. The distinction between relapse and recurrence makes conceptual sense, and clear operational criteria have been proposed by Frank et al.*
MDD also has a significant economic impact. Not only with regard to direct cost for treatment, but also for the sizeable indirect costs in terms of sick leave and early retirement. In 2005, the combined direct cost (outpatient care, drug cost and hospitalization) and indirect costs due to morbidity and mortality of depression in Europe was estimated at €120 billion.

A promising strategy to reduce the disabling effects of depression is prevention, both primary and secondary. This thesis will focus on secondary prevention strategies in patients at high-risk of recurrence MDD. In their review, Burcusa and Iacono discuss a number of hypotheses that have been offered as explanation for the high risk of recurrences. One of these is the vulnerability-accumulation (or scarring) hypothesis, which postulates that the experience of a depressive episode induces a lasting increase in vulnerability, which increases the risk of recurrence. One aspect of the scarring hypothesis is related to the phenomena of “kindling”, which assumes that with each subsequent episode, less stress is required to provoke a new episode.

A more biological explanation of scarring suggests ‘episode sensitization’. This means that after each new episode of depression, it takes a less dysphoric mood to produce the following episode. This change is believed to be encoded at the level of gene expression. It suggests that the neurobiology of this affective disorder is a dynamic process that changes over the course of the illness.

Many variables seem to be related to risk for recurrence of MDD. However, these presumed “risk factors” could actually be manifestations of an underlying premorbid susceptibility to MDD in general. So contrary to the scarring hypothesis, Burcusa & Iacono suggest that “…individuals at high risk for multiple episodes possess the necessary characteristics to make them prone to recurrent depression, and such characteristics exist even before their first episode.” More specifically, scarring theories do not take into account that the “cut-off” for MDD risk may have already been met even before the first episode.

It could be that individuals inherit a level of risk for recurrent MDD. If they are high in this underlying genetic vulnerability, they are also likely to have an earlier age at onset, a greater number of episodes, increased severity of the episodes, and greater comorbidity and risk for CVD. It is not yet clear how genetic and biological factors specifically contribute to the recurrent subtype of MDD and so these factors merit further investigation as hopefully genetic risk, in future, can be modified through environmental mechanisms in order to alter the course of recurrences.
In this thesis we consider the previously presented explanatory models for the high risk for recurrence, specifically: (I) the vulnerability-accumulation (or scarring) hypothesis and (II) the premorbid vulnerability hypothesis. Our aim is to investigate premorbid factors (e.g. genetics, childhood trauma) that are present before MDD onset, as well as biological factors that could play a premorbid role and/or are involved in vulnerability-accumulation (‘scarring’). These biological variables are collected in different stadia in the course of recurrent MDD.

Genetic factors
Recurrent MDD is thought to have a higher heritability than other forms of MDD. Individuals with recurrent MDD not only have greater numbers of episodes in their lifetime but also are more likely to experience stressful life events, display increased neuroticism, have a greater risk for earlier onset of co-morbid psychopathology, and show a family history of MDD. Therefore, value might be added to a diagnosis, such as recurrent MDD, if an underlying pathophysiology or biomarker can be identified.

Risk factors for recurrence
Identifying predictors for recurrence in recurrent depressed patients is important for a better understanding of the course of this disease. These risk factors can be divided into those that can be modified and those that cannot. Identification of dynamic, potentially modifiable, (neuro) biological risk factors for recurrence could be helpful particularly in the development of targeted preventive intervention. Currently well-known risk factors for recurrence are: clinical variables (age of onset of the first episode, severity of first episode, number of previous episodes, residual symptoms), family history, negative/extreme cognitions, personality traits (neuroticism), exposure to a stress-inducing environment (daily hassles), poor social support, and maladaptive coping techniques. However, these predictors explain only part of the variation in recurrence. In the DELTA study, only the following risk factors for relapse/recurrence were significant over a 5.5 year period: a high number of previous episodes, greater residual depressive symptomatology and psychopathology, and daily exposure to a stress-inducing environment.

MDD and cardiovascular disease: a collaborative relationship.
MDD is associated with significantly elevated risk of early death. Partly because MDD has a high risk for suicide, but also because it is significantly associated with a wide variety of chronic physical disorders including arthritis, asthma, cancer, diabetes, hypertension, chronic respiratory disorders, chronic pain conditions and CVD. This thesis will focus, in part, on the association between MDD and CVD as it is of considerable relevance to both the patient and public health with regard to the costs (personal and financial) of MDD.
Different explanatory mechanisms for the proposed MDD-CVD association:

(I) As a unidirectional relationship. MDD can be considered as a risk factor for CVD, with its associated financial costs, impairments, and increased mortality risk. This is supported by meta-analyses of longitudinal studies that show that MDD is a consistent predictor of the subsequent first onset of coronary artery disease and for stroke, diabetes, and myocardial infarctions. Based on the aforementioned findings, a number of mechanisms have been proposed to explain the prospective associations of MDD with CVD. These include a variety of health behaviors known to be linked to MDD, such as elevated rates of smoking and drinking, obesity, physical inactivity, low compliance with treatment regimens. However, the question remains of whether CVD-risk is premorbid present, due to an underlying pathophysiological mechanism, or that CVD can be caused by MDD and its recurrences and thereby of an accumulation of vulnerability (scarring).

(II) As a bidirectional relationship. Some CVD-risk factors, such as obesity, increase the risk of MDD and, in turn, MDD increases the risk for the development of obesity. This relationship could explain the observed association between MDD and CVD. However, pathophysiological mechanisms underlying the mutual association between MDD and CVD are complex and still largely unknown.

(III) Through a shared risk factor. A common risk factor of MDD and CVD is thought to be metabolic syndrome (MetS*). MetS is defined as a conglomerate of metabolic risk factors associated with the development of type 2 diabetes mellitus, coronary artery disease and increased cardiovascular mortality. Major depressive disorder (MDD) has been associated with an increased incidence of type 2 diabetes mellitus and cardiovascular disorders. Thereby providing a potential shared mechanism between depression and incident somatic conditions. Pan et al. found depression and MetS to be modestly associated (unadjusted OR = 1.42; adjusted OR = 1.34) in a systematic review. Although there is continuing debate regarding standardizing the MetS symptoms and diagnostic criteria, this clustering of risk factors is unequivocally linked to an increased risk for developing CVD. To avoid obfuscation this thesis will refer to the risk factors for MetS as CVD-risk factors. Assies et al. argue that oxidative stress represents a major common biological denominator underlying psychiatric disease (e.g. MDD) and CVD. The common risk factor model implies that (part of) the observed relation between MDD and CVD is not caused by a causal relation between the two conditions.

*Five criteria: waist circumference (WC) >102 cm in men; >88 cm in women, triglyceride levels (TG) ≥150 mg/dL (1.7 mmol/L), HDL-C <40 mg/dL (1.08 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women, blood pressure (BP) ≥135/ ≥85 mmHg and fasting blood glucose (GL) ≥100 mg/dL (≥5.6 mmol/L) or known diabetes.
In sum, because both recurrent MDD and CVD-risk factors constitute significant public health challenges, studying the association between these two conditions is of high relevance and importance. Particularly as evidence of critical (neuro)biological consequences of the supposed underlying condition of recurrent MDD is emerging. This thesis was produced both at the time of and in response to the scarcity of data available with respect to the interplay of recurrent MDD and CVD-risk factors. The goal of this thesis is to advance this burgeoning field by investigating some of the possible involved pathophysiological mechanisms that could mediate the manifestations of CVD-risk factors and stress in recurrent MDD.

Hypothalamic-pituitary-adrenal axis

An important biological system that might link MDD and CVD is the hypothalamic-pituitary-adrenal (HPA) axis. Dysfunction of the HPA-axis may contribute to the pathogenesis of both MDD and co-morbid CVD. This contribution may be mediated, at least partly, by the loss of glucocorticoid receptor-mediated negative feedback on inflammatory signaling. Furthermore, elevated cortisol levels in MDD could increase CVD-risk, since cortisol increases visceral fat, which is a CVD-risk factor.

Dysfunction of the HPA-axis during MDD has been called one of the most reliable finding in all of biological psychiatry. Hundreds of studies have compared HPA axis function between depressed and non-depressed individuals. However, few studies have examined the predictive value of HPA activity on course in recurrent and chronic depression (for a comprehensive meta-analysis see ). Moreover, knowledge of the degree of (hyper/hypo)activity and the clinical conditions under which it occurs remains incomplete.

It is debated whether abnormal HPA-axis activity in MDD-patients reflects a state (i.e occurs only during MDEs), and/or represents a persistent trait. The answer to this question has pathogenic implications. If HPA-axis abnormalities are state-dependent, they might be the consequence of epiphenomenal effects of depressive symptoms or underlying/accompanying daily hassles. Conversely, if HPA-axis abnormalities are a trait, they might be genetically regulated (endophenotype) and/or mediated by perinatal programming, traumatizing childhood life-events (CLEs) and/or or scarring-effects of previous MDEs.

A major liability of the HPA-axis theory of MDD is the difficulty of defining the association of stress to MDD. As discussed, about half of the patients have a single lifetime depressive episode, whereas the other half has a recurrent course. In addition various types of acute stress, early
childhood trauma, or long-term psychosocial problems may be involved and may lead to different responses of the stress (HPA-) system. Stress can be causative in some cases and secondary to depressed mood in others. There are also indications that stress and childhood trauma might affect the predictive value of the HPA-axis on evaluating risk of recurrence.

As discussed, the underlying pathophysiological mechanism of the recurrent nature of MDD and its association with CVD-risk needs further clarification. To help reveal whether HPA-dysfunction is a vulnerability in recurrent MDD and/or vulnerability-accumulation (scarring), we aim to investigate HPA-axis functioning in MDD-R patients.

**Fatty acid metabolism**

Severus et al. were the first to draw attention to the interaction between fatty acids (FA's), homocysteine, and increased mortality due to CVD-risk in MDD-patients. Current evidence suggests that a deficiency in membrane omega-3 (n-3) FA's may be a preventable risk factor for both CVD and MDD. There are several biological mechanisms that could potentially link the membrane n-3 FA deficiency observed in MDD to increased risk for CVD. In addition to clinical and preclinical evidence that n-3 FAs protect against cardiac arrhythmias, other mechanisms including enhanced platelet reactivity and aggregation, elevated triglyceride levels, and inflammation have been found.

The (cellular) membrane is a complex structure, composed primarily of a phospholipid bilayer and its constituent FAs, which provide scaffolding for proteins responsible for many key functions in the membrane. The membrane is also a natural intersection between genetic and environmental factors. Membrane defects, such as those induced by decreased docosahexaenoic acid (DHA) in phospholipids, can significantly alter a broad range of membrane functions. FAs are key components of (nerve) cell membrane phospholipids and synapses and are responsible for: signal transduction, ion transport and receptor sensitivity (e.g. for serotonin, dopamine, endocannabinoids). Therefore, alterations in key neurotransmitters involved in MDD can both be modified by- and contribute to oxidative stress and membrane dysfunction. This makes fatty acids (FAs) essential components of cell membranes.

Fatty acids can be classified into three families: saturated FAs, mono-unsaturated FAs and poly-unsaturated FAs (PUFAs). Unsaturated FAs have one or more double bonds between carbon atoms. The position of the double bond in the carbon chain is included in the name of the unsaturated FA. When the double bond is in position 6, the unsaturated FA is called an
‘omega-6 FAs’ and those with a double bond in position 3 are called ‘omega-3 FAs’, examples of omega-3 FA’s include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In a typical western diet, omega-6 FAs are much more abundant than omega-3 FAs. A high omega-6 to omega-3 ratio can alter cell membrane properties and increase production of inflammatory mediators because arachidonic acid, an omega-6 FA found in cell membranes, is the precursor of inflammatory eicosanoids, such as prostaglandins and thromboxanes. By contrast, omega-3 FAs are anti-inflammatory. Therefore, a high dietary omega-6 to omega-3 fatty ratio could promote neuroinflammation. Decreased omega-3 FAs concentration in the diet may also act by altering central nervous system cell membrane fluidity and phospholipid composition, which may alter the structure and function of the proteins embedded in e.g. neurotransmitter receptors 53, which can be the case in MDD. Furthermore, FAs alterations 54 have been implicated in both CVD and MDD. It is of note that this ratio has increased dramatically throughout history.

A genetic factor could underlie FA-alterations in MDD-R. This is suggested by the bimodal distribution of factors reflecting FA-metabolism in MDD-R patients 55 implying a dichotomous causal factor (e.g. a genetic mutation leading to altered enzymatic processing of dietary FA’s). An interesting candidate for the factor is the fatty acid-binding protein 2 (FABP2) gene. It is mostly expressed in small intestine enterocytes, where it codes for intestinal FABP, which is accountable for uptake of dietary FAs. A transition G to A at FABP2-codon 54 results in an amino acid substitution (Ala54 to Thr54) 56. This polymorphism is common, with a Thr54 allelic frequency of 30% in most populations, resulting in altered FABP FA-affinity. Homozygous Thr54-carriers show altered dietary FA-uptake, with increased postprandial concentrations of 14-18-carbon FA’s 57. As a result of the (patho)physiological role of FAs in metabolism, they have been suggested as an explanatory factor for the association of FABP2 with increased insulin resistance and FA-oxidation, which corroborates observations suggesting a role of the FABP2 Ala54Thr-polymorphism in CVD-etiology (e.g. obesity and atherosclerosis) 58, 59.

With respect to MDD, an important limitation of studies so far on FA levels is that they mostly addressed only n-3 and n-6 PUFA levels. They did not measure the whole FA spectrum. Neither were estimates of their respective desaturases and elongases routinely reported 49, 60, 61. Moreover, these studies focused mainly on patients suffering from a single depressive episode while data regarding the FA metabolism of patients with recurrent depression is currently lacking.
In this thesis we compare PUFA-levels in patients with MDD-R to PUFA-levels in non-depressed controls and we investigate whether PUFA-levels are associated to the current depressive status. Moreover, we investigate whether the Thr54-polymorphism in the \textit{FABP2}-gene is (I) more prevalent in MDD-R patients than in sex-and age-matched controls, (II) associated with observed alterations in FA-metabolism, and (III) associated with crucial CVD-risk factors, such as waist circumference.

One Carbon metabolism
Another biological pathway potentially underlying susceptibility to onset, symptomatology and recurrence of MDD is the folate-mediated one-carbon (1-C) metabolism\textsuperscript{62, 63}. An inconsistent pattern emerges when looking at the associations between MDD and the key constituents of the 1-C-cycle (homocysteine folate, vitamin B\textsubscript{6} and vitamin B\textsubscript{12})\textsuperscript{62, 64, 65}. The 1-carbon cycle is also associated with increased CVD-risk in patients with MDD\textsuperscript{66}. A meta-analysis concluded that each 5 micromol/L increase in homocysteine independently raised CVD-risk by approximately 20\%\textsuperscript{67}. In addition, a meta-analysis of prospective studies showed that folate level is inversely associated with CVD-risk\textsuperscript{68}.

The 1-C-cycle plays a central role in (I) the regulation of oxidative stress and (II) the generation of methyl groups for methylation of DNA, proteins, phospholipids and neurotransmitters\textsuperscript{69, 70}. A crucial enzyme in this pathway is 5,10-methylenetetrahydrofolate reductase (\textit{MTHFR}). A single nucleotide polymorphism (SNP) in the \textit{MTHFR} gene (C677T or rs1801133) results in the production of a thermolabile variant of \textit{MTHFR}, which is associated with decreased methylation capacity\textsuperscript{71} and increased oxidative stress. This genetically determined variation in 1-C-cycle activity is associated with increased stress sensitivity, due to a multitude of factors (e.g. a lower level of the important intracellular antioxidant glutathione). This genetic variation may therefore contribute to alterations in neurocognitive functioning and mood regulation, predisposing to either development and/or the adverse course of MDD\textsuperscript{72, 73}.

Evidence for the link between polymorphisms in the \textit{MTHFR} gene with MDD is thus far equivocal. Patients with the \textit{MTHFR} 677T genotype may be particularly prone to recurrent MDD as a result of a dysfunctional methylation metabolic pathway and/or long-lasting (methylation) effects of childhood traumatic stress. The association of the \textit{MTHFR} gene with recurrent MDD, however, has not yet been investigated. Studying genetic susceptibility to stress is of specific relevance in the context of MDD as stress is considered one of the main pathogenic factors involved in MDD recurrence.
Most genetic MTHFR studies in patients with MDD did not simultaneously measure the key constituents of the 1-C-cycle mentioned above (folate, vitamin B6, B12 and homocysteine). The MTHFR 677TT genotype and, to a lesser extent the 677CT genotype, is associated with a pattern of significant elevation in the circulating concentrations of the thiol amino acid homocysteine and a decrease in serum folate concentrations, which may parallel a similar reduction in 5-MTHF in the central nervous system (CNS).

Total homocysteine can be interpreted as a marker of oxidative stress resulting in neurological and vascular damage and as an interruption of the optimal biosynthesis of neurotransmitters. High homocysteine levels have been linked with MDD. Low folate concentrations have been found in patients with MDD compared to healthy controls. There is also evidence that vitamin B12 and vitamin B6 may be associated with depressive symptomatology. Nevertheless, not all studies have found significant associations between the 1-C-cycle constituents and depressive symptomatology. Explanations for the possible association of the 1-C-cycle constituents with recurrent MDD includes direct causation, reverse causation, coincidence, and having a common cause.

In this thesis, we will examine integratively all the 1-C components, while including the possible effects of medication and depressive state, and thereby attempt to provide more insight in the possible association between the MTHFR polymorphism and recurrent MDD. Additionally, we investigated if C677T MTHFR could be a potential predictor for depressive symptomatology and MDD recurrence in the context of traumatic stress during early life.

Medically Unexplained Physical Symptoms
Somatic symptoms, unattributed to a diagnosable medical condition, are common in patients with MDD. These medically unexplained physical symptoms (MUPS) show a wide variety of severity ranging from single, mild, and transient to a larger number of more chronic and extremely disabling symptoms. High levels of MUPS in MDD patients may greatly impair their quality of life and could increase the burden of depression, hinder full remission, and may hamper treatment response. Understanding the relation between MUPS and MDD is therefore of high relevance. A correlation between depression and MUPS has been reported in cross sectional studies but longitudinal studies investigating the temporal relationship between depression and MUPS are scarce.

Mutual underlying biological pathways could (partly) play a role in the relation between recurrent MDD and MUPS. The first candidate is fatty acid (FA)-metabolism as FA’s play multiple roles that
include: (I) participating in immune regulation (II) determining neuronal membrane stability, and
(III) being involved in neurotransmission and signal transduction. MDD is linked to lowered n-3
FAs levels and an imbalance between n-3/n-6 levels, which is viewed as harmful and might
be linked to somatic manifestations. A second candidate is the serotonergic pathway because
it is considered to play a role in both MDD and the development of pain symptoms. There is
some evidence for an association of longer 5-HTTLPR allele mutations with MUPS, while MDD
itself is related either with a longer 5-HTTLPR allele mutation or other 5-HTTLPR mutational
variants. Additionally, lowered n-3 FA status is related to serotonergic disturbances and could
be a pathway for mood and cognitive dysfunction in depression.

In this thesis, we assess whether MUPS is a predictor of recurrence in patients with recurrent
depression. This could be of clinically relevance, especially because MUPS may represent a
dynamic modifiable factor involved in recurrence. Additionally, the associations with FA- and
serotonergic pathways and MUPS will be explored.

1.1.2 Study used in this thesis (DELTA study)
This thesis is based on data from two research projects that were initiated and conducted by
the Program for Mood Disorders at the Academic Medical Center (AMC) located in Amsterdam,
Netherlands.

First, data was drawn from the DELTA (Depression Evaluation Longitudinal Therapy
Assessment) Study, which was initiated with a grant from the Health Research Development
Council, Department Prevention Program. The aim of this project was to assess the recurrence-
preventing effect of cognitive therapy (CT) in recurrent MDD in a randomized controlled trial

The second source of data for this thesis was drawn from an additional biological study within
the DELTA study entitled “Somatic, psychological and social course in patients with recurrent
MDD: development of clinical relevant models”, which received support from the Netherlands
Foundation for Mental Health

Studying recurrent-MDD is important because it represents a more biologically determined
MDD-subtype, which may be specifically linked to recurrence and CVD-risk. For this reason,
we expected these patients to deviate strongly from healthy controls in the (patho)physiolo-
gical and genetic mechanisms and to have more pronounced alterations in their course of
MDD. Moreover, this patient group can be considered characteristic for those patients particularly causing the large MDD-associated burden of disease. Interestingly, few in past studies have included this patient group.

A full description of DELTA has been provided by Bockting, The rhythm of depression.

In addition to these two research projects, we recruited an age and gender matched, healthy, non-depressed control group by advertising.
1.1.3 Aims and outline of this thesis (including figure)

The general aim of this thesis is to investigate aspects of biological- and pathophysiological mechanisms (including HPA-axis functioning, one-carbon and fatty-acid metabolism), the role of stress (childhood trauma, life events and daily hassles), and the impact of gene-environment (stress, childhood trauma) interactions associated with the course of recurrent MDD. This thesis aims to assess this in relation to: (I) clinical relevance, (II) underlying associated mechanisms and course of depression in terms of relapse and recurrence and (III) improvement of treatment strategies to prevent recurrences.

These aims resulted in the following research questions:

1  What is the relation between recurrent depression, being overweight, and obesity in patients with MDD-R and is this relation explained or modified by use of anti-depressant medication (AD)?

Chapter 2 describes the results of a study that focused on the relation between obesity and MDD-R and the association between long-term use of ADs and obesity. Additional data were derived from a large Dutch population-based study of time trends on obesity prevalence rates.

2a In what ways does the HPA-axis functioning differ between MDD-R patients and healthy controls?

2b Does this reflect a persistent trait or is this influenced by depressive state, stress or previous episodes associated with recurrence? c) Can this relation be modulated by cognitive therapy?

Chapter 3.1 describes the results of our longitudinal study that assessed the HPA-axis activity over time in highly recurrent MDD-patients. At the onset of the study, all patients were in remission and were matched with those in the healthy control group. The patients were followed-up prospectively at three months and two years, where MDD-recurrence and HPA-axis activity data was collected. This longitudinal design was chosen to distinguish between state and trait effects.

3  Do HPA-axis measures predict time to recurrence in MDD-R patients?

In chapter 3.2, we investigate whether HPA-axis measures predict time to recurrence in remitted recurrently depressed patients corrected for residual depressive symptoms. We also examine the role of stress (current daily hassles) and childhood trauma on the predictive value of the HPA-axis on recurrence.
4a Do polyunsaturated fatty acid (PUFA) levels and the n-6/ n-3 ratio differ between MDD-R patients compared to non-depressed controls?

4b Are these possible alterations state dependent, or do they reflect a trait (i.e. are they independent of the current depressive status)?

Chapter 4.1 describes our explorative study that comprised of 44 randomly selected subjects chosen out of a cohort of 134 patients with the recurrent form of MDD (MDD-R). In this study, we assessed homocysteine levels together with saturated fatty acids (FAs), monounsaturated fatty acids (MUfAs) and polyunsaturated FAs (PUFAs) of the omega n-3, omega-6 and omega-9 series in plasma and erythrocytes. Levels were compared with laboratory reference values.

We subsequently carried out a case-control study, which is described in chapter 4.2. The sample for this study consisted of 137 patients with MDD-R and 65 matched non-depressed controls.

5a What is the relationship of the Ala54Thr fatty acid-binding protein 2 (FABP2) polymorphism in recurrent depression?

5b Are fatty acid concentrations associated with the CVD-risk factor waist circumference?

In chapter 4.3, we investigate whether the Thr54-polymorphism in the FABP2-gene is (I) more prevalent in MDD-R patients than in sex-and age-matched controls, (II) associated with observed alterations in FA-metabolism, and (III) associated with the CVD-risk factor, i.e. waist circumference.

6 What is the prevalence of the MTHFR polymorphism and its relationship with 1-C-cycle components in MDD-R patients compared to matched controls and are the latter influenced by depressive state and/or AD use?

In chapter 5.1, we examine the MTHFR C677T polymorphism together with 1-C-cycle components (folate, homocysteine, vitamin B₆ and B₁₂) in clinically diagnosed patients with a high risk of recurrence of depression compared to age- and sex-matched healthy controls.

7a What is the association between recurrent MDD patients carrying the T allele with time to recurrence?

7b Is this association associated with having been exposed to traumatic childhood events (TCE)?

In chapter 5.2, we investigate the moderating effect of the C677T MTHFR variant on the association between traumatic childhood events (TCEs) and MDD recurrence in a 5.5-year follow-up study in a sample with recurrent MDD and, in an independent replication sample, on depressive symptomatology in healthy individuals from the general population.
8a What is the association between medically unexplained physical symptoms (MUPS) with time to recurrence in recurrent depression?

8b What is the association between MUPS with FA metabolism and the serotonin transporter gene?

In chapter 6, we assess the predictive value of medically unexplained physical symptoms (MUPS) on time to recurrence in recurrent depression. In addition, we examine the association between a sustained high level of MUPS and omega n-3 and −6 fatty acid (FA)-status as well as functional polymorphisms in the promoter region of the serotonin transporter gene (5-HTTLPR), to elucidate pathophysiological mechanisms that could explain the relations between MUPS and MDD.

To conclude, in chapter 7, the findings of this thesis are summarized and discussed and implications and suggestions for future directions are presented.
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