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NOVEL MECHANISMS IN DEPRESSION: FOCUS ON TELOMERE BIOLOGY AND EPIGENETIC REGULATION

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Novel Mechanisms in Depression: Focus on Telomere Biology and Epigenetic Regulation

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To my beloved family and friends.

ABSTRACT

Depression is a complex disorder with an average lifetime prevalence from 11.1% to 14.6%. It causes serious disability and is a significant public health problem worldwide. The etiology of depression is heterogeneous and multifactorial. Traditionally, researchers have tried to investigate depression from biochemical, genetic, environmental and behavioral perspectives. Since few biomarkers are available, diagnosis and treatment are still based on clinical assessment and are far from satisfactory. In recent years, depression has been proposed to be a state of "accelerated biological aging", with an increased risk of comorbidity with other ageing-related conditions such as diabetes, cardiovascular disease and dementia. There is accumulating evidence to support that depression itself is in fact a state that involves telomere dysfunction, a prominent feature in the ageing process. Epigenetic regulation, with an emerging role in a number of complex disorders, constitutes a fusion between the results of genetic, biochemical and environmental factors. The aim of this thesis was to investigate the pathophysiology of depression with a focus on mechanisms that are perturbed in telomere biology and epigenetic regulation. Specifically, in paper I and III: telomere length and the genetic variation in the *hTERT* gene were examined in relation to lithium treatment, to depression disorder and depressive episodes in bipolar disorder in human cohorts. In paper II, we used a genetic rat model of depression (FSL) to study hippocampal telomere length and telomerase activity, and investigated the mechanism of how lithium affects telomere length. The epigenetic mechanisms potentially involved in depression, specifically DNA methylation/hydroxymethylation and miRNAs were investigated in the prefrontal cortex region of the FSL rats in paper IV and V, respectively. The major finding from the thesis work includes 1) telomere lengths were decreased in saliva DNA from patients with adult depression 2) genetic variation in *hTERT* may influence the susceptibility to depression 3) telomeres and telomerase activity are dysfunctional in the hippocampus of the depressed FSL rats 4) long-term lithium treatment is associated with longer telomeres in bipolar disorder especially when therapeutically efficacious 5) lithium treatment may normalize hippocampal telomerase dysfunction through activation of β-catenin in the rat 6) sodium butyrate exerts antidepressant-like effect and the suggestive epigenetic effects may include DNA methylation changes that are mediated by the demethylation-facilitating enzyme TET1 in the rat 7) elevation of cytokine *Il6* in the prefrontal cortex is associated with depression-like states and may involve disturbance in let-7 biogenesis in the rat 8) physical exercise appears to normalize *Il6* and let-7 levels through regulatory processes upstream of primary miRNA transcription in the rat.

Keywords: telomere length, *hTERT*, telomerase activity, depression, FSL, lithium, TET, sodium butyrate, methylation, hydroxymethylation, miRNA, let-7

LIST OF PUBLICATIONS/MANUSCRIPTS

I. Martinsson L*, **Wei Y***, Xu D, Melas PA, Mathé AA, Schalling M, Lavebratt C†, Backlund L†.
Long-term lithium treatment in bipolar disorder is associated with longer leukocyte telomeres.
Transl Psychiatry. 2013 May 21;3:e261. doi: 10.1038/tp.2013.37.

II. **Wei Y**, Backlund L, Wegener G, Mathé AA†, Lavebratt C†.
Telomerase dysregulation in the hippocampus of a rat model of depression. Normalization by lithium.
Int J Neuropsychopharmacol. 2015: pyv002. doi: 10.1093/ijnp/pyv002.

III. **Wei Y**, Martinsson L, Liu JJ, Forsell Y, Schalling M, Backlund L, Lavebratt C.
***hTERT* genetic variation in depression.**
Submitted manuscript.

IV. **Wei Y**, Melas PA, Wegener G, Mathé AA, Lavebratt C.
Antidepressant-like effect of sodium butyrate is associated with an increase in TET1 and in 5-hydroxymethylation levels in the *Bdnf* gene.
Int J Neuropsychopharmacol. 2014 Oct 31;18(2). pii: pyu032. doi: 10.1093/ijnp/pyu032.

V. **Wei Y**, Liu JJ, Åberg E, Brené S, Wegener G, Mathé AA, Lavebratt C.
Elevation of *Il6* is associated with disturbed let-7 biogenesis in a genetic model of depression.
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*Contributed equally
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LIST OF ADDITIONAL PUBLICATIONS/MANUSCRIPTS

I. Backlund L, **Wei Y**, Martinsson L, Melas AP, Liu JJ, Mu N, Ekström T, Schalling M, Lavebratt C. **Lithium treatment is associated with DNA hypomethylation in leukocytes of bipolar disorder patients**. Molecular Neuropsychiatry. *Accepted manuscript*.

II. Fandino-Losada A, **Wei Y**, Åberg E, Sjöholm LK, Lavebratt C, Forsell Y. **Influence of serotonin transporter promoter variation on the effects of separation from parent/partner on depression.** J Affect Disord. 2013 Jan 25; 144(3): 216-224.

III. Melas PA, **Wei Y**, Wong CC, Sjöholm LK, Åberg E, Mill J, Schalling M, Forsell Y, Lavebratt C. **Genetic and epigenetic associations of MAOA and NR3C1 with depression and childhood adversities.** Int J Neuropsychopharmacol. 2013 Aug;16(7):1513-28. doi: 10.1017/S1461145713000102. Epub 2013 Mar 1.

IV. Melas PA, Lennartsson A, Vakifahmetoglu-Norberg H, **Wei Y**, Åberg E, Werme M, Rogdaki M, Mannervik M, Wegener G, Brené S, Mathé AA, Lavebratt C. **Allele-specific programming of neuropeptide Y (Npy) and epigenetic effects of physical activity in a genetic model of depression.** Transl Psychiatry. 2013 May 7;3:e255. doi: 10.1038/tp.2013.31.

CONTENTS

LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
ATM	ataxia telangiectasia mutated
ATR	ataxia telangiectasia and Rad3 related
BDNF	brain-derived neurotrophic factor
CRH	corticotropin-releasing hormone
DNMT	DNA (cytosine-5-)-methyltransferase
DROSHA	drosha, ribonuclease type III
DSM-IV/V	diagnostic and statistical manual of mental disorders
fMRI	functional magnetic resonance imaging
HDR	homology-directed repair
HIP	hippocampus
HPA	hypothalamic-pituitary-adrenal
IL-1β	interleukin-1β
IL-6	interleukin-6
MAOI	monoamine oxidase inhibitor
NaB	sodium butyrate
NHEJ	nonhomologous end joining
PET	positron-emission tomography
PFC	prefrontal cortex
qRT-PCR	quantitative real time-polymerase chain reaction
SSRI	selective serotonin reuptake inhibitor
TERC	telomerase RNA component
TERT	telomerase reverse transcriptase
TET	ten-eleven translocation protein
TNF-α	tumor necrosis factor α

1 INTRODUCTION

Depression was initially called "melancholia" which first appeared in ancient Mesopotamian texts written around 2000 B.C. It was documented as one of four humors in the old medical beliefs. From ancient Greeks and Romans, for thousands of years, depression has been debated back and forth as to whether it was best thought as a mental or physical disease. Nowadays we define depression, or a more clinical term "major depressive disorder", as a mental disorder characterized by pervasive and persistent low mood that is accompanied by low self-esteem and a loss of interest or pleasure in normally enjoyable activities [1]. However, it became the accepted view for scientists that depression can actually have many causes, and different approaches for treatment are considered to be important in helping depression patients "out of the blue". Depression is a commonly occurring and recurrent disorder. Specifically, the estimated lifetime prevalence is from 11.1% to 14.6% between different countries [2] and approximately 60% of people who had a first lifetime episode would develop a second one [3]. It is noteworthy that depression is highly comorbid with other medical diseases that associates with accelerated biological aging (e.g. cardiovascular disease and type 2 diabetes) [4-6] and recently mounting evidence seem to support the accelerated biological-aging conception in depression [7; 8]. The importance of studying depression is also reflected by the global projections of disease burden by World Health Organization (WHO) which ranked depression as the second leading cause of burden of disease in 2030 worldwide [9]. Therefore, from both individual and public health perspectives, depression is a problem of major significance and importance.

In the introduction section of the thesis, some major definitions and background information are introduced with the purpose to help everyone, also readers who are not working in the psychiatric field, to understand the topics studied in the constituent papers. I hope this thesis work can raise the awareness of people who have ignored or underestimated the significance of studying mental disorder.

"Success is not final, failure is not fatal: it is the courage to continue that counts."
— Winston Churchill

1.1 DEPRESSION

In this section, an overview of depression is provided which includes the symptoms and diagnoses criteria, the current view of etiology and relevant treatments for depression. A brief introduction of bipolar disorder is also included which contains the diagnoses and treatment options.

1.1.1 Symptoms and clinical diagnoses

Depressive disorders include a spectrum of disorders with the common features being the presence of sadness or irritable mood accompanied by somatic and cognitive changes that significantly affect the individual's capacity of functioning (DSM-V). Depression is a highly recurrent and dimensional illness with the affected persons moving in and out of diagnostic subtypes, such as Major Depression, Dysthymia and Minor Depression [10]. The symptoms and diagnostic criteria are determined on the basis of clinical interviews, by using diagnostic classification systems, including Diagnostic and Statistical Manual of Mental Disorders (DSM) and International Statistical Classification of Diseases and Related Health Problems (ICD), with no biochemical or physiological test available. According to DSM-IV (and also DSM-V), the main criteria include depressed mood or a loss of interest or pleasure in daily activities (anhedonia) consistently for at least a two week period. Other symptoms include significant change in weight and appetite, change in sleep (insomnia or hypersomnia), change in activity (psychomotor agitation or retardation), fatigue or loss of energy, guilt/worthlessness, diminished ability to think or concentrate and suicidal thoughts. General practitioners were able to make routine diagnoses of depression only in about 50% of the cases [11].

Depressive episodes are common in bipolar disorder (BD). Bipolar disorder originally called manic-depressive illness, is a mental disorder characterized by recurrent episodes of elevated mood and depression accompanied by changes in activity levels. Based on DSM-IV, BD type 1 is featured as alternating episodes of depression and mania, whereas BD type 2 is characterized by depression and hypomania. Other related diagnoses include cyclothymia, characterized by hypomania and subthreshold depression, and bipolar disorder not otherwise specified. Manic and depressive symptoms can also co-occur, so called "mixed states"[12]. Rapid cycling is a severe subtype of BD defined as four or more bipolar episodes per year, which is associated with treatment resistance and overall poorer prognosis [13]. BD affects ~1-2% of the population and this causes tremendous economic burden [14; 15]. In contrast to unipolar depression, the heritability of BD is high, up to 70~80%, and overlaps with that of schizophrenia in a number of common variants alleles [16]. Lithium is the first-line mood stabilizer in treating BD [16] however treatment response varies considerably and there is no predictive biomarker available [17]. Some studies suggest that lithium responsive-BD may be a distinct subtype of BD with genetic difference, however this hypothesis requires more investigation [18; 19]. In the thesis constituent papers, the study has been primarily focused on BD type 1 patients which the majority of study materials are collected from.

"I can't eat and I can't sleep.

I'm not doing well in terms of being a functional human, you know?"

— Ned Vizzini, *It's Kind of a Funny Story*

Depression by Yu Chen. Beijing, China. 2015.

1.1.2 Etiology of depression

Depression is a complex disorder, meaning that the etiology of depression is the interactive effects of multiple genes in combination with lifestyle and environmental factors, which is best summarized as a prototypical G×E interaction model [20]. Moreover, epigenetics provide a bridge through which environment can affect the genome and play considerable roles in the pathophysiology of depression and antidepressant action [21]. In this section, neuroanatomy and related theories of depression studied in the constituent papers are provided, including the monoamine hypothesis, the neurotrophins and neurogenesis hypothesis, the hypothalamic-pituitary-adrenal axis and stress response theory and the inflammation theory of depression. Other theories such as glutamate hypothesis of depression and circadian abnormality hypothesis of depression also received great attention from researchers. One should keep in mind that although these theories of depression have been debated for a long time, none of these theories sufficiently explains the pathophysiology and treatment of depression.

1.1.2.1 Neuroanatomy of depression

Depression is a disorder that involves several critical brain regions and associated circuits and neural pathways. Using brain imaging technologies, e.g. structural imaging (CT) and functional imaging (fMRI, PET), several brain regions in depressed patients have been identified with altered structures and activity. Specifically, reductions in grey-matter volume and glial density in the prefrontal cortex (PFC) and the hippocampus (HIP) have been implicated in depressed patients [22; 23]. Activity changes in depression is characterized by abnormalities in limbic system–cerebrocortical circuits with reduced activity in frontal cortical areas and hyperactivity in the amygdala [24; 25]. PFC and HIP are the two main brain regions investigated in the current thesis. PFC has been implicated in executive function, working memory, personality expression and moderation of social behavior [26-28]. Patients with PFC lesion showed depressive symptoms, suggesting that PFC is both critically and causally involved in depression [29]. HIP plays pivotal roles in cognitive function [30], mood regulation and memory formation [31]. In particular, substantial literature shows existence of adult neurogenesis in the subgranular zones of dentate gyrus in HIP [32] and the role of reduced neurogenesis in the pathophysiology of depression [33; 34].

1.1.2.2 The monoamine hypothesis of depression

The monoamine hypothesis of depression posits that depression is caused by a depletion of the monoamine neurotransmitters in the central nervous system, such as serotonin, norepinephrine or dopamine which structurally contain one amino group connected to an aromatic ring by a two-carbon chain [35]. In accordance with this theory, almost all established antidepressants act either by inhibiting neuronal reuptake of monoamines that leads to an increased concentration in the synaptic cleft (e.g. SSRIs such as fluoxetine) or by hindering the degradation of monoamines (e.g. MAOIs such as tranylcypromine). Although SSRIs and MAOIs are potent antidepressants, full and partial resistance to these drugs and their delayed onset of action suggest the cause of depression is far from being a simple

deficiency of central monoamines. Although it is not particularly studied in the thesis constituent papers, the monoamine hypothesis is regarded as the most clinically relevant neurobiological theory of depression.

1.1.2.3 Neurotrophins and neurogenesis hypothesis of depression.

Reduced volume in hippocampus and other forebrain regions in subsets of depressed patients have supported a popular hypothesis for depression, involving a decrease in neurotrophic factors −− a family of proteins that are responsible for the growth, maintenance and survival of neurons that also regulates plasticity of mature neurons within the adult brain [36]. These events may be causally linked via neurogenesis [37]. One of the most studied neurotrophic factors, also the one investigated in the thesis, is brain-derived neurotrophic factor (BDNF) which has been shown to play a key role in the regulation of neurogenesis in the hippocampus and to have antidepressant-like effects [38; 39]. A number of studies, both preclinical and clinical evidence, have supported the theory that precipitating factors such as chronic stress may decrease the BDNF neurotrophic support, which leads to significant atrophy of the hippocampus. This is detrimental to hippocampal function and ultimately leads to the development of depressive symptoms [40-43]. However, considerable studies have generated inconsistent data which reminds us to reassess this theory [44; 45]. A role of BDNF supported by more consistent reports is that in antidepressant treatment, which could be beneficial to the development of novel antidepressant drugs [46].

1.1.2.4 Dysregulation of the hypothalamic-pituitary-adrenal axis and stress response

Stress is commonly defined as a state of real or perceived threat to homeostasis. In order to maintain homeostasis and to increase the probability of survival, animals activate a complex set of behavioral and physiological responses involving the nervous, endocrine, and immune systems which are collectively known as the stress response [47; 48]. Numerous studies have linked stress and depression, as depression may be a cause and/or outcome of chronic stress and increased stress levels can significantly affect the duration and degree of symptoms of depression [49]. Psychosocial stressors implicated in depression includes e.g. early life adversities, divorce or serious marital conflict and death of a relative [50; 51]. The principle anatomical structures that mediate the stress response is commonly referred to as the hypothalamic-pituitary-adrenal (HPA) axis (Figure 1) [52]. Activation of the HPA axis will lead to the release of glucocorticoids, which exert profound effects with their receptors on several somatic organ systems and brain regions. A negative feedback loop exists where high glucocorticoid levels dampen the HPA-axis activity inhibits the secretion of ACTH from the pituitary and CRH from the hypothalamus [53]. Glucocorticoids are reported to regulate neuronal survival, neurogenesis and the sizes of hippocampus [54] and the two main receptors that are widely expressed in the brain include the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). A number of studies have reported that depressed patients have HPA axis hyperactivity and increased levels of cortisol in the saliva, plasma and urine [55]. The increased activity of the HPA axis is thought to be related, at least in part due to an impairment of GR-mediated negative feedback (glucocorticoid resistance) [56]. In

agreement with this notion, abnormal GR and GR-associated protein expression (through both genetic and epigenetic mechanisms) have been implied in the brain of depressed individuals and GR is suggested to be a target for antidepressant action [55; 57-59].

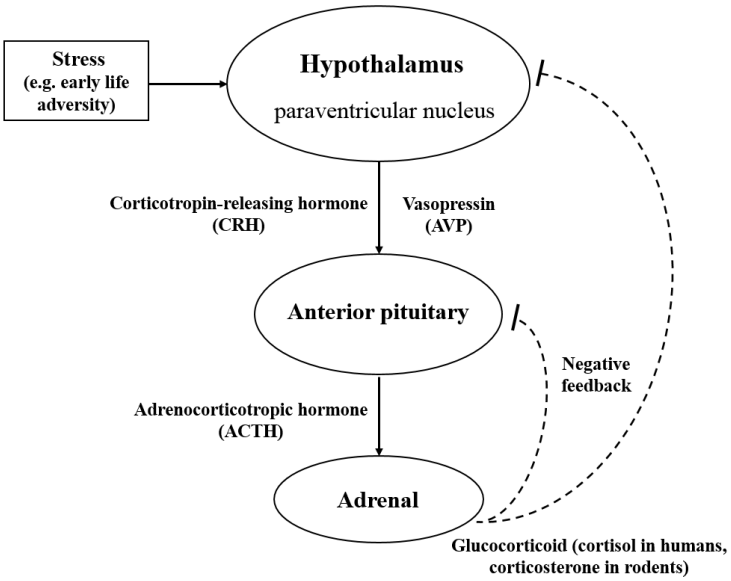

Figure 1. HPA axis.

1.1.2.5 The inflammation theory of depression

Increasing evidence suggests that activation of the immune system may play a critical role in the pathophysiology of depression. Increased levels of inflammatory biomarkers, including proinflammatory cytokines (such as TNF-α, IL-6 and IL-1β), acute phase proteins and chemokines have been reported in depressed patients [60; 61]. In addition to association between inflammatory markers and depression, several studies also demonstrate that both acute and chronic administration of cytokines (or cytokine inducers such as lipopolysaccharide or vaccination) can cause depressive symptoms [62; 63] and anti-inflammatory therapy may have clinical benefit for depressed patients [64; 65]. Cytokines in the periphery have been shown to access the brain by several routes and can interact with a number of pathophysiological domains involved in depression, including neurotransmitter metabolism (serotonin, norepinephrine, and dopamine), neuroendocrine function (HPA axis), and neural plasticity (neurotrophic support, neurogenesis and glutamate transportation) [66]. However, it remains unclear whether activation of inflammation pathways during depression originates primarily in the periphery or in the central nervous system. The source of immune activation in depression can be quite diverse, nevertheless, psychosocial stress has been regarded as a major factor particularly in medically healthy depressed individuals, which can activate the inflammatory response both peripherally and in the brain [67; 68].

1.1.3 Genetics and depression

Genetic components are considered to be important in the development of depression, as indicated by genetic epidemiology data from family and twin studies, and may provide important evidence about disease mechanisms. Family studies estimate that there is

approximately threefold increase in lifetime risk of developing depression among first-degree relatives of depressed probands [69]. Twin studies suggest a heritability of depression to be 37% and it is significantly higher in women than in men [69; 70]. Familial aggregation coupled with the high heritability of depression led to optimistic thoughts that molecular genetic techniques would reveal risk gene variants that have substantial influence on depression. Unfortunately, from linkage studies, candidate gene studies to genome-wide association studies, the process of identifying those gene variants have been slow and the results have been difficult to consistently replicate so far [71]. The possible explanation could be that depression is a complex and heterogeneous disorder where each susceptibility gene variant contributes only a small fraction of the overall genetic risk. In order to overcome these problems, increase of sample size and categorization by subtype of depression are among the most recommended suggestions [72]. Despite these obstacles, some promising and reproducible genetic findings have been generated using G x E model which incorporates environmental risks, such as the study of interaction effect of stressful life events and the variants in the serotonin system [73].

1.1.4 Epigenetics and depression

The term ‘epigenetics’ was initially coined by Waddington in the 1940s to address the question of how numerous distinct cell types were generated from the same genome [74]. The definition of epigenetics has changed over time, and now it refers to a stably heritable (mitotically and/or meiotically) phenotype resulting from changes in a chromosome without alterations in the DNA sequence [75]. By this definition, epigenetic markers mainly include DNA methylation and histone modifications. However, previously the definition of epigenetic modifications included also the effect of noncoding sequences such as microRNAs (miRNAs) [76]. Epigenetics has provided a mechanism by which environmental factors can affect chromatin structure that ultimately lead to persistent alteration in gene expression. Accumulating evidence suggests critical roles of epigenetic mechanism in neuronal plasticity, neurogenesis and neurological and psychiatric disorders [77; 78]. Epigenetics may help to explain some of the missing heritability in depression and provide new insights to understand the pathophysiology [79]. In the present thesis, two types of epigenetic modifications are investigated in relation to depression: DNA methylation/hydroxymethylation and miRNAs.

1.1.4.1 DNA methylation

DNA methylation was described as early as in 1948 [80], but it was not until 1969 that Griffith and Mahler suggested that these modifications may modulate gene expression [81]. DNA methylation includes methylation of cytosine (predominant form in mammals), adenine and guanine [82] and the primary form of mammalian methylated cytosine is in the context of CpG dinucleotides (5-methylcytosine, 5mC); 70 – 80% of CpGs are methylated. There is increasing evidence showing that cytosines in non-CpG sequences (i.e. CpH, H=A/T/C) are also frequently methylated, especially in the brain [83; 84]. In mammals, DNA methylation patterns are initially established by the de novo DNA methyltransferases 3

family (DNMT3A/3B) and maintained during cell division by the maintenance methyltransferase (DNMT1) which prefers hemi-methylated DNA [85; 86]. Although DNA methylation has been regarded as a stable epigenetic mark, growing evidence indicates that DNA demethylation can occur through both passive and active mechanisms [87]. Passive DNA demethylation refers to the loss of 5mC during DNA replication in the absence of functional DNMT1. By contrast, active DNA demethylation requires an enzymatic process which removes or modifies 5mC by ultimately breaking the carbon-carbon bond. A number of enzyme has been characterized recently which includes the ten-eleven translocation protein family (TET1-3), facilitating the oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [88]. The following demethylation process is often coupled with thymine DNA glycosylase (TDG)-mediated base excision repair (BER) pathways and deaminases which lead to unmodified cytosine (C). A brief summary is shown in Figure 2. Currently, it is generally accepted that cytosine methylation regulates gene transcription in a highly cell-type specific manner [89]. In general, a heavily methylated promoter region corresponds to inactive transcription. This gene silencing mechanism is thought to either be due to direct inhibition of transcription factor binding by DNA methylation or mediated by methyl-binding domain proteins that recruit various co-repressor complexes to methylated DNA [90]. 5hmC is regarded as an intermediate of demethylation, however the relatively high steady-state levels of 5hmCsuggest that 5hmC might also function as a stable signal that modulates binding of protein complexes to chromatin thus influences gene expression [91; 92]. In the present thesis, both 5mC and 5hmC were studied with a specific investigation of the *Bdnf* gene [93]. Other established functions of DNA methylation involves multiple cellular processes, such as DNA–protein interactions, cellular differentiation, transposable elements suppression, embryogenesis, X-chromosome inactivation and genomic imprinting [94; 95].

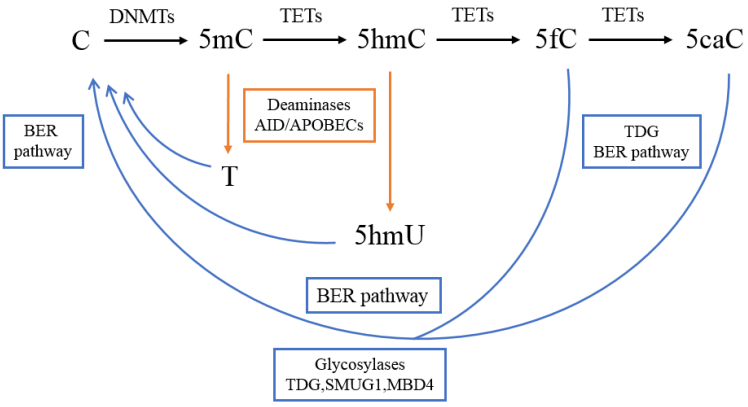

Figure 2. Active DNA demethylation.

1.1.4.2 MiRNA

MiRNAs are small, ~22 nucleotides long, non-coding RNAs that typically function as key post-transcriptional repressors of gene expression [96]. The biogenesis and expression of miRNAs is under strict temporal and spatial control and is involved in nearly all

developmental and pathological processes. Dysregulation of miRNAs have been associated with many human diseases including depression [97]. Most miRNAs derive from transcription of primary transcripts (pri-miRNAs) by RNA-PolII in the cell nucleus. Primary transcripts are processed by DROSHA/DGCR8 heterodimer into 60-80 nucleotides (nt) long precursors miRNAs (pre-miRNAs) which are released into cytoplasm. DICER together with its dsRNA-binding partner TRBP, further cleave pre-miRNAs into an approximately 22 nt double stranded mature miRNAs. Supported by the HSC70–HSP90 chaperone machinery, only one strand of the mature miRNA is incorporated into the RNA-induced silencing complex (RISC) while the other strand is degraded. The miRNA-RISC complex regulates target mRNA expression through mRNA degradation and/or translational repression [98]. A few miRNAs are produced by alternative pathways e.g. the 'mirtron pathway' that replaces standard miRNA biogenesis steps [99]. In the present thesis, the lethal-7 (let-7) miRNA family is for the first time investigated in the FSL/FRL rat PFC region, a study which focused on LIN28B-mediated biogenesis dysregulation. The let-7 family is highly conserved between species and consists of 12 genes encoding 9 distinct miRNAs (let-7a to let-7i and miR-98) [100]. There is increasing evidence suggesting the involvement of the let-7 family in inflammation and immune response, and the let-7 family has also been implicated in neuronal proliferation and differentiation and synaptic plasticity [101-104]. However, its role in relation to the pathophysiology of depression is less investigated.

1.1.5 Telomere biology and depression

Mammalian telomeres are protective DNA-protein structures located at the end of chromosomes. They shorten progressively during each cell division due to incomplete replication of linear chromosomes by conventional DNA polymerases, which often referred to as 'end-replication problem' [105]. Then a natural question is that by which mechanism telomeric DNA is maintained. This was answered by Blackburn and Greider who showed that telomeric DNA is synthesized by telomerase, being capable of compensating for this progressive telomere attrition [106]. One may also wonder how cells distinguish their natural chromosome ends from double-strand breaks elsewhere in the genome, i.e. how do cells solve the 'end-protection problem'. Shelterin is one of the most important and well-characterized telomere-associated protein complexes that are involved in protecting the chromosome ends from two DNA damage signaling pathways (ATM and ATR mediated cell cycle arrest) and two double-strand break repair pathways (NEHJ mediated chromosome fusion and HDR mediated chromosome recombination) [107]. In this section, telomere length and telomerase in relation to depression research are introduced.

1.1.5.1 Telomere length shortening in depression

Telomeres consist of tandem repeat DNA sequences (TTAGGG) and protective proteins. The telomeres are responsible for preventing the chromosome ends from activation of DNA damage response pathways and improper repair pathways [108]. Telomeres are shortened during every cell division, and critically shortened telomeres result in loss of telomere protection which lead to cell cycle arrest and apoptosis pathways. The length of telomeres is

suggested to indicate biological aging [109]. In recent years, a number of studies have reported shorter blood leukocyte telomere length (LTL) to be associated with depression, and the shorter LTL the more experienced depressive episodes in bipolar disorder [110-116], implying that depressive individuals may bear increased risk of dysfunctional telomeres and biological aging-related decline. For example, an average shortening corresponding to 6-10 years of accelerated biological aging has been estimated in blood leukocytes of depressive patients [117; 118]. But only a few studies have investigated telomere length in depressed brains [119; 120]. Telomere shortening has also been associated with psychological stress, oxidative stress and inflammation [121-123]. Adversity in childhood has been associated with shorter LTL in adulthood [124; 125].

1.1.5.2 Telomerase dysfunction in depression

Telomerase is a reverse transcriptase that consists of a catalytic reverse transcriptase subunit (TERT) and an RNA component (TERC) being template for DNA synthesis. Telomerase adds TTAGGG repeats to the chromosome ends and thereby counteracts the telomere shortening [126]. High level of telomerase expression is found in pluripotent stem cells, early stages of embryonic development and cancer cells, although telomerase activity is also present in normal adult stem cell compartments, such as lymphocytes in the bone marrow and peripheral blood, a subset of proliferating epithelial cells in the skin, the hair follicle, the gastrointestinal tract and endometrium and a subset of cells in the testis [127]. There is also detectable telomerase in adult brain regions where neurogenesis exists: the subgranular zone of the dentate gyrus in hippocampus and the subventricular zone of lateral ventricle. In addition to adding nucleotide repeats, telomerase has been reported to be involved in cellular protection and plasticity [128; 129]. In the murine hippocampus, inhibition of TERT expression induced neuronal excitotoxicity, apoptosis and a depressive-like behavior [130; 131], and in humans, telomerase activity in peripheral blood leukocytes associated positively with hippocampal volume of postmortem brains from depressed individuals [132]. Further, reduced *hTERT* expression was found in oligodendrocytes of white matter from postmortem depressed brains compared to corresponding tissue from control brains [120]. Accordingly, TERT overexpression was associated with [133] and promoted [134] adult neurogenesis, and the antidepressant fluoxetine upregulated telomerase activity [134].

1.1.6 Treatment

First-line treatment for moderate to severe depressive disorders includes pharmacotherapy (i.e. antidepressants such as SSRIs [135]), psychotherapy (e.g. cognitive behavioral therapy [136]), or a combination of both. Next-step treatment recommendations are switching drug or augmentation (e.g. in combination with lithium, antipsychotics or ECT), depending on patients response to the initial treatment. Maintenance therapy continues the approach that led to remission [137]. However, these medications are facing a number of difficulties, with a substantial proportion of patients showing poor clinical response and suffering from residual symptoms and side-effects. It also takes weeks to months before those drugs achieve clinical benefits [138]. Thus, there is a clear and urgent need for the development of novel

antidepressants with robust efficacy. For example, clinical trials have shown that ketamine acts rapidly and effectively for treatment-resistant depression patients and may serve as a novel antidepressant [139]. Pre-clinical evidence also suggests drugs targeting epigenetic mechanism such as sodium butyrate (NaB) and L-acetylcarnitine, which exhibit promising antidepressant-like effect in animal model [93; 140]. In addition, physical exercise, a non-pharmacological intervention, has been shown to alleviate depressive symptoms in persons affected by mild to moderate depression [141]. Molecular mechanism of lithium, NaB and physical activity were investigated in the thesis constituent papers in relation to depression therapy.

The last day of Robin Williams by Yu Chen. Beijing, China. 2015.

2 AIMS

The overall aim of the thesis is to increase the understanding of the pathophysiology of depression with a focus on telomere biology and epigenetic regulation. Such information may be critical in providing both preclinical and clinical evidence to develop better diagnostic markers and therapeutic approaches.

The specific aims of each constituent study in the thesis were listed below:

Study I To test whether lithium treatment in bipolar patients may influence blood leukocyte telomere length (LTL) and whether LTL may associate with lithium responsiveness, number of depressive episode as well as rapid cycling feature of bipolar patients.

Study II Based on the findings in **study I**, we used a genetic rat model of depression (FSL) to investigate whether telomere length was shorter, telomerase activity was changed in the depressed brain with a focus on the hippocampus region, and whether lithium treatment could reverse such processes.

Study III Based on the findings in **study I and II**, we investigated whether telomere length was shorter in adult depression by using less-invasive saliva DNA samples and whether genetic variation in the functional subunit of telomerase (*hTERT*) was associated with depression and number of depressive episodes in bipolar disorder type I.

Study IV To examine histone deacetylase inhibitor NaB's putative antidepressant-like efficacy in relation to DNA methylation changes in the prefrontal cortex region of FSL rat model of depression.

Study V To investigate whether the FSL rat model of depression had elevated levels of the proinflammatory cytokine *Il6* in the PFC region and whether this inflammation state was associated with disturbed miRNA let-7 expression, in turn influenced by alterations in miRNA biogenesis. We then explored if physical exercise would lower the elevated *Il6* levels in the PFC region of the FSL rats, through the rescue of let-7 expression.

3 MATERIALS AND METHODS

In this section, an overview of the materials and methods used in the thesis' constituent papers are introduced. If not otherwise stated, all generated data were analyzed using appropriate statistical methods. All experiments and materials were approved by relevant ethical committees.

3.1 ANIMAL STUDY

All animal studies in this thesis were performed by using a genetic rat model of depression-like behavior: the Flinders Sensitive Line (FSL) and its controls, the Flinders Resistant Line (FRL). The animal studies included analyses of behavior tests and treatment effects, gene and protein expression, telomere length and telomerase activity, DNA/protein interaction and epigenetic modifications.

3.1.1 The FSL genetic rat model of depression

The FSL/FRL rats were generated as a genetic model by selective breeding towards sensitivity to the anticholinesterase agent diisopropyl fluorophosphate (DFP). Compared to FRL, the FSL is genetically more sensitive to DFP and partially resembles human depression, thus it is referred to as a depression-like model [142]. The FSL strain exhibits good face validity for depression (e.g. psychomotor retardation, reduced appetite/loss of weight, sleep disturbances, impaired emotional memory and immune abnormality), satisfies the criterion of construct validity (e.g. abnormal neurochemical systems including serotonergic, dopaminergic and neuropeptide Y), and has a high predictive validity for a number of either known antidepressant drugs (e.g. tricyclic and SSRI) or novel drugs that have antidepressant effect potentially for later on use (e.g. sodium butyrate) [142; 143].

3.1.2 Behavior tests and treatments

One type of behavior experiment and three types of treatment interventions were used. The behavior experiment performed was the behavioral despair test (a.k.a. the Porsolt forced swimming test; FST), which is commonly used to observe FSL's exaggerated immobility (floating) behavior in a water cylinder that reflects a despair state analogous to human depression. FST is also a measure of effectiveness of antidepressants [144]. The expected antidepressant-like effect is a decrease of the immobility time after intervention. Three types of treatment interventions were introduced: oral administration of lithium, intraperitoneal (i.p.) injection of HDACi (sodium butyrate) and physical exercise (running wheel).

3.1.3 RNA and protein analyses

The RNA molecules included both coding (mRNA) and non-coding (miRNA and pri-miRNA) RNA. The expression levels of targeted RNA (converted into the form of complimentary DNA; cDNA) were detected primarily by using quantitative Real-Time PCR (qRT-PCR), which is a technique that simultaneously amplifies and quantifies cDNA [145]. The protein analyses were performed by immunoblotting (western blotting) using specific

antibody to detect and quantify targeted protein levels (e.g. TET1, BDNF, β-catenin, DROSHA and LIN28B)[146].

3.1.4 RNA and protein interaction analyses

In order to investigate RNA/protein interaction related to LIN28B and pri-let-7 transcripts *in vivo*, RNA immunoprecipitation (RIP) was performed using PFC region from FSL/FRL rats. RIP involved tissue lysis, followed by an immunoprecipitation stage with an antibody that targeted LIN28B. After RNA-protein complexes were isolated by magnetic beads, the RNA of interest was purified, which then was followed by cDNA conversion and qRT-PCR quantification [147].

3.1.5 Telomere length and telomerase activity analyses

Telomere length (TL) was determined as relative values using qRT-PCR technique by calculating the ratio of telomere repeat copy number to single copy gene copy number [148]. Based on the enzymatic property of telomerase, which is adding telomeric repeats (TTAGGG) to the chromosome ends, the real-time telomeric repeat amplification protocol (RT-TRAP) [149] was used to detect telomerase activity in the hippocampus region of FSL/FRL rats.

3.1.6 Epigenetic analyses

The epigenetic analyses included DNA methylation/hydroxymethylation (5mC/5hmC) and histone modification experiments. DNA methylation/hydroxymethylation analyses were performed both globally (producing an average value representing the whole genome) and within specific regions of the target genes. Global DNA methylation/hydroxymethylation was assessed using a sandwich-based ELISA method while region-specific DNA 5mC/5hmC was assessed using a magnetic assay capable of isolating DNA fragments either with 5mC or a modified 5hmC.

3.2 HUMAN STUDY

The human studies presented in this thesis were performed mainly from PART study and bipolar study. It includes genetic and telomere analyses.

3.2.1 The PART study

PART (Psykisk hälsa, Arbete, RelaTioner) is a longitudinal population-based study of mental health, work and relationships conducted in Stockholm County, Sweden. It started 1998 and questionnaires were sent out to individuals randomly selected from Stockholm city council registers. The questionnaire includes demographic, socioeconomic and somatic health data, negative life events, smoking, illicit drug use and screening instruments for psychiatric disorders including the Major Depression Inventory (MDI), Sheehan Patient-Rated (Panic) Anxiety Scale, the Yale-Brown Obsessive-Compulsive Scale, symptoms of social phobia and agoraphobia according to Marks and Mathews (1979), eating disorders according to Beglin and Fairburn (1992), the World Health Organization Short Disability

Assessment Schedule (WHO DAS-S) and hazardous alcohol use according to Alcohol Use Disorder Identification Test (AUDIT). Epidemiological data have so far been collected three times: wave I (1998-2000), wave II (2001-2003) and wave III (2010-2011). A subgroup of individuals were selected for psychiatric interviews using Schedules for Clinical Assessment in Neuropsychiatry (SCAN) by experienced psychiatrists and one psychologist. During the period 2006-2007, saliva DNA were asked for from 5527 PART II participants including all individuals with a depression or anxiety diagnosis and a random number of individuals who had no psychiatric diagnosis or psychopathological symptoms in any of the two waves. Blood samples were collected in a subgroup of individuals, which yielded in total 88 samples. The schematic chart with detailed number of participants is shown below. For more information about PART study see Hällström et al [150] and www.folkhalsoguiden.se.

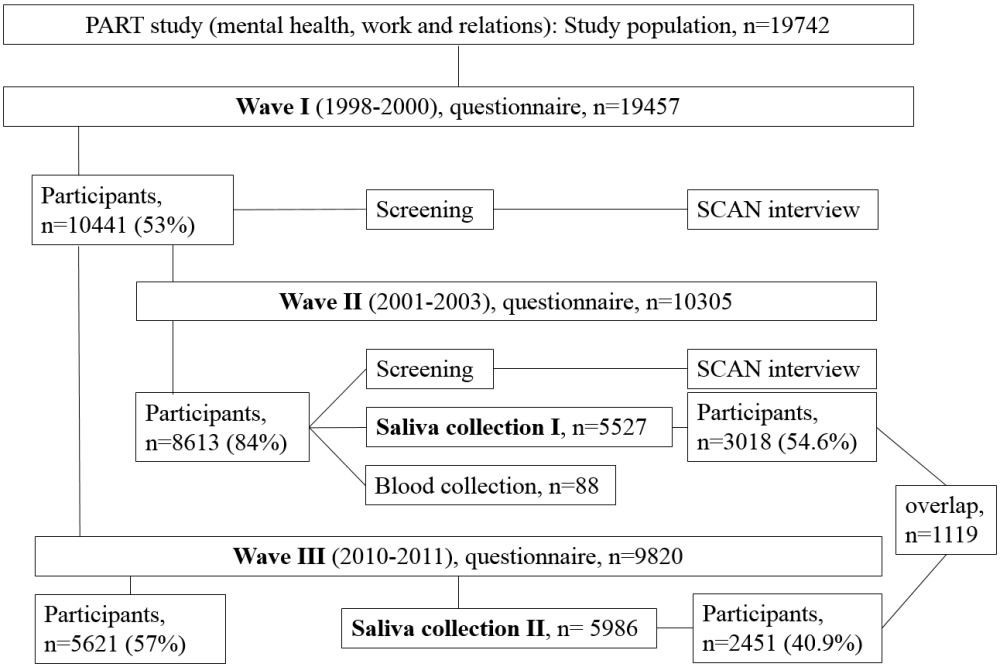

Figure 3. Schematic chart of PART study.

3.2.2 The bipolar study

Patients with a clinical diagnosis of bipolar disorder (BD) were consecutively recruited from the Unit of Affective Disorders at Psychiatry Southwest, Huddinge Hospital, Stockholm. Life-time manic and depressive symptoms were assessed by a psychiatrist specialized in BD or by a trained psychiatric nurse using the modules for mania and depression in the SCAN. On the basis of these assessment patients were considered as fulfilling the diagnostic criteria for BD type 1, 2, or not otherwise specified (NOS). The symptoms as well as the number of manias and depressive episodes, rapid cycling and mixed episodes were assessed, including the age of onset of mania and depression. Lithium response was measured according to the Alda-Scale. In previous studies, lithium responders (LiRs) were those who scored ≥ 7 and non-LiRs were those who scored ≤ 6 (range 0–10 points) [151].

3.2.3 Genetic and telomere analyses

Genetic analyses were performed for single nucleotide polymorphism (SNP) in human *TERT* gene (rs2736100 A/C). SNP genotyping utilized TaqMan assay, which was a PCR-based reaction that amplified the region included the SNP site. Allele discrimination is achieved using probes linked to fluorophore at 5' end and a quencher molecule at 3' end [152]. During the PCR amplification, if the allele-specific probe is perfectly complementary to the SNP allele, the fluorophore would separate from the quencher due to degradation by 5'-nuclease activity of the Taq polymerase, generating a detectable signal that could be read in qRT-PCR machine. Based on the conservation of telomere sequence between mammals (TTAGGG tandem repeat) [153], the method used for human telomere analyses was the same as rats, which was described in section 3.1.5 with minor modification.

4 RESULTS AND DISCUSSION

In this section, the major findings are integrated from the thesis constituent papers. More details and comprehensive descriptions are given in the full papers which could be found in the back of the printed thesis.

4.1 TELOMERE DYSFUNCTION IN HUMAN DEPRESSION

4.1.1 Shorter saliva telomere length in self-reported adult depression

To date, most of the reports on telomere length (TL) in depression have been performed on peripheral blood leukocytes. In paper III, we used whole saliva DNA, a less invasive DNA source, and showed that adults with a history of depression had shorter TL compared to age-matched controls, by using samples (n = 662) from the PART cohort. In this analyses, a number of parameters previously reported to be associated with LTL were adjusted for including age, sex, alcohol use, number of somatic diseases and number of childhood adversities. In agreement with, and supported by previous studies, the majority of DNA extracted from whole saliva actually originates from blood leukocytes, which is different from buccal cell collection techniques [154; 155].

4.1.2 Telomere length associated negatively with increasing number of depressive episodes in bipolar disorder

In paper I, a significant effect of number of depressive episodes on blood leukocytes telomere length (LTL) was found in bipolar disorder (BD) patients. Specifically, the LTL marginal mean was reduced 0.075 units per depressive event after adjusting for age and sex in the linear model. This effect was stronger in males than in females. This is in line with the only previous study of TL in BD, which found that BD type 2 and number of depressive episodes were associated with shorter LTL however in a smaller sample size [116].

4.1.3 Rs2736100 polymorphism is associated with depression and the number of depressive episodes in bipolar disorder type I

SNP rs2736100, located in the intron 2 of the *hTERT* gene, was previously associated with shorter LTL through the risk allele A in a genome-wide meta-analysis [156]. In paper III, we tested the hypothesis that rs2736100 associates with depression in PART cohort. There was no association between rs2736100 genotype and depression in the whole material (n = 2026), adjusted for age, sex, and experience of childhood adversity. However when stratified for childhood adversity, an established risk factor for depression in adulthood [157; 158], homozygosity for the ‘short LTL’-risk allele A was significantly associated with higher risk of depression compared to the AC/CC genotypes in the group without experience of childhood adversity (rs2736100: P = 0.010, OR = 1.51, 95% CI = 1.10-2.05; sex: P < 0.001; age: P = 0.007). That this relationship was detected only in those without experience of childhood adversity might be explained by the fact that early adversity has previously been associated with depression and shorter LTL in adulthood [124; 125; 159; 160]. Hence, early adversity might conceal an rs2736100-depression relationship.

We and others previously showed that LTL was associated with the number of depressive episodes and lithium response in BD [115; 116]. We tested whether SNP rs2736100 associated with the number of depressive episodes within BD1, considering the patients' response to lithium treatment. In patients responding well to lithium treatment (LiR), the risk allele A showed dominance, that is the AA/AC genotype significantly associated with higher number of depressive episodes compared to the CC genotype (AA/AC vs CC: $F = 10.9$, $P = 0.001$; sex: $F < 0.001$, $P = 1.00$; years since onset of first depressive episode: $F = 16.8$, $P < 0.001$; age: $F = 0.36$, $P = 0.55$; ANCOVA). We also observed a similar effect in A/C allelic model, with the A allele conferring a risk for increased number of depressive episodes. That the finding in BD patients was confined to the LiR group may reflect a possibly higher lithium functionality in the LiR group, and the fact that lithium upregulates *hTERT* [161], which in turn may upregulate the functionality of rs2736100.

4.2 TELOMERE DYSFUNCTION IN THE FSL RAT MODEL OF DEPRESSION

4.2.1 Shorter hippocampal telomere length in the depressed FSL rat model

Shorter telomeres in leukocytes were reported to be associated with major depression. However, it is not clear whether the same holds true for the respective brains. A study from Szebeni et al showed that oligodendrocytes but not astrocytes from depressed individuals displayed shorter TL and decreased *TERT* expression compared to corresponding postmortem white matter (frontal and temporal lobes) from control brains [162]. Since hippocampus is pivotal in cognitive function [30], mood regulation and memory formation [31] and it is a region that expresses telomerase activity also in adulthood, in paper II we tested whether there is telomere dysfunction in the hippocampus of the depressed FSL rat. We found that the FSL had shorter hippocampal TL compared to the control line FRL.

4.2.2 Decreased *Tert* and telomerase activity in the FSL hippocampus

Shorter telomeres may result from a decreased telomerase activity. The expression of the catalytic subunit (TERT) of telomerase is stringently regulated, and of the several splicing forms the full length mRNA correlates positively with telomerase activity [163; 164]. The *Tert* transcript is highly conserved between human and rodents [164], thus enabling translational studies in rodent models. In paper II, we explored if the shorter TL in the FSL hippocampus could reflect a reduced telomerase activity. The *Tert* levels were reduced in the FSL compared to the FRL rats. Consistent with the downregulated *Tert* expression, telomerase activity was lower in the depressed FSL hippocampi. Telomerase overexpression has been suggested to promote adult neurogenesis in the hippocampus [133; 134]. Substantial literature shows existence of adult neurogenesis, particularly in the dentate gyrus [32] and the role of reduced neurogenesis in the pathophysiology of depression [33; 34]. Interestingly, chronic mild stress in mice resulted in decreased TERT levels and telomerase activity as well as reduced neurogenesis in hippocampus and a depression-like behavior. In contrast, fluoxetine and intrahippocampal infusion of 3-azido-deoxythymidine (AZT)

reversed these effects leading the authors to suggest that hippocampal telomerase plays a role in depression-like behaviors, possibly by regulating neurogenesis [134].

4.3 INFLUENCE OF LITHIUM TREATMENT ON TELOMERE LENGTH AND TELOMERASE ACTIVITY

4.3.1 Longer telomere length in bipolar patients and good Li-responders

In paper I, we found that LTL correlated positively with length of lithium treatment (duration ≥30 month) in BP patients. We also found significantly longer LTL in BP patients (n = 202) when compared to healthy controls (n = 135). This significance could also be seen in lithium monotherapy (n = 39) in comparison with healthy controls. Furthermore, LTL seemed to reflect lithium response indicated by that those with good response (LiR, n = 31) had 10% longer LTL compared with those with no or partial response (non-LiR).

4.3.2 Lithium increases *Tert* and telomerase activity in the FSL hippocampus

In paper II, we tried to explore the mechanism how lithium protects against TL shortening. We conducted a 6-week treatment with either Li_2SO_4 or vehicle admixed to the FSL rat chow. Both *Tert* expression and telomerase activity were increased in the hippocampi from the lithium treated FSL (FSL-Li) compared to the FSL-vehicle group. But hippocampal TL was not statistically increased in FSL-Li group. A similar lack of TL change despite telomerase upregulation was also reported by Wolkowitz et al [114]. Explanations could be that TL changes much slower than telomerase activity [165; 166]; our previous study found that long-term lithium treatment (≥30 months) in patients diagnosed with bipolar disorder correlated positively with LTL [115].

4.3.3 Potential mechanism of lithium's effect on telomere biology

Lithium was previously shown to inhibit GSK-3β [167], which results in retention of β-catenin [168]. Lithium-induced upregulation of β-catenin was shown to upregulate *hTERT* transcription in cancer cell lines [169] however no such studies have been done in the brain region. Lithium has also been reported to promote expression of BDNF which, in turn, enhances *Tert* expression [170]. In addition, BDNF was reported to modulate telomerase activity in embryonic hippocampal neurons [170], In paper II, we investigated levels of putative mediators, β-catenin and BDNF, of lithium's effect on telomerase activity, both in naïve FSL/FRL and FSL-vehicle/FSL-Li groups. We found decreased BDNF levels in naïve FSL compared to FRL, which may in part underlie the reduced telomerase activity in FSL brain. But we didn't see any level difference in β-catenin between naïve rats. Interestingly, when we measured the β-catenin levels in FSL-Veh and FSL-Li hippocampi, β-catenin levels were significantly higher in the FSL-Li group, but lithium didn't seem to influence BDNF levels. The duration of lithium treatment has been implied to influence BDNF changes, e.g. an increase in BDNF was found after 14 days, but not 28 days, while the treatment duration in our study was 42 days [171].

4.4 EPIGENETIC FINDINGS IN THE FSL RAT MODEL

4.4.1 Sodium butyrate affects DNA methylation in the FSL prefrontal cortex (PFC)

The epigenetic drug sodium butyrate (NaB) showed antidepressant-like effects in preclinical studies [172; 173]. Research has focused on its role as a histone deacetylase inhibitor, but there is also evidence that NaB affects DNA methylation [174-176]. In paper IV, chronic intraperitoneal administration of NaB had antidepressant-like effects in the FSL and was accompanied by increased levels of TET1 in the PFC region. Hydroxylation of 5-methylcytosine (5mC) by TET proteins were previously shown to lead to the formation of 5-hydroxymethylcytosine (5hmC), which can then mediate active DNA demethylation [177-179]. This mechanism has important implications for studies of postmitotic tissues where cell division and DNA replication have ceased, such as the brain region we studied here. In addition, 5hmC has been found to be most abundant in the brain where it is particularly enriched in active genes, indicating a crucial role for this DNA modification in neuronal gene expression and memory formation [92; 180]. In paper IV, the TET1 upregulation was associated with an increase of 5hmC and a decrease of 5mC in *Bdnf* gene. These epigenetic changes were associated with a corresponding BDNF overexpression. These findings are in line with two recent studies showing that TET1 overexpression in the mouse hippocampus also leads to increased *Bdnf* expression [177; 180].

4.4.2 Inflammation and disturbed let-7 biogenesis in the FSL prefrontal cortex

Elevation of the proinflammatory cytokine IL-6 has been implicated in depression, however the mechanism remains elusive [181-183]. Previous study showed that let-7 family directly inhibited IL-6 expression, which may act as an immunorepressor [184]. In paper V, we found elevation of *Il6* in PFC region of FSL, which was associated with overexpression of LIN28B and downregulation of let-7 miRNAs (including let-7b, let-7c, let-7f, let-7i and miR-98). LIN28B is an RNA-binding protein that selectively represses let-7 synthesis. Also DROSHA, key enzyme in miRNA biogenesis, was downregulated in the FSL PFC. The let-7 family plays an important role in early neurodevelopment. However, other roles of let-7 family in the adult brain have been less investigated despite the fact that let-7 is known to be upregulated in later developmental stages and is one of the most abundant miRNA families in the adult brain [185; 186]. Thus, the results indicate upregulated *Il6* levels in FSL PFC by let-7 downregulation through LIN28B and DROSHA dysregulation. Let-7 can also inhibit LIN28B translation by binding to the 3′ UTR target sites, creating a double negative feedback loop [187]. Noteworthy, let-7 could also be regulated in a LIN28B-independent way, e.g. through epigenetic mechanisms such as DNA and histone methylation [188]. It is possible that let-7 dysregulation can lead to disturbances also in other pathophysiological processes because a miRNA often has multiple target genes. The fact that we observed that FSL PFC had decreased DROSHA levels, suggests a disturbed miRNA biogenesis probably not only in let-7 but also in a variety of other miRNAs. In line with this hypothesis, a recent

study has demonstrated a general reduction of miRNA expression in the PFC from depressed suicidal subjects [189]. Previous studies have shown that physical activity in the form of wheel-running exerts antidepressant-like effect in the FSL rat model [190; 191]. We found that physical activity reduced *Il6* levels and selectively increased let-7i and miR-98 expression in the FSL PFC, which were independent of *Lin28b* and *Drosha* changes. However, upstream primary miRNA transcription was increased in the FSL-runners, implying that other mechanisms (e.g. epigenetic) are involved in regulating let-7 expression in response to physical activity.

5 SUMMARY AND CONCLUDING REMARKS

In this section a summary of conclusions from each constituent paper, as well as general concluding remarks, are provided.

- In bipolar patients, long-term lithium treatment was suggested to protect against telomere shortening especially when therapeutically efficacious (i.e. in patients who responded well to lithium). The shorter telomere length associated with a history of higher number of depressive episodes (**Paper I**).
- Shorter telomere length and dysfunctional telomerase activity in hippocampus was associated with a depression-like state in the FSL rat. Lithium treatment normalized the hippocampal telomerase dysfunction, possibly through the activation of β-catenin (**Paper II**).
- Saliva telomere length was decreased in adult individuals with a history of depression and genetic variation in *hTERT* may influence the susceptibility to depression (**Paper III**).
- Sodium butyrate exerted antidepressant-like effect in the FSL and the suggestive epigenetic effects of sodium butyrate may include DNA methylation changes that are mediated by demethylation-facilitating enzymes like TET1 (**Paper IV**).
- Elevation of proinflammatory cytokine *Il6* in prefrontal cortex region of the FSL was associated with disturbance of let-7 family biogenesis. Physical activity could reduce the cortical *Il6* levels possibly through regulating miRNA expression (**Paper V**).

Depression is a complex disorder with multiple genetic, epigenetic, behavioral and environmental risks that contribute to the heterogeneity of the illness. The understanding of the pathophysiology of depression has evolved substantially over years and researchers have realized the importance of utilizing multidisciplinary approaches to unravel the neurobiological bases for depression. Focusing beyond the traditional pathophysiological theories of depression, telomere biology may provide new insights to the development of predictive markers for depression and to the understanding of the neuroprotective effects of lithium. Expanding the knowledge about epigenetic dysregulations in depression may provide novel mechanism and therapeutic possibilities. In addition to some psychotropic drugs with epigenetic effects e.g. valproic acid, there are many epigenetic drugs that have lately received much attention, however still more preclinical and clinical support is needed before targets in psychiatric disorders are identified.

In summary, the studies in this thesis work contribute to increase the understanding of molecular mechanisms in depression and mood disorder treatment.

6 FUTURE PERSPECTIVES

A number of questions, related to the results in the studies of this thesis, remains to be answered. Here, the major perspectives for future work are listed.

- The telomere length and telomerase activity were measured in a homogenate of hippocampus tissue which may include neurons, glia, vascular and immune cells. Since cell types exhibit different telomere vulnerability towards cellular stress, it is important to investigate telomere dysregulation at a cell-type specific level.
- The causal relationship of the association between dysfunctional telomeres and depression symptoms is elusive. Support from additional human studies that genetic variation in *hTERT* associates with depression and the number of depressive episode in bipolar disorder would suggest that telomerase activity influences the risk for depression and the number of depressive episodes in bipolar disorder.
- Leukocyte telomerase activity was reported to correlate positively with SSRI responsiveness in depressed cases with low baseline telomerase activity [114], suggesting that telomerase activation might be beneficial in antidepressant treatment. The molecular mechanisms underlying leukocyte telomerase changes in response to SSRI is not clear. However, it was recently shown that leukocyte telomerase activity correlated with hippocampal volume, suggesting that this activity indexes a neuroprotection or neurogenesis in the hippocampus of depressed individuals, possibly in part through a correlated telomerase activity in the hippocampus [192]. Telomerase is suggested to promote neurogenesis, however the detailed mechanism is still elusive. Telomerase may influence neurogenesis through extra-telomeric functions, and telomerase may thereby be implicated in depression through extra-telomeric functions. Our previous finding showed that bipolar patients who responded well to lithium treatment had longer leukocyte telomere length. Whether this could reflect leukocyte telomerase activation are planned to be studied in newly diagnosed bipolar patients. We showed that lithium upregulated telomerase activity in the hippocampus of depressed rats.
- Telomeres consist of DNA and protective protein complexes. A key protective complex is called shelterin and it includes TRF1, TRF2, TPP1, TIN1, POT1 and RAP1. Dysfunctional shelterin leads to insufficiant telomerase docking and increased DNA damage response which may result in cell cycle arrest [107]. Memebers of shelterin e.g. RAP1 was shown also to have extra-telomeric functions such as regulating gene expression [193]. The role of RAP1 is planned to be investigated in the FSL rat model.
- The understanding of epigenetic regulation is increasing rapidly. Recently a number of studies showed that non-CpG methylation, primarily produced by DNMT3A, are abundunt and more dynamic than CpG methylation in adult brain [83]. DNMT3A is suggested to play a role in neurogenesis by modifying methylation in neuronal genes [194]. It would be interesting to investigate the function of DNMT3A in the depressed brain and the biological role of non-CpG methylation in depression.

"There are two ways to live: you can live as if nothing is a miracle;
you can live as if everything is a miracle."
"Logic will get you from A to B. Imagination will take you everywhere."
— Albert Einstein

Brilliant imagination by Yu Chen. Beijing, China. 2015.

- We showed that the HDACi NaB can affect DNA methylation, however it is not clear how mechanistically epigenetic markers interact with each other, e.g. the order by which histone acetylation and/or DNA methylation predominantly influence one another. This emphasizes the importance of elucidating in detail how epigenetic drugs like NaB, and the structurally similar valproic acid, exert their mood stabilizing effects.
- We found disturbed biogenesis in the *Il6*-targeting let-7 miRNA family in frontal cortex region of the FSL rats. In addition to LIN28B dysfunction, we also noticed a decreased DROSHA expression which implied a general reduction of miRNA biogenesis in frontal cortex region in depression states. In agreement, one study showed that most of the miRNAs were found to be decreased in the postmortem brain from drug-free depressive subjects [189]. In addition, a recent finding reported that enoxacin, an antibiotic drug that has miRNA production enhancement property, exhibited antidepressant-like effect in preclinical research (Smalheiser et al. 2014). MiRNAs show high stability in human paraffin-embedded tissues and plasma samples; a number of studies from cancer research have suggested the possibility that miRNA expression may be a useful tool to identify disease states and subtypes. It would be interesting to examine this idea also in depression. Thus, a comprehensive miRNA profiling is planned to be performed in the FSL frontal cortex. Furthermore, we found that physical activity reduced *Il6* levels and selectively increased let-7i and miR-98 expression in the FSL frontal cortex, which was independent of *Lin28b* and *Drosha* changes but associated with upstream primary miRNA transcription changes, implying that other mechanisms (e.g. epigenetic) are involved in regulating let-7 expression in response to physical activity. The reason to altered primary miRNA transcription is planned to be investigated in FSL frontal cortex.

"By three methods we may learn wisdom:

First, by reflection, which is noblest;

Second, by imitation, which is easiest;

and third by experience, which is the bitterest."

— Confucius

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