Pharmacogenetic Testing to Reduce Depression and Anxiety in Adults Treated with Low-Dose Lithium

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Abstract

Mental illnesses, including major depressive disorder, bipolar disorder, and generalized anxiety disorder, are leading causes of disability in the United States, affecting about one-third of Americans and one-fifth of Kansas City residents. A substantial number of patients with bipolar disorder are misdiagnosed with major depression. Clinical evidence supports the use of both pharmacogenetics and lithium for treating mood disorders and anxiety. This research aimed to determine whether lithium effectively decreases depression and anxiety in adults with a risk allele on *CACNA1C* genotypes. The study design was correlational. Fifty patients were treated at a nurse practitioner-owned clinic specializing in psychiatric services in Prairie Village, Kansas. Chart review was used to assess pharmacogenetics in guiding treatment with lithium therapy for depression and anxiety. Adults over 18 diagnosed with major depressive disorder, bipolar disorder, or generalized anxiety disorder presenting with an abnormality in the *CACNA1C* gene single-nucleotide polymorphism rs1006737 were included in this research. Patients were assessed by a nurse practitioner using the PHQ-9 for depression and GAD-7 for anxiety before and after treatment with low-dose lithium (≤ 600 mg/d). There was a significant reduction in depression and anxiety for all *CACNA1C* genotypes. Pharmacogenetic-guided treatment is an emerging field. This study could aid in establishing genetic testing as an effective clinical tool for treating depression and anxiety using lithium, an inexpensive and widely available medication.

Keywords: major depressive disorder, bipolar disorder, generalized anxiety disorder, *CACNA1C, ANK3,* pharmacogenetics, lithium, chaos theory, spectrum disorders

Pharmacogenetic Testing to Reduce Depression and Anxiety in Adults Treated with Low-Dose Lithium

Mental illness in the United States has considerable societal, economic, and personal costs. Misdiagnosis of major depressive disorder (MDD), bipolar disorder (BD), and generalized anxiety disorder (GAD) may lead to inappropriate and inadequate treatment. Genetic testing for mental health disorders has recently emerged as a tool to aid in medication management, which may help alleviate this problem, and improve pharmaceutical treatment.

Significance of Topic

The National Alliance on Mental Illness (NAMI) estimated that 8.4% of Americans over the age of 18 years have MDD, 2.8% have BD, and 19.1% have an anxiety disorder (National Alliance on Mental Illness [NAMI], 2022). About 6.1% experienced symptoms of generalized anxiety disorder (Centers for Disease Control and Prevention [CDC], 2020).

The costs to the US economy are substantial. According to Potter (2019), MDD has an annual cost of over $67 billion. Depression is the most common cause of disability affecting the workplace, and 7.7% of people with MDD were unemployed in 2018 (Greenberg et al., 2021; World Health Organization [WHO], 2021). These costs are exacerbated by the loss of about 5% of workers to suicide (Greenberg et al., 2021). Bipolar disorder costs over $195 billion annually (Bessonova et al., 2020), is the sixth leading cause of disability in the United States, and increases the risk of suicide (Moore, 2013). The cost of anxiety disorders, when direct medical and indirect expenses are combined, is over $240 billion annually (Konnopka & König, 2019).

Local Issue

Major depression, bipolar disorder, and anxiety substantially impact the Kansas City metropolitan area. According to a study from the Health Care Foundation of Kansas City,

untreated mental illness costs the greater metropolitan area $624 million annually (Health Forward Foundation [HFF], 2022). The same study reported that 6.6% of the local population have MDD, 2.7% have BD, and 10.2% have an anxiety disorder (HFF, 2022).

Costs to the sufferers of these mental health disorders are considerable. In addition to depressed mood, disability, and loss of work, people with MDD may present with anhedonia or loss of interest in pleasurable activities (Potter, 2019). Those with bipolar disorder, in addition to disability and loss of productivity, experience oscillations in mood that alternate between depression and euphoria (Moore, 2013). People with GAD lose productivity, have a lower quality of life, and worry incessantly (Brown & Tung, 2018; Plummer et al., 2016).

The United States lacks mental healthcare services, with 96% of all counties reporting insufficient providers (Thomas et al., 2009) and about 56% of the workers nearing retirement age (Association of American Medical Colleges, 2016), which is expected to exacerbate the problem. Nurse practitioners increasingly provide mental health care (Cai et al., 2022).

Diversity Impacts

Healthcare disparities are present in mental health research, access, diagnosis, and treatment. White Americans are more likely to participate in research, and minority groups are more likely to have limited access due to financial and educational disparities (NAMI, 2022). Black Americans with BD are more often misdiagnosed, mistreated, and untreated than other groups (NAMI, n.d.). Hispanic Americans with BD seek treatment less often, are more likely to be undertreated, and are most likely to suffer from depression (NAMI, n.d.; Rodriquez et al., 2018). Asian Americans are diagnosed with BD at a higher rate but are less likely to comply with pharmaceutical treatment (NAMI, n.d.). White Americans are more likely to suffer from anxiety (Vilsaint et al., 2019).

Problem and Purpose

Treatment-resistant depression (TRD) occurs when a patient does not respond to two or more antidepressant medication trials (Jaffe et al., 2019). One factor leading to TRD is the misdiagnosis of BD as MDD, which occurs in more than 40% of cases (Menezes et al., 2019; Stiles et al., 2018; Undurraga et al., 2019). Most mental health diagnoses rely on a trial-and-error approach using primarily subjective data in medication selection (Manchia et al., 2020; Rakofsky, 2018). Recent studies on the physiological causes of mental illness make genetic testing an option with the potential to improve the treatment of depression (Manchia et al., 2020). The calcium-regulator gene *CACNA1C* (see Appendix A for definitions) has been linked to BD, TRD, and anxiety, particularly in the context of childhood trauma (Lazary et al., 2021; Undurraga et al., 2019); therefore, genetic testing provides an objective tool to aid in the predisposition to certain mental health disorders that have traditionally relied on subjective measures (Manchia et al., 2020; Rakofsky, 2018). If depression, BD, and anxiety share a genetic basis and BD is often misdiagnosed as MDD, then treatment with lithium, a first-line treatment for BD, may effectively reduce depression and anxiety in patients affected by MDD and TRD. Ineffective treatment based on misdiagnosis is a gap in healthcare for patients with mood disorders and anxiety.

The purpose of this research is to determine whether low-dose lithium effectively decreases depression and anxiety in adults with a risk allele on *CACNA1C* genotypes treated at a nurse practitioner psychiatric clinic in Prairie Village, Kansas. The secondary purpose is to determine if the number of *A* alleles affects depression and anxiety scores before or after treatment with lithium. The primary null hypothesis is that there is no difference in depression and anxiety scores after low-dose lithium treatment compared with scores at baseline. The

secondary null hypothesis is that the number of *A* alleles does not affect depression and anxiety scores before or after treatment with low-dose lithium.

Barriers and Facilitators

Vassar and Holzmann (2013) identified barriers associated with medical chart reviews. The first barrier was formulating a poor research question that the available data could not answer. The second barrier involved sampling issues, including insufficient sample sizes or need to correctly consider the sampling strategy. A third barrier was failing to operationalize study variables that could be adequately measured (Vassar & Holzmann, 2013). Three of the barriers involved the reliability of the data collection, including varying data collection strategies employed by research team members, usage of different data tables, and the lack of a written procedure. The seventh barrier was the need to define inclusion and exclusion criteria. The final barrier identified was the need for a plan to address confidentiality and ethical concerns.

Chart review has important research facilitators. First, the raw data have previously been collected and stored, expediting the research timeline (Worster & Haines, 2004). Second, chart review allows research of questions inappropriate for randomization due to ethical considerations (Worster & Haines, 2004). Withholding treatment to patients who qualify for lithium therapy would be unethical if done for randomization in an RCT.

Review of the Evidence

Inquiry

In adults over 18 years of age diagnosed with a mood disorder or generalized anxiety disorder with the genotypical presentation of a risk allele on the *CACNA1C* gene, does low-dose lithium decrease depression and anxiety compared with baseline at a nurse practitioner clinic in Prairie Village, Kansas?

Search Strategy

The literature search was conducted using the PubMed database accessed through the University of Missouri-Kansas City library, the American Psychological Association (APA) Clinical Guidelines Website, and the CPIC Clinical Practice Guidelines website. Keywords included Boolean combinations of depression, unipolar depression, major depressive, resistant depression, anxiety, bipolar, bipolar 2, spectrum, biomarker, *CACNA1C*, lithium, and augmentation.

The initial search of the databases and practice guideline website repositories yielded 871 results. Automated search limiters included the English language and full-text articles with an abstract from 2005 to 2022. During the screening of abstracts, articles were excluded for lack of relevancy, including articles on multiple gene interaction effects; studies of genetic variations among subpopulations; disorders linked to *CACNA1C* unrelated to BD; depression, anxiety, or relevant psychiatric conditions; the effect of *CACNA1C* on physiology and anatomy unrelated to psychiatric disorders; and non-genetic biomarkers of mental illness.

Literature reviews, integrative reviews, letters to the editor, study protocols, and grey literature were excluded. Particular articles were excluded due to the saturation of the evidence for a topic. Thirty-three articles were chosen for the inquiry. These included 10 evidence level I articles encompassing three clinical guidelines; seven systematic reviews or meta-analyses of randomized control studies (RCTs); three level II RCTS; two well-designed quasi-experimental studies (level III); 14 cohort, case-control, or cross-sectional correlational studies (level IV); three level V studies including two systematic reviews or meta-analyses of level IV studies; one cross-sectional descriptive study; and one level VI cross-sectional descriptive study (see Appendix B for PRISMA diagram).

Evidence by Themes

The *pharmacogenetics of mood disorders* was the first theme that provided the rationale for targeting the *CACNA1C* gene for study. The concept of *mood spectrum disorders* was the second theme, providing the emerging evidence that depression, bipolar disorder, and anxiety disorders might be better understood as mental health states existing along a multidimensional continuum that may share genetic causes and treatment modalities. The third theme was the evidence that lithium is an effective therapy for unipolar depression, treatment-resistant depression, bipolar depression, and anxiety (see Appendix C).

Pharmacogenetics of Mood Disorders

Pharmacogenetics, also known as pharmacogenomics, personalized medicine, and precision medicine, is the use of genetic information to guide drug therapy, thereby reducing subjectivity in treatment decisions (Holden et al., 2019). Personalizing medications requires identifying genetic biomarkers such as protein expression, methylation, and genetic polymorphisms associated with disease states (Menezes et al., 2019). Bipolar disorder and MDD share genetic biomarkers, the most prominent being BDNF, a gene that codes for brain-derived neurotrophic factor. This knowledge could aid in making more accurate differential diagnoses, leading to more effective treatment for both disorders (Menezes et al., 2019).

The *CACNA1C* gene is a genetic biomarker for psychiatric disorders, including BD, MDD, anxiety, schizophrenia (SCZ), psychosis, and autism (Dedic et al., 2017; Guo et al., 2020; Mallas et al., 2016; Nurnberger et al., 2014; PGCPGBDWG, 2011). The gene, located on human chromosome 12 (Mallas et al., 2016), codes for building part of voltage-gated channel proteins that regulate the flow of calcium in and out of neurons. Calcium channels, in turn, regulate neurotransmitter secretions, mRNA transcription for protein expression, and lymphocyte actions

leading to immunodeficiencies, autoimmune syndromes, and the inflammatory response (Trebak & Kinet, 2019). The *CACNA1C* single-nucleotide polymorphism (SNP) rs1006737 is the most-studied *CACNA1C* variant affecting bipolar disorder (Liu et al., 2020). The gene functions normally if this allelic genotype contains two *G* alleles (*G*/*G*). In contrast, the *A* allele, also called the risk allele, impairs functioning in heterozygous carriers (*G*/*A*) or homozygous (*A*/*A*) individuals (Green et al., 2010; Guo et al., 2020).

In a case-control study by Green et al. (2010), individuals cataloged in a genome-wide area association study (GWAS) database were enrolled and evaluated for psychiatric disorders. The *CACNA1C* risk allele was strongly associated with unipolar depression, BD, and SCZ, prompting the authors to conclude that the *A* allele increased the risk of these disorders. In a meta-analysis of 6,104 individuals from three GWAS, no gene was strongly associated with MDD; however, *CACNA1C* was among 4% of genes associated with MDD, BD, and SCZ (Wray et al., 2012). The authors stated that this result agreed with previous findings that depression is highly multi-factorial with low heritability (Wray et al., 2012).

The *CACNA1C A* allele increases risks to early brain development due to adverse childhood events (ACEs), resulting in mood disorders (Tesli et al., 2013). In an RCT by Dedic et al. (2017), *CACNA1C* gene expression due to adverse events in early development was found to affect the limbic system of mice, altering emotional response, motivation, and cognition. The authors conjectured that similar changes could result in psychiatric disorders and impair the human stress response (Dedic et al., 2017). Smedler et al. (2022) studied MRI brain images of 87 people with BD. In people with reduced expression of the *CACNA1C* gene, ACEs were associated with alterations of the hippocampus and mammillary bodies. These brain regions function in memory formation and are associated with anxiety behaviors (Smedler et al., 2022).

In a study of monozygotic twins, *CACNA1C* was associated with depression and early-life stress in humans (Córdova-Palomera et al., 2015). In a study of fMRI images of the brain of 58 adolescents, the *CACNA1C* risk allele influenced changes in amygdala structure and function in participants who had experienced early childhood trauma by increasing amygdala reactivity, a region of the limbic system linked to the fear response (Sumner et al., 2015). In a larger GWAS of 1,825 individuals, *CACNA1C* was found to be the gene most strongly associated with both ACEs and depression by regulating the endocannabinoid pathway for the expression of the BDNF gene (Lazary et al., 2021). Calcium regulation is integral to the function of CNS GABA receptors, and anxiolytics, including benzodiazepines, bind to GABA receptors to reduce anxiety (Lazary et al., 2021).

Beyond the limbic system, *CACNA1C* is associated with altered development and functioning in the cerebrum. In an MRI study of patients with BD, reduced *CACNA1C* expression was associated with thinning of the frontal and parietal cortices (Smedler et al., 2020). A correlation analysis using fMRI scans by Liu et al. (2020) found that the *CACNA1C* gene was significantly correlated with depression arising from the prefrontal cortex. Tecelão et al. (2018) found that the risk allele was associated with changes in neuronal tracts in the frontal, temporal, and occipital lobes associated with verbal fluency and task completion.

The identification of the risk allele with changes in brain anatomy and physiology resulting in major psychiatric disorders has made the *CACNA1C* gene a potentially effective biomarker for pharmacogenetic targeting in precision medicine (Dedic et al., 2017; Guo et al., 2020; Lin et al., 2017; Tecelão et al., 2018). Calabrò et al. (2019) found that *CACNA1C* was strongly associated with treatment-resistant depression. This finding is consistent with data

suggesting that treatment-resistance depression is also observed when individuals with bipolar disorder are misdiagnosed with MDD (Menezes et al., 2019).

Lithium, the first-line drug of choice for BD, is partially regulated by *CACNA1C* and affects the glutamate receptor network (Higgins et al., 2015). Patients carrying abnormal *CACNA1C* alleles treated with lithium had more positive recovery outcomes related to cognitive functions, including attention, verbal and visual working memory, and planning (Lin et al., 2017). The *CACNA1C* gene has been identified as a pharmacogenetic biomarker. Lithium has been cited as a potentially effective medication to treat BD and TRD. However, pharmacogenetics clinical guidelines for the therapeutic use of lithium based on the *CACNA1C* risk allele have yet to be published (CPIC, 2022).

Mood Spectrum Disorders

Major depressive disorder, BD, and anxiety disorders have traditionally been diagnosed and treated as separate illnesses (Angst et al., 2018). Misdiagnosis is a major factor in developing treatment-resistant depression (Menezes et al., 2019). Changes in brain function, such as in amygdala structure and function in early adolescence, affect multiple psychiatric conditions, including BD, MDD, and schizophrenia, providing a biological basis for the spectrum (Green et al., 2010; Sumner et al., 2015). The development of psychiatric syndromes due to shared physiology demonstrates the pleiotropy for *CACNA1C*, in which one gene affects multiple traits (Smedler et al., 2022).

In a cohort study of 1,978 individuals diagnosed with BD or MDD, Angst et al. (2018) found that one-fifth of all participants diagnosed with major depression were found to have manic syndrome, and one-fourth exhibited manic symptoms. The authors recommended that instead of thinking of depression and BD as binary categories, think of these disorders as existing

on a spectrum of mood disorders with six subtypes: MDD, MDD with manic symptoms, MDD with manic syndrome, bipolar I, bipolar II, and unipolar mania with minor depression (Angst et al., 2018).

In a well-designed quasi-experimental study of 148 participants divided into people diagnosed with BD and a healthy control group, BD was inherited as a quantitative bipolar spectrum (Evans et al., 2005). This spectrum was expressed as different temperaments categorized as dysthymic, cyclothymic, hyperthymic, irritable, or anxious (Evans et al., 2005). These results suggested that the same underlying heritable traits may cause different forms of depression, BD, and anxiety behaviors (Evans et al., 2005). In a study of 650 people diagnosed with either MDD or BD, hypomanic symptoms occurred between depressive episodes in those with MDD in numbers comparable to people with BD (Benazzi, 2006). The evidence did not support the classification of MDD and BD into binary categories but instead as part of a multidimensional spectrum (Benazzi, 2006). Additionally, a dose-response relationship was found between hypomanic symptoms in MDD and a family history of BD. The more relatives with BD in the family tree, the greater the number of hypomanic symptoms (Benazzi, 2006).

Titone et al. (2018) found that having an anxiety disorder affects the occurrence of depressive episodes. An anxiety disorder diagnosis predicts a shorter time before the first depressive episode and a longer time before the onset of hypomanic or manic episodes (Titone et al., 2018). Anxiety comorbid with BD or MDD is not only a risk factor for depression but also substance use disorder (SUD) and suicide (Titone et al., 2018). Suicide has also been linked to the *CACNA1C* gene. Individuals with MDD and homozygous for the risk allele have a 44.6% risk of suicide compared with 38.7% of individuals without the alleles (Smedler et al., 2020).

Angst et al. (2018) suggested that assumptions are changing, moving from a binary mode of thinking about psychiatric disorders to a multidimensional spectrum view. This change in perspective can set the stage for more accurate diagnoses and more effective drug therapies. These improvements could lead to preventative therapy for BD, substance abuse, and suicide (Angst et al., 2018).

Lithium Treatment of Mood Disorders and Anxiety

According to clinical practice guidelines published by the APA, lithium is the first-line gold standard drug therapy for BD (APA, 2002). Although there is no cure for BD, the APA has established three recommendations: psychiatric measures, acute treatment, and maintenance treatment (APA, 2002). First, the primary care provider (PCP) should establish a therapeutic relationship with the patient (APA, 2002). Lithium plus an antipsychotic should be used for acute severe manic illness, although valproate plus an antipsychotic is also acceptable (APA, 2002). Lithium as monotherapy is preferred for manic patients who are not severely ill (APA, 2002). Lithium or lamotrigine monotherapy should be initiated for depressive episodes, and an antidepressant may be added for severe depression (APA, 2002). The third recommendation is that alternatives for maintenance treatment are lithium, valproate, lamotrigine, carbamazepine, or oxcarbazepine monotherapy (APA, 2002).

Clinical guidelines for lithium treatment of bipolar disorder published by the *Journal of Affective Disorders* recommend lithium plus an antipsychotic for acute mania (Malhi et al., 2017). Lithium monotherapy is recommended for depressive episodes and as a prophylactic treatment for BD (Malhi et al., 2017). Lithium has also been recommended as an effective prophylactic against suicide in unipolar depression and BD (Malhi et al., 2017). This

recommendation was consistent with a systematic review by Undurraga et al. (2019) of RCTs that found that lithium therapy was preventative of depression-associated suicide.

Including lithium as a recommended treatment for unipolar depression is one of the few differences between the guidelines published by APA and the *Journal of Affective Disorders* (APA, 2002; Malhi et al., 2017). Kleeblatt et al. (2017) conducted a systematic review and meta-analysis of five RCTs to determine the efficacy of using off-label medications to augment the treatment of unipolar depression. The authors concluded that while most pharmaceuticals had mixed or poor results, pindolol, ketamine, testosterone, estrogen, and modafinil had potential. However, lithium and antipsychotics remained the drugs of choice (Kleeblatt et al., 2017).

Vázquez et al. (2021) performed a systematic review and meta-analysis to determine the effectiveness of combination treatments for MDD, including 49 RCTs with 8,104 participants across all studies. The study compared various second-generation antipsychotics versus lithium as adjuncts to various traditional antidepressants. Depression outcomes were measured by the Hamilton Depression Rating Scale (HDRS17) or the Montgomery Åsberg Depression Scale (MADRS). Adverse reactions were measured by the number needed to treat (NNT) versus the number needed to harm (NNH). The authors found that lithium was the most effective and best-tolerated adjunctive and was faster acting in treating MDD than antipsychotics. The study was prompted by the increasing prevalence of TRD and the need to find better treatment regimens (Vázquez et al., 2021).

A systematic review and meta-analysis of RCTs was performed by Edwards et al. (2013) to determine whether lithium plus an antidepressant was more effective in treating depression than a selective serotonin reuptake inhibitor (SSRI) alone. The meta-analysis included 12 studies and made pairwise comparisons between SSRIs plus antipsychotics, SSRIs plus lithium, and

SSRIs alone. The results showed that treatment with SSRI augmented with lithium was more effective than using an SSRI alone. Treatment with an SSRI plus lithium was more effective than treatment with an SSRI plus an atypical antipsychotic (Edwards et al., 2013).

Strawbridge et al. (2018) conducted a systematic review and meta-analysis of 25 RCTs that evaluated interventions for treatment-resistant depression that augmented antidepressants. Therapies assessed included antipsychotics, mood stabilizers, anxiolytics, thyroid medications, and cognitive behavioral therapy (CBT). The authors recommended two medications, aripiprazole, and lithium. However, medications plus CBT were more effective than psychotherapy or drugs alone (Strawbridge et al., 2018).

Dold et al. (2018) investigated comorbidities, psychiatric symptoms, and depression outcomes associated with augmentation and combination therapies in 1,410 patients diagnosed with MDD. Lithium and second-generation antipsychotics (SGAs) were found to be effective adjuvant medications for TRD. However, due to the adverse event profiles of the SGAs, lithium was recommended. Undurraga et al. (2019) conducted a systematic review of RCTs to evaluate whether lithium effectively treats unipolar depression. Thirty-nine RCTs with 1,605 participants diagnosed with MDD were examined. Lithium was an effective adjunct to antidepressant therapy for acute depression and prophylactic treatment for depression. Undurraga et al. (2019) concluded that there was insufficient research to determine whether lithium is an effective monotherapy for unipolar depression.

Despite supporting evidence from clinical guidelines, systematic reviews, meta-analyses, and RCTs, lithium remains underused as a treatment for depression (Dold et al., 2018; Post, 2017). This hesitancy may be due to the need to monitor lithium serum levels to avoid adverse drug effects (ADEs). Lithium ADEs include hyperparathyroidism, hypothyroidism, impaired

kidney function, and weight gain (Shine et al., 2015; Tondo et al., 2017). The social stigma associated with lithium use may be another reason lithium is underused (Undurraga et al., 2019). Additionally, there is little interest by pharmaceutical companies in funding research for a drug which is an element of the universe, is inexpensive, and cannot be patented (Undurraga et al., 2019). However, cost may also be a facilitating factor in why patients might choose lithium. In a systematic review of the economics of antidepressants, Edwards et al. (2013) found lithium to be an inexpensive and cost-effective treatment for depression.

Lithium is an effective treatment for BD and TRD. The *CACNA1C* gene is associated with these psychiatric disorders and was one of 10 genes associated with lithium regulation in the glutamate receptor network (Higgins et al., 2015). The *CACNA1C* gene affects cognitive recovery after treatment with lithium (Lin et al., 2017). Additionally, there is evidence that lithium may be protective of cerebral function, may reduce dementia, may lower the risk of cancer for patients with BD, and reduces the risk of suicide and total general mortality (Undurraga et al., 2019).

Strength of Evidence

The evidence supported the inquiry themes (see Evidence Grid, Appendix D). One clinical practice guideline supported the pharmacogenetics of mood disorders theme, two level I systematic studies (Menezes et al., 2019; Nurnberger et al., 2014), and three level II studies. The spectrum disorders theme was supported by one level I systematic review (Menezes et al., 2019), one level III well-designed quasi-experimental study (Evans et al., 2005), and five level IV case-control or cohort studies (Angst et al., 2018; Green et al., 2010; Smedler et al., 2020; Sumner et al., 2015; Titone et al., 2018). The evidence for using lithium as an effective first-line treatment for BD and an off-label treatment for unipolar depression is well-established in clinical

guidelines published by the American Psychological Association and the *Journal of Affective Disorders* (American Psychological Association [APA], 2002; Malhi et al., 2017). Additionally, five level I systematic reviews provided evidence for using lithium to treat unipolar depression (Edwards et al., 2013; Kleeblatt et al., 2017; Strawbridge et al., 2018; Undurraga et al., 2019; Vázquez et al., 2021).

Limitations Found in the Literature

Robust evidence from systematic reviews and meta-analyses established the *CACNA1C* gene as a major biomarker for BD. One assumption for the present inquiry is that BD and MDD are part of a spectrum of mood or limbic system disorders. While one systematic review was found, no level II studies were discovered to support this proposition. This limitation arises due to the common use of genome-wide association studies for analyzing correlations between diagnoses. These studies analyze datasets of genetic information and do not include randomized control groups. These studies can be used to find correlations between genes and disease when withholding medical treatment in an RCT would be unethical. Another limitation was the large volume of integrative reviews and expert opinion articles excluded from the evidence synthesis.

Gaps in the Literature

A significant gap in the literature was the lack of studies similar to the proposed inquiry. The closest approximation was a level IV case-control study by Lin et al. (2017) that assessed the effects of the *CACNA1C* genotypes on response to antidepression treatment on cognitive recovery attributes such as attention, planning, and memory. This study was similar to the proposed inquiry in testing for the effects of lithium based on *CACNA1C* gene presentation. However, the study measured outcomes related to cognitive function rather than mood and anxiety (Lin et al., 2017). Finally, Lin et al. (2017) studied different *CACNA1C* SNPs than the

rs1006737, which is the focus of the current research. Another gap is that there are no published clinical guidelines for the *CACNA1C* risk allele related to lithium treatment to the knowledge of this researcher (CPIC, 2022; PharmGKB, n.d.). A gap also exists in research on lithium for treating anxiety, even though anxiety with irritability has been indicated as a component of bipolar I (Suppes et al., 2017). However, one study showed that the response of anxiety to lithium could be anxiolytic or anxiogenic depending on gene expression, epigenetics, and environmental conditions (Smagin et al., 2021).

Summary of Evidence

Level I evidence exists for the three themes of this inquiry, including using pharmacogenetics in treating mood disorders; MDD, BD, and anxiety existing along a multidimensional mood spectrum; and lithium as an effective therapy for both BD and unipolar depression. A significant limitation of the evidence was that only one systematic review supported the spectrum theme. Finally, there is a lack of studies similar to the proposed inquiry. No studies were found evaluating the use of pharmacogenetics to study the effects of *CACNA1C* genotypes on levels of depression and anxiety. Additional gaps include the lack of clinical guidelines for the *CACNA1C* gene and the use of lithium to treat anxiety.

Theory

The chosen theoretical framework was chaos theory. Founded by Edward Lorenz, a Massachusetts Institute of Technology meteorologist, chaos theory was first used to predict weather patterns (Oestreicher, 2007). The proposition that a patient's genotype affects the response to drug therapy corresponds to the central concept of chaos theory, the *butterfly effect*; the concept that the initial conditions of a complex system determine its outcomes (Demir et al., 2019). The butterfly effect was operationalized as the initial conditions of the allelic variants of

the *CACNA1C* gene, either an *A* (adenine) or a *G* (guanine) allele (Mallas et al., 2016). Therefore, if initial genotypic conditions are calculated, outcomes of dynamic systems may be *predictable*, an important concept in chaos theory. Predictability was operationalized in the present study as the metabolism of lithium based on genotype (Oestreicher, 2007). Metabolism in this sense refers to the classical biochemical usage of the sum of all chemical reactions that affect how a medication is processed in the body, since lithium is an element of the period table and is not broken down by catabolic processes.

Nonlinear dynamic systems, the concept that some systems are repeating complex systems that change over time, was the second concept in chaos theory used in the inquiry (Oestreicher, 2007; Warren et al., 1998). Nonlinear systems include diverse phenomena such as neuronal firing, embryonic development, weather systems, economics, epileptic seizures, the cardiac cycle, and hospital system management (Haigh, 2002; Holden, 2005; Oestreicher, 2007). Relevant to the study of lithium therapy is that pharmaceutical metabolism in the body has been described as a nonlinear dynamic system (Tsatsaris et al., 2011). Nonlinearity was operationalized in the present study as the expression of depression and anxiety in spectrum disorders (Ahmadlou et al., 2010).

Chaos theory was chosen as the guiding framework for this inquiry due to the explanatory power of the theory. First, the pharmacokinetics and pharmacodynamics of drug metabolism are nonlinear, self-organizing systems sensitive to initial conditions (Grabowski et al., 2016; Pillai et al., 2019). Second, gene expression is nonlinear, ranging from no expression to full penetrance based on initial conditions. These initial conditions include specific genotypic alleles, gene interactions, the cellular environment, and epigenetic factors (Guo et al., 2019; Tsatsaris et al., 2011). Third, chaos theory has been adapted for nursing, including research on

nurse-patient relationships and environmental variables (Demir et al., 2019). Finally, even though chaos theory can be complex, it can be used to explain the inquiry in simple terms. That is, the effect of lithium on depression and anxiety depends on the initial condition of the genotype (see Appendix E for the theory diagram).

Methods

IRB Approval

The University of Missouri-Kansas City Institutional Review Board (IRB) determined that this study was exempt in the category of secondary research involving identifiable private information or biospecimens (see Appendix F). The clinic owner provided site approval (see Appendix G), and the project was approved by the UMKC School of Nursing and health sciences (see Appendix H). The study used chart review of electronic data recorded in the Valant electronic health record system at the project site clinic. No intervention was performed or variables manipulated for this research. All data accessed by the student investigator was de-identified data. Patient risks were determined to be minimal.

Ethical Considerations

Evidence from the literature shows that the healthcare community ostracizes people with mental illness (Clement et al., 2014). Although legislation has been passed to make access to mental health services and treatment equitable with physical healthcare, parity has not been achieved (CMS, n.d.; CF, 2020). Because the research population included people with mental illness, they were considered a vulnerable population. All participants had undergone genetic testing using the Genomind Professional PGx Express testing kit. Without insurance or Medicare, this test had an out-of-pocket expense of $2,000 (Genomind, 2022a). Most insurance plans cover all but about $400, and Medicare and Medicaid cover the entire cost (Genomind,

2022a). Therefore, except for individuals who qualify for these government programs, the cost may have been prohibitive to those of lower socioeconomic status.

Minorities may be disadvantaged because these groups are often not included in the research, including GWAS studies specifically used as evidence for this project, and are often less likely to seek care due to barriers to access or mistrust of the healthcare system (Clark et al., 2019). Most participants were patients seen by telehealth. Telehealth reduces geographical barriers to care, but may create new barriers, such as access to a computer or a cell phone and the skills needed to use these devices (Kruse et al., 2016).

The research involved chart review and all data were collected and used only for the intended purpose of this study without distribution to other parties for any reason (Kruse et al., 2016). No participant was excluded from the data analysis based on race, ethnicity, sexual or gender identification, disability, or socioeconomic status (Shivayogi, 2013). A potential conflict of interest was that the student investigator participated in a student clinical rotation at the research site where patients may have also been participants. However, the research was double-blind for both the student investigator and the patient population.

Funding and Return-on-Investment

External funding was not sought (see Appendix I for the cost table). The student investigator and nurse practitioner volunteered time, travel, and printing expenses to conduct the project. Telehealth website access, Genomind testing kits, and patient services were conducted as the standard of care at the clinic and did not require additional funding. The scheduling assistant did not perform any actions for the project outside what would be performed as part of usual patient care. The instruments used to measure the healthcare outcomes of depression and anxiety, the PDH-9 and GAD-7, were free of charge by the copyright holder (Pfizer, Inc, 2022).

There is evidence that pharmacogenetics research has a positive return on investment (ROI) for the healthcare system. A cost-effective study was conducted that included 2,000 patients with MDD divided into two groups (Davenport, 2022). One group was genetically tested, and the second group received traditional treatment with antidepressants selected without genetic testing. The fiscal impact on the healthcare system included costs of pharmaceuticals, hospitalization, psychiatric therapy, and loss of productivity. The incremental cost-effectiveness ratio (ICER) for the patients who underwent pharmacogenetic testing was $64,000 per quality-adjusted-life year, reflecting a substantial increase in savings and effectiveness (Davenport, 2022). Perlis et al. (2018) conducted a case-control analysis of insurance health claims comparing outcomes of people with a mood or anxiety disorder who had undergone genetic testing with the Genomind test kit with people who had not been tested. Over six months, $1,984 was saved on individuals who had been tested (Perlis et al., 2018). Savings were due to reduced hospitalizations and emergency department visits (Perlis et al., 2018).

Setting and Participants

The setting was a privately-owned nurse practitioner clinic in Prairie Village, Kansas, specializing in general psychiatric healthcare. The clinic was staffed by one nurse practitioner and one scheduling assistant. The number of participants was 50, representing care at the clinic from June 2019 through January 2023. All participants meeting inclusion and exclusion criteria during specific times, including retrospective and prospective, were included in the study. The inclusion criteria were all adult patients over 18 diagnosed with a mood disorder or an anxiety disorder with an *A* risk allele presenting on the *CACNA1C* rs1006737 SNP and who completed pre-test and post-test screening for depression and anxiety. Exclusion criteria included patients

who were ineligible for lithium treatment based on comorbidities, patients who have failed a previous trial on lithium, and new patients currently on lithium during patient intake.

Research Implementation

The research consisted of chart review analysis to determine whether statistical associations existed between the *CACNA1C* rs1006737 SNP risk allele *A* and depression and anxiety following treatment with lithium. In addition to demographic data such as age, race, and gender identification, data analyzed included depression and anxiety scores measured by standardized instruments before and after initiating lithium therapy. Additionally, *CACNA1C* and *ANK3* genotypes for patients were collected by the research team that included the student investigator and the nurse practitioner.

Protocol

The research consisted of data collected over two time periods. For the retrospective arm of the study, the nurse practitioner provided de-identified chart review data to the student investigator for patient records starting in June 2019 and ending in January 2023. During this arm of the study, the time interval between pre-test and post-test was not standardized and was determined by the clinical judgment of the nurse practitioner. The prospective study began when IRB approval was obtained on June 4, 2022 (see Appendix J). During this arm of the study, the time interval between pre-test and post-test was limited to four to six weeks following post-test. Other than the variance in the time interval length, the protocol was the same for both arms of the study. The nurse practitioner provided de-identified chart review data to the student investigator (see Appendix K for the implementation diagram). The student investigator entered data into an SPSS 22 data spreadsheet. Because no new intervention or process was introduced

for the study, no extra time was required of the nurse practitioner, the patients, or the scheduling assistant.

Standard of Care

The nurse practitioner screened each patient with the Patient Health Questionnaire-9 (PHQ-9) and the Generalized Anxiety Disorder-7 (GAD-7) for depression and anxiety, respectively, at the establishing visit. These scores served as a baseline for depression and anxiety levels. Patients may have been receiving treatment with other therapies, such as antidepressants and anxiolytics, before and after starting lithium.

All patients at the clinic were genetically tested to determine polymorphisms of the *CACNA1C* rs1006737 allele, among 23 other psychiatric genes (Genomind, 2022b). The nurse practitioner ordered each participant's Genomind genetic test kit and provided education on using the kit. Test kits were mailed to each participant by Genomind. Patients collected a saliva sample and returned it to the testing lab in a pre-addressed, postage-paid envelope. The nurse practitioner used the genetic results as one decision tool in a comprehensive diagnostic process to determine appropriate pharmaceutical therapy. Patients started on lithium as the new drug therapy were reevaluated for depression and anxiety using the PHQ-9 and GAD-7 instruments by the nurse practitioner during office telehealth and in-office visits.

Study Design

The study used an observational, correlational design with one cohort. The study consisted of retrospective and prospective data collected from chart review, including pre-test- and post-test data following the start of low-dose lithium treatment. Chart review was used to assess patients from June 2019 through January 2023 as part of the retrospective study, and from June 2022 through January 2023 prospectively to include newly established patients.

Validity

The internal validity of the project was maintained through the consistent application of protocols, including standards of care at the clinic and the interaction of the student researcher with the nurse practitioner. The same instruments were used to measure depression and anxiety throughout the study for all pre-tests and post-tests. The only interviewer was the nurse practitioner, who followed the standard of care of reading the instrument questions verbatim. The student investigator never had access to the protected health information of the participants. Another potential internal threat to validity was the variance in the timing of the post-test administration. There was the potential for an external maturation threat in the prospective study, but none of the participants dropped out.

Research Outcomes

The health outcomes measured were levels of depression and anxiety before and after treatment with low-dose lithium. The primary outcome of the research measured was whether low-dose lithium treatment based on genetic testing of the *CACNA1C* gene reduced levels of depression and anxiety compared with baseline levels. To the knowledge of the student investigator, this is the first study to assess the effects of lithium on depression and anxiety for people with the *CACNA1C* gene risk allele. The effect of the *CACNA1C A* allele on the levels of depression and anxiety before and after low-dose lithium treatment was also evaluated in the study. Additionally, the prevalence of the *A* allele in the sample population was compared with the general population (see Appendix L for the outcomes table).

Measurement Instruments

The Patient Health Questionnaire-9 (PHQ-9) and the Generalized Anxiety Disorder-7 (GAD-7) are instruments for measuring depression and anxiety, respectively (see Appendix M).

These interview schedules had fixed-alternative questions that were scored as rating scales. The PHQ-9 depression scale ranges from 0 (no depression) to 27 (severe depression). The GAD-7 anxiety scale was used to measure anxiety, ranging from 0 (no anxiety) to 21 (severe anxiety). The nurse practitioner used these tools as part of the standard of care. The instruments are questionnaires owned by Pfizer and made available free of charge and without copyright restriction or need for permission (Pfizer, 2022). The nurse practitioner used the questionnaires to interview the participants during telehealth office visits. The combined questionnaire interviews take approximately five minutes.

In a systematic review of depression and anxiety measurement scales by Kroenke et al. (2010), the PHQ-9 was found to have high validity, with specificity ranging from a Cronbach-alpha of .77 to .88 and sensitivity ranging from .88 to .94. The PHQ-9 also demonstrated high reliability, ranging from .86 to .89. The GAD-7 was found to have high validity, with a Cronbach-alpha score of .89 for specificity and .82 for sensitivity. The GAD-7 exhibited high reliability with a Cronbach-alpha of .92 (Kroenke et al., 2010).

Quality of Data

An *a priori* power analysis was performed using G*Power software to assess the sample size needed to achieve .80 statistical power with a medium effect size (.50) and an α error probability of .05 (Brysbaert, 2019). Separate analyses were made for one-way ANOVA tests (N = 34) and repeated-measures ANOVA calculations accounting for within-measure effects (N = 12), between-measures effects (N = 26), and interaction effects (N = 12). Therefore, to evaluate all desired effects, a sample size of at least 34 was indicated by the power calculation (see Appendix N for sample size calculations). Descriptive statistics included demographic information regarding age, race, gender identification, and depression and anxiety scores for

each pre-test or post-test measurement. These data are important in determining the generalizability of the data to other populations. Additionally, the *CACNA1C* risk allele is correlated with age, cortical thinning in the brain, and cognitive decline (Dedic et al., 2017; Soeiro-de-Souza et al., 2017). The risk allele is also correlated with sex. People identifying as women have a higher risk of affective disorders, and men have higher emotional lability and depressive symptoms (Strohmaier et al., 2012). No studies investigated the effects on genderqueer and nonbinary communities, illustrating a research gap (Scandurra et al., 2019).

Information obtained from the chart review included genotypes for *CACNA1C* and *ANK3* and pre-test and post-test scores for levels of depression and anxiety. Depression and anxiety levels were recorded from PHQ-9 and GAD-7 scores. For the retrospective study, the interviews were previously administered. As part of the standard of care for the prospective study, the interviews were conducted before starting lithium therapy (pre-test) and four-to-six weeks after beginning treatment (post-test). New patients were added to the study on a rolling basis as they became established with the nurse practitioner. Interviews were held during private telehealth visits and conducted by the nurse practitioner.

Analysis Plan

Statistical analyses of hypotheses were performed using the SPSS v22.0 statistical software package. Demographic statistics were collected and analyzed as frequency distributions for age, gender identification, and genotype. Time-dependent measures analyzing differences between pre-test and post-test scores were assessed with multi-factorial analysis of variance (ANOVA) with repeated measures. Whenever significant main or interaction effects were found, Bonferroni post hoc tests were conducted to determine simple effects. Statistical significance was defined as $p < .05$. Differences between sample means without a time component were

assessed by one-way factorial ANOVA, including different levels of *CACNA1C* genotypes and the effect of post-test administration time interval (Boisgontier & Cheval, 2016). The *CACNA1C* genotypes were analyzed for Hardy-Weinberg equilibrium and compared to the general population using a Chi-square analysis. De-identified data were housed in the secure REDCap software on the UMKC server.

Results

Data Collection

The nurse practitioner conducted the chart review for the retrospective arm of the study at the clinic. The earliest chart data collected for the retrospective study was from June 6, 2019, and the final date was January 27, 2023. The administration of the pre-test was on the establishing visit for each patient. The administration of the post-test for the retrospective chart review was not standardized because these patients were seen at the clinic prior to the initiation of the research. For the prospective arm, there was an attempt to standardize the administration of the post-test at six weeks, but due to the unpredictability of patient schedules, this was not always achievable. The first chart data collected for the prospective study was on August 17, 2023, and the final chart data was collected on January 17, 2023. Only patients who met the inclusion criteria were included in the study. The student investigator met with the nurse practitioner in person. The nurse practitioner read de-identified data from patient charts to ensure that the student investigator was blind to protected patient information. The student investigator recorded these data into an SPSS datasheet (see Appendix O for the data collection template).

Sample Size Determination

A one-way ANOVA was performed to determine whether the timing of the post-test had any statistical effect. There was no significant effect of time on post-test depression levels (p =

.136) or post-test anxiety levels (p = .633). Therefore, retrospective and prospective data were pooled to achieve a sample size that would reach a statistical power of .80 based on *a priori* power calculations. The combined data provided a sample size of 51. A Mahalanobis distance was calculated to check for outliers. One patient was omitted due to a Mahalanobis distance of 20.82, which was more than twice the distance from other data points and had a *p-value* of .003, leaving a sample size of 50, exceeding the 34 people necessary to achieve the desired power.

Setting and Participants

The setting was a nurse practitioner-owned psychiatric clinic in Prairie Village, Kansas. Patients were seen via the Spruce telehealth system. The mean age was 36.66 years, ranging from 18 to 74 years of age. Most participants were White (94%) and identified as female (76%). Low-dose lithium was defined as ≤ 600 mg/day. Seven patients took 600 mg/day, and 43 patients took 300 mg/day. The frequency of the *CACNA1C GA* genotype (one risk allele) was 33 patients and 17 genotypes had two *A/A* risk alleles (see Appendix P for demographic data).

Statistical Analysis of Outcomes

Depression

A repeated-measures ANOVA was performed to determine the effect of lithium treatment on levels of depression and anxiety based on the *CACNA1C* genotype (see Appendix P for statistical analysis tables). The *ANK3* genotypes were also put into the model because this gene was potentially a confounding variable. There was a significant 66% reduction in depression (p < .001) as measured by PHQ-9 scores from pre-test to post-test following low-dose lithium treatment (see Appendix Q for figures), with a large statistical effect (η^2 = .728). For *G/A* genotypes the pre-test mean was 12.91 and the post-test mean was 4.27 following low-dose lithium treatment. The pre-test *A/A* genotypes had a pre-test mean of 9.47 and a post-test mean of

3.29. The average pre-test PHQ mean was 11.19 and the average post-test mean was 3.78. There were no statistical interaction effects between depression, *CACNA1C*, and *ANK3.* There was a significant simple effect in pairwise comparisons of the *CACNA1C* genotypes on pre-test depression ($p = .032$). A Bonferroni adjustment was made for multiple comparisons. A post hoc one-way ANOVA was performed and found a significant main effect of *CACNA1C* on pre-test depression ($p = .033$). The *G/G* genotypes had a mean pre-test PHQ-9 score of 12.91, compared to 9.47 for *A/A* genotypes.

Anxiety

There was a significant 65% reduction in anxiety from pre-test to post-test following low-dose lithium treatment ($p < .001$). This effect was statistically large ($\eta^2 = .728$). For *G/A* genotypes the pre-test mean was 13.27 and the post-test mean was 5.06 following low-dose lithium treatment. The *A/A* genotypes had a pre-test mean of 12.18 and a post-test mean of 3.47. The average pre-test mean was 12.73 and the average post-test mean was 4.27. There were no interaction effects between anxiety, *CACNA1C*, and *ANK3.* There was a significant simple effect in pairwise comparisons of the *CACNA1C* genotypes on pre-test and post-test anxiety ($p = .044$), but no main effect of *CACNA1C* was found in a post hoc one-way ANOVA analysis. No interaction effects were found between *ANK3* and post-test anxiety. A post hoc one-way ANOVA was performed to determine if there was a main effect of *ANK3* on post-test anxiety, and no significant main effect was found. A Bonferroni adjustment was made for multiple comparisons.

Hardy-Weinberg Equilibrium

To assess whether the *CACNA1C* genotypes were in Hardy-Weinberg Equilibrium (HWE), data from patients with a *G/G* genotype and subject to the same standard of care were

pooled with the sample population to determine percentages of *G* and *A* alleles. The *CACNA1C* rs1006737 genotype frequencies did not deviate significantly from HWE assessed by Chi-square analysis (χ^2 = 0.233, df=2, p = .890). The *A* allele frequency in the patient population was .48, 60% higher than the .30 expected in the general population (SNPedia, 2023).

Discussion

Successes

Treatment of patients with at least one *CACNA1C A* risk allele with low-dose lithium significantly reduced levels of depression and anxiety as measured by the PHQ-9 and GAD-7, respectively. In addition to these primary healthcare outcomes, the study team made additional insights. The patient population had a significantly higher proportion of *A* alleles than expected in the general population. Therefore, the study provided clinical evidence that the *CACNA1C* gene is a biomarker for mood disorders and anxiety. The effect of *CACNA1C* genotype on untreated depression as measured by pre-test PHQ-9 scores showed significant differences in levels of depression based on the number of *A* alleles, with *G/A* genotypes having significantly higher initial depression scores than *A/A* genotypes.

Study Strengths

The first strength of this study was the sample size of 50, which exceeded 34, the number needed to achieve a power of .80 for all effects analyzed in the statistical models. Second, the PHQ-9 and the GAD-7 instruments used to measure depression and anxiety were free to use, did not require permission from the copyright holders, and had high validity and reliability. Third, because this study relied on chart review and depended only on collaboration with the nurse practitioner at the clinic, the organization of data collection and coordination with the clinic was smooth. Finally, the prospective study did not have any loss of participants due to attrition.

Results Compared to the Evidence from Literature

No research studies were found in the literature search that focused on the effects of low-dose lithium on depression and anxiety in people with at least one *CACNA1C* risk allele. Hence, this study appears to be the first to assess this pharmacogenetic treatment protocol. The closest approximation was a study by Lin et al. (2017) that examined the effect of lithium on aspects of cognition, including attention and memory. However, this study excluded the *CACNA1C* rs1006737 and is therefore not directly comparable. One study examined the effect of the *CACNA1C* genotype on lithium response using the Alda scale but did not address specific health outcomes (Paul et al., 2021). The authors concluded that *CACNA1C* genotype polymorphisms did not significantly affect response to lithium. However, the article was a letter to the editor, the lowest type of scientific evidence, did not evaluate the same health outcomes of depression and anxiety, and included only the Indian population, which is known to have a lower incidence of the *A* allele. In a systematic review and meta-analysis of 234 studies on the effectiveness of lithium on bipolar disorder, Fountoulakis et al. (2022) found that lithium should still be considered the gold standard for treatment for bipolar disorder and BD subtypes. The present study is consistent with those findings. However, the present study evaluated the effectiveness of low-dose lithium, whereas the literature reviewed in Fountoulakis et al. only included studies using lithium dosages in so-called therapeutic levels above 600 mEq/lt.

Limitations

Internal Validity

The study internal validity was threatened by the lack of randomization and equivalent control groups, resulting in selection bias (Mitchell, 1985). The effectiveness of lithium on health outcomes was evaluated before and after treatment. However, the inclusion criteria precluded

patients who had not completed a post-test, minimizing attrition of participants or missing data that could have resulted in maturation threats (Mitchell, 1985).

Because the genotype for the sodium-channel regulator gene *ANK3* is known to influence the response to lithium in people with bipolar disorder (BD), this gene could have been a confounding variable and a threat to internal validity (Gottschalk et al., 2017). This gene was also used in treatment decisions at the clinic during the research.

Initially, the research team planned to analyze the retrospective study separately from the prospective one. This approach was because the timing of the administration of the pre-tests, especially in the retrospective study, had a wide variance because they had been administered based solely on clinical need. The prospective study, however, was planned to have a six-week interval between pre-test and post-test. Due to uncontrollable aspects of patient scheduling, the prospective interval varied from 3.5 to 18 weeks. Additionally, neither arm of the study achieved the minimum desired sample size of 34, which would have further threatened internal validity and weakened statistical power.

External Validity

Consecutive sampling was used, which was a threat to external validity, potentially limiting the extent to which the outcomes may be generalized to people in other settings (Mitchell, 1985). The generalization and transferability of the study may be limited by the lack of diversity in the sample population, mostly Whites who identified as female. The study site was located in the wealthiest county in Kansas (United States Census Bureau [USCB], 2023), which may limit the transferability of the treatment regimen to areas of lower socioeconomic status due to the co-pay that is required for genetic testing depending on insurance coverage provider.

Sustainability

The study has the potential for high sustainability. The project inquiry originated from the owner of the project site clinic, who provided site approval and collaborated in the research development. The inquiry evaluated clinical standards of care. The standard of care analyzed has continued after the completion of the research and is expected to be sustainable.

Efforts to Minimize Study Limitations

Consecutive sampling was used. Minimizing this threat when studying mental health is challenging because placing a subset of patients into a control group, thereby denying treatment, is unethical. Lithium therapy preceded the health outcomes, minimizing temporal threats to validity (Mitchell, 1985). The retrospective and prospective arms of the study were combined to make it possible to achieve the desired sample size to reach a power of .80. To determine if the variance of the time interval between pre-test and post-test administration was a threat to internal validity, a one-way ANOVA was used to determine the main effect of the interval length between testing. Because this effect was nonsignificant, the threat of a small sample size was minimized.

Polymorphisms of the *ANK3* genotype (rs10994336 SNP) affect mood disorders and anxiety. This gene was also used in genetic testing at the clinic in treatment decisions. Therefore, the *ANK3* genotypes of the participants were included in the analysis to reduce threats to construct validity (Genomind, 2022b; Gottschalk et al., 2017; Mitchell, 1985; Roby, 2017).

Interpretation

Expected and Actual Outcomes

The nurse practitioner had been using the *CACNA1C* gene as a biomarker to identify patients with a predisposition to mood disorders and anxiety and treating them with low-dose

lithium for about six years prior to the start of the study. Based on her observations, there was an expectation that there would be a reduction in depression and anxiety following low-dose lithium therapy. The results of this study were consistent with those expectations, as evidenced by the significant reductions in the post-test PHQ-9 and GAD-7 means. There were no expectations of the size of these effects. However, there were large statistical effects based on the partial eta squared scores of .728 for depression and .810 for anxiety (Richardson, 2011). Because large effects are associated with the greater applicability of findings, the results may translate into practical uses in other clinical settings.

The inclusion of *CACNA1C* in genetic testing panels is based on large GWAS studies that have found correlations between the gene and mood disorders (Genomind, 2019). Therefore, a population of patients with mood disorders would be expected to have a higher frequency of this allele than average. This prediction was consistent with the sample population, which had a 60% higher incidence of the *A* allele, providing clinical collaboration for what would be predicted from GWAS studies.

There was one unexpected finding. Because the *A* risk allele is associated with an increased chance of having BD and since depression is a major symptom of BD, it was expected that patients with two *A* alleles would have higher levels of untreated depression than *G/A* individuals. However, untreated patients with one *G* had significantly higher depression in the PHQ-9 pre-test scores than those with the *A/A* genotype. However, this was consistent with a study by Dam et al. (2022). That study used the Hamilton Depression rating scale and found that *G/G* and *G/A* genotypes had higher levels of depression than *A/A* genotypes. A review of the research on *CACNA1C* and depression concluded that the *A* allele had a protective effect against elevated levels of depression yet increased anxiety (Moon et al., 2018). Therefore, while

counterintuitive, this study supports previous findings from the literature that the *A* allele has a complex relationship with bipolar disorder, depression, and anxiety. The allele increases the odds of being bipolar and heightens anxiety and is associated with lower levels of baseline depression.

Effectiveness and Revisions of Research

This research found that the clinic treatment protocol using pharmacogenetics to help start patients on low-dose lithium significantly reduces depression and anxiety in patients with at least one *CACNA1C A* risk allele. The research protocol itself was effective, and there were no obstacles that delayed or complicated the process. Early in the timeline, there was thought of extending the research to multiple clinical sites. However, this would have made coordination among multiple providers and maintaining standardized protocols difficult. In the end, the sample size was sufficient for the desired statistical power, making additional sites unnecessary, especially for the limited timeframe for the research.

Expected and Actual Impact on Health Systems, Cost, and Policy

This study assessed the effectiveness of a standard of care at a private psychiatric clinic and found no direct effects of the research on the patient population. However, there is the potential for impacts on healthcare. First, low-dose lithium is gaining acceptance for having health benefits for the whole body similar to exercise (Fajardo et al., 2023). This research supplies evidence that low-dose lithium can be effective at treating mood disorders and anxiety without the risks associated with higher blood levels. Second, there are currently no clinical guidelines regarding using the *CACNA1C* gene as a guide for lithium treatment; therefore, this research has begun to fill a gap in the literature. According to a systematic review and meta-analysis of 234 studies by Fountoulakis et al. (2022), lithium is a low-cost and effective medication that is preferable to more expensive antipsychotics, without the associated movement

disorder adverse reactions. If this protocol is transferable to other clinical settings, treatment of depression and anxiety could become safer and less expensive. The cost was minimal, including travel to the clinical site and office supplies. The PHQ-9 and GAD-7 are free of charge and without copyright restriction from Pfizer and are embedded in many EHR systems by default, and as of this writing, a 60-day supply of 300 mg lithium costs about $7 (Epocrates, 2023).

Conclusion

Mood disorders, including depression and anxiety, affect over one-third of Americans, are the leading causes of disability in the United States, and result in substantial societal, economic, and individual costs. Bipolar disorder is often misdiagnosed as major depressive disorder, leading to ineffective drug therapy and treatment-resistant depression.

Pharmacogenetics has the potential to help guide drug therapy based on personal genetic profiles and more effectively treat mood disorders and anxiety in clinical practice. Although lithium is commonly used for treating bipolar disorder, this research may show the effectiveness of lithium therapy in treatment-resistant depression, major depressive disorder, and anxiety.

Medical treatment of mental health has traditionally been a trial-and-error approach. Multiple medications with adverse effects are often prescribed based solely on subjective clinical experience. Pharmacogenetics provides an objective measure to aid in medication selection. The *CACNA1C* calcium-regulator gene has been linked to bipolar disorder, major depression, and anxiety. It is potentially an excellent biomarker for pharmacogenetics to improve the quality of life for people suffering from these debilitating mental illnesses.

Further Study of Intervention

Beyond this initial study, further research is needed to evaluate using *CACNA1C* as a biomarker for treating depression and anxiety with lithium. Studies examining the effectiveness

of adjunctive medications to lithium, such as lamotrigine, could help determine the effectiveness of pharmaceutical combination therapy. This study only assessed the *G/A* and *A/A* genotypes. Studies comparing these genotypes with *G/G* individuals could provide more insight into the interactions of the *G* and *A* alleles with lithium, depression, and anxiety. Additionally, studies of gene-by-gene interaction effects on these disorders might increase the precision of pharmacogenetics. For instance, the *CACNA1C* genotypes only had a statistically significant effect on pre-test anxiety when *ANK* genotypes were included in the model, and the interactions between these genes might provide further insight into pharmacotherapy selection.

Dissemination

This research was presented as a speech at the World Nursing Congress on April 10, 2023, in San Francisco, California. This research will be presented to the University of Missouri-Kansas City School of Nursing and Health Studies on May 5, 2023, and through publication in a scientific journal if accepted.

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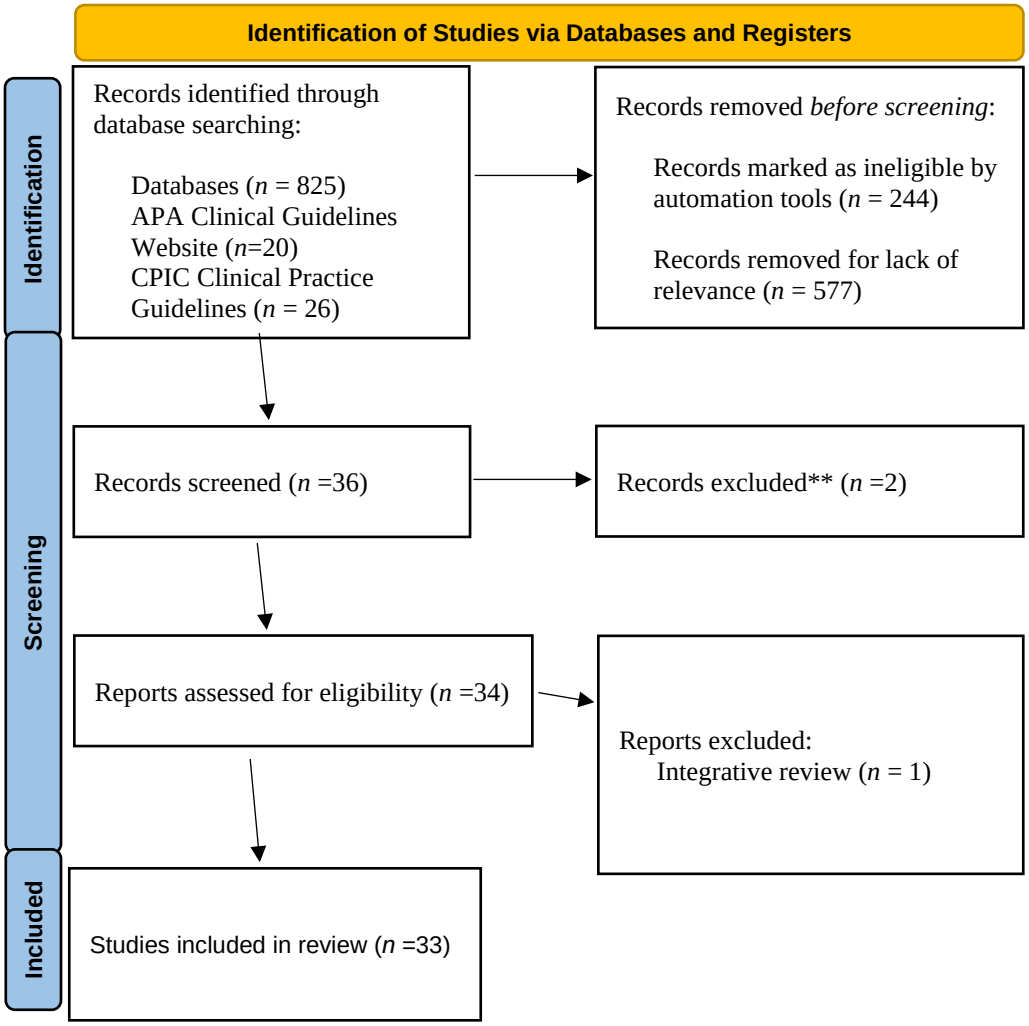
Appendices

Appendix A: Definition of Terms Table

TERM	DEFINITION
Pharmacogenetics	How individual genetics affects medication response (Mallas et al., 2016).
Mood Spectrum Disorders	Certain mental illnesses classified as separate disorders may be different expressions of the same underlying disorder ranging from no symptoms to pathological (Angst et al., 2018).
Chaos Theory	A highly mathematical scientific theory used to predict patterns in complex systems such as the weather, economics, and drug metabolism (Oestreicher, 2007).
The Butterfly Effect	The initial conditions of a complex system determine its outcomes (Demir et al., 2019).
Nonlinear Dynamic Systems	Certain systems, such as metabolism, are repeating complex systems that change over time (Oestreicher, 2007).
CACNA1C	A gene that codes for protein channels in neuronal cell membranes that regulate the flow of calcium (Mallas et al., 2016).

Appendix B: PRISMA Diagram



Adapted from Moher D, Liberati A, Tetzlaff J, Altman DG. The PRISMA Group (2009). *Preferred Reporting Items for Systematic Reviews and Meta-Analyses:* The PRISMA Statement. PLoS Med 6(7): e1000097.

Appendix C: Synthesis of Evidence Table

Pharmaco-genetics of Mood Disorders	Spectrum Disorders	Lithium in Treatment of Mood Disorders	First Author, Year, Title, Journal	Purpose	Research Design, Evidence Level & Variables	Sample & Sampling, Setting	Measures & Reliability (if reported)	Findings	Limitations & Usefulness
X	X		Menezes (2019). Behavioural Brain Research	Determine genetic biomarkers to aid in differential diagnosis of MDD and BP.	Systematic review. I. Not reported (systematic review).	N = 27 studies about genetic biomarkers for major depressive disorder (MDD) or bipolar depression (BD). Participants from all included studies were between 18-65 years of age. Settings not specified (systematic review).	The review reported whether each study reported measures of validity.	BD is often misdiagnosed as MDD because they have been historically viewed as binary diseases, but biomarkers suggest they are linked genetically, and this can aid in differential diagnosis and treatment.	Some of the included studies did not include the severity of MDD or BD and did not control for variables including BMI and drug use. Some studies had small sample sizes.
X	X		Green (2010). Molecular Psychiatry.	Test whether BD, MDD and schizophrenia are associated with the CACNA1C A allele.	Case control. IV. CACNA1C SNPs and diagnoses of SCZ, BP, Unipolar, or control.	N = 1,868 BD patients if British white people diagnosed with BD. Not reported	BADDS, GAS	The CACNA1C A allele increases the risk for BD, SCZ, and MDD.	Contributes to understanding of pathogenesis of psychiatric disorders and towards better diagnoses and classifications of these disorders.
X	X		Smedler (2020). CACNA1C polymorphism and brain cortical structure in bipolar disorder.	Analyze brain changes in people with CACNA1C A alleles.	Cross-sectional. IV. Independent: CACNA1C alleles. Dependent: Brain anatomy.	N = 87 participants with bipolar disorder genotyped for the A	Not reported	Patients with a CACNA1C genotype containing the risk allele A had thinner cortical	This study did not have a control group and could not differentiate between

			Journal of Psychiatry & Neuroscience: JPN			allele of the CACNA1C gene who had also undergone MRI scans. Northern Stockholm Psychiatric Unit, Stockholm, Sweden.		brain regions that are altered in BD.	correlation and causation, so it is unknown whether have the A risk allele causes thinning of brain regions.
X	X		Sumner (2015). Journal of Child and Adolescent Psychopharmacology.	Examine whether genetic variation in the CACNA1C gene influences amygdala structure and function early in development.	Cohort study. IV. Amygdala volume, amygdala activation, CACNA1C SNPs.	N = 59 adolescents and young adults. All females were postmenarchal. Boston Children's Hospital.	Not reported	CACNA1C influences brain structure and function in early adolescence.	Subpopulation may not generalize to general population.
X		X	Lin (2017). Scientific Reports	Investigate effect of CACNA1C SNPs on recovery of executive functions.	Case control. IV. CACNA1C SNPs, attention, processing, memory, set shifting, and planning.	N = 192 inpatients and outpatients diagnosed with BD and suffering a major depressive episode. Convenience sampling through advertisements Guangzhou Brain Hospital, Jinan University	HAM-D, HAM-A, BPRS, YMRS	CACNA1C variants affect recovery after treatment.	The study was naturalistic, and the choice of medications were not controlled. Sample size was relatively small

X		X	Higgins (2015). Pharmacogenomics.	Analyze genotypes that mediate lithium response.	Cross-sectional descriptive study. VI. CACNA1C SNPs, glutamate neuron signaling.	N = 23,312 SNPs analyzed from GWAS data. Genome database.	Not reported.	CACNA1C was one of 10 genes associated with lithium regulation in the glutamate receptor network.	Some subsamples in the genomic database used small sample sizes.
X			Clinical Pharmacogenetics Implementation Consortium (2022). Clinical Guidelines Website (NIH)	A reference for all clinical guidelines for pharmacogenetics.	Clinical Practice Guideline. 1.	Not applicable. Not applicable. Not applicable	Not applicable	The CACNA1C gene is not listed in the clinical guidelines.	Pharmacological treatment guidelines based on CACNA1C genotypes is not available.
X			Nurnberger (2014). JAMA Psychiatry	Identify genes that increase risk of BP.	Meta-analysis. I.	N = 4 Genome-Wide Association Studies (GWAS). Not reported (meta-analysis).	Not reported (meta-analysis).	Three genes showing the strongest association with BD were CACNA1C, GNG2, and ITPR2.	Some included studies included analysis of genes in only the -cis regulation and not -trans.
X			Dedic (2018). Molecular Psychiatry.	Investigate how CACNA1C affects risk of psychiatric disorders.	RCT. II. Mice with SNPs knocked out of CACNA1C gene, stress response.	N = 4,023 mice. Not reported.	Hippocampal electrophysiology readings, chronic social stress.	CACNA1C expression affects the limbic system in development. (ADEs) can result in psychiatric disorders and impaired stress response.	Mice studies may not transit to humans.
X			Guo (2020). Replicated risk CACNA1C variants for major psychiatric disorders may serve as potential therapeutic targets for the	Using the CACNA1C gene as a marker for therapeutic targeting of depression in multiple	RCT. II.	N = 134 Europeans. No additional information was provided. UK Brain Consortium	Not reported	Among nine genes evaluated, the CACNA1C gene was most strongly associated with psychiatric disorders.	The modest sample size was cited as being the only limitation in the study.

			shared depressive endophenotype. Journal of Neuroscience & Cognitive Studies	psychiatric disorders.					
X			Smedler (2022). Proceedings of the National Academy of Sciences.	Investigate the cellular mechanism by which CACNA1C contributes to psychiatric disorders.	RCT. II. Prescence of CACNA1C SNPs in knockout mice, anxiety behaviors, and brain development.	N = 16 mice, 8 with normal CACNA1C gene and 8 with abnormal gene. Not specified.	Not reported	Knockout CACNA1C genes causes structural changes in the limbic system, including in the hippocampus and mammillary bodies, resulting an anxiety behavior.	Not reported
X			Calabrò (2019). Clinical Psychopharmacology and Neuroscience.	Identify genetic factors that play a role in MDD.	Well-designed quasi-experimental study. III. Presence of SNPs (39), signs and symptoms of MDD	N = 568, including a control group of 326 healthy people and 242 patients with MDD. All were of Korean ancestry. Sampling was patients consecutively admitted to the hospital. St. Mary's Hospital, Seoul, South Korea.	Mini-International Neuropsychiatric Interview (MINI), HAMD.	The CACNA1C gene and its haplotypes were associated with depression treatment resistance (p = 0.012).	The study had a small sample size. All participants were of Korean descent and findings may not be generalizable to other settings or ethnicity groups.
X			Córdova-Palomera (2015). Transitional Psychiatry	Evaluate whether DNA methylation differs in twins	Case-control. IV. Psychological disorder diagnosis,	N - 34 monozygotic twins. Not reported.	SCID-I, BSI	CACNA1C associated with depression and early-life stress.	The sample size, heterogeneity, and ack of DNA methylation

				by mental illness.	methylation states of CACNA1C.				analysis may limit the generalizability.
X			Lazary (2021). Neuroscience Letters	Test associations between genes, childhood trauma, and affective disorders.	Cohort study. IV. CACNA1C SNPs, depression, anxiety.	N = 1825 participants from the general population recruited from general practice providers, distance study programs, and public advertisements. Studies were carried out in two locations, Budapest, Hungary, and Manchester, England.	BSI-ANX, BSI-DEP, CAQ	The CACNA1C gene was associated with ADEs and depression (p = 0.00016).	The sample size was cited as a limitation, as was the fact that participants from the two research sites differed by age groups.
X			Liu (2020). CACNA1C Gene rs11832738 Polymorphism Influences Depression. Frontiers in Psychiatry.	Determine whether the CACNA1C gene influences depression centers in the brain.	Cross-sectional. IV.	N = 182 participants of over 18 years old of Chinese Han ethnicity diagnosed with acute MDD.	HAMD-24	Correlation analyses were performed and found that CACNA1C was correlated to depression (r = -0.224, p = 0.017) arising from the prefrontal cortex of the brain. Brain fMRI imaging was used to determine areas of the brain activated during depression.	Brain imaging studies are limited by the fact that participants thoughts and emotions cannot be controlled for during scanning. The second limitation was the use of two sites to collect imaging and DNA samples, potentially introducing differences in instrumentation

									and data collection.
X			Mallas (2017). Genes, Brain, and Behavior.	To assess the effect of the CACNA1C A alleles in brain tracts.	Case control. IV. Diagnosis of BD, SCZ, and controls, CACNA1C genotype, brain anatomy.	N = 230 participants, with 63 diagnosed with schizophrenia (SZ), 43 with bipolar disorder, and 124 in the control group. The setting was not specified, but recruitment was carried out in South London and Maudsley, England.	Not reported	The CACNA1C and ZNF804A genotypes associated with both bipolar disorder and schizophrenia increase susceptibility to psychosis.	The authors did not address limitations of this study.
X			Psychiatric GWAS Consortium Bipolar Disorder Working Group (2011). Nature Genetics.	To identify genes common to autism, ADHD, BD, MDD, and SCZ.	Case control. IV. Genotype SNPs, psychiatric diagnoses	N = 47,035. Genome database	Not reported	Strong evidence supports CACNA1C and ODZ4 association with BD.	Not reported
X			Tecelão (2018). Genes, Brain, and Behavior	To examine whether abnormal verbal fluency is affected by CACNA1c SNPs.	Case control. IV. CACNA1C SNPs and brain region activation.	N = 174 English white people diagnosed with BD, SCZ, or control. Not reported.	Not reported	CACNA1C influences brain activation related to verbal fluency and brain imaging provides evidence that the gene is implicated in both SCZ and BD.	Relatively small sample sizes within each ANOVA group.

X			Tesli (2013). PLOS One	Investigate differences in amygdala due to influence of CACNA1C gene polymorphisms.	Case control. IV. SCZ, BD, MDD, Anxiety.	N = 250, 66 BD, 61 SCZ, and 123 healthy controls from Northern Europe between 18 and 65. Psychiatric inpatient and outpatient hospitals in Oslo.	SCID for DSM-IV, YMRSm IDS, PANSS	CACNA1C affects amygdala activity during emotional processing in SCZ and BD.	Not reported
X			Holden (2019). Personalized Medicine	Study public perception of personalized medicine	Systematic review of quantitative and qualitative level IV studies. V. Public perception of personalized medicine.	N = 21 studies with 9,507 participants. Not reported (systematic review). Not reported (systematic review).	MMAT Mixed Methods Appraisal Tool	The public is generally accepts PM, although concerns exist relating to costs and discrimination.	The authors had to define personalized medicine and explain the concept to participants, possibly introducing bias. The studies reviewed used different study designs, although efforts were made using MMAT to correct for this.
X			Wray (2012). Molecular Psychiatry.	Examine GWAS studies to determine genes associated with MDD.	Meta-analysis of cross-sectional descriptive studies. V. MDD, genotypes.	N = 6,104 cases, 2,431 diagnosed with MDD, 3,673. University of Edenborough.	Not reported	CACNA1C was in the top 4% of genes associated with MDD, BD, and SCZ. MDD has much lower heritability, is highly multi0factorial, and no single gene shows strong association with	Genotypes were obtained using different techniques in different study sites, sometimes due to economic constraints.

								MDD, unlike the highly heritable BD and SCZ.	
	X		Evans (2005). Journal of Affective Disorders.	Investigate the genetic spectrum for bipolar disorder.	Well-designed quasi-experimental study. III. Diagnosis of bipolar, dysthymic, cyclothymic, hyperthymic, irritable, or anxious, familial relatedness.	N = 148 including 85 people with BD and 63 control subjects. Convenience sampling through advertisements. UCSD Mental Health Clinical Research Center.	TEMPS-A, TCI-125	Bipolar disorder is inherited as a quantitative bipolar spectrum that is expressed in a variety of temperaments.	Participants were recruited through advertisements. Patients were in different states of mania or depression during the study.
	X		Angst (2018). Bipolar spectrum in major depressive disorders. European Archives of Psychiatry & Clinical Neuroscience	To evaluate the prevalence of people diagnosed with MDD who fall withing the bipolar spectrum.	Cohort study. IV. BP, MDD, family history, age of onset, manic symptoms, depression symptoms, main symptoms, onset of disorders, anxiety and panic symptoms, alcohol, tobacco, drug use, suicide.	N = 591 participants between 19-20 years of age screened for depression, neurotic, and psychosomatic syndromes. Note: the participants were the same cohort studied in #7 above. In the homes of residents of Zurich, Switzerland.	SPIKE, BBI	One-fifth of all participants who had a diagnosis of major depressive disorder were found to found to have manic syndrome, and one-fourth exhibited manic symptoms.	Due to the longitudinal nature of the study, all participants were between 20-50 years of age, and this may limit generalizability. Another limitation was underrepresentation of individuals with bipolar disorder.
	X		Titone (2018). Psychiatry Research	Examine effects of anxiety onset and future	Case-control. IV. Anxiety, depressive episodes,	N = 244 diagnosed with cyclothymia	BDI, HMI, GBI, SADS-L, SADS-C	Anxiety increases risks for SUD and depression.	All anxiety-related disorders were grouped together, and

				manic episodes.	hypomanic and manic episodes,	or BD. Temple University			anxiety severity was not assessed
	X		Benazzi (2006). European Archives of Psychiatry and Clinical Neuroscience	Assess distribution of hypomanic symptoms shared by BD and MDD.	Cross-sectional descriptive. V. Number of hypomanic symptoms between depressive episodes and family history of BD.	N = 389 BP 2 and 261 MDD. Outpatient psychiatry private practice in northern Italy.	SCID-CV, GAF, family history screen.	The evidence did not support the categorial classification of MDD and BD into a bimodal system, but instead as part of a multidimensional spectrum.	A lack of staff to take interviews may have reduced validity.
		X	American Psychological Association (2017). APA Report.	Establish clinical practice guidelines for the treatment of bipolar disorder.	Clinical Practice Guideline. 1.	N = 124 patients with bipolar disorder. Five RCTs were selected as evidence for guidelines. Setting not specified (clinical practice guideline).	Not reported (clinical guideline).	Recommendations for lithium use (clinical guideline).	The RCTs often included participants with poorly defined diagnoses different studies used different exclusion criteria. Some included patients with bipolar and unipolar depression, and some excluded unipolar depression.
		X	Mahli (2017). Journal of Affective Disorders	Clinical guidelines for using lithium for BD.	Clinical Practice Guideline. 1. Not reported (clinical guideline).	N = 13 papers included in review. Not reported (clinical guideline).	Not reported (clinical guideline).	Lithium monotherapy is recommendations for use with BD.	Evidence not similar to real-world.
		X	Edwards (2013). Lithium or an atypical drug in the treatment of treatment-resistant	Is lithium an effective adjunct medication for depression?	Systematic review of RCTs. I. Independent: Lithium, SSRIs, Dependent: Depression	N = 12 studies in pairwise meta-analysis. Variable due	Not reported.	Lithium plus an SSRI was more effective than treatment using an SSRI alone.	The primary limitation noted was that the study comparing treatment with an SSRI plus

			depression. Health.			to study being systematic analysis of different studies.			lithium with an SSRI plus an atypical antipsychotic did not use participants with treatment-resistant depression.
		X	Kleeblatt (2017). European Neuropsychopharmacology	Determine efficacy of off-label augmentation in unipolar depression.	Systematic review. I. Not reported (systematic review).	N = 5 RCTs. Studies that did not provide direct evidence for the question, which had low quality, or were reviews were excluded. Not reported (systematic review).	Not reported (systematic review).	Lithium augmented with SGAs are effective for depression.	Some non-indexed studies or those not in English may have been overlooked.
		X	Strawbridge (2019). The British Journal of Psychiatry	To quantify evidence fir augmentation interventions for TRD.	Systematic review and meta-analysis. I.	N = 238 RCTs included. RCTs worth at least 10 participants and a minimum of one augmentation treatment with TRD. Not reported (Systematic review and meta-analysis).	Used Cochrane Risk of Bias (RoB) tool to evaluate each study.	Lithium is an effective treatment for TRD.	TRD was inconsistently defined across studies.
		X	Undurraga (2019). Lithium treatment for	Determine whether lithium is	Systematic review. I. Independent: lithium various	N = 39 studies, there were N =	Not reported	Lithium is effective as an adjunct in	Not reported

			unipolar major depressive disorder. Journal of Psychopharmacology	effective in treating. unipolar depression.	adjunct antidepressants. Dependent: depression.	1605 participants, who were adults diagnosed with unipolar Major Depression Disorder (MDD) depression without a major medical or neurological illness. Randomized, double-blind. Variable due to study being systematic analysis of 39 different studies.		treating unipolar depression. The use of lithium as a treatment for unipolar depression is infrequent and limits the amount of data available to be analyzed. Some of the studies did not use a placebo as a comparator, providing ambiguity in result interpretation.	
		X	Vasquez (2021). Journal of Psychopharmacology.	To determine efficacy and tolerability of combination treatments for MDD.	Systematic review and meta-analysis. I.	N = 49 RCTs with 8104 participants. Not specified (systematic review and meta-analysis).	OR NNT, NNH, HDRS, MADRS	Lithium acts faster as an adjunct in treating MDD than other adunctives. Lithium is underutilized as an effective treatment for affective disorders and reduces suicide risk.	Many antidepressants used with lithium were older TCAs. Dosing was not optimized for all treatments. There was poor reporting of adverse events in some studies.
		X	Dold, (2018), Acta Psychiatrica Scandinavica.	Investigate augmentation therapies for MDD.	Cross-sectional. IV.	N = 1,410 adults over 18 with a diagnosis of major	HAM-D, MADRS. Inter-rater reliability was noted as not	Augmentation of standard antidepressant therapy with another medication,	All participants were outpatients at psychiatric treatment centers and may not be

						depressive disorder.	being calculated	especially lithium, is recommended for patients with treatment-resistant depression.	generalizable to populations in different settings. It is unknown if there were differences among clinical sites. Drug therapies were not stratified.

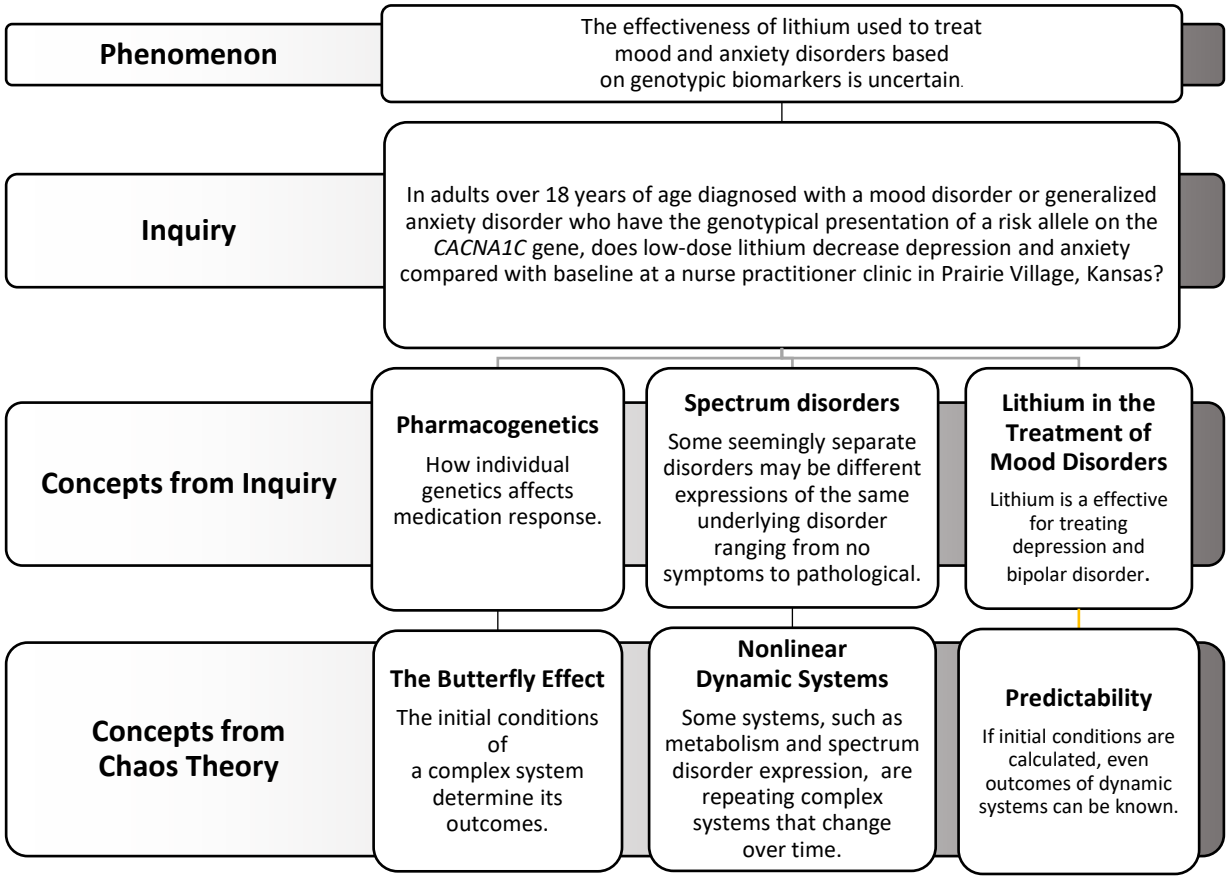
Appendix D: Evidence Grid

First Author, Year	Pharmacogenetics of Mood Disorders	Spectrum Disorders	Lithium in the Treatment of Mood Disorders
Clinical Pharmacogenetics Implementation Consortium, (2022)	X		
Smedler, (2022)	X		
Lazary, (2021)	X		
Guo, (2020)	X		
Smedler, (2020)	X	X	
Liu, (2020)	X		
Menezes, (2019)	X	X	
Calabrò, (2019)	X		
Holden, (2019)	X		
Dedic, (2018)	X		
Tecelão, (2018)	X		
Lin, (2017)	X		X
Mallas, (2017)	X		

Sumner, (2015)	X	X	
Córdova-Palomera, (2015)	X		
Higgins, (2015)	X		X
Nurnberger, (2014)	X		
Tesli, (2013)	X		
Wray, (2012)	X		
Psychiatric GWAS Consortium Bipolar Disorder Working Group, (2011)	X		
Green, (2010)	X	X	
Vasquez, (2021)			X
Strawbridge, (2019)			X
Undurraga, (2019)			X
Angst, (2018)		X	
Titone, (2018)		X	
Dold, (2018)			X
American Psychological Association, (2017)			X

Mahli, (2017)			X
Kleeblatt, (2017)			X
Edwards, (2013)			X
Benazzi, (2006)		X	
Evans, (2005)		X	

Appendix E: Theory to Application Diagram



Appendix F: IRB Approval Letter

UMKC Institutional Review Board
University of Missouri-Kansas City

5319 Rockhill Road
Kansas City, MO 64110
816-235-5927
umkcirb@umkc.edu

August 03, 2022

Principal Investigator: Lyla Jo Lindholm
Department: Nursing - General

Your IRB Application to project entitled "Pharmacogenetic Testing to Reduce Depression and Anxiety in Adults Treated with Lithium" was reviewed and determined to qualify for IRB exemption according to the terms and conditions described below:

IRB Project Number	2092206
IRB Review Number	379760
Initial Application Approval Date	August 03, 2022
IRB Expiration Date	N/A
Level of Review	Exempt
Project Status	Active - Exempt
Exempt Categories	45 CFR 46.104(d)(4)
Risk Level	Minimal Risk

Approved Documents
- Site approval documentation.
- Existing tool used in patient care-1
- Existing tool used in patient care-2

The principal investigator (PI) is responsible for all aspects and conduct of this study. The PI must comply with the following conditions of the determination:

1. No subjects may be involved in any study procedure prior to the determination date.
2. Changes that may affect the exempt determination must be submitted for confirmation prior to implementation utilizing the Exempt Amendment Form.
3. The Annual Exempt Form must be submitted 30 days prior to the determination anniversary date to keep the study active or to close it.
4. Maintain all research records for a period of seven years from the project completion date.

If you are offering subject payments and would like more information about research participant payments, please click here to view the UM system Policy on Research Subject Payments: https://www.umsystem.edu/oei/sharedservices/apss/nonpo_vouchers/research_subject_payments

If you have any questions, please contact the IRB at 816-235-5927 or umkcirb@umkc.edu.

Thank you,
UMKC Institutional Review Board

Appendix G: Project Site Approval Letter

JENNIFER REED NP

Jennifer Reed
FNP-BC, PMHNP-BC
8016 State Line Road, #205
Prairie Village, Kansas
66208

05/01/2022

Dear Sir/Madam,

I will be conducting a research project with Michael Dickerson, DNP student at UMKC starting summer 2022, pending IRB approval. I give permission for this research project to be conducted in my practice, Jennifer Reed NP LLC located at 8016 State Line Rd., #205, Prairie Village, KS, 66208.

Please contact me directly for any questions 816-508-7600.

Sincerely,

Jennifer Reed FNP-BC, PMHNP-BC

Appendix H: UMKC Faculty Project Approval Letter

UMKC School of Nursing and Health Studies

July 10, 2022

UMKC DNP Student: Michael Dickerson

Congratulations. The UMKC Doctor of Nursing Practice (DNP) faculty have approved your DNP project proposal, *Pharmacogenetic Testing to Reduce Depression and Anxiety in Adults Treated with Lithium.*

Sincerely,

Cheri Barber, DNP, RN, PPCNP-BC, FAANP
Clinical Assistant Professor
DNP Program Director
UMKC School of Nursing and Health Studies barberch@umkc.edu

Lyla Lindholm, DNP, RN, ACNS-BC
Clinical Assistant Professor, DNP Faculty
MSN-DNP Program Coordinator
UMKC School of Nursing and Health Studies lindholml@umkc.edu

Debbie C. Pankau DNP, APRN, FNP-BC
Clinical Assistant Professor
DNP Project Course Faculty
UMKC School of Nursing pankaud@umkc.edu

DNP Faculty Mentor: Lyla Lindholm
UMKC School of Nursing and Health Studies

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• nurses@umkc.edu
an equal opportunity/affirmative action institution

Appendix I: Cost Table

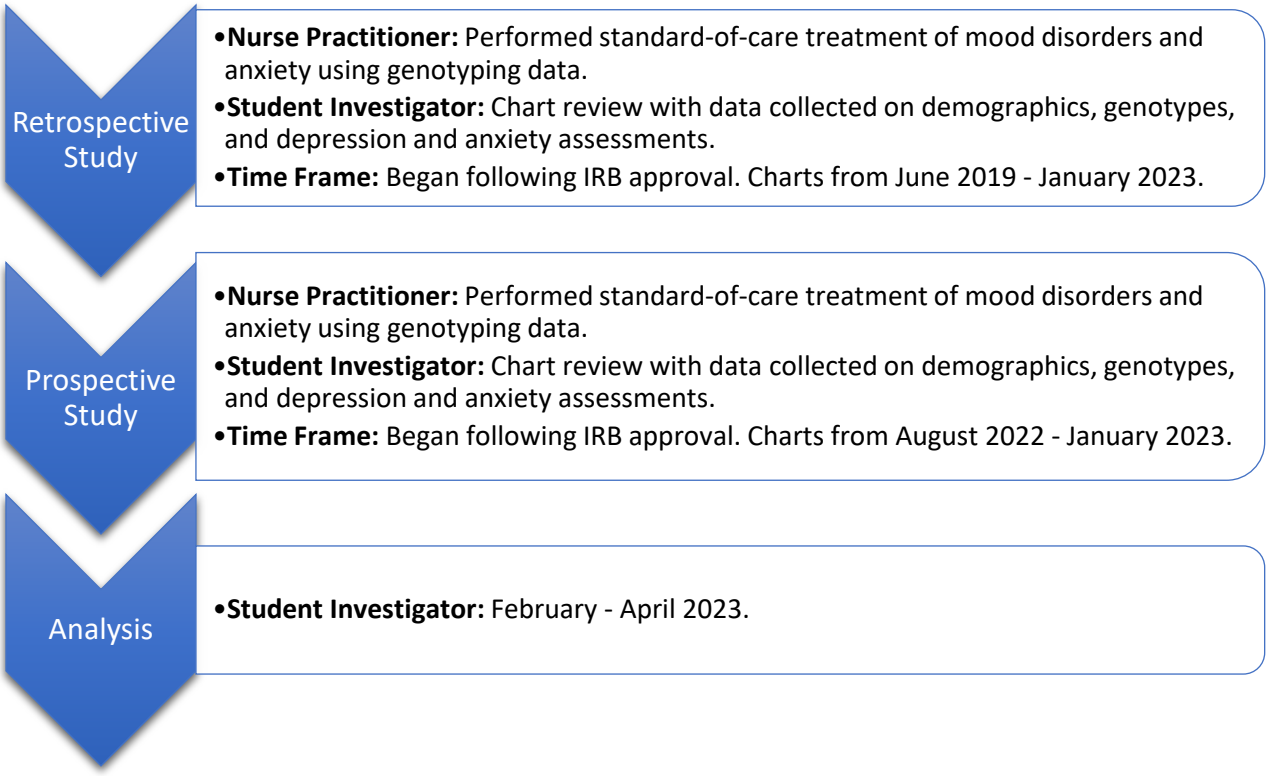
Budget for DNP Project			
Item	Cost/Unit	Quantity	Amount
Reimbursement for time for nurse practitioner	Donated		$0
Reimbursement for time for student investigator	Donated		$0
Gasoline for student investigator to and from project site	$3/gallon	80 gallons	$360
Printer Paper	$25/case	1	$25
Ink	$0.10/page	600	$60
Total Cost			$445

Note: Costs associated with standard operating procedures at the clinic that generated data were not included in the cost table because they would have occurred in absence of the project.

Return on investment (ROI) for the healthcare system: A cost-effective study was conducted that included 2,000 patients with Major Depression Disorder (MDD) divided into two groups (Davenport, 2022). One group was genetically tested, and the second group received traditional treatment with antidepressants selected without genetic testing. The fiscal impact on the healthcare system included costs of pharmaceuticals, hospitalization, psychiatric therapy, and loss of productivity. The incremental cost-effectiveness ratio (ICER) for the patients who underwent pharmacogenetic testing was $64,000 per quality-adjusted-life year, reflecting a substantial increase in savings and effectiveness (Davenport, 2022).

Appendix J: Project Timeline

Appendix K: Research Implementation Flow Chart



Appendix L: Logical Flow of Outcomes Table

	State Outcome	**Measurement**	**Tool validity and reliability if survey or similar***	**Permission Need**	**Statistical Analysis Test**
Primary Outcomes For *CACNA1C* genotypes after lithium therapy	Decreased depression	PHQ-9	Validity has a selectivity Cronbach-alpha of 0.77 to 0.88, sensitivity ranging from .88 to .94. Reliability from .86 to .89 (Kroenke et al., 2010).	Not required (Pfizer, 2022).	Repeated measures mixed ANOVA
	Decreased anxiety	GAD-7	Validity: Cronbach-alpha score of .89 for specificity and .82 for sensitivity. Reliability: Cronbach-alpha of .92 (Kroenke et al., 2010).	Not required (Pfizer, 2022).	Repeated measures mixed ANOVA
Number of risk *A and T* alleles and interactions between *CACNA1C* and *ANK3*.	Decreased depression	PHQ-9		Not required (Pfizer, 2022).	Repeated measures mixed ANOVA
	Decreased anxiety	GAD-7		Not required (Pfizer, 2022).	Repeated measures mixed ANOVA
Impact of *A* and *T* alleles on pre-test and post-test scores.	Decreased depression	PHQ-9		Not required (Pfizer, 2022).	One-way ANOVA.
	Decreased anxiety	GAD-7		Not required (Pfizer, 2022).	One-way ANOVA.
Impact of time interval between pre-test and post-test.	Decreased depression and anxiety.	PHQ-9 and GAD-7.		Not required (Pfizer, 2022).	One-way ANOVA.
Hardy-Weinberg Equilibrium of genotypes.					Ch-square analysis.
Demographics		NA	NA	NA	Descriptive for cohort.

Appendix M: Measurement Instruments PHQ-9 and GAD-7

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9)

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✔" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

FOR OFFICE CODING 0 + _____ + _____ + _____

=Total Score: _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult
☐	☐	☐	☐

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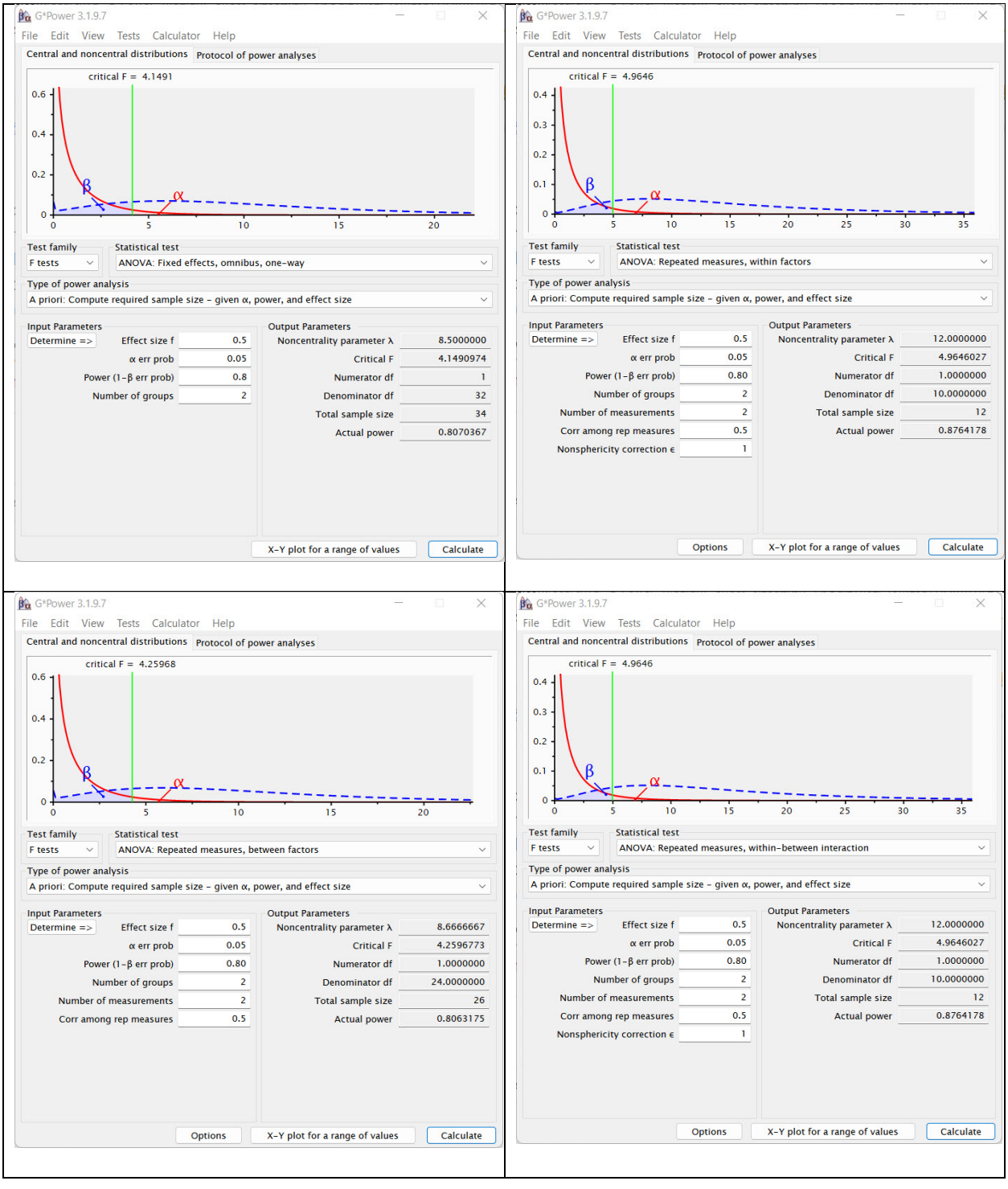
GAD-7

Over the last 2 weeks, how often have you been bothered by the following problems? *(Use "✔" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Feeling nervous, anxious or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it is hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid as if something awful might happen	0	1	2	3

(For office coding: Total Score T____ = ____ + ____ + ____)

Developed by Drs. Robert L. Spitzer, Janet B.W. Williams, Kurt Kroenke and colleagues, with an educational grant from Pfizer Inc. No permission required to reproduce, translate, display or distribute.

Appendix N: Sample Size Calculations



Appendix O: Data Collection Table Templates

Table O1
SPSS Data View Table Template

ID	Gender	Age	Race	CACNA1C	ANK3	PrePHQ9	PostPHQ9	PreGAD7	PostGAD7	Weeks	Dose	MAH_1	Prob_Mah	Delta_Dep	Delta_Anx
1															
2															
3															
4															
5															

Table O2
SPSS Variable View Table Template

	Name	Type	Width	Decimals	Label	Values	Missing	Columns	Align	Measure	Role
1	ID	Numeric	8	0	ID	None	None	8	Right	Scale	Input
2	Gender	Numeric	8	0	Gender	{1, Male}...	None	8	Right	Nominal	Input
3	Age	Numeric	8	2	Age	None	None	8	Right	Scale	Input
4	Race	Numeric	8	0	Race	{1, Caucasi...	None	8	Right	Nominal	Input
5	CACNA1C	Numeric	8	0	CACNA1C	{0, GA}...	None	8	Right	Nominal	Input
6	ANK3	Numeric	8	0	ANK3	{0, CC}...	None	8	Right	Nominal	Input
7	PrePHQ9	Numeric	8	0	PrePHQ9	None	None	8	Right	Scale	Input
8	PostPHQ9	Numeric	8	0	PostPHQ9	None	None	8	Right	Scale	Input
9	PreGAD7	Numeric	8	0	PreGAD7	None	None	8	Right	Scale	Input
10	PostGAD7	Numeric	8	0	PostGAD7	None	None	8	Right	Scale	Input
11	Weeks	Numeric	8	0	Weeks	None	None	8	Right	Scale	Input
12	Dose	Numeric	8	0	Dose	{150, 150}...	None	8	Right	Ordinal	Input
13	MAH_1	Numeric	11	5	Mahalanobis Di...	None	None	13	Right	Scale	Input
14	Prob_Mah	Numeric	8	4		None	None	10	Right	Scale	Input
15	Delta_Dep	Numeric	8	2		None	None	11	Right	Scale	Input
16	Delta_Anx	Numeric	8	2		None	None	11	Right	Scale	Input

Appendix P: Tables

Table P1

Demographic and Medication Dose Data of Sample Population with GA or AA Genotype

	Age			Gender		Race				Weeks			Lithium Dose (mg)		
n	Mean	Minimum	Maximum	Male	Female	Caucasian	Black	Hispanic	Asian	Mean	Minimum	Maximum	150	300	600
50	36.66	18.00	74.00	12	38	46	1	1	1	43	4	171	1	42	7

CACNA1C Genotype Distribution	
GA	AA
Count	Count
33	17

Table P2

Effect of Time Interval between Post-Test and Pre-Test on Depression and Anxiety

Effect of Time on Post PHQ-9 Scores (One-Way ANOVA)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	561.903	36	15.608	1.766	.136
Within Groups	114.917	13	8.840		
Total	676.820	49			

Effect of Time on Post GAD-7 Scores

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	485.980	36	13.499	.884	.633
Within Groups	198.500	13	15.269		
	684.480	49			

Table P3

Change in Depression after Treatment with Low-Dose Lithium (Repeated-Measures Mixed ANOVA)

Tests of Within-Subjects Effects

Source		Type III SS	df	Mean Square	F	Sig.	Partial Eta Squared
Depression	Greenhouse-Geisser	1115.308	1.000	1115.308	122.927	**.000**	**.728**
Depression * CACNA1C	Greenhouse-Geisser	26.266	1.000	26.266	2.895	.096	.059
Depression * ANK3	Greenhouse-Geisser	8.882	1.000	8.882	.979	.328	.021
Depression * CACNA1C * ANK3	Greenhouse-Geisser	1.033	1.000	1.033	.114	.737	.002

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
CACNA1C	133.915	1	133.915	4.064	.050	.081
ANK3	32.377	1	32.377	.982	.327	.021
CACNA1C * ANK3	9.104	1	9.104	.276	.602	.006

Pairwise comparisons of depression and *CACNA1C* genotype interactions.

Effect of *CACNA1C* on PHQ-9 Score Change from Pre-Test to Post-Test after Treatment with Low-Dose Lithium

						95% Confidence Interval for Difference	
Depression	CACNA1C	CACNA1C	Mean Difference in Genotypes	Std. Error	Sig.	Lower Bound	Upper Bound
PHQ-9 Pre-Test	GA	AA	3.755	1.695	**.032**	.344	7.167
PHQ-9 Post-Test	GA	AA	1.450	1.174	.223	-.914	3.814

Descriptive Statistics

	CACNA1C	ANK3	Mean	Std. Deviation	N
PHQ-9 Pre-Test	GA	CC	12.76	6.109	17
		CT	13.06	4.123	16
	AA	CC	9.92	5.760	12
		CT	8.40	4.722	5
PHQ-9 Post-Test	GA	CC	5.00	4.770	17
		CT	3.50	3.307	16
	AA	CC	4.00	2.796	12
		CT	1.60	1.817	5

Tests of Model Assumptions

Box's Test of Equality of Covariance Matrices

Box's M	9.47
F	.93
df1	9
df2	1887.88
Sig.	.49

Levene's Test of Equality of Error Variances

	F	df1	df2	Sig.
PHQ-9 Pre-Test	.937	3	46	.43
PHQ-9 Post-Test	3.441	3	46	**.02**

Table P4

Change in Anxiety after Treatment with Low-Dose Lithium (Repeated-Measures Mixed ANOVA)

Table . Change in Anxiety after Treatment with Low-Dose Lithium (Repeated Measures Mixed ANOVA)

Tests of Within-Subjects Effects

Source		Type III SS	df	Mean Square	F	Sig.	Partial Eta Squared
Anxiety	Greenhouse-Geisser	1533.088	1.000	1533.088	196.630	**.000**	.810
Anxiety * CACNA1C	Greenhouse-Geisser	6.399	1.000	6.399	.821	.370	.018
Anxiety * ANK3	Greenhouse-Geisser	29.676	1.000	29.676	3.806	.057	.076
Anxiety * CACNA1C * ANK3	Greenhouse-Geisser	3.160	1.000	3.160	.405	.528	.009

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
CACNA1C	62.111	1	62.111	2.529	.119	.052
ANK3	49.015	1	49.015	1.996	.164	.042
CACNA1C * ANK3	6.737	1	6.737	.274	.603	.006

Pairwise Comparisons of Anxiety and *CACNA1C* Genotype Interactions.

Effect of *CACNA1C* on PHQ-9 Score Change from Pre-Test to Post-Test after Treatment with Low-Dose Lithium

						95% Confidence Interval for Difference	
Anxiety	CACNA1C	CACNA1C	Mean Difference in Genotypes	Std. Error	Sig.	Lower Bound	Upper Bound
GAD-7 Pre-Test	GA	AA	1.204	1.415	.399	-1.644	4.051
GAD-7 Post-Test	GA	AA	2.341*	1.128	**.044**	.071	4.612

Descriptive Statistics

	CACNA1C	ANK3	Mean	Std. Deviation	N
GAD-7 Pre-Test	GA	CC	13.35	4.690	17
		CT	13.19	4.636	16
	AA	CC	12.33	3.367	12
		CT	11.80	5.263	5
GAD7 Post-Test	GA	CC	5.94	4.235	17
		CT	4.13	3.008	16
	AA	CC	4.58	3.728	12
		CT	.80	.837	5

Tests of Model Assumptions

Box's Test of Equality of Covariance Matrices

Box's M	17.773
F	1.752
df1	9
df2	1887.888
Sig.	.073

Levene's Test of Equality of Error Variances

	F	df1	df2	Sig.
GAD-7 Pre-Test	.604	3	46	.615
GAD-7 Post-Test	3.562	3	46	**.021**

Table P5

Effect of *CACNA1C G*enotypes on Depression before and after Low-Dose Lithium Treatment

PHQ-9 Pre-Test One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	132.657	1	132.657	4.842	**.033**
Within Groups	1314.963	48	27.395		

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
.000	1	48	.999

PHQ-9 Post-Test One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.745	1	10.745	.774	.383
Within Groups	666.075	48	13.877		

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
4.927	1	48	**.031**

Descriptive Statistics for One-Way ANOVA

PHQ-9 Pre-Test

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GA	33	12.91	5.162	.899	11.08	14.74	2	26
AA	17	9.47	5.375	1.304	6.71	12.23	3	24
	Ave	11.19						

PHQ-9 Post-Test

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GA	33	4.27	4.133	.719	2.81	5.74	0	15
AA	17	3.29	2.733	.663	1.89	4.70	0	10
	Ave	3.78						

Table P6

Demographic and Medication Dose Data of Sample Population with GG, GA, or AA Genotype

Demographics

	Age			**Gender**		**Race**				**Weeks**			**Dose**	
n	Mean	Min	Max	Male	Female	Caucasian	Black	Hispanic	Asian	Mean	Min	Max	300	600
69	37	18	74	13	56	62	3	1	3	47.69	4.00	170.86	61	8

Allele Frequencies

	Worldwide Population	Sample Population
G	.70	.52
A	.30	.48

Table P7
Hardy-Weinberg Chi-Square Analysis

Genotype Counts

	Observed N	Expected N	Residual
GG	20	19.0	1.0
GA	32	34.0	-2.0
AA	17	16.0	1.0
Total	69		

Test Statistics

	Genotype Counts
Chi-Square	.233[a]
df	2
Asymp. Sig.	.890

Appendix Q: Figures

Figure Q1

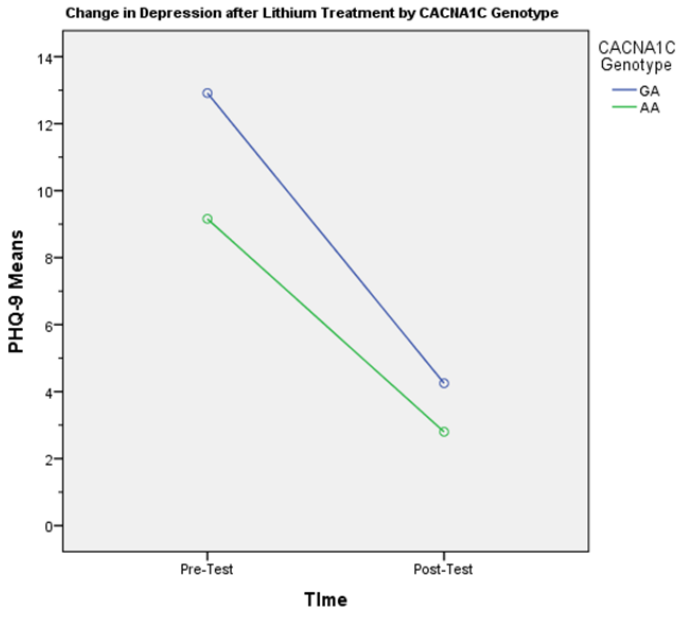


Figure Q2

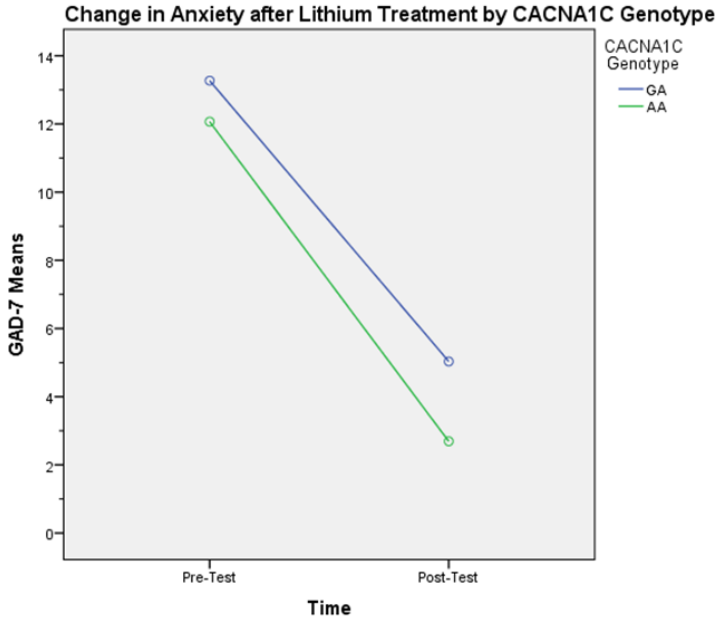
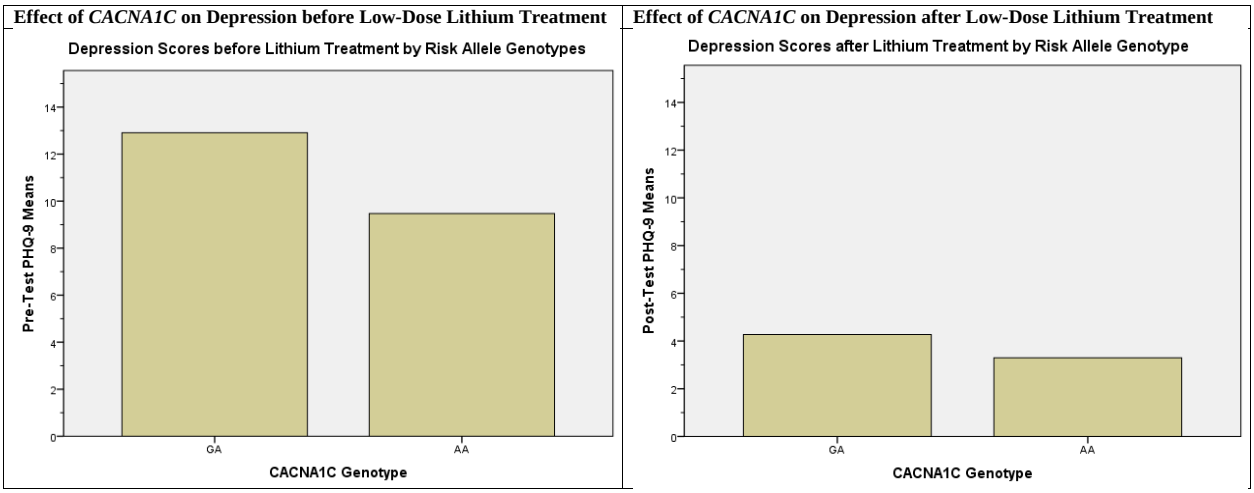


Figure Q3


Effect of *CACNA1C* on Depression before Low-Dose Lithium Treatment
Depression Scores before Lithium Treatment by Risk Allele Genotypes
Pre-Test PHQ-9 Means
0
2
4
6
8
10
12
14
GA
AA
CACNA1C Genotype
Effect of *CACNA1C* on Depression after Low-Dose Lithium Treatment
Depression Scores after Lithium Treatment by Risk Allele Genotype
Post-Test PHQ-9 Means
0
2
4
6
8
10
12
14
GA
AA
CACNA1C Genotype

Appendix R: Executive Summary

In the US, 8.4% over age 18 suffer from major depressive disorder (MDD), 2.8% from bipolar disorder (BD), and 19.1% from anxiety. The cost to the economy is over $502 billion yearly due to medical expenses, disability, lost productivity, and suicide. Treatment for mood disorders and anxiety is often trial-and-error, leading to treatment-resistant depression. Bipolar disorder is misdiagnosed as MDD over 40% of the time. Pharmacogenetics is emerging as being effective in personalizing drug selection to individual metabolism.

Purpose

The purpose of this research was to determine whether low-dose lithium (defined as ≤ 600 mg/day) effectively decreased depression and anxiety in adults with a risk allele on *CACNA1C* genotypes treated at a nurse practitioner psychiatric clinic in Prairie Village, Kansas. The secondary purpose was to determine if the number of *A* alleles affected depression and anxiety scores before or after treatment with lithium.

Methods

The study design was quantitative and correlational with a single cohort with two groups differing by the number of risk alleles. Chart review was accessed by a nurse practitioner who owned a private psychiatric clinic in the greater Kansas City area. Consecutive sampling was used to select patients over 18 years old who had been diagnosed with a mood disorder or anxiety and treated with low-dose lithium from June 2019 through January 2023. The nurse practitioner verbally relayed demographic, genotypic, and assessment scores to the student investigator who recorded the de-identified information in a SPSS spreadsheet. Pre-test and post-test depression and anxiety were measured using the PHQ-9 and GAD-7 assessment tools,

respectively. The SPSS 22 statistical software package was used to analyze the data with a combination of repeated measures ANOVA, one-way ANOVA, and chi-square tests.

Findings

The sample size of 50 achieved a statistical power of greater than .80 for all ANOVA tests, with alpha < .05. Low-dose lithium significantly decreased depression by 66% ($p < .001$) and anxiety by 65% ($p = < .001$). There was a significant difference in pre-test levels depression levels based on *CACNA1C* genotype ($p = .033$). The *A* allele frequency was 60% higher (48%) in this population than found in general population (30%). The timing of post-test was not significant for depression ($p = .136$) or anxiety ($p = .633$).

Recommendations

Low-dose lithium significantly decreased anxiety and depression compared with baseline. The *CACNA1C* genotype significantly affected levels of untreated depression. The *A* risk allele was 60% more common than in the general population, consistent with GWAS studies that predicted this allele as a biomarker for mood disorders. This study provides clinical evidence that the *CACNA1C A* risk allele is a useful biomarker that can be used to determine treatment of mood disorders and anxiety with low-dose lithium. Precision medicine has the potential to revolutionize the treatment of depression and anxiety by moving away from trial-and-error drug selection, improving mental health, and reducing cost of treatment.