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BIOLOGY OF LITHIUM RESPONSE IN BIPOLAR DISORDER – GENETIC MECHANISMS AND TELOMERES

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BIOLOGY OF LITHIUM RESPONSE IN
BIPOLAR DISORDER —
GENETIC MECHANISMS AND TELOMERES

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my family and friends

ABSTRACT

Background: Bipolar disorder is a common, chronic and severe mental illness, causing suffering and large costs. Lithium treatment is the golden standard and works in 2/3 of patients, of which 50% are called lithium responders. There is strong evidence that both bipolar disorder and the degree of lithium response are highly heritable, although many mechanisms are unknown. Short telomere length has been found in both somatic and psychiatric disorders, but little is known about telomeres in bipolar disorder and nothing about telomeres and lithium treatment. A few studies limited in size have reported an increased risk of kidney cancer in lithium-treated patients. These findings have led to warnings and changes in policies for lithium treatment, although clinicians and researchers have disputed it. There is thus a clinical need for large-scale studies and reliable evaluations of the cancer incidence in lithium-treated bipolar patients.

Aims: The overall aim of this thesis is to increase the knowledge of the genetic mechanisms in bipolar disorder and in lithium response. Specifically, it aims to find genetic associations to lithium response and to investigate how telomere length is related to bipolar disorder and lithium treatment. Additionally, it explores the overall and site-specific cancer incidence in bipolar disorder and lithium treatment.

Methods: Study I is a GWAS of lithium response. Study II is a retrospective study of telomere length in lithium-treated bipolar patients. Study III is an association study of a genetic variant in the *hTERT* gene (previously associated with short telomeres) and depression, and a retrospective cohort study of telomere length in depression. Study IV is a longitudinal study of telomere length in lithium-treated bipolar patients. Study V is a register study of overall and specific cancer incidence in lithium-treated bipolar patients compared to the general population.

Results: I) A single locus with four linked common gene variants on chromosome 21 coding for long, non-coding RNAs, which might be important for brain gene regulation, was associated with lithium response. **II)** Leukocyte telomeres were 35% longer in bipolar patients compared to healthy controls and correlated positively with length of lithium treatment in patients who had had lithium for more than 2.5 years. Lithium responders had 10% longer telomeres than non-responders. Short telomeres were associated with a larger number of depressive episodes. **III)** A genetic variant in the *hTERT* gene was associated with the number of depressions in bipolar type 1 patients responding to lithium and with unipolar depression. Telomere length was shorter in depressed patients without previous childhood trauma. **IV)** The total reduction of leukocyte telomere length between tests was, for bipolar patients, an average of 2.1% per year versus 3.7% per year in healthy controls. Age at baseline had a positive effect while total time on lithium between tests had a negative effect on the leukocyte telomere length reduction independent of other confounders. In bipolar patients there was no association between the total number of leukocytes or leukocyte subtypes and leukocyte telomere length at follow-up. **V)** There was no increase in the overall

or site-specific cancer incidence in lithium-treated patients in the age span of 50-84 years (N=2 393) compared to the general population (N=2 593 011).

Conclusions: Association of lithium response to a genomic region containing long, noncoding RNA with potential importance for gene regulation in the brain adds a new piece of knowledge to the heritability of lithium response in bipolar disorder. Results must be replicated and translated into a biological context. The new finding of a decelerating effect of lithium treatment on telomere shortening suggests that lithium might have operative effects on telomere biology, which also has potential importance for lithium response and should be investigated further. An important clinical implication of the lack of difference in cancer incidence between lithium-treated bipolar patients and the general population is that recently added warnings for renal cancer in patients with long-term lithium treatment is unnecessary and ought to be omitted from the current policies.

Key words: Bipolar, lithium response, GWAS, DNA, telomere length, telomerase activity, *hTERT*, cancer incidence, register study

LIST OF SCIENTIFIC PAPERS

- I. Liping Hou*, Urs Heilbronner*, Franziska Degenhardt*, **Lina Martinsson** (48), John R. Kelsoe**, Martin Alda MD**, Marcella Rietschel**, Francis J. McMahon**, Thomas G. Schulze** (*first/**last authorship).
Common Genetic Markers for Lithium Response in Bipolar Disorder, Genomewide Association and Prospective Validation Study Implicate a Long Noncoding (lnc) RNA in Response to Lithium for Bipolar Disorder. The Lancet. 2016, *In press*.
- II. **Lina Martinsson**, Ya Bin Wei, Dawei Xu, Philippe A Melas, Aleksander A Mathe, Martin Schalling, Catharina Lavebratt, Lena Backlund.
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- III. Ya Bin Wei, **Lina Martinsson**, Jia Jia J Liu, Yvonne Forsell, Martin Schalling, Lena Backlund, Catharina Lavebratt.
hTERT genetic variation in depression. Journal of affective disorders. 2016;189:62-9.
- IV. **Lina Martinsson**, Vincent Millischer, Ya Bin Wei, Birgitta Lindberg R, Inger Römer, RN, Harvest Gu, Claes Göran Östensson, Martin Schalling, Catharina Lavebratt, Lena Backlund.
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- V. **Lina Martinsson**, Jeanette Westman, Jonas Hällgren, Urban Ösby, Lena Backlund.
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LIST OF ABBREVIATIONS

BD	Bipolar Disorder
BD1	Bipolar Disorder type 1
BD2	Bipolar Disorder type 2
DPR	Drug Prescription Register
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders IV
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders IV Text Revised
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5
LCM	Life Charting Method
LiR	Lithium Responder
LTL	Leukocyte Telomere Length
MDD	Major Depressive Disorder
NonLiR	Non Lithium Responder
NPR	National Patient Register
RC	Rapid Cycling
SCR	Swedish Cancer Register
SCZ	Schizophrenia
SNP	Single Nucleotide Polymorphism
TA	Telomerase Activity
TERT	Telomerase Reverse Transcriptase
TL	Telomere Length

1 INTRODUCTION

I was fascinated when I first heard about the DNA helix and messenger RNAs in 8th grade biology class. A few years later in high school, I decided to do my high school master exam in Genetics. That was about the same time the ambitious HUGO project began mapping out human DNA. My interest in genetic research was awakened, and led me to become a medical doctor.

My way through medical school at Karolinska Institutet (KI) finally led to the Unit for Affective disorder at Psychiatry South West. Working as a junior psychiatrist there, I realized that I had found my niche. The ward at this unit was well organized, effective and smart. I found that the team members had high academic skills and clinical competence and they both enjoyed and excelled at working with patients with bipolar disorder. Every day during my residency period at this unit, I met interesting patients with histories of severe bipolar disorder. After years of trial and error, most of them had found an effective treatment, often lithium in monotherapy or in combination with other mood stabilizers. The lack of knowledge of the causes of bipolar disorder and of the mechanisms of treatments both frustrated me and sparked my interest. I decided to focus on mood disorders and become a specialist in bipolar disorder.

I clearly remember my first week in the psychiatric in-patient ward, where I started working as a senior psychiatrist. In particular, I remember one young woman who had an episode of acute mania. As I left the ward on Friday afternoon, she was euphoric and loud and exhibited sexually explicit behavior. Over the weekend her demeanor changed radically, and as I entered the corridor on Monday morning she was calm and responsive. I was stunned by how quickly she had responded to the lithium salt tablets! She had two relatives with mood disorders and at least one of them had a history of the same quick response to lithium treatment, information that further ignited my curiosity.

Dr Lena Backlund had already started recruitment and phenotyping of bipolar patients with access to medical records and sampling of DNA from blood. Lena introduced me to her research and her ideas about the genetic mechanisms of lithium response in bipolar disorder. I was also invited to an inspiring meeting with the bipolar group at the Center for Molecular Medicine (CMM) at KI. When Lena asked me to be her doctoral student I was overwhelmed by enthusiasm. The journey had begun.

2 BACKGROUND

2.1 BIPOLAR DISORDER

Although both the clinical impression and previous research support a familial etiology of bipolar disorder, there is still a huge knowledge gap in this research area.

2.1.1 History

Hippocrates described the symptoms of bipolar disorder (BD) already around 400 BC as Melancholia and Mania stemming from disturbances in the balance of black and yellow bile. Around 1850, Falret and Bailarger formulated the concept that depression and mania could be different manifestations of the same illness. Emil Kraepelin defined “Manic Depressive Illness” in the beginning of the twentieth century, which also included recurring depressions. Bleuler broadened Kraepelin’s concept by using subcategories and using the term “Affective illness”. In 1957, Leonhard introduced the more narrow concept of bipolar disorder, which was further developed by Angst and Perris in the 1960s and formally incorporated in the diagnostic system of DSM III in 1980 (1). The diagnoses studied in the present thesis are based on the later developments of DSM III; i.e. DSM-IV, which in turn has been further developed into DSM-IV-TR and DSM-5 (Figure1 and 2).



Depressive episode	Manic episode	Mixed episode
<p>A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.</p> <ol style="list-style-type: none"> 1. Depressed mood 2. Markedly diminished interest or pleasure 3. Appetite or weight changes 4. Insomnia/hypersomnia 5. Psychomotor agitation/retardation 6. Fatigue/loss of energy 7. Feelings of worthlessness/excessive/inappropriate guilt 8. Diminished ability to think/concentrate 9. Recurrent thoughts of death/suicidal ideation <p>B. The symptoms do not meet the criteria for a Mixed Episode.</p> <p>C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.</p> <p>D. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism).</p> <p>E. The symptoms are not better accounted for by bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than two months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.</p>	<p>A. A distinct period of abnormally and persistently elevated, expansive, or irritable mood, lasting at least 1 week (or any duration if hospitalization is necessary).</p> <p>B. During the period of mood disturbance, three (or more) of the following symptoms have persisted (four if the mood is only irritable) and have been present to a significant degree:</p> <ol style="list-style-type: none"> 1. Inflated self-esteem/grandiosity 2. Decreased need for sleep 3. Talkativeness 4. Flight of ideas/subjective experience that thoughts are racing 5. Distractability 6. Increased goal-directed activity/psychomotor agitation 7. Excessive involvement in pleasurable activities that have a high potential for painful consequences <p>C. The symptoms do not meet the criteria for a Mixed Episode.</p> <p>D. The mood disturbance is sufficiently severe to cause marked impairment in occupational functioning /in usual social activities/relationships with others/to necessitate hospitalization to prevent harm to self or others/there are psychotic features.</p> <p>E. The symptoms are not due to the direct physiological effects of a substance.</p>	<p>A. The criteria are met both for a Manic Episode and for a Major Depressive Episode nearly every day during at least a 1-week period. (At least 2 manic symptoms during depression or two depressive symptoms during mania).</p> <p>B. The mood disturbance is sufficiently severe to cause marked impairment in occupational functioning or in usual social activities or relationships with others, or to necessitate hospitalization to prevent harm to self or others, or there are psychotic features.</p> <p>C. The symptoms are not due to the direct physiological effects of a substance or a general medical condition.</p>

Figure 1. DSM-IV criteria for bipolar episodes. Used with permission of Lena Backlund.

	DSM-IV-TR	DSM-5
Change in criterion A (main criteria) for mania and hypomania	Emphasis on change in mood	Emphasis on changes in activity and energy as well as mood.
Change in definition of mixed episodes	Defined as a special episode type, requiring that the individual simultaneously meet full criteria for both mania and major depressive episode.	Defined as a specifier to be added to episodes of mania, hypomania or depression, requiring the presence of at least three symptoms of depression in concert with an episode of mania/hypomania or at least three manic/hypomanic symptoms in concert with an episode of major depression.
Change in the category for borderline cases of Bipolar II	Can be included in a category without specific criteria.	Includes a category with specific criteria, requiring a past history of a major depressive disorder and hypomanic episodes meeting all criteria for hypomania except the duration criterion (i.e., at least 4 consecutive days) or too few symptoms of hypomania to meet criteria for the full bipolar II syndrome, although the duration is sufficient at 4 or more days.
Anxiety in bipolar and depressive disorder	Can only be addressed by adding an additional anxiety diagnosis.	Can be addressed by adding a specifier for anxious distress to the diagnosis of bipolar or depressive disorder.

Figure 2. Differences in DSM-IV-TR and DSM-5. Adapted from Highlights of Changes from DSM-IV-TR to DSM-5. American Psychiatric Society, 2013. Used with permission of Mats Adler.

2.1.2 Clinical features of bipolar disorder

Bipolar disorder is a common, chronic and severe mental illness, causing suffering and large costs (2). BD is commonly divided into two main types; BD type 1 (BD1), and BD type 2 (BD2). BD1 is more severe and is usually characterized by alternating episodes of depressions and manias, while BD2 is characterized by depressions and hypomanias (mild manias) (3). Treatment of bipolar disorder focuses on acute stabilization from a manic or a depressive state into a symptomatic recovery with euthymic (stable) mood and on maintenance treatment, in which the goals are relapse prevention, reduction of subthreshold symptoms, and enhanced functioning (4).

The worldwide prevalence of BD is 2-5% depending on which definitions of the disorder are used (5-7). The aggregate lifetime prevalence is 0.6% for BD1, 0.4% for BD2, 1.4% for subthreshold BD, and 2.4% for bipolar syndrome. The debut of BD usually occurs in the age of 13-30, commonly with the first episode in adolescence (7). BD1 usually debuts earlier than BD2 (7). Rapid cycling (RC) is an unstable form of BD1 or BD2, defined as ≥ 4 episodes in a 12 months period. (8). A mixed episode (ME) is a severe type of bipolar episode according to DSM-IV, which means a depression with manic symptoms or a hypomania/mania with depressive symptoms at the same time. (3) BD is evenly distributed between genders, although manias are more common in men and depressions and RC in women (6).

The mortality of bipolar patients as a result of somatic disorders, such as cardiovascular disorder, is significantly increased. The suicide risk is twenty-fold compared to the general population (9) and as many as about 15% die from suicide (1). BD often coexists with other psychiatric illnesses, such as anxiety, ADHD, Borderline personality syndrome and substance abuse (10). Bipolar patients often suffer from cognitive effects, such as impaired concentration and thought disturbances both during episodes and during the free intervals without affective symptoms (11). Neuroimaging studies of MRI scans show that the volume of the prefrontal cortex is reduced in BD compared to healthy controls, especially in individuals who had experienced several manic episodes (12). The number of depressive episodes in BD has been associated with reduced hippocampal volume (13). Furthermore, the hippocampi of bipolar patients often show a reduced number of neurons (14, 15). The role of immune activation in BD has been discussed recently. Cerebrospinal fluid (CSF) levels of the proinflammatory cytokine interleukin (IL)- 1β were found in several studies to be increased in patients with BD or schizophrenia (SCZ). Furthermore, elevation of brain kynurenic acid (KYNA), which is produced in astrocytes as an end-metabolite of the kynurenine pathway of tryptophan metabolism, has been associated with psychotic symptoms (16) and cognitive deficits (17) in BD. Recently, a genome-wide association (GWA) study of kynurenic acid in cerebrospinal fluid found support for an association with a common variant associated with reduced SNX7 expression and positive psychotic symptoms and executive function deficits in BD (18).

A method for clinical measurements of symptoms in bipolar disorder – The life-charting method

In order to systematically investigate symptoms and get a descriptive picture of the long-term course of bipolar disorder, clinicians have developed various methods and tools. One such method is called “The life-charting method” (LCM), and has been computerized to give a graphical illustration of life-events, episode frequency and duration, as well as the severity of episodes and treatments (19, 20). In Sweden, a LCM program was developed (21) and further refined by my main supervisor for prospective life-charting and statistical analysis (22).

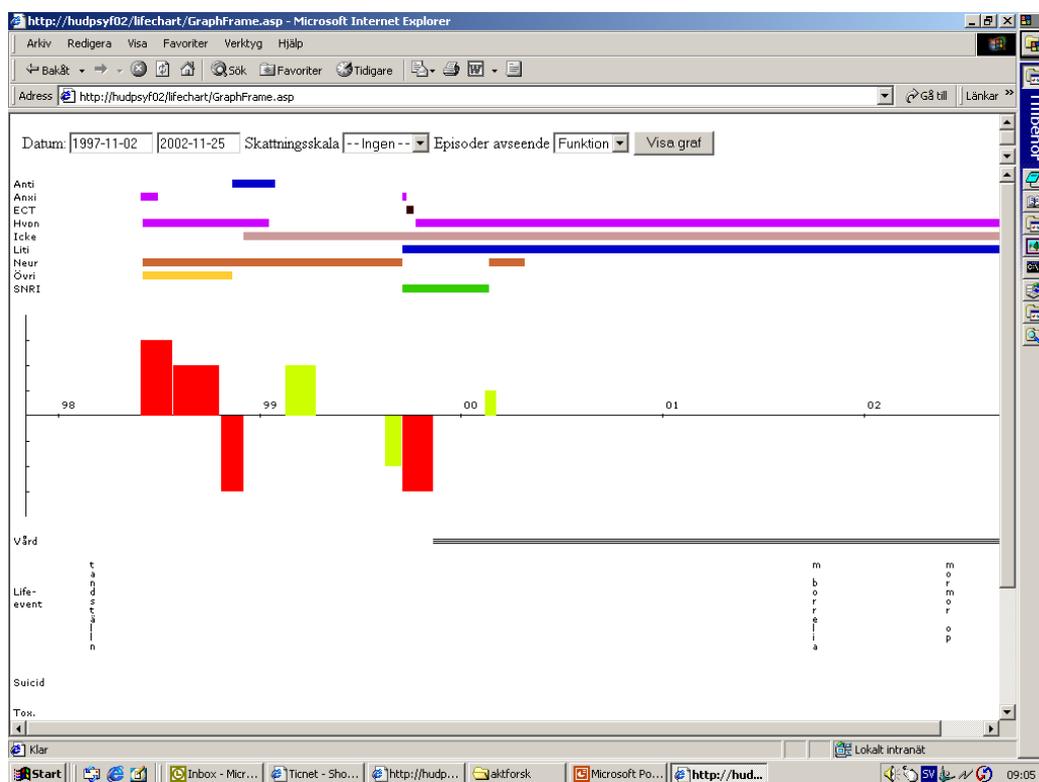


Figure 3. The life-charting method. Figure used with permission of Lena Backlund.

2.1.3 Genetics of bipolar disorder

Psychiatrists have been convinced for many years that BD is a highly heritable disorder. Twin- and adoption studies confirm that there is a familial aggregation of BD and that most patients have a family history with at least one first degree relative with some type of the disorder. The risk of illness for first-degree relatives of bipolar patients is nearly ten times greater than for the general population (23, 24). The heritability for BD is high and has been estimated to 75-93% (25). Although the clinical impression and these findings support a familial etiology of BD, there is still a huge knowledge gap in this research area (26). During the last decade, large GWA studies have been established and presented significant findings in SCZ (27) and in BD (28) in which samples of over 10 000 subjects are often required to reach significance. Genome-wide significant associations have been reported at several common polymorphisms, including variants within the genes CACNA1C, ODZ4, and NCAN (29). Evidence is strong that many risk alleles of small effect are involved in BD (29). Individual genes in the etiology of BD have been difficult to find and replicate, probably due to the wide heterogeneity of BD, the large number of genes involved and the overlapping clinical symptoms with other psychiatric disorders (26).

2.2 LITHIUM TREATMENT

The most important factor for a good prognosis in bipolar disorder is effective pharmacological prophylactic treatment with lithium as the first choice.

2.2.1 History

Lithium is a soft silver-white metallic basic element with atomic number 3 in the Periodic Table and it belongs to the alkaline group. Its Greek name (lithos) means “stone”. Lithium does not exist freely in nature, but appears in ion form in compounds (30). There are no lithium receptors and no known essential biological functions of lithium in the human body (31). It has been suggested that lithium was one of few elements present already during the Big Bang.

Lithium was discovered on Utö in the Stockholm archipelago in 1800, and identified in 1817 in the laboratory by Arfwedson.. The Australian psychiatrist John Cade discovered lithium’s effect on BD in 1949; by that time called “manic depressive illness” (32). Ever since then, lithium salts have been used for mood stabilizing treatment, especially in BD. Today, lithium is still regarded as the cornerstone of long-term therapy for bipolar disorder (33).

2.2.2 Lithium treatment in bipolar disorder

The most important factor for a good prognosis in BD is effective pharmacological prophylactic treatment, and lithium is the first choice among several mood stabilizers (1). Lithium also has a unique role compared to other psychopharmacological drugs in suicide prevention, providing protection both to individuals who respond well and individuals who respond only partially to the treatment (34, 35). Furthermore, lithium also has an important function in combination with other antidepressants in the treatment of major depressive disorder (MDD) (36). Aside from the wide usage of lithium in BD and MDD, investigations from the past decade have also focused on lithium’s effects on a number of other central nervous system disorders, including Alzheimer’s disease, multiple sclerosis, amyotrophic lateral sclerosis, Huntington’s disease and stroke (glutamate neurotoxicity). (37). Interestingly, lithium-treated patients with spinocerebellar ataxia (“Machado-Joseph disease”) presented less severe progressions compared to a placebo in a recent study (38, 39).

Fifty years after John Cade’s revolutionary discovery of lithium’s effect on manic depressive illness (32), the Canadian psychiatrist Paul Grof invented the term excellent lithium responders for patients who responded to lithium monotherapy by having no further episodes of the illness and who were able to live totally normal lives despite the chronic illness (40).

In long-term prospective follow-ups on bipolar patients on lithium maintenance treatment, about one third of the subjects do not experience any bipolar episodes (33). However 40-50% of bipolar patients relapse within two years of lithium treatment (41). Other mood stabilizing medications, such as Valproic acid, Antiepileptics or Neuroleptics, are used for those patients as alternative treatments in combination with lithium or in monotherapy. Unfortunately, to date there are no clinical tools available to predict the effect of pharmacological prophylactic

treatment in BD, which often means several years of therapeutic trials and suffering for the patients before improvement can be achieved. It would be of high clinical value if individuals who are responsive to lithium could be identified before the first medication is introduced.

2.2.3 Possible mechanisms of action

When a bipolar patient responds to lithium, the effect often is clinically obvious. Mania, and in some cases even depression, can almost be “switched off” by lithium over a short period of time. However, the key mechanism (or mechanisms) of action are still unclear (42). Over time, *in vitro* and *in vivo* studies of lithium suggested different possible mechanisms, which may act separately or together (43). Lithium has effects on monoaminergic systems, neurotrophic factors, neuropeptides and the arachidonic acid cascade. Lithium has also been found to enhance neuroprotective proteins and pathways (such as Bcl-2 and the Wnt signaling pathway), and to inhibit the key enzymes glycogen synthase kinase-3 (GSK-3; isoforms a and b) and phosphatidylinositol phosphatases, either directly or indirectly by affecting protein complexes that can modify the Akt/GSK-3 signaling (36, 42-48). Furthermore, lithium has gene regulatory functions via inducing effects on nuclear receptors (49).

In the body, the small lithium ions enter all cells, replace sodium ions, compete with magnesium binding and have been found to exert a variety of inhibitory and facilitatory effects on enzymes and signaling systems. Many proteins in the brain are dependent on small positive ions, such as sodium, potassium and magnesium for their function. If one of those positive ions is replaced by the smaller lithium ion, the function of the affected protein might be changed. Proteins sensitive to ion replacements by lithium and proteins with functional changes that have high biological downstream effects, for instance GSK-3 β and brain-derived neurotrophic factor (BDNF), are of great importance for lithium mechanisms (37, 50, 51).

In summary, there is robust evidence that lithium improves clinical symptoms by facilitating neural plasticity (42, 48). Biochemically, the neurotrophic and neuroprotective effects of lithium stem mainly from the increased expression of BDNF and the inhibition of GSK-3 β (37, 50, 51). The broadening of lithium’s therapeutic range in later years has emerged as a consequence of studies showing that lithium is neuroprotective and anti-inflammatory, and that it also affects cell proliferation, neurogenesis, neuronal plasticity and hippocampal volume. (52-55). Some suggested molecular and cellular effects of lithium mechanisms are listed in Figure 4. Whether any of those mechanisms is key in treating the actual mood disorders or just contributing to wanted or un-wanted side effects is still an unsolved issue. Any new insights of how these mechanisms are involved in BD and other disorders would be of great importance for solving the mystery of lithium response in BD.

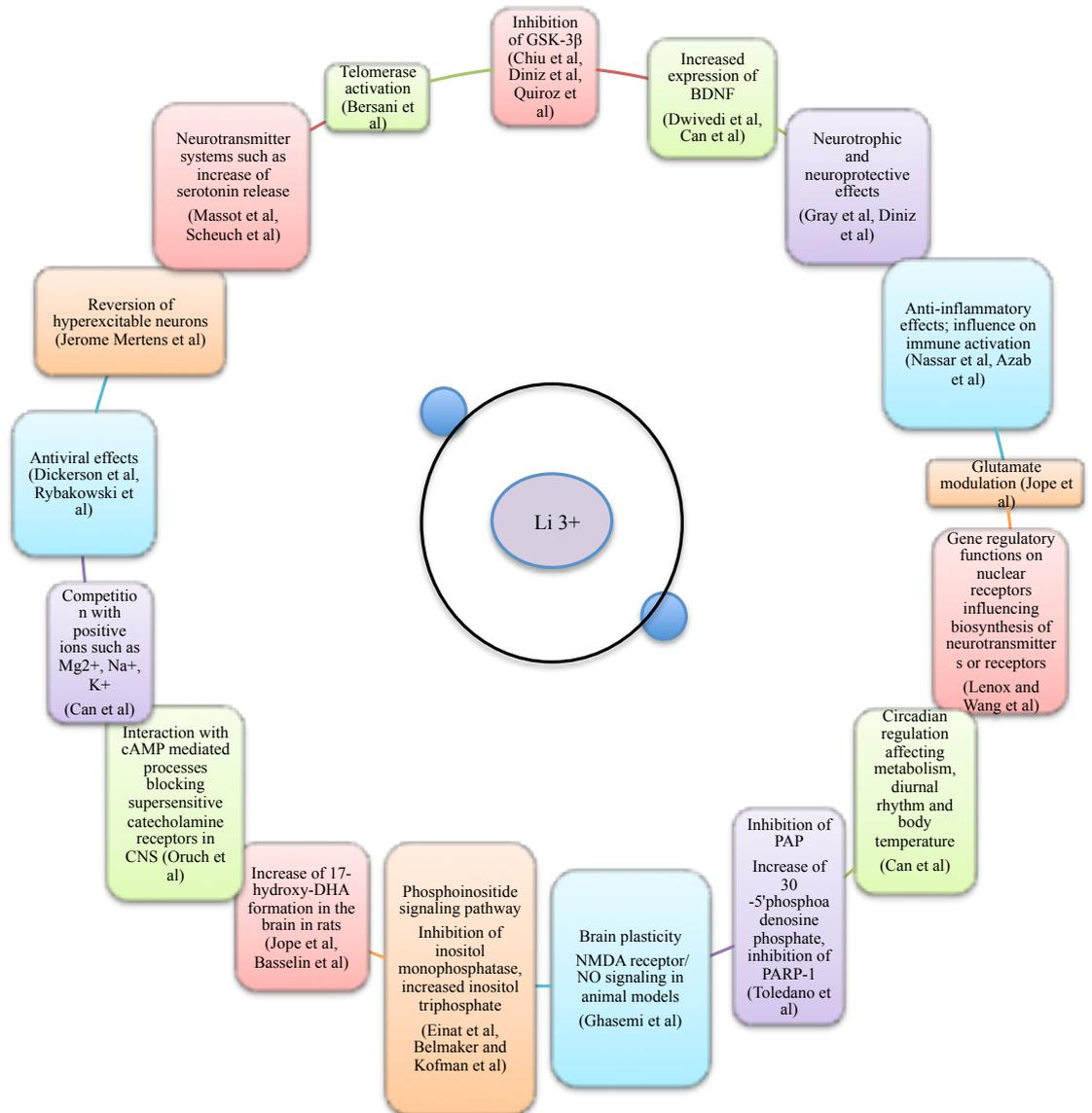


Figure 4. Molecular and cellular mechanisms of lithium.

2.2.4 Genetics of lithium response

Previous research has shown that not only the vulnerability to BD, but also the ability to respond to lithium is heritable (56, 57). A modern strategy is to analyze the association between these genes (candidate genes) and subgroups of the disease, i.e. phenotypes, in a homogeneous population. Such phenotypes are characterized by specific symptoms that can represent different biological variants of the disease, such as bipolar patients who respond well to lithium (58). Some clinicians claim that such phenotypes should be considered a separate disorder (1).

For example, a number of candidate genes related to neurotransmitters (e.g., 5HTT, DRD1), intracellular signaling (e.g., INPP1, CREB1), neuroprotection (e.g., BDNF, GSK-3 β), circadian rhythms (e.g., Rev-Erba), and other pathogenic mechanisms of BD (e.g., glucocorticoid receptor, DISC-1) were found to be associated with lithium response (26). Poor Li-response has been associated with the presence of two short arms of the serotonin transporter gene 5-HTT (59). A Swedish research group demonstrated that the expression of the clock gene PER1 in fibroblasts was affected by lithium and VPA (60). Although some of these findings have been replicated, for example, the CREB family of genes (61, 62) and synapse-related genes (63, 64) the majority of reported genetic associations to lithium response have not been reproduced (65). One possible reason for the lack of replication is the inclusion of different ethnicities between studies, for example, studies of single-nucleotide polymorphisms (SNPs) in the brain-derived neurotrophic factor (BDNF) gene (66-68).

GWAS

Another approach is hypothesis-free Genome Wide Association Studies (GWAS) that provides an observational study design of association between a phenotype, such as lithium response, and over 100 000 common genetic variants (SNPs). Previous GWAS found associations for SNPs in the CACNA1C, ANK3 and ODZ4 genes to BD (69-71). A GWA study of lithium response searching for new candidate genes showed significant association with variants in the PLCG1 gene and the region on chr10p155 (57). Other GWA studies of lithium response showed associations with GADL1, DGKH, GRIA2, ACCN1, SESTD1 and clock genes (65, 72-77).

Unfortunately, the explanatory power for the candidate genes and the genetic variations significantly associated with lithium response is low (26). So far, these findings cannot provide enough knowledge to fully explain the underlying genetic mechanisms of either BD or lithium response. What is more, there have been methodological problems, since the methods for measuring lithium response have not been performed in similar ways in the different study samples (78). Lately, larger sample sizes have been used in genetic studies with some significant findings of new genes in BD, although there is still a wide knowledge gap to fill. One possible solution to the problem is to use well-defined samples of lithium response. Such an approach might reach a higher level of significance. Furthermore, since little of the strong heritability of BD and lithium response has been explored in previous

studies, it is relevant to expand our thinking and hypothesize that other biological mechanisms might influence lithium response.

2.2.5 Measurements of lithium response

In order to increase the quality and precision of phenotyping the “true” lithium responders in samples of bipolar patients, Canadian researchers introduced the Alda-scale (79). This scale is a rating tool for the trained clinician that allows a quantitative, retrospective assessment of the quality of the prophylactic lithium response in the patient (79). The scale was named after Dr. Martin Alda, who was instrumental in its construction. By using the Alda-scale, each individual’s severity of disease before and after introducing the treatment is assessed. Treatment duration, compliance issues and additional medication can be controlled for resulting in a total Alda-score of 0-10, as described in the figure text below. Lithium Responders (LiR) are defined as Alda-score 7-10 and individuals who do not respond so well are defined as non-Responders (non-LiR) scoring 0-6. This method of phenotyping LiR and non-LiR provides a more robust way of defining the sub-sets of the study samples. The Alda-scale has been used and evaluated within an international research collaboration of lithium response studies, the ConLiGen (78, 80). The estimations of lithium response rate in this thesis are based upon the Alda-scale (Figure 5).

Name: _____ Date: _____ Drug: _____ Evaluated By: _____

Criterion A

The criterion A is used to determine an association between clinical improvement and the treatment. The rating should apply to the period of treatment considered adequate in duration and dosage. The illness activity should be judged by frequency, severity, and duration of episodes.

10 = Complete response, no recurrences in the course of adequate treatment, no residual symptoms, and full functional recovery
9 = Very good response, no recurrences, but the patient may have minimal residual symptoms (transient anxiety, sleep disturbance, dysphoria, irritability) not requiring any intervention
8 = Very good response. Illness activity reduced by more than 90%
7 = Good response. Illness activity reduced by 80 - 90 %
6 = Good response. Reduction in activity of illness by 65 - 80%
5 = Moderate response. Reduction in illness activity by 50 - 65%
4 = Moderate improvement. Reduction in illness activity by 35 - 50%
3 = Mild improvement. Reduction of illness activity by 20 - 35%
2 = Mild improvement. Reduction of illness activity by 10 - 20%
1 = Minimal improvement. Reduction of illness activity by 0 - 10%
0 = No change or worsening

A Criterion Score: _____

Criteria B

The criteria B are used to establish whether there is a causal relationship between clinical improvement and the treatment. Score 0, 1 or 2 points for each item:

B1: Number of episodes off the treatment.

0 = 4 or more episodes
1 = 2 or 3 episodes
2 = 1 episode

B1: _____

B2: Frequency of episodes off the treatment.

0 = Average to high, including rapid cycling
1 = Low, spontaneous remissions of 3 or more years on average
2 = 1 episode only, risk of recurrence cannot be established

B2: _____

B3: Duration of the treatment.

0 = 2 or more years
1 = 1 - 2 years
2 = Less than 1 year

B3: _____

B4: Compliance during period(s) of stability.

0 = Excellent, e.g. documented by drug levels in the therapeutic range
1 = Good, more than 80% levels in the therapeutic range
2 = Poor, repeatedly off treatment, less than 80% levels in the therapeutic range

B4: _____

B5: Use of additional medication during the period of stability.

0 = None except infrequent sleep medication (1 per week or less); no other mood stabilizers, antidepressants or antipsychotics for control of mood symptoms
1 = Low-dose antidepressants or antipsychotics as an "insurance", or prolonged use of sleep medication
2 = Prolonged or systematic use of an antidepressant or antipsychotic

B5: _____

B Criteria Score: _____

Total Scale Score: _____
(Subtract B from A)

© Martin Alda, 2002

Figure 5. The Alda-scale. Criterion A rates the degree of response to adequate lithium treatment from 1 to 10. Criteria B1–B5 assess the causal relationship between the treatment and the improvement by detailing the frequency of episodes when lithium was not used (B1 and B2), treatment duration, compliance during euthymic periods on lithium, and use of additional medications during stable periods (B3, B4, and B5, respectively). The total score is obtained by subtracting B from A and ranges from 0 to 10. The Alda-scale thus allows for either a categorical assessment (i.e., below or above some cutoff point) or a dimensional assessment (which includes periods off the treatment) of the lithium response. Used with permission of Martin Alda.



“Lithium Response” painted by Therese Richter, 2015.

2.3 TELOMERE BIOLOGY AND BIPOLAR DISORDER

Just a small piece of the strong heritability of BD and lithium response has been explored by genetic studies. Therefore, one may hypothesize that other biological mechanisms might influence lithium response in BD.

2.3.1 Telomere biology

Telomeres are DNA-protein complexes composed by DNA tandem repeats of TTAGGG/CCCTAA that cap the ends of linear eukaryotic chromosomes and thereby protect the DNA from damage, much like aglets on a shoelace. The telomeres erode progressively with each cell division. The length of the telomeric sequence; the telomere length (TL), declines with age, finally signaling cellular senescence resulting in apoptosis (81).

Telomerase is a reverse transcriptase that consists of a catalytic reverse transcriptase subunit (TERT) and an RNA component (TERC) forming the template for DNA synthesis. This enzyme adds TTAGGG repeats to the chromosome ends and thereby counteracts the telomere shortening. Despite this repair of the chromosome ends, telomeres get shorter in each cell division due to the “end replication problem”. When telomeres reach a critical shortness, the cell ceases to proliferate and enters a state called “replicative senescence” or can become genomically unstable. Senescent cells malfunction in cell-specific ways. For example, the tumour suppressor protein, p53, might be activated, which inhibits oxidative defense mechanisms promoting mitochondrial damage and senescence or apoptosis (82). Pre-cancerous cells with too short telomeres can also become genomically unstable through DNA end-to-end fusions and can promote cancer progression (83).

Most normal human somatic cells have very little, if any, detectable telomerase, explaining their susceptibility to finite limits on cellular division. By contrast, germ-lineage cells, stem cells, progenitor cells, many rapidly dividing cells and cancerous cells typically have high telomerase activity (TA) (84). There is also detectable telomerase in adult brain regions where neurogenesis exists: the subgranular zone of the dentate gyrus in the hippocampus and the subventricular zone of the lateral ventricle. Interestingly, in addition to protecting telomeres by adding nucleotide repeats, telomerase has been reported to be involved in cellular protection and plasticity (85, 86) and has recently been proposed as a target of action for psychopharmacological drugs such as lithium (87).

Chromosomes and telomeres are sensitive to exposure of oxidative stress and inflammation. Thus, loss of telomere length can serve as a cellular “mitotic clock” that limits the number of cell divisions and cellular life span. This knowledge was awarded the Nobel Prize in Physiology or Medicine in 2009 to Blackburn, Greider and Szostak (88). Subsequently, TL and TA have become modern biomarkers of cellular aging. TL is a stable and slow biomarker and TA is sensitive, quickly changing and is considered unstable. TL has been reported to correlate strongly between tissue types, such as leukocytes, skeletal muscle, skin, subcutaneous fat and cerebral cortex within an individual, although the absolute TL varies

across tissue types (89). Thus, it is plausible that leukocyte telomere length (LTL) is correlated with TL in certain brain tissues (83). Leukocytes are easily available from peripheral blood samples and commonly used as a DNA source for TL measurements (Figure 6)

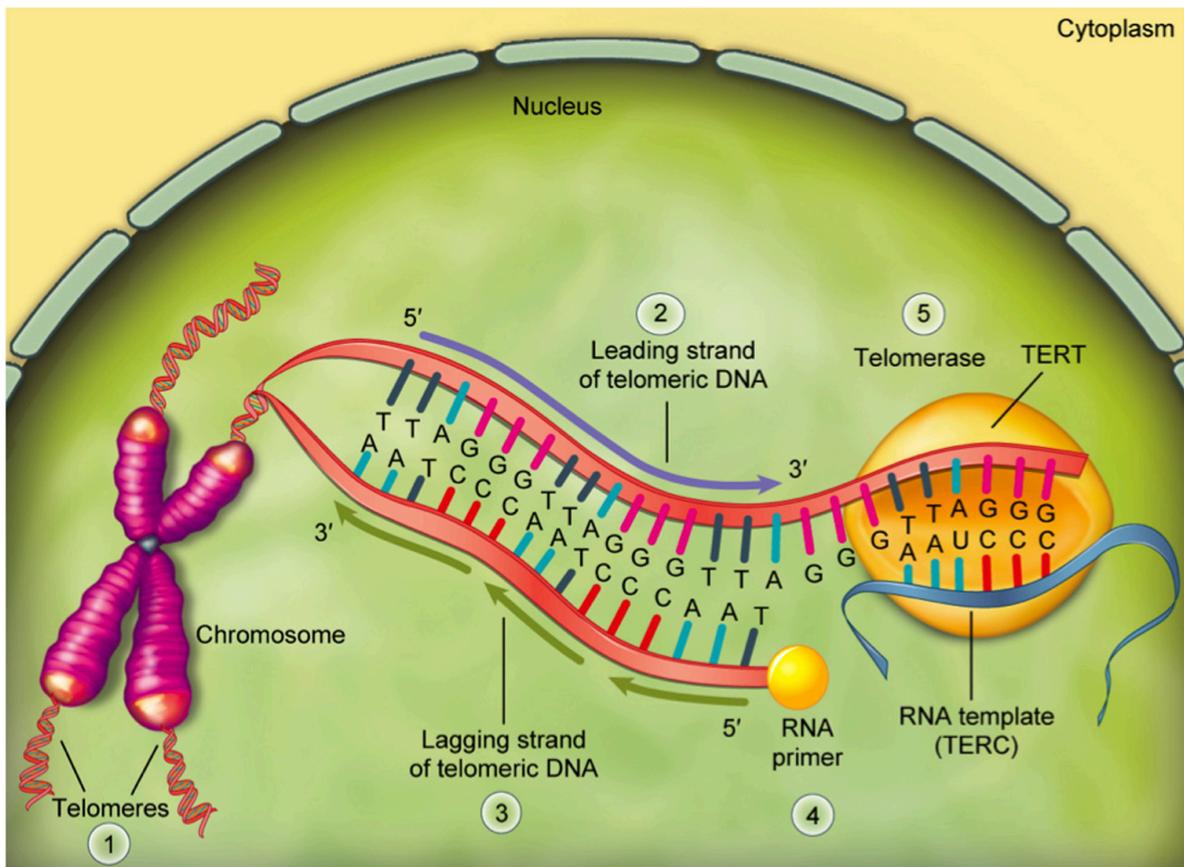


Figure 6a. Telomere biology. Used with permission of the publisher and authors, Lindqvist et al 2015 (83).

2.3.2 Telomere length in somatic diseases

LTL maintenance is reported to be affected by age and gender (90) and by environmental factors, such as physical activity, cigarette smoking, obesity and stress (91). Short LTL in humans has been associated with serious medical illnesses including cardiovascular disease, diabetes, and cancer (92-95). There are some studies linking shorter LTL with premature mortality (93, 96, 97) and a reduction in years of healthy living (98). Additionally, short telomeres correlate to early life stress, depression and hypocortisolemia suggesting that short TL might be a cumulative indicator of stress in life (99). In contrast, and consistent with the aforementioned factors that shorten telomeres, physical exercise and effective treatment can prevent telomere shortening and rebuild telomeres probably through TA (100, 101).

In summary, short TL and unbalanced TA have become modern markers for age and burden of disease, although TL that is too long will also cause DNA damaged in the cell due to

chromosomes sticking together, which has been associated with some types of cancer (83, 87, 102). Biological mechanisms providing balance in TL are established in the cell to maintain “optimal” TL (103, 104).

2.3.3 Telomere length and telomerase activity in psychiatric diseases

Telomere length and telomerase activity were not studied in BD and lithium treatment prior to the start of this doctoral study.

2.3.3.1 Telomere length

During the last decade, several studies have reported shorter leukocyte telomere length (LTL) to be associated with depression (99, 105-110). Short LTL has also been associated with psychological stress, oxidative stress and inflammation (111-113). Adversity in childhood has been associated with shorter LTL in adulthood (114, 115). Several studies have found association of short LTL to SCZ (116, 117) and to poor response to treatment (118). Interestingly, a correlation between LTL and medication was suggested in two studies of SCZ (119, 120).

2.3.3.2 Telomerase activity

While TA in psychiatric illnesses remains poorly studied, it has been better described in the context of psychological stress (111). A small-scale study of MDD found relatively lower pretreatment TA and relatively greater increase in TA during treatment in individuals with superior antidepressant responses (108). Similar results of increased TA have been found in studies of individuals with schizophrenia with significantly decreased TA compared to unaffected individuals (121). Combat-related PTSD (122) had unaltered peripheral blood mononuclear cell (PBMC) basal TA compared to controls. Preclinical animal studies suggest that hippocampal TA in mice is involved in the regulation of “depression-like” behaviors and possibly “antidepressant-like” mechanisms, perhaps by regulating adult neurogenesis in the dentate gyrus (123). An overexpression of *Tert* was associated with (124) and promoted adult neurogenesis, and fluoxetine upregulated TA, suggesting that SSRIs can increase TA (123). In humans, TA in peripheral blood leukocytes associated positively with hippocampal volume of postmortem brains from depressed individuals (125). Interestingly, activation of telomerase has recently been suggested to be a possible mechanism of action for psychopharmacological drugs (87). Reduced human *TERT* (*hTERT*) expression was found in oligodendrocytes of white matter from postmortem depressed brains compared to corresponding tissue from brains of controls (126). Genetic variation of the *hTERT* gene, i.e. the single nucleotide polymorphism (SNP) rs2736100, was shown by a meta-analysis to be associated with LTL in cohorts of primarily healthy adults (127). Whether or not this SNP rs2736100 also associates with mood disorders had not been investigated before the start of this doctoral study.

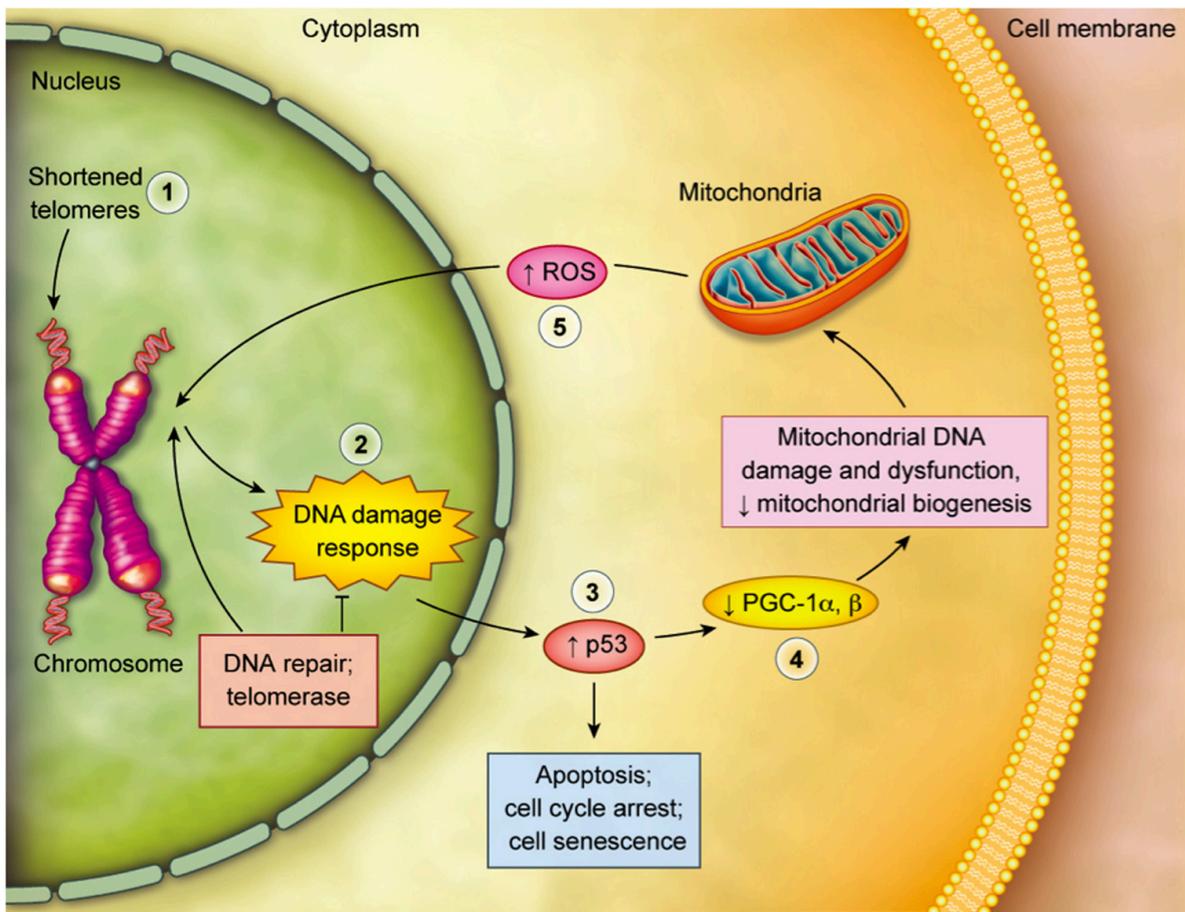


Figure 6b. Cellular effects of telomere shortening. Critical shortening of telomeres can lead to apoptosis, cell cycle arrest or genomic instability. When telomere length is sufficiently shortened [1] or when telomere integrity is sufficiently challenged, classic DNA damage responses (DDR's) [2] are initiated. A major effector of the DDR is the tumor suppressor protein p53 [3], which is activated upon telomere damage. This can lead to cell cycle arrest (“replicative senescence”), cellular senescence and apoptosis; this is most likely to affect cells turning over rapidly, such as blood cells (Sahin et al., 2011). Cellular death and senescence can give rise to stem cell dysfunction, degenerative diseases and tissue death. Were it not for p53 activation, telomere-damaged cells could survive, and their genomic instability could give rise to cancerous cells. Activation of p53 can also damage cells turning over slowly, such as those in heart and brain, by directly decreasing the expression of peroxisome proliferator-activated receptor gamma, coactivator-1 alpha and beta (PGC-1 alpha and PGC-1 beta) [4], the master regulators of mitochondrial function and biogenesis (Sahin et al., 2011). Such effects on mitochondrial number and function can also decrease cellular viability by decreasing cellular energy production and by releasing excessive amounts of free radicals such as reactive oxygen species (ROS) [5], which further damage telomeres and other cellular components. Figure and figure legend used with permission from the publisher and the authors of Lindqvist et al 2015 (83).

2.3.4 Telomere length in bipolar disorder and lithium treatment

At the starting point of this doctoral study, there was one small study of BD2 and TL suggesting short TL in BD2 in line with previous findings for MDD and SCZ (109). No research had been done on telomere biology and lithium treatment.

2.3.5 Telomeres and cancer

Short TL and unbalanced TA have been associated with several cancer types (83, 95, 102). In contrast, longer TL has also been associated with human breast cancer, colorectal cancer cell lines and human embryonic kidney cells (102). Mutations that increase expression of the *hTERT* gene by two-fold caused large increases in risks of certain cancers (83, 128, 129). By controlling the telomere function, cancer cells can avoid cell death and thereby force themselves to live longer.

2.4 CANCER IN BIPOLAR DISORDER AND LITHIUM TREATMENT

If lithium has any effect on cancer; overall or in any specific organ, it is of great clinical importance to detect and understand that mechanism.

Previously, both BD and lithium treatment have been proposed to increase cancer incidence (130, 131). However, the role of BD as a comorbidity factor in cancer is inconclusive, since results from studies on cancer mortality and comorbidity are inconsistent (132, 133). Bipolar patients constitute a risk group of various somatic comorbidities such as cancer, dementia and coronary heart disorders (134-136). Lifestyle factors influencing cancer risk (such as alcohol intake, food behavior patterns, obesity, somatic disorders, social group belonging, smoking habits, and lack of physical activity) are important and might increase the cancer risk in BD. Furthermore, the presence of BD might delay the diagnosis of cancer and other somatic diseases. However, results from previous research concerning the overall comorbidity or mortality of cancer for bipolar patients are still inconsistent (9, 130, 132, 137, 138) and knowledge of cancer incidence in specific organs is lacking.

Some recent studies have reported enhanced risk for solid renal tumors in long-term lithium-treated patients (139-142). However, clinical impressions from experienced psychiatrists suggest the opposite (143, 144). Additionally, two recently published register studies from Denmark showed that the incidence of renal and upper urinary tract (RUT) cancer was not increased in lithium-treated patients (145, 146). The first study included subjects exposed to lithium (N=24 272) and subjects with bipolar disorder (N = 9 651) and a randomly selected sample (N=1 500 000) from the Danish population (134). The second study included cancer matched cases (N=6 477) and cancer-free controls (N=259 080) with data on lithium use from 1995 to 2012 from the Danish Prescription Registry (135).

On the other hand, some recent studies argue that lithium, beyond its mood stabilizing effects, also might provide mechanistically protective effects via GSK-3 β pathways associated with less risk of cancer for several cancer types (147-150), because reduced GSK-3 β activity reduces cell proliferation and tumor growth (151). Since lithium treatment frequently becomes life-long in BD1, increased risks or protective effects are of significant clinical importance. European policies of lithium treatment have recently been changed with added warnings of increased kidney cancer, although the evidence is unclear. Large studies with robust data of overall and site-specific cancer incidence in lithium-treated bipolar patients are needed in order to evaluate whether there is any increased cancer risk for patients using lithium.

From studies of the neuroprotective pathways of lithium, it is well known that lithium (directly and also via inhibition of GSK-3 β) activates β -catenin (37, 43, 152). In parallel, the cancer research field has suggested that β -catenin can stimulate telomerase and thereby affect the TL in the cancer cell (43, 102, 104). Specifically, transcription of *hTERT* has been found to be activated by β -catenin, which binds to and removes repressor T-cell factor 4 (TCF4) from the *hTERT* promoter, resulting in increased TA and longer TL in human cancer cell lines and human embryonic kidney cells (102). Whether or not lithium treatment in BD affects the overall cancer incidence risk has previously not been investigated.

2.5 SUMMARY OF NEEDS AND GAP OF KNOWLEDGE

Below is my summary of the lack of knowledge and what is needed within the three research areas 1) heritability of lithium response, 2) telomere length in bipolar disorder and lithium response and 3) cancer in bipolar disorder and lithium treatment.

2.5.1 Genetics of lithium response

2.5.1.1 Unknown genetic mechanisms behind causes of bipolar disorder

The causes of bipolar disorder are still unknown, although it is a well-known fact that several genetic factors are involved. So far, however, genetic studies have only managed to explain a small piece of the complex etiology. Genetic studies with sharper focus on specific phenotypes of the broad spectrum of bipolar disorder, such as lithium response (LiR), are needed. More knowledge of how environmental factors influence the DNA and proteins, such as immune activation factors, may be the key to new insights in this area.

2.5.1.2 Lithium response

One third of bipolar patients respond very well to lithium treatment, but today there is no way of predicting who will respond. Lithium probably has intracellular effects on protein functions by replacing other positive ions, but the mechanisms are still not fully understood. More knowledge in this field might provide a desirable biomarker for lithium response that would offer possibilities to test for lithium response before starting a treatment. There is clear evidence that genetic factors are involved not only in BD, but also in lithium response, although candidate genes have been difficult to find and replicate. There is need to create international collaborations in order collect well-defined samples that are large enough to provide adequate power to find candidate genes of lithium response. DNA sequencing of large families affected by BD with responsiveness to lithium may be a possible way to find genetic keys to lithium response.

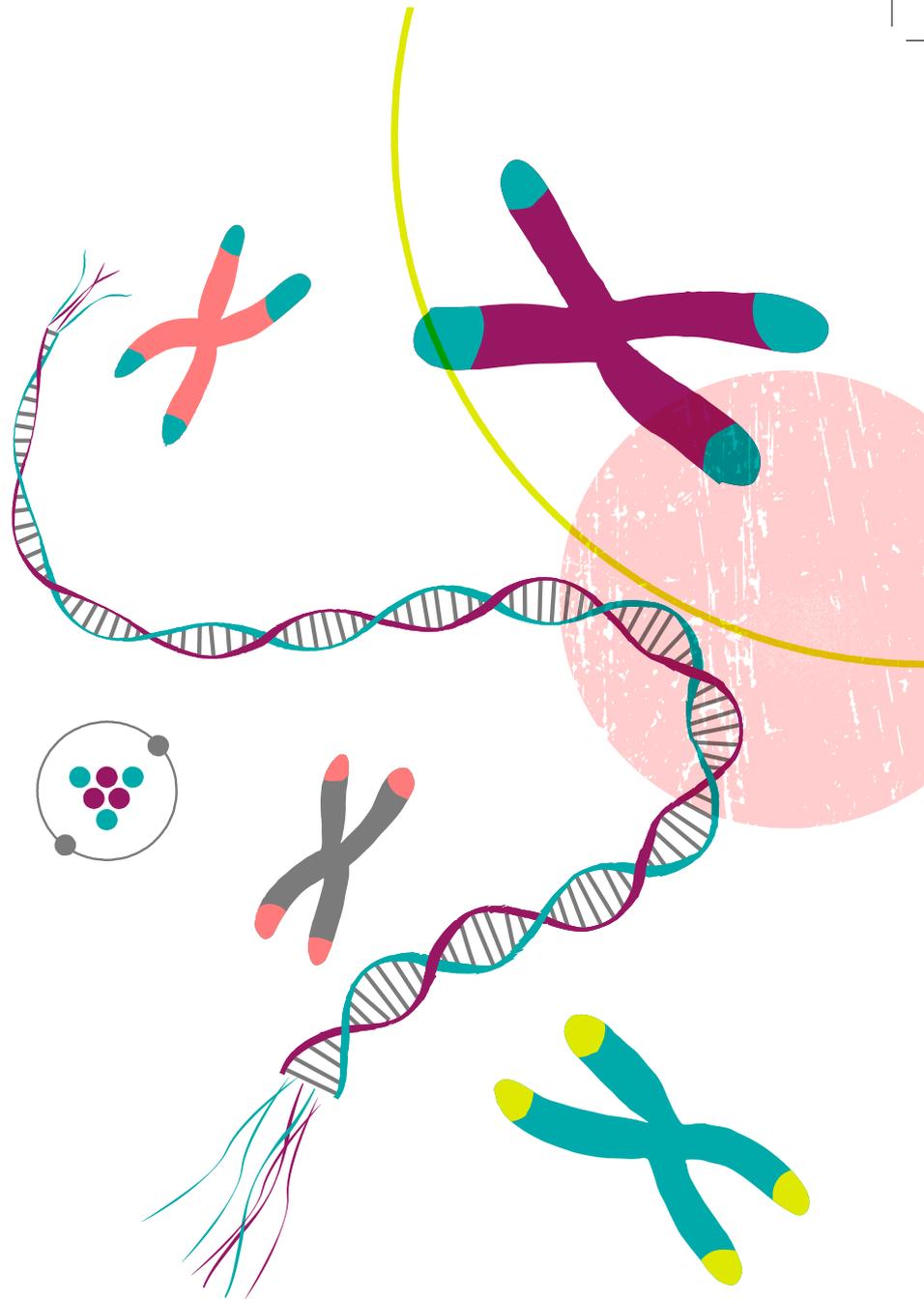
2.5.2 Telomere length in bipolar disorder and lithium response

Since genetic studies of BD and LiR have been limited in explaining heritability, it is necessary to do additional studies of other genetic mechanisms that might be involved. One approach is to study TL and TA, which have become modern biomarkers for cellular aging and exposure to metabolic stress. Psychiatric disorders, such as schizophrenia, MDD, anxiety disorders and chronic stress have been associated with short telomeres, but TL in BD was only sparsely investigated before the start of this doctoral study. Response to Antidepressants in MDD and antipsychotics in schizophrenia is associated with changes in TA, although to date no research has been done on TL or TA in relation to lithium treatment or LiR. Such

studies of telomere biology might offer new possible hypotheses of lithium's mechanisms and the etiology and heritability behind BD and LiR.

2.5.3 Cancer in bipolar disorder and lithium treatment

Somatic disorders are common in BD, but previous studies of cancer incidence in BD show inconsistent results. Some studies of limited size reported increased kidney cancer incidence in lithium-treated patients, although the clinical impression is not in support of these findings. Additionally, two recently published register studies of kidney cancer found no risk increase in lithium-treated patients. If lithium has any effect on the risk of developing cancer overall or in any specific organ, it is of great clinical importance to detect and understand that mechanism. Swedish registers provide the possibility to conduct observational studies of overall cancer incidence in different diseases and treatments, but no such studies have been published.



Biology of Lithium Response in Bipolar disorder– Genetic mechanisms and Telomeres; Cancer incidence and Lithium treatment. Illustratör: Jens Magnusson, 2016.

3 AIMS AND RESEARCH QUESTIONS

3.1 AIMS

3.1.1 Overall Aims

The overall aim of this thesis is to increase the knowledge of the biological mechanisms behind lithium response in bipolar disorder.

This thesis explores new hypotheses of genetic mechanisms that might be involved in bipolar disorder and lithium response through a Genome Wide Association Study and three studies of telomere length in humans. It also investigates the cancer risk in bipolar disorder and lithium treatment in a register study.

3.1.2 Specific Aims

The specific aims of each study in the thesis are:

- Study I** to test whether there are any genetic polymorphisms associated with lithium response in bipolar disorder by conducting Genome Wide Association Study of a large international cohort of lithium-treated bipolar patients; ConLiGen, to which our research group has contributed with a large sample.
- Study II** to assess blood leukocyte telomere length in bipolar disorder compared to healthy controls; to test whether lithium treatment and the number of episodes may influence the telomere length and to investigate whether the telomere length may be associated with lithium responsiveness.
- Study III** to investigate whether genetic variation in the functional subunit of telomerase (*hTERT*) is associated with depression; to replicate measurements of leukocyte telomere length in depression and to determine telomere length by using less-invasive saliva DNA samples.
- Study IV** to investigate how leukocyte telomere length changes over time with long-term lithium treatment in a prospective follow-up of bipolar individuals; to study whether other factors may prospectively influence the leukocyte telomere length shortening and to evaluate whether leukocyte type distribution affected the leukocyte telomere length.
- Study V** to study the overall and site-specific cancer incidence in BD with and without lithium treatment compared to the general population.

3.2 RESEARCH QUESTIONS

This thesis comprises three different research areas to which the five studies belong. Within each research area, the following questions have been asked:

3.2.1 Genetics of lithium response

Are there any common gene variants (SNPs) associated with lithium response in bipolar disorder?

3.2.2 Telomere length in bipolar disorder and lithium response

How is the leukocyte telomere length affected by exposure to bipolar disorder and to lithium treatment?

Is the leukocyte telomere length associated with lithium response?

Is depression associated with the genetic variant (SNP rs2736100); a genetic variant previously associated with short leukocyte telomere length?

How does leukocyte telomere length develop prospectively in lithium-treated bipolar patients?

Is leukocyte telomere length dependent on leukocyte type distribution at sampling?

3.2.3 Cancer incidence in BD and lithium treatment

Is the overall cancer risk or the cancer risk in specific organs increased in BD with or without lithium treatment?

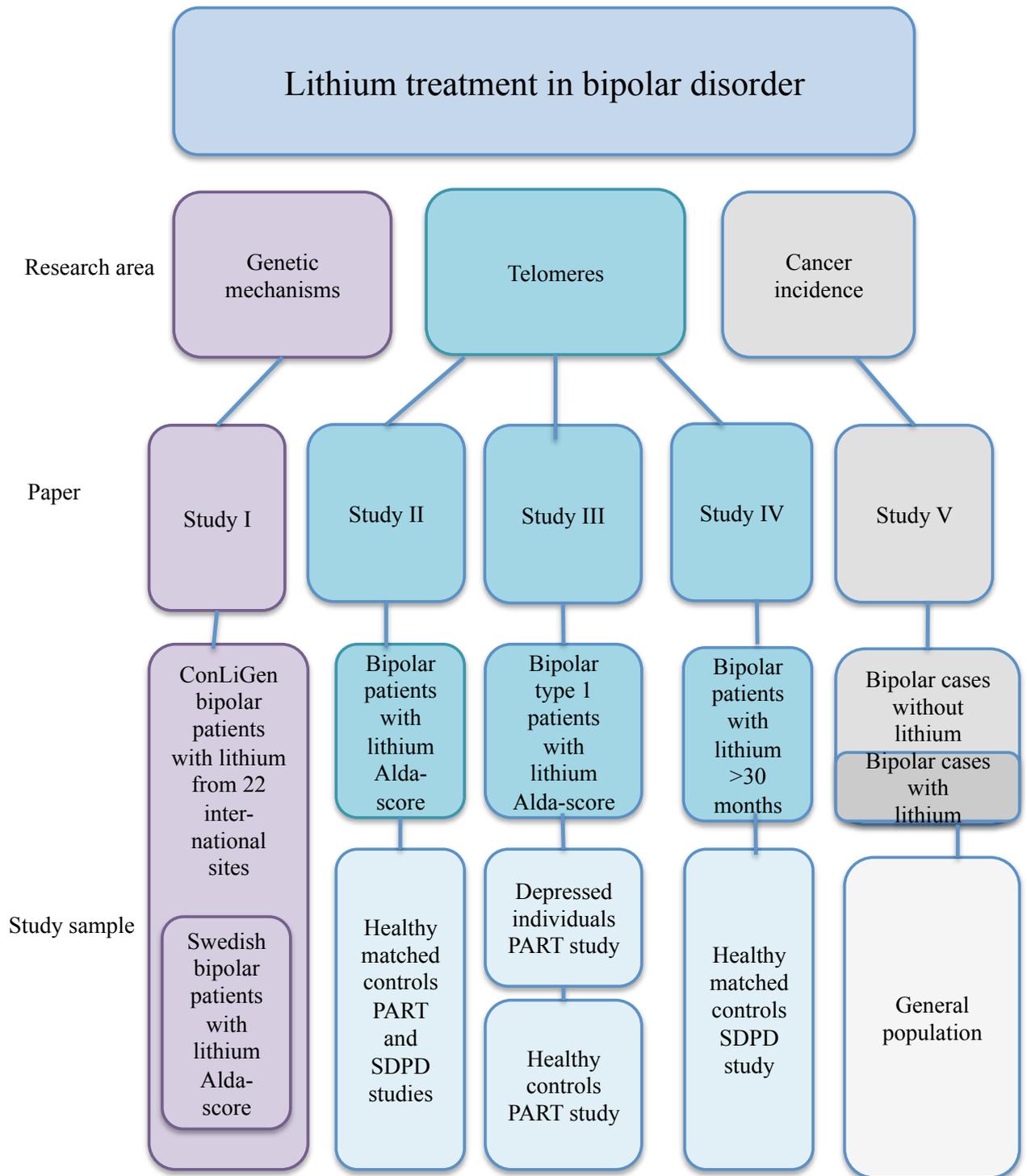


Figure 7. Thesis Architecture.

4 SUBJECTS AND METHODS

4.1 OVERVIEW OF STUDIES

Research Area	Genetics of lithium response	Telomere length in bipolar disorder and lithium response			Cancer incidence in bipolar disorder and lithium treatment
Paper	Study I	Study II	Study III	Study IV	Study V
Aims	To study genetic variations in lithium response.	To study telomere length in bipolar disorder, lithium and lithium response.	a) To study telomere length in depression in leukocytes and saliva, b) to study association between a <i>hTERT</i> SNP rs2736100 and depression in MDD and BD1.	To investigate telomere length in 10-year prospective follow-up of lithium treatment in bipolar patients, and healthy controls.	To compare cancer incidence in bipolar disorder with and without lithium treatment to general population.
Research Questions	Are there any common gene variants (SNPs) associated with lithium response in bipolar disorder?	How is leukocyte telomere length affected by exposure to bipolar disorder and to lithium treatment? Is the leukocyte telomere length associated with lithium response?	How is telomere length in leukocytes and saliva affected in depression? Is depression associated with the genetic variant (SNP rs2736100), a genetic variant previously associated with short leukocyte telomere length?	How does leukocyte telomere length develop prospectively in lithium-treated bipolar patients? Is leukocyte telomere length dependent on leukocyte type distribution at sampling?	Is the overall cancer risk or the cancer risk in specific organs increased in BD with or without lithium treatment?
Design	GWAS study	Retrospective cohort study	a) Retrospective cohort study b) Genetic association study	10 year prospective cohort study; Life Charting Method	Register study of 3 linked registers (Swedish Cancer Register, National Patient Register and Drug prescription

					register)
Population	N=2 563 patients from 22 international sites (ConLiGen). (N=525 from our Swedish bipolar cohort).	Bipolar patients (N=256), healthy matched controls (N=135), subsets of RC/nonRC (N=97/94) and LIR/nonLiR (N=65/65) within BD cohort.	Relative TL was measured in saliva from age-matched depressed individuals and controls in the PART cohort (N=662) , rs2736100 was genotyped in 436 depressed individuals, 1 590 controls, and 368 BD1 patients with Alda-score.	Lithium-treated bipolar patient cohort (N=55), N=100 healthy age-matched controls.	Population N=2 593 011, Age-span 50-84 in bipolar patients (N= 5 442) with lithium (N=2 393) and without lithium treatment (N=3 049) from July 2005 to December 2009.
Main outcome measures	Association of various genetic variants and lithium response according to the Alda-scale.	Relative blood leukocyte telomere length.	Relative blood leukocytes telomere length, Saliva telomere length. Genetic association of depression and SNP rs2736100 in <i>hTERT</i> -gene.	10-year description of development in: Relative blood leukocyte telomere length in relation to blood cell count at sampling.	Overall and organ specific cancer incidence rate ratios (IRRs).
Data analyses .	Imputation to common SNPs in relation to lithium response PLINK v1.0727, logistic regression (dichotomous phenotype) linear regression (quantitative phenotype).	ANCOVA, Mann–Whitney U-tests, linear regression, Spearman’s correlation test . (SPSS).	ANCOVA (SPSS).	Chi-2 tests for dichotomous variables ; independent samples t-tests for continuous variables, Spearman’s rank correlation, multiple linear regression analyses. One way repeated measures ANOVA, multiple linear regression (SPSS).	Poisson regression models estimating IRRs with 95% confidence intervals. (SAS version 9.2.).

4.2 GENETICS OF LITHIUM RESPONSE

4.2.1 Study I

Genetic variants associated with response to lithium treatment in bipolar disorder: a genome-wide association study

Why did we do the study?

Previous research has failed to fully explain the underlying genetic mechanisms of BD and LiR (33). There is a need for comprehensive genetic studies of large well-defined samples of lithium response in order to search for new gene variants associated with lithium response.

Study Design

A genome-wide association study (GWAS) of lithium response was arranged and managed by the International Consortium on Lithium Genetics (ConLiGen), in which I am one of many members.

Hypotheses

We hypothesized that there is an association between lithium response and common gene variants. The study design allows for a hypothesis-free search for such associations.

Study participants

N=2563 lithium-treated bipolar patients collected from 22 participating sites of ConLiGen from four continents (Europe, America, Asia and Australia). 525 of those were patients with available Alda-score who had been recruited from our bipolar cohort in Sweden.

Laboratory methods

Phenotyping

A DSM-III or DSM-IV diagnosis of a bipolar spectrum disorder was required, along with data on gender and total score on the Alda scale. All patients in whom response could be reliably evaluated were included. Patients were required to have taken lithium treatment for at least 6 months with no additional mood stabilizer. Comorbid conditions were not among the exclusion criteria.

Genotyping

DNA was extracted from peripheral blood samples. Samples were genotyped at the NIMH, Life & Brain Center at the University of Bonn, or Broad Institute using either Affymetrix or Illumina SNP arrays according to the manufacturers' protocols. Quality control and imputation were carried out in batches corresponding to distinct SNP arrays and ethnicities.

Statistical analysis

Data from over 6 million common single nucleotide polymorphisms (SNPs) were tested for association with categorical and continuous ratings of lithium response of known reliability. Association testing was carried out separately in European-ancestry and Asian-ancestry samples. Both the categorical and quantitative response phenotypes were analyzed. By using PLINK v1.0727, the association between allele dosages and the dichotomous phenotype was evaluated by logistic regression, and the association between allele dosages and the quantitative phenotype was evaluated by linear regression.

Ethical considerations

Written informed consent was obtained from all participants. Ethics approval was given from "Abteilung für Genetische Epidemiologie in der Psychiatrie, Zentralinstitut für Seelische Gesundheit" in Mannheim, Germany, DN 2010-206N-MA. Ethics approval was also given for the Swedish samples from the Regional Ethical Review Board in Stockholm, Sweden, in accordance with the Helsinki Declaration of 1975. For those patients, both written and verbal informed consent was obtained during a visit with a specialized psychiatric nurse during euthymic state. All individuals had full capacity to consent. The procedure was documented in the research protocol, fulfilling the Swedish legal requirements, DN 01-389 and 04-056.

4.3 TELOMERE LENGTH IN BIPOLAR DISORDER AND LITHIUM RESPONSE

4.3.1 Study II

Long-term lithium treatment in bipolar disorder is associated with longer leukocyte telomeres

Why did we do the study?

Previous studies of BD and LiR have been limited in explaining heritability. There is therefore a need for additional studies of other genetic mechanisms that might be involved. Other psychiatric disorders have been associated with short telomeres and response to treatment has been associated with changed TA, but TL in BD and lithium treatment has not been investigated.

Study design

In order to investigate TL in BD and lithium treatment, we performed a retrospective cohort study of LTL in bipolar patients treated with lithium compared to healthy controls matched for age and gender (Study II).

Hypotheses

We hypothesized that:

- 1) BD, in line with previous studies of, for example, SCZ and depression, would have shorter LTL than healthy controls, especially rapid cyclers (RC), who have a severe type of the disorder.
- 2) Lithium treatment might influence LTL, so that there would be a correlation between LTL and amount of time on lithium treatment and
- 3) Lithium responsiveness might influence telomere length so that LTL in LiR would differ from LTL in non-LiR.

Study participants

The bipolar cohort

Patients with a clinical diagnosis of bipolar disorder (BD) were consecutively recruited from the Unit of Affective Disorders at Psychiatry Southwest, Huddinge Hospital in Stockholm. Lifetime depressive and manic symptoms were assessed by a senior psychiatrist specialized in BD or by a trained psychiatric nurse using the modules for depression and mania in the SCAN based on DSM-IV criteria as has been previously described (153). On the basis of these assessments, patients were considered to fulfill 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, RC and mixed episodes were assessed, including the age of onset of mania and depression. Lithium response was measured according to the Alda-scale (78). In previous studies, LiR were those who scored ≥ 7 and non-LiR were those who scored ≤ 6 (range 0–10 points) (78). In this study, at inclusion, we choose to leave out those who scored 6 in order to optimize the dichotomous and well-defined groups.

Healthy controls

Healthy non-obese controls were selected from two longitudinal population-based studies in Stockholm, Sweden: the Diabetes Prevention Program (154) and the PART study of mental health (155). Two cohorts were selected according to predefined diagnostic and treatment response criteria: Set I consisted of patients with excellent LiR and sex and age-matched patients with none or partial LiR (non-LiR), all with therapeutic serum concentration (0.5–0.9 mmol/L) during a period of at least 3 months before DNA sampling. Set II consisted of RC patients and matched patients without RC (non-RC). Set III (the combined bipolar cohort of Set I+Set II) was compared to healthy controls matched for age and gender.

Laboratory methods

Telomere length

Peripheral blood samples were collected in EDTA tubes and DNA was extracted followed by purification. Relative leukocyte telomere length LTL was determined using quantitative real-time PCR technique by calculating the ratio of telomere repeat copy number (TTAGGG tandem repeat) to single copy gene copy number according to Cawthon et al (156) and Kananen et al (157). Samples from BD patients and healthy controls were assayed in the same 384-well plate and analyzed at the same time.

Statistical analysis

Differences in clinical characteristics between study groups were assessed using Pearson's chi-square test or the Mann–Whitney U-test. LTL dependence on sex and age was determined using analysis of covariance (ANCOVA). Correlation between LTL and age was determined using nonparametric Spearman's correlation test. Partial correlation between LTL and duration of lithium treatment, controlled for age and gender, was also determined using nonparametric Spearman's correlation test. Difference in duration of lithium treatment between LiR and non-LiR groups was determined using nonparametric Mann–Whitney U-test. Dependence of LTL on duration of lithium treatment and on number of depressive episodes was assessed using linear regression while adjusting for age and sex; partial Cohens eta square (η^2) indicates effects. Residuals from linear regression analyses were controlled for normality. Dependence of LTL on the dichotomous variables RC and response to lithium treatment was tested using ANCOVA adjusted for age and sex. Similarly, differences in LTL between BD patients on lithium and healthy controls were assessed in the sex- and age-matched sample (age range: 33–77 years) using ANCOVA, while adjusting for sex and age, and then verified using nonparametric Mann–Whitney U-tests. A p-value of 0.05 was regarded as statistically significant. The analyses were performed using IBM SPSS Statistics v. 20 (IBM Corporation, Armonk, NY, USA).

Ethical considerations

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden, in accordance with the Helsinki Declaration of 1975. For patients, both written and verbal informed consent was obtained during a visit with a specialized psychiatric nurse during euthymic state. For controls, verbal or written informed consent was obtained according to procedures approved by the ethical committee. All individuals had full capacity to consent. The procedure was documented in the research protocol, fulfilling the Swedish legal requirements, DN 01-389, 04-056, 91-164 and 04-528.

4.3.2 Study III

hTERT genetic variation in depression

Why did we do the study?

The novel findings in Study II suggested that lithium affects TL (158). These results gained support from an animal study done by colleagues in our group, showing that lithium-fed rats had increased TA in their hippocampi in the brain (159). Furthermore, according to the findings in humans in Study II, depressed rats without lithium treatment had shorter TL in the same brain region (159).

Study Design

Based on these findings, we designed a combined study of: 1) Genetic association study of the genetic variation (SNP rs2736100) in the *hTERT* gene and depression in MDD and BD1, 2) a retrospective cohort study of TL in MDD.

Hypotheses

The allele A of the SNP rs2736100, located in the intron 2 of the *hTERT* gene, was previously associated with shorter LTL (127). Given the association of TL with depression, we expected rs2736100 to associate with depression. LTL was previously associated with the number of depressive episodes and lithium response in BD (109). We tested whether SNP rs2736100 associated with the number of depressive episodes within BD1, considering the patients' response to lithium treatment. We hypothesized that 1) rs2736100 is associated with unipolar and bipolar depression and 2) LTL is associated with the depression.

Study participants

Human subjects with depression and healthy screened controls

Individuals with a history of depression and those without (controls) were identified using self-reported questionnaires from the well-characterized population-based cohort in Stockholm, Sweden: the PART study of mental health (155).

Bipolar cohort

Patients (N=789) with a clinical diagnosis of BD were consecutively recruited from the Unit of Affective disorders at Psychiatry Southwest, Karolinska University Hospital Huddinge between 2003 and 2010. Venous blood DNA was obtained at the time of interview from 736

unrelated BD patients. Lithium response was measured according to the Alda-Scale. Patients with DNA included in Study III were all those with a BD1 diagnosis (N=613), with a scorable lithium response (N=444) and data available on the age at onset of the first depressive episode and the number of depressive episodes prior to the interview (N=368).

Laboratory methods

Relative TL was measured in saliva from age-matched depressed individuals and controls in the PART cohort (N=662) using qRT-PCR (as described above in Study II in Laboratory methods). rs2736100 was genotyped in 436 depressed individuals, 1590 controls, and 368 patients with BD1.

Statistical analysis

The normality of the data and the homogeneity of the variance were tested using the Shapiro–Wilk and the Levene’s tests, respectively. The difference in age between two groups was assessed using the Mann–Whitney U test. The difference in mean between multiple groups was determined using the Kruskal–Wallis test. Homogeneity in the distribution of frequency data between groups was examined using the Pearson’s χ^2 test or the Fisher’s exact test. Correlation between saliva TL and age was tested according to Spearman. Dependence of TL on depression was tested using ANCOVA adjusted for age, sex, alcohol use, and occurrence of somatic disease and experience of childhood adversity. Moderation of the relationship between depression and TL by rs2736100 in the individuals without childhood adversity was tested using the latter model with the addition of rs2736100 and the rs2736100*depression factor. In patients with BD, dependence between number of depressive episodes and rs2736100 was tested using ANCOVA, adjusted for variables previously proposed to influence LTL: sex, age at sampling, and the number of years since onset of the first depressive episode, all of which was then stratified for lithium response.

Ethical considerations

Both the PART study and the BD study were approved by the Regional Ethical Review Board in Stockholm, Sweden in accordance with the Helsinki Declaration as revised in 1989. In the PART study, written consent was obtained from all the participants. Both written and verbal informed consent was obtained for patients with BD while in a euthymic state. All individuals had full capacity to consent. The procedure was documented in the research protocol, fulfilling the Swedish legal requirements, DN 01-389, 04-056, 91-164 and 04-528.

4.3.3 Study IV

A prospective study of long-term lithium treatment and telomere length in bipolar disorder

Why did we do the study?

Our findings in Study II and III supported our hypothesis of lithium's influence on TL. However, prospective studies of TL had not yet been done. Based on results in Study II and III, replications by others (159, 160) and conclusions from metaanalyses and reviews in the field (87, 161), we conducted a prospective study of TL.

Study Design

We performed a 10-year prospective cohort study of leukocyte telomere length change in lithium-treated bipolar patients compared to age-matched healthy controls using the Life Charting Method.

Hypotheses

We hypothesized that 1) positive TL maintenance is associated with exposure time on lithium treatment and 2) good lithium response in bipolar disorder influences the LTL change over time and that 3) TL is independent of the current leukocyte type distribution at sampling.

Study participants

The Life-charting method

Patients with a clinical diagnosis of bipolar disorder (BD) were consecutively recruited from the Unit of Affective Disorders at Psychiatry Southwest, Huddinge Hospital, Stockholm following the same procedure as in Study II and III and were then included in the prospective study. After inclusion, bipolar patients (N=100) were investigated twice a year by a trained nurse who registered any symptoms of bipolar episodes, as well as treatments and life events using the Life Charting Method (LCM) as has been previously prescribed (22). Participants were asked to give detailed information about heredity, socio- demographics, important life events, affective episodes and treatments during their entire life span. In addition to number of episodes and part (%) of time in episodes, a calculated variable called "the Accumulated Burden of Mood Swings" (ABMS) was used. ABMS is a combination of the accumulated duration and the severity of episodes measured as functional disability as previously described (21, 162). Based on ABMS, the affective episodes were categorized into three groups according to symptoms and functional disability related to the extent of sick-leave

(21, 162). Information from all medical records was added to the program. Any discrepancies between the information derived from interviews and the medical records were clarified by telephone. Lithium response was measured according to the yearly reduction of numbers and/or severity of episodes after the beginning of lithium treatment, controlled for the duration of lithium treatment (defined as months on treatment with serum concentration of 0.5-0.9 mmol/L), the age of onset of BD, the number of years with BD before treatment, the duration of lithium treatment before baseline DNA sampling and the presence of other mood stabilizers, antidepressants, neuroleptics or sleep medication according to the Alda-scale (78, 79). Lithium responders (LiR, n=26) were those who scored ≥ 7 and non-responders (nonLiR, n=29) were those who scored ≤ 6 (range 0-10 points) (40). At the final point of follow-up of LTL (LTL2), differential leukocyte count and high-sensitive C-reactive protein (hsCRP) were determined at the same time by the Karolinska University Laboratory according to standard GLP protocols. The reference value for hsCRP was < 3 mg/L.

Descriptives of the bipolar sample

From the bipolar patients who were life charted for more than 10 years, 79 individuals who had lithium maintenance treatment with lithium serum-concentrations of 0.5-0.9 mmol/L for a total period of at least 30 months (158) and DNA available from baseline were included into the study. In total, 55 patients completed the study period with a mean duration of 11.0 years (median= 11.3 years). Two patients had hsCRP levels higher than the reference value of < 3 mg/L but those were not considered very high. Those patients were re-tested after two weeks and showed normal values, which indicated that these cases did not have a chronic inflammation, and were therefore kept in the analysis.

Healthy controls

A total of 100 unrelated Swedish non-obese subjects with normal glucose tolerance at baseline were selected by matching with the bipolar patients for age from the same longitudinal population-based studies in Stockholm, Sweden: the Diabetes Prevention Program (154) used in Study II.

Blood sampling procedure

Peripheral blood samples were collected from bipolar patients and controls in EDTA tubes twice with an approximate 10-year time interval; the first sample in 2004 and the second sample in 2015. Genomic DNA from bipolar cases was extracted using a standard SDS-urea procedure followed by purification using Illustra NAP-5 (GE Healthcare, Buckinghamshire, U.K.) whereas the DNA from controls were extracted using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany). DNA concentration was quantified with NanoDrop ND-1000 Spectrophotometer (Nano-Drop Technologies Inc., Wilmington, DE, USA).

Laboratory methods

Telomere length

Relative LTL was determined by quantitative real-time PCR as described above in Laboratory methods in Studies II and III.

Ethical considerations

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden in accordance with the Helsinki Declaration of 1975. For patients, both written and verbal informed consent was obtained at a visit with a specialized psychiatric nurse during euthymic state. For controls, verbal or written informed consent was obtained according to procedures approved by the ethical committee. All individuals had full capacity to consent. The procedure was documented in the research protocol, fulfilling the Swedish legal requirements, DN 164/100, 01-389, 91-164 and 04-528.

4.4 CANCER INCIDENCE IN BIPOLAR DISORDER AND LITHIUM TREATMENT

4.4.1 Study V

Lithium treatment and cancer incidence in bipolar disorder

Why did we do the study?

Results from previous research of cancer incidence in BD are inconsistent (132, 133). Lithium has been suggested to increase the cancer risk in some studies (139-142) and to protect against cancer in others (147-151). Unbalanced TL is associated with increased cancer risk (102). Based on this knowledge, in combination with the findings of long TL in Study II, III and IV recently supported by others (159, 160) we asked the question: does lithium affect cancer risk in bipolar patients?

Study Design

We conducted a nationwide Swedish register study of incidence rate ratios of total cancer and site-specific cancer in the age-span 50-84 in bipolar patients (N= 5 442) with (N=2 393) and without lithium treatment (N=3 049) from July 2005 to December 2009 compared to the general population (N=2 593 011) using linked information from The Swedish Cancer Register, The National Patient Register (NPR) and The Drug Prescription Register (DPR).

Study participants

Based on the NPR, 18 660 subjects who had been admitted to the hospital between January 1, 1987 and June 30, 2005 with a main diagnosis of BD were identified. Of these, 2 245 had previously been diagnosed with schizophrenia and were consequently excluded. Subjects who had died (N=4 004), emigrated (N=623) or who had been diagnosed with cancer before the start of this study (N=1 271) were identified and then excluded. Cancer incidence is low in early life and there were too few cases in the group 85 or older. Therefore, patients younger than 50 or older than 84 years (N=4 459) were excluded. By linking individuals to DPR, lithium use among patients could be determined. Patients were stratified into two exposure groups based on their lithium purchasing habits: “Bipolar patients with lithium treatment” and “bipolar patients without lithium treatment”. Finally, the total bipolar population of the study was comprised of 5 442 patients, 2 393 lithium users and 3 049 who did not fulfill lithium prescriptions during the study period. Each person was followed from July 1, 2005 until December 31, 2009, or until the date of death or the date of diagnosis of cancer, depending on which came first, and thus the follow-up period was 4.5 years.

Incidence rate ratios (IRRs) adjusted for age and gender of first cancer and site-specific cancer diagnosis between 2005-07-01 and 2009-12-31 were calculated in bipolar patients compared to the general population. Since lithium treatment is known to affect certain organs specifically with different clinical side-effects, cancer incidence in these organs was compared in detail case by case in order to detect any small difference in tendency between cases in the bipolar cohort with lithium and those without lithium and the population.

Statistical analysis

For patients with BD and for the general population, the number of person-years at risk and the number of cause-specific incident cases of cancer were calculated. Data were stratified by gender and 5-year age groups. Poisson regression models were used to compare incidence rates in patients with BD to the general population rates by estimating IRRs with 95% confidence intervals. The logarithm of the person-years of follow-up was used as offset parameter. Results were adjusted for potential confounding by including sex and age as covariates in the models at the start of follow-up. Analyses were done using SAS version 9.2.

Ethical considerations

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden in accordance with the Helsinki Declaration of 1975, DN 03-466 and 02-410.

5 RESULTS AND MAIN FINDINGS

5.1 GENETICS OF LITHIUM RESPONSE

5.1.1 Results of Study I

Genetic variants associated with response to lithium treatment in bipolar disorder: a genome-wide association study

Gene polymorphisms of long non-coding RNA on chromosome 21 are associated with lithium response in bipolar disorder.

A single locus with four linked SNPs (rs79663003, rs78015114, rs74795342, and rs75222709; minimum $p=3.31E-09$) met corrected genome-wide significance criteria for association with lithium response. The associated region contains two genes of long, non-coding RNA (lncRNA). There is evidence that lncRNAs are important players in the gene regulation in the brain and several studies suggested that they play a role in BD, circadian rhythms, neurogenesis and aging (163-166) (Figure 8a, b, c).

Summary of findings in Study I

- **A single locus of four linked common gene variants (SNPs) on chromosome 21 is associated with lithium response in this large cohort of bipolar patients from 22 international sites.**
- **The associated gene region contains long, non-coding RNAs (lncRNAs) that might be biologically important for the gene regulation in the brain in BD.**

5.2 TELOMERE LENGTH IN BIPOLAR DISORDER AND LITHIUM RESPONSE

5.2.1 Results of Study II

Long-term lithium treatment in bipolar disorder is associated with longer leukocyte telomeres

35% longer leukocyte telomeres in BD patients compared to healthy controls.

The mean value of LTL was 34.5% higher in BD cases (N=202) compared to healthy controls (n = 135) $p < 0.0005$ adjusted for sex and age. This significance could also be seen in lithium mono-therapy (N=39) in comparison with healthy controls. Not only LiR, but also nonLiR individuals (n = 60) had longer LTL compared to controls $p < 0.0005$ (Figure 8a).

Lithium responders had 10% longer telomeres than non-responders.

LTL was 10% longer in the LiR patients compared to in the nonLiR patients after adjusting for all the parameters with potential to influence LTL (LiR vs nonLiR; $p = 0.047$, partial $\eta^2 = 0.13$) (Figure 8a).

Leukocyte telomere length correlated positively with duration of lithium treatment in patients who had had lithium for more than 2.5 years (≥ 30 months).

LTL correlated positively with duration of lithium treatment (duration ≥ 30 months) in BD patients ($p = 0.044$, Spearman's $\rho = 0.24$) and was also positively dependent on lithium treatment duration as determined by linear regression (n = 72; lithium treatment duration: standardized (std) beta = 0.29, $t = 2.2$, $p = 0.031$). Lithium duration did not differ between the LiR and the nonLiR groups. In the whole of Set I, with a lithium duration range of 3-468 months, LTL was not significantly dependent on lithium treatment duration (n = 121) (Figure 8b).

Short telomeres associated with the number of depressive episodes

There was a tendency for shorter LTL in the RC cases compared to the age- and sex-matched nonRC cases (Set II), although it was not statistically significant. When assessing the combined group of RC and nonRC cases, there was a significant effect of the number of depressive episodes on LTL, with an effect size similar to that of age ($p = 0.007$). The LTL (R.Q.) marginal mean was reduced 0.075 units per depressive event after adjusting for age

and sex assuming a linear model; stronger in males than in females. Since there was an effect of the number of depressive episodes on LTL, the LTL dependence on lithium treatment duration was corrected for the number of depressive episodes. However, the number of depressive episodes was not found to influence the association between LTL and lithium treatment duration ($p = 0.62$). For manic/hypomanic episodes, no dependence between LTL and number of episodes was found (Figure 8c).

Summary of findings in Study II

- **35% longer leukocyte telomeres in BD patients compared to healthy controls. This significance remained in those with lithium mono-therapy compared to healthy controls.**
- **Lithium responders had 10% longer telomeres than non-responders.**
- **Leukocyte telomere length correlated positively with duration of lithium treatment in patients who had had lithium for more than 2.5 years.**
- **Short telomeres associated with the number of depressive episodes.**

Figure 8a

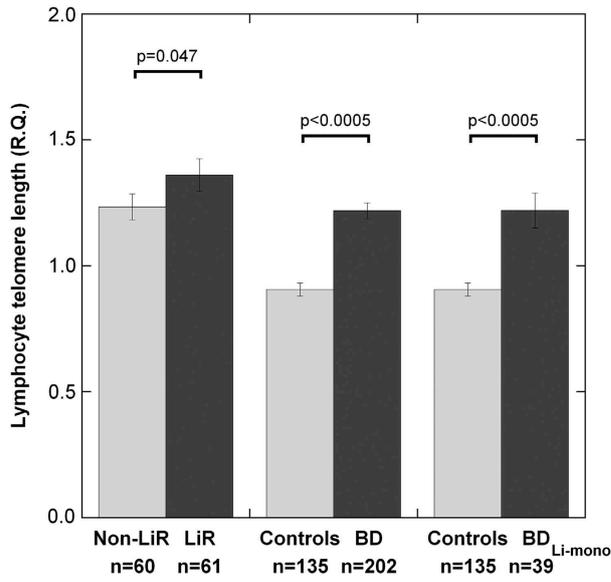


Figure 8b

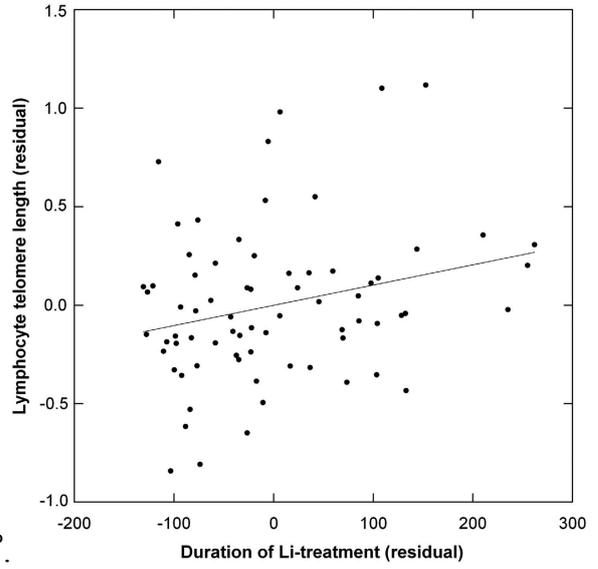
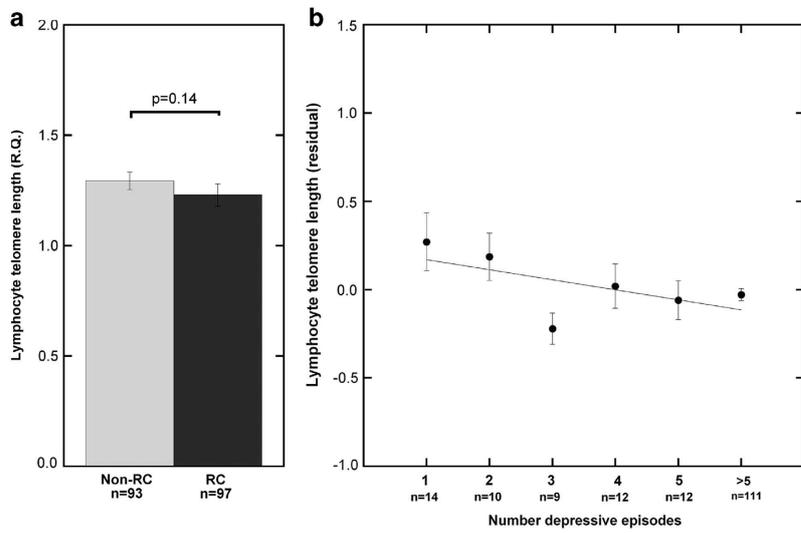


Figure 8c



5.2.2 Results of Study III

hTERT genetic variation in depression

rs2736100 polymorphism was associated with depression

In the group without any experience of childhood adversity, the distribution of the three genotypes (AA/AC/CC) differed between the depressed and the controls ($P=0.013$, logistic regression adjusted for age and sex). There was no association between rs2736100 genotype and depression in the whole material. Homozygosity for the “short LTL”-risk allele A was associated with higher risk of depression compared to the AC/CC genotypes (rs2736100: $p=0.010$, OR = 1.51, 95% CI = 1.10–2.05; sex: $p=0.001$; age: $p=0.007$). In A/C allelic model however, the A allele was not significantly more common in those with depression than in controls.

rs2736100 polymorphism was associated with the number of depressive episodes in bipolar type 1 patients

In the LiR group, rs2736100 associated with the number of depressive episodes. That is, the distribution of the three genotypes (AA/AC/CC) differed between the patient groups defined by the number of depressive episodes, after adjusting for parameters previously suggested to influence LTL (AA vs AC vs CC: $F_{1/45.9}$, $P_{1/40.004}$; sex: $F_{1/40.002}$, $P_{1/40.97}$; years since onset of first depressive episode: $F_{1/417.4}$, $P_{0.001}$; age: $F_{1/40.50}$, $P_{1/40.48}$; ANCOVA). The risk allele A showed dominance, that is the AA/AC genotype significantly associated with a higher number of depressive episodes compared to the CC genotype (AA/AC vs CC: $F_{1/410.9}$, $P_{1/40.001}$; sex: $F_{0.001}$, $P_{1/41.00}$; years since onset of first depressive episode: $F_{1/416.8}$, $P_{0.001}$; age: $F_{1/40.36}$, $P_{1/40.55}$; ANCOVA). There was a similar effect in A/C allelic model, with the A allele conferring a risk for an increased number of depressive episodes. No association between the number of depressive episodes and the rs2736100 was found in the nonLiR group.

Saliva TL was shorter in those with a history of depression

The individuals with depression had significantly shorter saliva TL compared to the controls, after adjusting for age, sex and alcohol use, occurrence of somatic disease and experience of childhood adversity. Age was associated with TL in the control group such that higher age corresponded to shorter TL ($P=0.031$). However, in the depressed sample, there was no association between age and TL.

Summary of findings in Study III

- **rs2736100 polymorphism (a genetic variant) previously associated with short TL was associated with depression.**
- **rs2736100 polymorphism was also associated with the number of depressive episodes in bipolar type 1 patients responding very well to lithium treatment.**
- **Saliva telomere length was shorter in those with a history of depression without any previous trauma compared to healthy controls.**

5.2.3 Results of Study IV

A prospective study of long-term lithium treatment and telomere length in bipolar disorder

LTL shortening was slower in lithium-treated bipolar patients than in healthy controls

A one-way repeated measures Anova was conducted to compare LTL between baseline and follow-up. In both bipolar patients and controls there was a significant reduction in LTL during the study period (bipolar patients: $n = 55$; LTL = 1.28 vs. 1.07 relative quantity; Wilk's Lambda = 0.60, $F(1, 30) = 19.8$, $p < 0.001$, multiple partial Eta squared = 0.397; controls: $n=100$; LTL = 2.83 vs. 2.04 relative quantity; Wilk's Lambda = 0.879, $F(1, 13) = 18.2$, $p < 0.001$, multiple partial Eta squared = 0.0121). The total reduction of LTL between baseline and follow-up for bipolar patients was 26% during an average time of 11.0 years (2.1% per year) versus 33% during 9.0 years for controls (3.7% per year; $t = -4.006$, $p = 0.01$).

Total time on lithium had a decelerating effect on telomere shortening independent of confounders

In the bipolar sample ($n=55$), multiple linear regression analysis was applied to study predictors for difference of LTL from baseline to follow-up. Using the LTL changes measured as the difference of LTL (baseline- minus follow-up) as a dependent variable, age at baseline had a positive effect ($t = 2.23$, $p = 0.031$) while the total time on lithium between tests had a negative effect on the LTL reduction ($t = -2.03$, $p = 0.049$; table 2). Neither smoking nor severity of illness (the number of episodes, severity of episodes, and duration of episodes) had significant impact on the LTL change (table 2). Using the ratio of LTL (baseline/follow-up) as a dependent variable, the age at baseline remained positively associated ($t = 2.17$, $p = 0.017$) while time on lithium between tests was not significant but showed a trend towards a negative association ($t = -1.86$, $p = 0.071$). The lack of association between smoking, alcohol use, age or the severity of illness and the LTL reduction still remained. In controls, using the difference in LTL between follow-up and baseline as a dependent variable, neither age, BMI, smoking nor physical activity at baseline were associated with LTL change.

LTL was not dependent on the number of leukocytes or the leukocyte types

In the bipolar patients ($n=55$), using a Spearman's correlation analysis, there was no association between the number of leukocytes (total leukocytes or leukocyte subtypes) and LTL at the follow-up ($\rho < -0.265$, $p > 0.055$). This was verified using a multiple linear

regression analysis (total leukocytes $p = 0.792$; lymphocytes $p = 0.789$; monocytes $p = 0.717$; neutrophils $p = 0.749$; eosinophils $p = 0.460$; basophiles $p = 0.661$).

Summary of findings in Study IV

- **Leukocyte telomere length shortening over 10 years was slower in lithium-treated bipolar patients than in healthy controls**
- **Lithium treatment had a decelerating effect on telomere shortening independent of other confounders**
- **Relative leukocyte telomere length was not dependent on the number of leukocytes or the leukocyte types at sampling**

5.3 CANCER INCIDENCE IN BIPOLAR DISORDER AND LITHIUM TREATMENT

5.3.1 Results of Study V

Lithium treatment and cancer incidence in bipolar disorder

No increase in overall cancer risk in bipolar patients in the age span of 50-84 years with or without lithium treatment compared to the general population

There was no difference in risk of overall cancer, neither in bipolar patients with lithium treatment compared to the population (IRR 1.04, 95% CI 0.89-1.23) or in bipolar patients without lithium treatment compared to the population (IRR 1.03, 95% CI 0.89-1.19). The study comprised 2 593 011 subjects (10 992 446 person-years) in the general population in the age-span of 50-84 years. N=2 393 bipolar patients with lithium treatment and N=3 049 without lithium treatment. There were N=327 (6.0%) cancer cases in bipolar patients and N=166 443 (6.4%) cases in the general population. In bipolar patients with lithium treatment, there were 142 (5.9%) cancer cases compared to 185 (6.0%) in those without lithium treatment.

Increased cancer risk in three specific organs in bipolar patients without lithium treatment

The cancer risk was significantly increased in the digestive organs (C15-NC26) in bipolar patients without lithium treatment (IRR 1.47, 95% CI 1.12-1.93), but there was no significant increase in bipolar patients with lithium compared the population (IRR 1.34, 95% CI 0.96-1.87). In the respiratory system and the intrathoracic organs (C30-C39), the cancer risk was significantly increased in bipolar patients without lithium treatment (IRR 1.72, 95% CI 1.11-2.66), but not in bipolar patients with lithium compared the population (IRR 1.23, 95% CI 0.68-2.22). The cancer risk was significantly increased in endocrine glands and related structures (C73-C75) in bipolar patients without lithium (IRR 2.60, 95% CI 1.24-5.47), but not in bipolar patients with lithium (IRR 1.41, 95% CI 0.45-4.36) compared to the population. No differences in risk were found in site-specific cancers in skin, breast, female genital, male genital, cancer, urinary, eye, brain and central nervous system, malignant neoplasms, secondary and ill-defined, lymphoid, haematopoietic or related tissue in bipolar patients compared to the population regardless of lithium exposure. When comparing cancer types from these sites case by case, no specific cancer type was substantially overrepresented.

Summary of findings in Study V

- **There was no increase in overall cancer risk in bipolar patients in the age span of 50-84 years with or without lithium treatment compared to the general population.**
- **There was an increased cancer risk in three specific organs in bipolar patients without lithium treatment. Those organs were: 1) the digestive organs 2) the respiratory system and intrathoracic organs and 3) the endocrine glands.**

6 DISCUSSION

6.1 GENETICS OF LITHIUM RESPONSE

6.1.1 Discussion on main findings in Study I

In Study I, we posed the question of whether there were any common gene variants associated with lithium response in the ConLiGen cohort. The most important finding was the association of lithium response and four common gene variants linked to a region on chromosome 21 containing two genes of long, non-coding RNA. This finding suggests a novel potential mechanism of action for lithium. I consider this pathway biologically interesting, since lncRNAs seem to be important in the regulation of genes in the brain (164). Interestingly, several studies suggested that lncRNAs play a role in BD, circadian rhythms, neurogenesis and aging (163-166). In an independent, prospectively followed clinical sample presented in Study I, the identified genetic markers also helped predict relapse during lithium treatment. However, in order to weigh the importance of this finding and understand the relationship between lncRNAs and lithium response, further translational studies are needed.

There is a wide knowledge gap about the genetics of lithium response in bipolar disorder (33, 65). Many genetic association studies of lithium response have been conducted, but samples were small, and findings have been difficult to replicate (43, 167). Four genome-wide association studies (GWAS) of lithium response were published before study I, each of them comprising different loci (65, 72, 76, 168). If replicated and translated into studies of biological functions, these findings from GWAS might provide important insights for the biology of lithium response in BD.

6.1.2 Strengths and limitations of Study I

Limitations

Study I had several limitations. The most important limitations included: 1) ConLiGen relies on retrospective ratings of treatment response, which lack precision and are subject to recall bias. This is a common problem in this type of study, however, and the members of ConLiGen have tried to minimize it by rating lithium response using a well-validated instrument (79) previously shown to be reliable (80); 2) The findings are only associated gene variants that must be replicated and then translated into biology. Previous GWAS of psychiatric traits including lithium response have had difficulties attaining these replications and continuing further into translational studies. One must also bear in mind that the explanatory power of each associated SNP is very low; 3) as for any GWAS of a complex disorder such as BD, the sample size is important. The ConLiGen sample size appears small when compared to larger GWAS of categorical disease phenotypes, such as BD and SCZ mentioned above (27, 28) where samples around 10 000 subjects are often required.

However, with narrower phenotypes like lithium response, samples of 2500 cases are considered relatively large (169).

Strengths

An important strength of Study I was the variety of patients from a range of ancestries and clinical settings. This is representative of real-world clinical situations, where patients present at various stages of BD and with a spectrum of illness severity. Another strength is the use of the valuated Alda-scale, which makes the ratings of lithium response more robust and the results more convincing compared to other studies relying on clinical estimations or on self-rated lithium response only.

6.1.3 Implications of Study I

Study I concludes that a deeper understanding of the mechanisms behind lithium response can be achieved through international collaborations that combine clinical expertise with large-scale genetic studies. The study identified new genetic markers for lithium response that also showed predictive value in a prospective clinical sample. This implication is causally uncertain, since the allelic variation at the associated SNPs still has to be linked to expression or function of the transcripts. Further studies are needed to evaluate whether there is real clinical use of the suggested markers and also to understand their biological context.

The results of Study I do not change any policy or practice, although they constitute a piece of a larger puzzle and represent a clear step forward towards greater knowledge of the biology of lithium treatment and BD. Every positive finding means more hope for bipolar patients and must be reported and acknowledged.

6.2 TELOMERE LENGTH IN BIPOLAR DISORDER AND LITHIUM RESPONSE

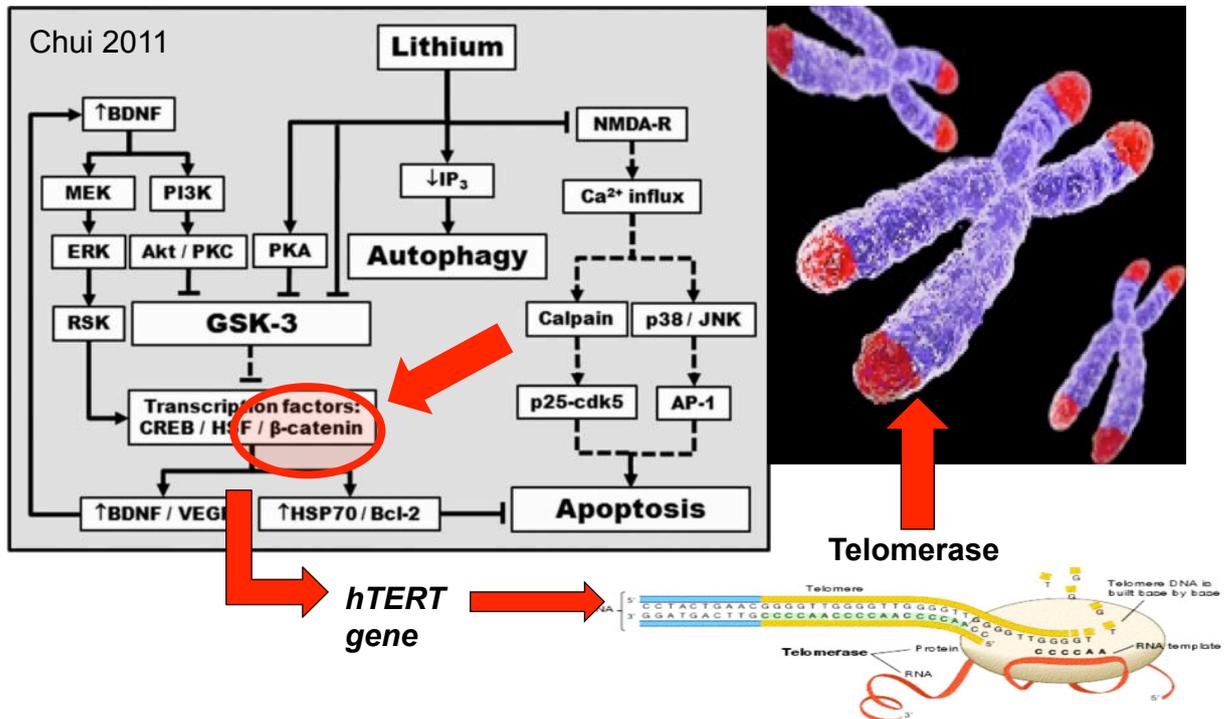
6.2.1 Discussion on main Findings in Studies II, III and IV

The most important and surprising finding in Studies II-IV was the new discovery that lithium associates with longer leukocyte telomeres in Study II and the important replication of that phenomenon in the prospective Study IV, where each subject was its own control. The association of long-term lithium treatment with long telomeres in Study II was especially pronounced in lithium responders, although this effect did not appear in Study IV, possibly due to the small sample size. As expected, and in line with previous studies, short TL was associated with depression in Studies II and III and the short-TL-risk-allele of *hTERT* was associated with depression, both in MDD and BD1. Study II was the very first study of TL in BD1 and in lithium treatment. Only one small study on BD2 had previously investigated TL and found short TL. In line with previous research on psychiatric disorders, one would have expected the TL to be short in our bipolar sample as well. When we stratified our sample by treatment, we saw the positive correlation between lithium and LTL. Lithium is known to induce leukocytosis (170, 171) but could this explain the huge difference in relative LTL between patients with BD and lithium treatment and healthy controls? The growing evidence of lithium's neuroprotecting effects, and previous results from studies on the effects of antidepressants on telomerase (108) led us into discussions and further reading of the literature in search of an explanatory mechanism. Lithium is known to activate β -catenin (directly and via inhibition of GSK3 β). In the literature of cancer and telomere biology, we found that betacatenin, an important protein of the Wnt pathway that regulates the coordination of cell-cell adhesion and gene transcription (172), increases the TA (102). Dysfunction of betacatenin has been implicated in several psychiatric disorders (87) We hypothesized that this is one possible mechanism for lithium to activate the telomerase and thereby increase the TL, although several molecules in lithium's pathways may be involved. These mechanisms have also recently been reviewed and discussed by others (87, 161) and must be further investigated.

After Study II was published, several studies have replicated its findings (83, 87, 161, 173). Most importantly, an animal study done by my colleagues investigating TA in lithium-fed rats confirmed our hypothesis that lithium activates telomerase in the brain (159). It also supported our finding in Studies II and III that depression is associated with short telomeres (159), by demonstrating short telomeres and reduced *Tert* expression and TA in the hippocampi of Flinders Sensitive Line rats with depression-like behavior (a genetic model of depression), compared to Flinders Resistant Line rats. The same study also found that lithium administration for 6 weeks significantly increased *Tert* expression and TA in the hippocampi of the "depressed" rats, thereby normalizing their baseline abnormalities. Recently, one study of TL in postmortem human brains from people who had suffered different psychiatric disorders was published, showing short telomeres in the hippocampus (160). Furthermore, a Brazilian group performed a study of TA in lithium-treated bipolar patients, although the study did not show any significant change, possibly due to its short time frame (174). Several

studies that investigated TL in BD and treatment have suggested longitudinal studies (161), but to date no such follow-up has been published. To our knowledge, Study IV provides the first long-term result of TL change in BD and lithium treatment.

Lithium's possible effects on telomere biology



Adapted from Chui 2011, Chuang 2011, Zhang 2012

Figure 9. Effects of lithium on telomere biology.

6.2.2 Strengths and limitations in Studies II, III and IV

Limitations

The most important limitations in Studies II-IV included 1) the lack of a control group of bipolar patients without lithium treatment. Lithium is the golden standard for BD1, which was the dominant diagnosis of our cohort. However, selecting such controls in Sweden would be strongly biased, since only a few BD1 cases never tried lithium for certain reasons (36, 175); 2) confounders previously associated with short LTL, such as cigarette smoking, obesity, inflammation and somatic diseases could not be controlled for in Studies II and III since data were not available, but those were controlled for in Study IV and had no significant impact on LTL prospectively. Furthermore, influence of those confounders would probably affect the results in the opposite direction and contribute to shorter TL in bipolar patients

compared to controls; 3) “the number of depressive episodes” and “the number of manic or hypomanic episodes” larger than six was indicated as ‘6’ in studies II and III but not in Study IV; 4) TL was measured in a peripheral tissue and not in the brain where lithium is considered to exert its therapeutic action. However, this is an established method and there are studies suggesting that TL correlates between blood and brain (89, 176-179); 5) we cannot rule out the possibility that the individuals with depression from the PART study may include some bipolar disorder patients, since mania/hypomania symptoms data were not available from our questionnaires; 6) recall bias of childhood adversity existed in the PART cohort but was limited (180); 7) the sample size in Study IV was small and the some patients dropped out before follow-up. However, considering the long study period, the number of subjects fulfilling the inclusion criteria and contributing with annual follow-ups and a second sample of TL was relatively large; 8) in line with previous studies of LTL in psychiatric disorders, blood cell types were not controlled for in either patients or controls in Study II or III. However, cell types were controlled for in bipolar patients in Study IV a, since distributions of leukocytes might have different TLs (181, 182); 9) data for most added medications and food was not available in Studies II and III and could not be controlled for, but in Study IV many but not all medications could be controlled for. For example, there is preliminary evidence that statins, certain hormones and caffeine may impact LTL (183-185). 10) A limitation of Study IV was the small sample size. However, considering that case drop-outs and exclusions due to different clinical events are common in these types of long-term studies, the number of bipolar patients fulfilling the long study period was quite large and clinical examinations were frequent (twice yearly). 11) Another limitation was the high mean age of the patients, although two controls matched for age were selected for most of the cases. 12) The usage of different procedures for DNA-extraction in bipolar cases and controls is a weakness and might provide different LTL between bipolar cases and controls. We dealt with this methodological limitation by measuring the change in individual LTL relative baseline ratio. Also, the measurements were performed at the same time for bipolar patients and controls, with baseline and follow-up samples in the same plate.

Strengths

Some important strengths of studies II-IV were 1) the timing of Study II (contributing with the first finding of a correlation between lithium and long TL), the timing of Study III (supporting the mechanistic pathway of lithium via *hTERT* activation in close relation with the animal study of increased TA in hippocampi of lithium-treated rats (159)) and the timing of Study IV, although not published; presenting the first results of a longitudinal of LTL change in lithium-treated bipolar patients that support our previous findings. 2) As a quality control, patients with BD on lithium monotherapy were studied as a subgroup in Study I, which gave support to the data showing that longer LTL after lithium treatment was not a consequence of other drugs; 3) the relatively large study samples of Studies II and III; 4) the long study period in Study IV with frequent clinical examinations and where both bipolar

patients and healthy controls constituted their own personal control of LTL change; 5) differential leukocyte count was controlled for at follow-up in the bipolar patients in Study IV, thereby precluding that the relative LTL change in the patients would be significantly dependent on the distribution of leukocyte types, as has previously been argued with regard to lithium's suggested induction of leukocytosis (170, 171); 6) chronic inflammation as a confounder of LTL at follow-up was excluded by measuring hsCRP in Study IV and 7) several potential confounders were controlled for in this study, such as age, gender, smoking, alcohol abuse, BMI, chronic somatic diseases, time on lithium before baseline and number, severity and part (%) of time of depressions and hypomanias/manias.

6.2.3 Implications of Studies II, III and IV

Study I was the very first study to report an association between lithium treatment in BD patients and LTL. Studies II and IV added support to this association and several studies from different parts of the world have replicated this finding. However, it remains unclear whether telomere biology has any importance for lithium's effects in BD or for lithium response. Interestingly, telomerase has been proven to have other important tasks in the cell, meaning that this enzyme is responsible not only for making telomeres longer, but also for antioxidant effects in the mitochondria (186). Additionally, telomerase has recently been suggested as a possible mechanism of action for psychopharmacological interventions by drugs including lithium (87). For example, the TA pattern in lithium treatment or a long TL from heritage may work as predictors of good lithium response. In that case, TA and TL may function as longed-for clinical biomarkers for lithium response in the future. F

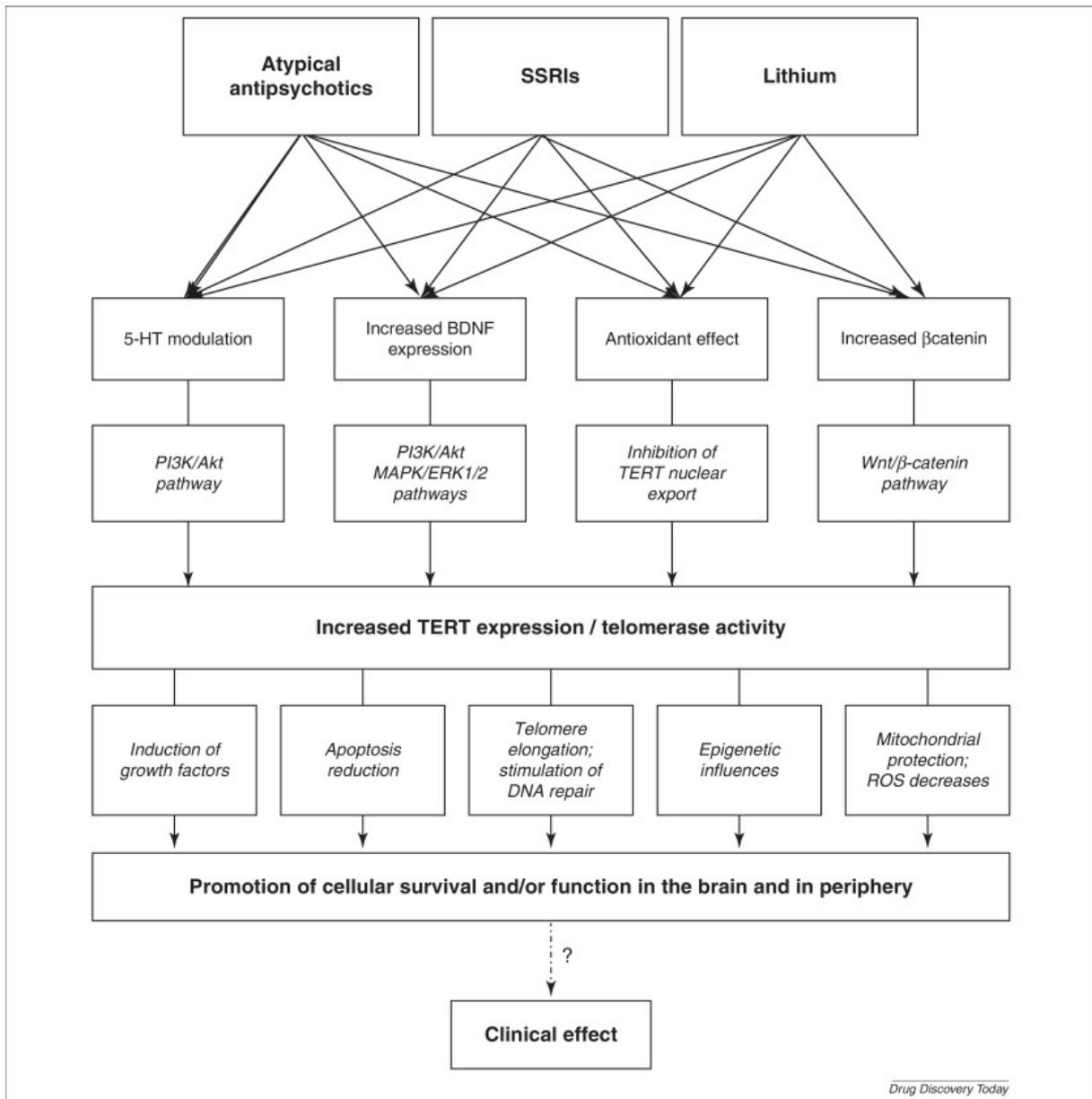


Figure 10. Some telomerase or *TERT*-related pathways that can link psychiatric medications with their clinical effects. Lithium can modulate telomerase activity. Summary of: (i) proposed mechanisms by which psychopharmacological interventions can modulate telomerase activity or *TERT* expression (top); and (ii) proposed mechanisms by which telomerase activation or *TERT* expression can promote cellular survival and/or function in the brain and periphery, eventually contributing to the clinical effects of these interventions (bottom). *Abbreviations:* Akt, protein kinase B; BDNF, brain-derived neurotrophic factor; ERK1/2, extracellular signal-regulated kinases 1 and 2; 5-HT, serotonin; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-3-OH kinase; ROS, reactive oxygen species; SSRIs, selective serotonin reuptake inhibitors; *TERT*, telomerase reverse transcriptase. Figure used with permission of the publisher and the authors, Bersani et al 2015 (87).

6.3 CANCER IN BIPOLAR DISORDER AND LITHIUM TREATMENT

6.3.1 Discussion on Main Findings in Study V

BD is a group at high risk of somatic comorbidities, although previous studies have been inconsistent. The recent alarms for increased kidney cancer in lithium-treated bipolar patients (139-142), together with our new hypothesis of lithium's possible influence on TL from Studies II-IV, gave rise to the question of whether lithium treatment might affect the cancer incidence in BD. The most important finding in Study V was that neither the total cancer risk nor any risk of specific cancer differed from the general population. Importantly, when focusing on cancer incidence in specific organs, there was no difference between lithium-treated patients and the general population in contrast to the small studies reporting increased cancer risk in kidneys with lithium treatment. These findings are in line with results from two register studies recently published which show no significant risk increase of kidney cancer in lithium-treated patients (145, 146). Interestingly, bipolar patients without lithium treatment showed increased cancer incidence in three specific organs, but causality could not be investigated with the present design.

6.3.2 Strengths and limitations

Limitations

The most important limitations of Study V included: 1) When comparing risk subgroups in bipolar patients (those using lithium compared to those not using lithium), the number of cancer cases were few with wide confidence intervals; 2) the assessment of lithium exposure was made in the DPR in the same time frame as the assessment of the outcome as opposed to before. However, we assumed that lithium-treated patients between the ages of 50 and 84 were likely exposed to lithium for several years before inclusion in the study; 3) the many confounding factors influencing cancer incidence (such as alcohol intake, food behavior patterns, obesity, somatic disorders, social group belonging, smoking habits, and physical activity). Such factors might influence the results when comparing the general population to bipolar patients, and also when comparing lithium-treated patients to other bipolar patients, due to different lifestyles (134, 187). After my half-time control, our group had a discussion on how to deal with these confounding factors, but it remained that these factors could not be controlled for in this study; 4) the presence of BD might delay the diagnosis of cancer and other somatic diseases, which might decrease the cancer incidence rate in the bipolar group with the present design (137); 5) misclassification of exposure to treatment because individuals who were classified as patients without lithium could have had lithium treatment before inclusion in the study, since register data was only available since 2005; 6) it is unclear whether the bipolar patients in this cohort have delayed seeking help, or to what extent the follow-ups at psychiatric ward units compensate for this matter. We assumed that treatment

was continuous for one year once lithium was prescribed. Although this could not be controlled for, most patients collected 3-4 prescriptions per year; 7) the design of this study meant that the incidence of specific cancer types commonly occurring before the age of 50 could not be studied.

Strengths

The most important strength of Study V was the large size of the cohort and timing of the study. When we decided to conduct the study, there was an obvious knowledge gap to fill. Interestingly, at the time of acceptance for publication of the article, the clinical guidelines for lithium had already been changed with added warnings of kidney cancer. Therefore there is currently a great need for trustworthy information regarding the issue of whether lithium increases the cancer risk in BD. The importance of Study V has developed from generally interesting to clinically relevant for lithium-treated patients.

6.3.3 Implications of Study V

Recently, the Swedish guidelines for prescription and use of Lithionit, (the only lithium tablets on the Swedish market) was changed on www.FASS.se. Below “side-effects” and “warnings and caution” there is now a new text inserted about microcysts, onkocytomas and renal cell cancer with reference to a few small studies suggesting increased cancer in lithium treatment (139-142). However, clinicians treating bipolar disorders around the world have argued that their impression is that cancer is uncommon in lithium-treated patients (143, 144). Furthermore, they claim that these studies are too small to be able to detect any true risk of kidney cancer caused by lithium (143, 144). I agree with the experienced psychiatrists who doubt that lithium causes cancer. Two recently published register studies from Denmark stated, in line with results in Study V, that renal cancer was not increased in lithium-treated patients (145, 146). A considerable strength of Study V is the large sample size. Even a small increase of the cancer risk would probably have been detected in long-term treated bipolar patients in the studied age span. I do trust in our results, which show no specific cancer risk increase in the kidneys of bipolar patients compared to the general population. Policies might change because of the warnings of lithium’s possible effect on cancer. I have realized the importance and urgency of presenting the findings in Study V, thereby contributing data to the discussion and refuting the previous unsound results and preventing their potentially harmful clinical consequences. Despite lithium’s powerfully beneficial effect on one third of bipolar patients, its uniqueness in suicide prevention and its cheap ecologic properties, lithium treatment has experienced a bad reputation throughout the years. These rumors might be due to the severe side effects caused (36) when lithium was used in higher serum-concentrations and on effective PR from the industries marketing competing mood stabilizers and antipsychotics of the last decades. It is of high clinical importance to avoid fear of lithium

treatment. My recommendation is to continue lithium treatment and trust in evidence from large studies like Study V and others (145, 146).

Psychiatric disorders, including bipolar disorder, also bring with them a large burden of additional somatic disorders (188). Considering this, one would expect larger differences in cancer risk between bipolar patients and the general population. Together with my supervisor, I have speculated as to the possible protective and harmful mechanisms. Are there any protective mechanisms against cancer in bipolar disorder or in lithium treatments as have been suggested? Such protecting effects (147-151) might reduce the over-risk of developing cancer in our sample. The difference in cancer risk in three specific organic systems between lithium-treated patients and bipolar patients found in study V might be due to factors such as different lifestyles of the subgroups or weaknesses in the study design. On the other hand, it is possible that lithium treatment in itself provides some protection. Biological studies are needed to test lithium's effect on the cells from different organic systems.

7 CONCLUSIONS

This thesis presented novel findings from three research areas within the field of bipolar disorder and lithium treatment: *Genetics, Telomere length and Cancer incidence*.

Genetics of lithium response in bipolar disorder

Study I showed association of a single locus with four linked common gene variants (SNPs) on chromosome 21 with lithium response in a large cohort of bipolar patients. The associated gene region contains long, non-coding RNAs with potential importance for the gene regulation in the brain in BD.

This finding added a new piece to the big puzzle of heritability of lithium response in bipolar disorder and should be replicated and translated into a biological context. Additionally, Study I demonstrated the great value of international multicenter collaborations.

Telomere length in bipolar disorder and lithium treatment

Study II found that that long-term lithium treatment was associated with long LTL. In individuals treated for more than 30 months, LTL was 35 % longer than in healthy controls. Lithium responders had 10% longer LTL than non-responders. In individuals treated longer than 30 months, LTL correlated positively with time on lithium treatment. Short telomeres associated with the number of depressive episodes. This was the very first study on telomere length in lithium-treated patients, implying that lithium might affect the telomere attrition.

Study III found that the “short-telomere-polymorphism” of the *hTERT*-gene (rs2736100) is also associated with the number of depressive episodes in bipolar type1 lithium responders and with unipolar depression. Saliva telomere length was shorter in those with a history of depression without any previous trauma compared to healthy controls. This study added further support to the hypothesis that depression is linked to telomere biology.

Study IV found a significant decrease of LTL in both bipolar patients and controls over 10 years. Lithium had a significant decelerating effect on LTL independent of other confounding factors. LTL was not dependent on total number of or type of leukocytes at sampling. This study was the first longitudinal follow-up on TL change with lithium treatment.

Findings in Studies II, III and IV in this thesis suggest that lithium decelerates telomere shortening. Telomere biology might be of importance in lithium response and has to be studied further.

Cancer in bipolar disorder and lithium treatment

Study V showed that the overall cancer incidence was not increased by lithium treatment. For cancer in digestive organs, respiratory system and intrathoracic organs and the endocrine glands, the risk was increased in bipolar patients without lithium treatment.

Biological in vitro studies are needed to test lithium's effect on human cells from different organic systems. A clinical implication of lack of difference in cancer incidence is that recently added warnings of renal cancers with long-term lithium treatment are unnecessary and should be changed in the current policies.

8 FUTURE PERSPECTIVES

Several questions related to the findings of this thesis remain. I suggest the following focuses of future studies:

Genetic factors of lithium response

- Replication of associated polymorphisms with lithium response and functional studies of the gene transcripts.
- Larger samples of GWAS of lithium response and stringent ratings of patients to increase accuracy when finding additional genetic factors.

Lithium and telomeres

- Prospective studies of drug-naïve bipolar patients and analyses of the TA patterns – before and after days, weeks and months – compared to other medications, since telomerase might be a useful biomarker of lithium response.
- Studies of neurons derived from fibroblasts (through iPSC) from lithium responders compared to other subtypes of BD. From skin biopsies, a new type of neuroepithelial stem cells (NES) derived from patient derived induced pluripotent stem cells (iPSC) can be transformed into mature human neurons. These neurons can be analysed by immunofluorescence to determine neuronal composition, neuronal connectivity by virus transmission between neurons, neural arborisation and synaptic composition. Additionally, complete transcriptomic analyses by RNA sequencing can be performed. Lithium can be added to the culture media of differentiated neurons thereby allowing lithium-exposed neurons to be studied.
- Investigation of the “moonlighting enzyme activities” of telomerase and its possible effect on neurogenesis. This mechanism might in fact be an important key involved in lithium response (87).
- Investigation of cell type-specific telomere dysregulation in BD in the brain, since results regarding cell types-specific TL and TA in the brain are sparse.
- Replication of association of *hTERT* and lithium response in human studies in order to weigh the importance of previous results.
- Comparison of TL and TA in BD1 cohorts without lithium treatment, for example in other countries than Sweden.

- Further test of lithium-regulated molecules with a role in telomere biology, for association to lithium response, e.g. common gene variants of Akt1 (87).

Lithium and cancer

- In vitro studies of lithium's effect on cells in relation to cancer, in order to test the hypothesis that lithium might protect cells from cancer.
- Replication of register studies on cancer incidence in different organic systems in lithium-treated cohorts to provide replicated and robust evidence that lithium does not cause cancer in any part of the body or increase the cancer risk.

Other aspects of lithium mechanisms

- Randomized Clinical Trials (RCT) of lithium treatment in other disorders, such as mild cognitive impairment in Alzheimer's disease and spinocerebellar ataxia. Lithium is proved to have effects that might protect from disorders including Alzheimer's disease (37), although controlled intervention studies in early stages have not been performed. Lithium-treated patients with spinocerebellar ataxia ("Machado-Joseph disease") presented less severe progressions compared to placebo in a recent study (38, 39). Lithium's neuroprotective effects and its influence on telomere biology could be important factors in diseases other than mood disorders.
- RCTs of lithium treatment and add-ons of anti-inflammatory drugs, since inflammation and diabetes type 2 have been linked to bipolar disorder and lithium (152, 189).
- Trials of lithium treatment in combination with regular physical activity, because obesity and diabetes type 2 have been linked to bipolar disorder and less response to lithium (190).

9 SVENSK SAMMANFATTNING

Litiumrespons och dess biologi vid bipolär sjukdom - genetiska mekanismer och telomerer

Bakgrund: Bipolär sjukdom är en vanlig, kronisk och allvarlig psykisk sjukdom som orsakar stort lidande och höga kostnader. Litiumbehandling är förstahandsval och fungerar hos 2/3 av patienterna, varav hälften med utmärkt effekt kallas "litiumreponders". Starka bevis finns för att såväl bipolär sjukdom som litiumrespons är ärftligt, men mekanismerna är okända. Korta telomerer har konstaterats vid flera psykiatriska sjukdomar, men telomerlängd har inte undersöks vid litiumbehandling hos individer med bipolär sjukdom. Små studier som rapporterat ökad njurcancer hos litiumbehandlade patienter har nyligen lett till ökade varningar och förändrade behandlingsrekommendationer för litium, trots motargument från kliniker och forskare inom området. Det finns ett stort kliniskt behov av stora registerstudier som på ett tillförlitligt sätt kan utvärdera om litium ökar cancerrisken eller ej.

Syfte: Det övergripande syftet var att öka kunskapen om genetiska mekanismer vid bipolär sjukdom och litiumrespons. Specifika syften var att leta efter nya genetiska associationer vid litiumrespons, att undersöka telomerlängden vid bipolär sjukdom och litiumrespons och att undersöka cancerincidensen vid bipolär sjukdom och litiumbehandling.

Metod: Studie I är en genomvid associationsstudie om litiumrespons. Studie II är en retrospektiv kohortstudie om telomerlängd hos litiumbehandlade bipolära patienter. Studie III innehåller dels en associationsstudie av en genvariant av *hTERT*-genen (tidigare associerad med kort telomerlängd) och depression och dels en retrospektiv kohortstudie av telomerlängd i saliv vid depression. Studie IV är en longitudinell studie av telomerlängd av litiumbehandlande bipolära patienter. Studie V är en registerstudie om cancerincidens hos bipolära patienter med och utan litium jämfört med normalbefolkningen.

Resultat: I) Ett genområde på kromosom 21 innehållande "long, non-coding RNA" med betydelse för hjärnans genreglering var associerat med litiumrespons. **II)** Den relativa telomerlängden i vita blodkroppar var 35 % längre i litiumbehandlade bipolära patienter jämfört med friska kontroller och korrelerade till tid på litiumbehandling för dem som haft litium i mer än 2,5 år. Litiumreponders hade 10 % längre telomerer än icke-reponders. **III)** Den studerade genvarianten var associerad med antalet depressioner hos patienter med bipolär sjukdom typ I och hos individer med unipolär depression. Telomerlängd från saliv var kortare hos dem med unipolär depression utan föregående barndomstrauma. **IV)** Den relativa telomerlängden i vita blodkroppar minskade signifikant under 10 år hos både bipolära patienter och friska kontroller. Åldern vid studiestart hade signifikant påskyndande och litiumbehandlingens längd tid hade emellertid signifikant inbromsande effekt på telomerlängden oberoende av andra confounders. Antalet och sorten av vita blodkroppar vid provtagningstillfället hade ingen inverkan på telomerlängden i vita blodkroppar. **V)** Ingen

ökad förekomst av total eller organspecifik cancer hos litiumbehandlade bipolära patienter jämfört med befolkningen.

Slutsatser: **1)** Associationen mellan litiumrespons och genvarianter innehållande ”long, non-coding RNA” med betydelse för hjärnans genreglering, är en ny pusselbit för kunskapen om litiumrespons vid bipolär sjukdom. Resultaten måste replikeras och studeras vidare i biologiska sammanhang. **2)** Den nya upptäckten litiumbehandling har en inbromsande effekt på telomerförkortning talar för att telomerbiologi kan innefatta viktiga mekanismer som är inblandade vid litiumrespons och behöver studeras vidare. **3)** Vi föreslår med stöd av resultaten från vår registerstudie om cancer att de onödiga och missvisande varningarna för ökad cancerförekomst hos litiumbehandlade patienter ändras i nuvarande behandlingsrekommendation.

Nyckelord: Bipolär, litiumrespons, GWAS, DNA, telomerlängd, telomeras, telomerasaktivitet, *hTERT*, cancerincidens, registerstudie

10 PERSONAL REFLECTIONS

This chapter consists of a few reflections on what I learned from the studies.

Study I

The Alda-scale

Our group contributed with one third of the total sample to the international consortium of ConLiGen (78, 80). By participating in Study I, I learned how to use the Alda-scale and how to interpret its results (79). I worked closely with my supervisors and the psychiatric research nurse and took part in ConLiGen's strategic and structured valuation of the Alda-scale (80). In parallel with Study I, I worked as a clinician in the in-patient and out-patient ward of the Affective Unit at Psychiatry South west. In my everyday clinical work, I met the individuals who were included in our bipolar patient cohort in person. Gradually, as my clinical experience and knowledge of BD and lithium treatment grew, I understood the usefulness of the Alda-scale, both clinically and academically.

The GWAS era

Study I was administered during the era of big steps forward for GWAS with the trend of cooperation rather than competition in large studies. Although I was only one of many co-authors, it was educational for me to gain insights of how a GWAS can be managed. I was impressed by the sophisticated management skills of the main authors in Study I. At the same time, I also had the opportunity to attend two international congresses in the field of psychiatric genetics (WCPG 2012 in Hamburg and 2013 in Boston) and two clinical congresses (APA 2010 in New Orleans and 2015 in Toronto) where I followed the development of GWAS collaborations in different psychiatric disorders. During those years, large GWAS were established and succeeded in presenting significant findings, primarily in SCZ (27) but also in BD (28). Another insight was the great importance of a well-defined sample resulting in clinically relevant phenotypes and generalizable findings.

Studies II, III and IV

Participating in Studies II-IV was very valuable for me both as a psychiatrist and as a researcher. As it stood clear that bipolar patients in Study II had unexpectedly long telomeres compared to healthy controls, we first thought it was some kind of artifact. After recalculations, we suspected that lithium was involved. During monthly seminars with the research group, I was responsible for a concrete part of the design, analyses, discussions and interpretations of these studies. I was involved from discussions of the very first idea until

writing and the happy celebration of completing Studies II and III. I collaborated closely with competent nurses in rating the patients' lithium response with the Alda-scale. This gave me deepened clinical insights into lithium response and fluctuations of BD. I appreciated the Alda-scale as a useful instrument with both dichotomous and continuous rating of lithium response. Furthermore, the Alda-score can be communicated to the patient and thereby initiate relevant clinical discussions and decisions about treatment for BD. To obtain knowledge of the laboratory methods and procedures, I was mentored in the molecular lab at CMM for two weeks during the preparation of TL measurements by my skilled colleague, Dr Wei. I also experienced the importance of planning and agreeing on a study and on the documentation of its results before conducting the study.

Study V

Study V contributed to my doctoral studies with a study design different from the ones in Studies I-IV and taught me about registers. The authors of the study collaborated closely in designing the study. Although I independently wrote the article, I got helpful support from the other authors. I appreciated the routine of writing a meeting protocol when designing the study, defining research questions, methods, next steps and authorship of the study. Additionally, Study V reminded me that a result without significant differences might turn out to have a clinically positive result with important implications.

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