# The Introduction of Methylation Testing for the *CACNA1C* Gene

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**Abstract**

One in four adults are diagnosed with a psychiatric disorder. Psychiatric genetic counseling, a rather new discipline of genetic counseling, assesses the familial history of a mental illness and analyzes the risk to transmit the psychiatric disorder to offspring. Psychiatric disorders are controlled by a multitude of genes, *i.e.* are polygenic, and are mediated by several environmental parameters, *i.e.*, are multifactorial, including, but not limited to, trauma, stress, abuse, smoking, and maternal physical or mental illness during pregnancy. Due to the complexity of their etiologies, there are no genetic tests specific for psychiatric disorders. This manuscript proposes using gene methylation, a mechanism for gene silencing or inactivation, as a marker for psychiatric disorders, with the *CACNA1C* gene as the model. The *CACNA1C* gene encodes for the alpha subunit of the CaV1.2, an L-type voltage-gated calcium ion channel, which controls the calcium ion flow into neurons of the brain. Methylation at the promoter of the *CACNA1C* gene inactivates the gene which negatively affects both brain functionality and an individual's electrochemical balance. Methylation of the *CACNA1C* gene is a risk factor in bipolar disorder and schizophrenia, as this negatively affects brain development. Transgenerational inheritance of traumatic stress, as shown in the offspring of Tutsi women in the Rwanda genocide experience, involved inheritance of a methylation imprint. Acknowledging the role of methylation as a transgenerational epigenetic mechanism in inheritance, it is suggested that gene methylation be used by the genetic counselor as a biomarker to screen for psychiatric disorders, using the *CACNA1C* gene as the model. Furthermore, utilizing gene therapy protocols, *e.g.,* CRISPR-Cas9. elimination of methylation from the *CACNA1C* gene, either in germline cells, the zygote, or in cells of the early embryo, would decrease the likelihood of transmitting/inheriting a psychiatric disorder. Another approach to lessen the transmission of psychiatric disorders due, in part, to

gene methylation, would be pharmaceuticals, such as DNA methyltransferase (DNMT) inhibitors.

## I. Psychiatric Genetic Counseling

Genetic counseling is the process by which healthcare providers, termed genetic counselors, work to identify the individual or familial risk of an individual or an offspring having a genetic disorder by collecting a family history and analyzing the information. Genetic counselors then use this information to help families understand and make informed decisions regarding their genetic health (Genetic Alliance, 2009).

Within genetic counseling, there are many specialties in which a genetic counselor can practice. These include, but are not limited to, prenatal, preconception, cancer, pediatrics, and most recently psychiatrics. Typically, genetic counseling appointments consist of the genetic counselor evaluating the patient's risk for a certain disorder based on a familial medical history. Additionally, genetic testing is offered to give the patient additional information about their risk. The genetic counselor explains the genetic testing process, how the test works, and the emotions that the patient may experience (Dailey *et al.,* 1995).

Psychiatric genetic counseling aims to provide genetic counseling for those whose offspring are at risk for a psychiatric disorder or for those already diagnosed with a disorder. This specialty within genetic counseling is incredibly niche and currently exists only in the Adapt Clinic located in the British Columbia Women's Hospital and headed by Dr. Jehannine Austin, Ph.D., M.S., C.G.C.. This niche is despite the fact that one in four adults suffer from a psychiatric disorder. (Andrade, 2019).

Psychiatric disorders are controlled by many genes, *i.e.*, are polygenic traits, over which are superimposed environmental factors that mediate the expression of the disorder. Such traits are termed to be multifactorial, polygenic traits. Whereas simple genetic screening is possible for single-gene disorders*, e.g.*, Tay-Sachs disease where the *HEXA* gene is mutated, currently, there is no genetic testing to identify or assess risk for a psychiatric disorder.

An obvious question is what tools are available to psychiatric genetic counselors to assess the risk factors of a psychiatric disorder. The role of a psychiatric genetic counselor is to first develop a family history of mental illness and then collect and analyze the information regarding known causes of the mental illness. Therefore, the role of a psychiatric genetic counselor is to educate the patient on the etiology of the psychiatric disorder and provide direction and strategies for the patient to alleviate the occurrence of the disorder. Guidance is provided by the psychiatric genetic counselor to educate family members who may be affected by the mental illness or who may have children affected. Another aspect of psychiatric genetic counseling focuses on preparation for the future as well as providing emotional support and validation, rather than a computer-calculated assessment of risk (Austin, 2019).

The unique aspect of this manuscript is directed around the idea that as psychiatric genetic counseling evolves, genetic screening for gene methylation, a process that mediates the expression of various mental and psychiatric disorders, should be integrated into the initial consultation between the genetic counselor and a patient with a family history of mental illness when evaluating whether or not this mental illness will be transferred to the patient's offsprings. An example of a gene that methylation testing needs to be developed for is the *CACNA1C* gene.

Recent research has assessed that the methylated form of the *CACNA1C* gene is a major risk gene for psychiatric disorders such as bipolar disorder and schizophrenia (Starnawska & Demontis, 2021).

## II. The *CACNA1C* Gene

The *CACNA1C* gene encodes for the formation of an ion channel, known as CaV1.2, a L-type voltage-gated calcium ion channel that mediates the number of calcium ions entering the cell in response to a proton gradient across the membrane. Calcium ions participate in many important cellular functions such as muscle contraction, regulation of intercellular electrical activity, and gene activation/inaction. The CaV1.2 L-type voltage-gated calcium ion channel allows for these essential functions by ensuring the proper amount of calcium ions enter the cell. In particular, the CaV1.2 channel encoded by the *CACNA1C* gene is connected to the genes that aid in the development of the fetal brain and bones. CaV1.2 specifically assists in the functioning of both neurons in the brain, as well as cardiomyocytes, the cells responsible for heart contraction (Hofmann *et al.,* 2014).

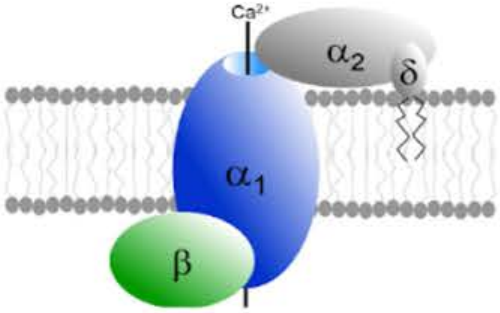


**Figure 1:** Depicted above is the structure of the CAV1.2 calcium ion channel when working properly. The alpha-1 transmembrane structure is seen with a calcium ion passing through the tunnel into the cell.

The structure of the CaV1.2 L-type voltage-gated calcium ion channel is composed of four subunits: alpha-1, alpha-2/delta, gamma, and beta. These subunits exist in a 1:1:1:1 ratio.

Distinctly, the *CACNA1C* gene encodes for the alpha-1 subunit of the CaV1.2 calcium ion channel (Figure 1). The alpha-1 subunit forms the tunnel through which ions enter the cell and is composed of twenty-four integral protein segments, which span the entirety of the cell membrane. Within the alpha-1 subunit are binding sites, where most drugs and regulatory circuits bind, as well as voltage sensors that monitor the amount of voltage entering the cell through this tunnel. The alpha-1 subunit of the CaV1.2 L-type voltage-gated calcium channel encoded by the *CACNA1C* gene is located on the *p* arm of chromosome 12, specifically at 13.33-spot 13 subsections 33. However, the *CACNA1C* gene has various isoforms resulting from alternative splicing, a process that produces diverse mRNAs and subsequently diverse proteins from one gene via the assembly of multiple exons in varying order. This process allows for different versions of the CaV1.2 L-type voltage-gated calcium ion channel to be present in humans (Hofmann *et al.*, 2014). The difference between each isoform is crucial in analyzing mutations in the *CACNA1C* gene, including the methylated form of the gene that will be discussed further.

## III. Importance of Calcium Ion Channels

It is well researched that mutations in the *CACNA1C* gene are linked to diseases that affect the heart such as Brugada Syndrome, Timothy Syndrome, and Short QT Syndrome. This is a result of the role the *CACNA1C* gene plays in controlling calcium ion flow. When mutated, the structure of the L-type voltage-gated calcium ion channel is altered, changing the heart's electrical system and the proper functioning of the brain (Moon *et al.,* 2021).

To add, calcium channels are essential for synaptic plasticity (Figure 2): the ability of synapses, in response to usage or disusage in their activity, to strengthen or weaken (Kreutz, 2012). In patients with polygenetic psychiatric disorders such as bipolar disorder and schizophrenia, one of the hallmarks is that the synaptic plasticity has been disrupted (Forsyth & Lewis, 2017). The disruption of calcium signaling, as well as intracellular calcium imbalance, is attributed to the methylated version of the *CACNA1C* gene (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011) which is explored later in this manuscript.

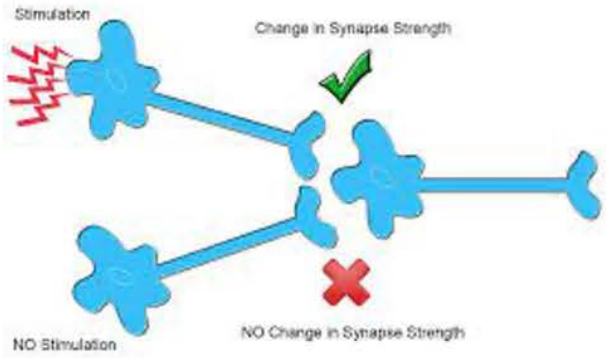


**Figure 2:** Demonstration of synaptic plasticity

## IV. Polygenic, Multifactorial Disorders

Each gene in an individual exists in two copies, one carried on the person's maternal chromosome and the other on the person's paternal chromosome. At times, a gene may carry a mutation, which causes it to lose function (*i.e.,* known as a loss-of-function allele). However, sometimes only one copy of the gene is defective. If the defect in one copy of the gene is not noticeable in the person, that allelic form of the gene is said to be recessive. If, on the other hand, one defective form of the gene causes an observable effect, the defective gene is termed dominant. The above information forms the basis of Mendelian genetics, in which the trait/disorder is governed by one pair of genes, To analyze phenotype, or the appearance, of the

expected progeny from a mating between two individuals, a Punnett square is constructed. Genes controlling the phenotype are not linked *i.e.,* their transmission is independent of one another.

A Punnett square is constructed by recording the genotypes, or genetic variations of a gene transmitted in the sperm and egg and using that information to determine the potential phenotypic variations in the offspring. The Punnett square is designed to include the various genotypes carried by the egg and sperm, allowing for the determination of the phenotypic and genotypic ratios in the offspring. For example, consider a mating between two heterozygotes, hybrid for the genes, *R* and *r* (Figure 3). Using the Punnett square, it is predicted that such a mating would yield the 1:2:1 genotypic ratio, 1*RR*: 2*Rr* 1*rr*. The capital letter (*R*) indicates the dominant allele, meaning this phenotype is always exhibited. The lowercase letter (*r*) indicates that this allelic form of the gene is recessive, meaning that this variant is masked when paired with a dominant allele. This is because while each dominant trait contributes to the phenotype, each recessive gene does not. Therefore, the recessive form of the gene is not observed unless an individual carries two doses of the recessive allele. The above is the overall theme of Mendelian inheritance, but there are many variations upon this theme. One mode of inheritance is autosomal recessive, which occurs when an individual has two recessive alleles (*rr*). Both recessive alleles are necessary for the individual to exhibit the trait. In an autosomal dominant pattern of

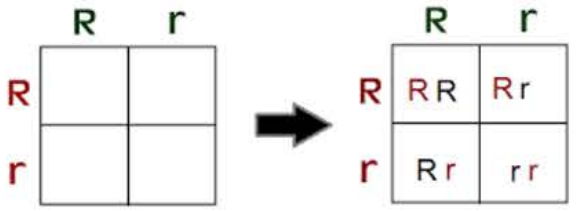


**Figure 3:** Using the parental genotypes of Rr and Rr, a 1:2:1 ratio is predicted as possible outcomes for an offspring.

inheritance, only one dominant allele is necessary for the person to exhibit the trait (either *Rr* or *RR*).

For traits or disorders controlled by a plethora of genes and environmental elements, the range of phenotypes in the offspring varies continuously, generating a bell-shaped curve. Such quantitative traits are characterized as polygenetic. For quantitative traits, it is impossible to use a Punnett square for risk assessment and, instead, statistical methods are commonly employed.

As quantitative traits are controlled by multiple genes, when genes affecting the quantitative traits are recognized, they are called quantitative trait loci (QTL). QTLs are able to be identified based on the location of the genes affecting a quantitative trait on a specific chromosome. QTLs are established based on their proximity to already known unique identifiers, called markers. (Brooker, 2021, p. 712-715).

## V. Determination of Genetic Contributions

### Heritability Value

There are many ways in which researchers distinguish between environmental and genetic components of a polygenic, multifactorial disorder. One is determining the heritability (H) value, which ranges from 0 – 1. H values greater than 0.5 indicate a strong genetic component as the cause of a disorder. To calculate the H value, the researcher compares the ratio of observed phenotypic variations to the expected variations for a population of related individuals (Sanfilippo *et al.,* 2019). For bipolar disorder and schizophrenia, H values were calculated for

identical, or monozygotic, twins raised by their biological parents as well as identical twins who were separated and raised by adopted parents. Such studies allowed for determining if the disorders were caused by genetic or environmental factors. It was established that while both bipolar disorder and schizophrenia have large genetic components, bipolar disorder has a stronger genetic component than schizophrenia (Gejman *et al.,* 2010).

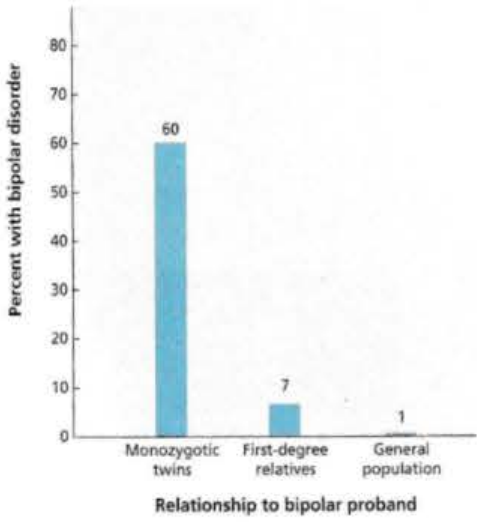


**Figure 4:** The graph above highlights the risk of developing bipolar disorder based on family relation. The high risk of monozygotic twins indicates a high genetic component.

In bipolar disorder, the concordance rate, or the rate at which a trait is present in identical twins, is around 60% (Kerner, 2014) and the heritability value is estimated to be around 85% (McGuffin *et al*., 2003) (Figure 4). This indicates that the genetic components play a larger role in the diagnosis of bipolar disorder than the environmental components. For schizophrenia, it is known that the concordance rate in monozygotic twins is

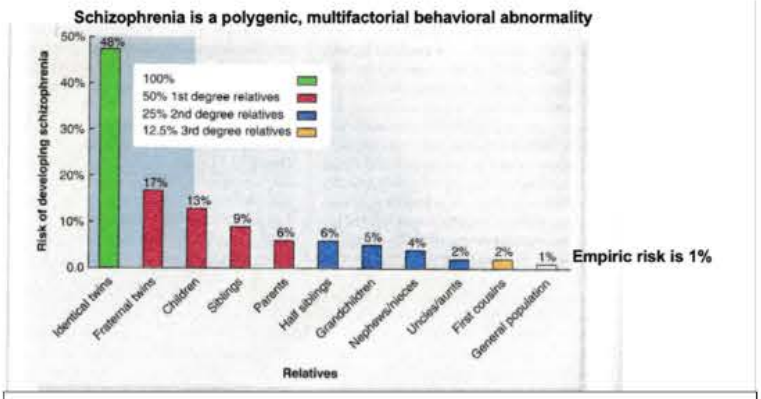


**Figure 5:** The graph above highlights the risk of developing schizophrenia based on family relation. The high risk of monozygotic twins indicates a high genetic component.

around 50% and the heritability value is 81% (Sullivan *et al.,* 2003) (Figure 5). This indicates that there are equally strong environmental components and genetic components that come into play when one is diagnosed with schizophrenia.

Contrastingly, when dizygotic, or fraternal twins, were raised by their biological parents versus dizygotic twins who were separated and raised by adopted parents, the concordance value was said to be around 8% for bipolar disorder (Smoller & Finn, 2003) and around 10% for schizophrenia (Imamura *et al.,* 2020) (Table 1). The low rate at which the trait was present indicates a weak environmental component and strong genetic onset. A large component of the genetic inheritance is due to the methylation that is passed from parent to offspring.

| | Concordance | |
|---|---|---|
| Trait | MZ (identical) twins | DZ (fraternal) twins |
| Acne | 14% | 14% |
| Alzheimer disease | 78% | 39% |
| Anorexia nervosa | 55% | 7% |
| Autism | 90% | 4.5% |
| Bipolar disorder | 33–80% | 0–8% |
| Cleft lip with or without cleft palate | 40% | 3–6% |
| Hypertension | 62% | 48% |
| Schizophrenia | 40–50% | 10% |

**Table 1:** Comparison of concordance values of monozygotic twins and dizygotic twins.

**Threshold Model**

Multifactorial polygenic disorders are best described using the threshold model, which is a plot of the frequency of the disorder within a population versus the genetic liability for the disorder being analyzed. A point within the bell-shaped curve is reached at which the genetic liability is

so great (*i.e.*, at the threshold) that the disorder is now manifested. Environmental factors are of most concern near the threshold level (Figure 6).

The threshold model takes into account the effects of the deleterious genes and the environmental factors which influence the point at which a person will become affected by the disorder (Frisen, 2002). For one to overshoot the threshold value and be affected by the disorder, these multiple components – defective genes and environmental factors – are summed. Examples of environmental factors include abuse, smoking, maternal virus infections or obstetric complications, and exposure to toxins during childhood (Schmitt *et al.,* 2014). The threshold model can be used for psychiatric disorders such as bipolar disorder and schizophrenia.

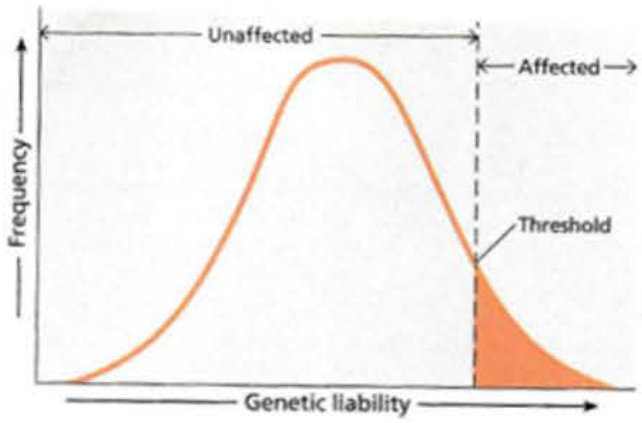


**Figure 6:** Depicted above is an example of the threshold model. The shaded region indicates the point at which manifestation of the disorder occurs.

## VI. Bipolar Disorder

Bipolar disorder is a psychiatric disorder that affects around 3% of the world (National Institute of Mental Health, 2017). It is classified as a mood disorder that involves intense mood swings and changes in energy levels. Within bipolar disorder, there are multiple types. Bipolar disorder I

is categorized by a long-lasting manic episode, during which the person is extremely elated and with much energy, followed by depressive episodes, during which the person is extremely hopeless and sad. Bipolar disorder II is categorized by depressive episodes and hypomanic episodes, a less severe version of a manic episode. The third type of bipolar disorder, cyclothymic disorder, involves both depressive episodes as well as hypomanic episodes. However, the episodes last for a shorter duration of time and are less intense (Medline Plus, 2021). While bipolar disorder is thought to be mostly genetic, there are environmental factors that play a role. It is indicated that the older the parent, the greater the chance of offspring with bipolar disorder (Chudal *et al.*, 2014). However, the most important environmental factor that influences bipolar disorder is childhood trauma. This includes instances of physical, emotional, or sexual abuse. Additionally, other stressful life events such as losing a job or the death of a loved one are thought to be environmental risk factors for bipolar disorder (Menculini *et al.,* 2020).

## VII. Schizophrenia

Schizophrenia is a psychiatric disorder that affects less than 1% of the population. It involves behavioral abnormalities as well as cognitive issues such as hallucinations and psychosis due to impairment of neurological functions. Symptoms of schizophrenia fall into three categories: positive, negative, and cognitive. Positive symptoms are when something is added to a person's behavior, such as delusions; negative symptoms are when something is removed, such as an individual distancing from social environments; and cognitive symptoms relate to an individual's thoughts, such as disorganized thinking (Canadian Mental Health Association, 2018).

Schizophrenia is a late-onset disease that typically exhibits itself in one's early twenties and thirties. It is thought to be due to a combination of genetics and environmental stressors (Batinic *et al.,* 2019). An individual whose mother experienced complications in pregnancy or birth has an increased risk of being affected with schizophrenia at a later age. This was correlated with hypoxia, or the lack of oxygen, often experienced by these individuals.

Furthermore, viral, bacterial, and parasitic infections both during pregnancy and in individuals post-birth are thought to increase one's risk of schizophrenia. Specifically, *Toxoplasma gondii* has been shown to increase the odds of schizophrenia by 80% when found latent inside an individual (Robinson & Bergen, 2021). *T. gondii,* a common pathogenic protozoan, is a single-cell parasite that causes toxoplasmosis. As *T. gondii* typically remains latent, the infected person will be asymptomatic and unaware of the entry of the pathogen. However, despite the latency, *T. gondii* can regulate the behavior of its host and is implicated in altering the gene activity and many biological pathways in the host (Sutterland *et al*., 2015). Therefore, there is a high correlation between individuals that are hosting the latent form of *T. gondii* and these individuals developing schizophrenia at a later age. Childhood trauma such as financial struggles, abuse, or neglect are also environmental factors that increase the risk of schizophrenia (Robinson and Bergen, 2021). The older the paternal age, the larger the risk of an offspring being affected with schizophrenia (Miller *et al.,* 2011).

## VIII. Genome-Wide Association Study (GWAS)

A Genome-Wide Association Study (GWAS), or Whole Genome Study, scans the unique markers of a genome with the purpose of recognizing if any specific variants or single nucleotide polymorphisms (SNPs) are associated with a trait or disorder (Chang *et al.,* 2018).

When searching for risk loci that may be associated with schizophrenia, the GWAS identified one hundred and eight genes that may be implicated in this disorder (table 2). Many of those genes are those that assist in neurological functioning. The etiology and mechanisms that lead to an individual having schizophrenia are still being discovered, despite extensive research over the past century (He, 2020). With bipolar disorder, as well, sixty-four loci have been found via GWAS to be associated with this disorder (table 1). Within those sixty-four loci, many were found to be related to synaptic signaling pathways and genes that assist with brain function (Mullins, 2021). With the addition of GWAS, new genes that may be risk alleles are constantly being uncovered. The whole-genome study has allowed researchers to gain better insight into the genes affected in an individual with polygenic disorder.

In psychiatric disorders, many risk loci are related to genes coding for voltage-gated calcium ion channels. Being that the *CACNA1C* gene affects synaptic plasticity as well as the regulation of genes associated with development of the brain, hypermethylation of this gene is a risk factor in both bipolar disorder and schizophrenia (Bigos, 2010).

Hertzberg *et al*. (2015), in a combination of a GWAS with genomic data from post-mortem patients diagnosed with schizophrenia, found a significant amount of calcium ions within the risk genes for schizophrenia. This indicates that the correlation between schizophrenia and highly

elevated calcium levels is statistically significant. Additionally, a study by Harrison *et al*. (2021) indicated a larger amount of calcium ions in the platelets and lymphocytic cells of patients with bipolar disorder compared to the healthy controls.

Typically, a low amount of free calcium ions are found in the cytoplasm due to the strict calcium ion regulation done by the voltage-gated calcium ion channels. However, when these channels are methylated, an influx of calcium ions flow into the cytoplasm, consequently disrupting neurological pathways necessary for development (Heyes *et al.,* 2015).

## IX. Methylation

Recently, there has been research regarding the correlation between the *CACNA1C* gene in its hypermethylated form and bipolar disorder and schizophrenia

Although these disorders are polygenic as well as multifactorial, the new research indicated that the *CACNA1C* gene was a major susceptibility gene within the multiplicity of genes that cause these psychiatric disorders (Starnawska and Demontis, 2021). Methylation occurs when methyl ($-CH_3$) groups are added

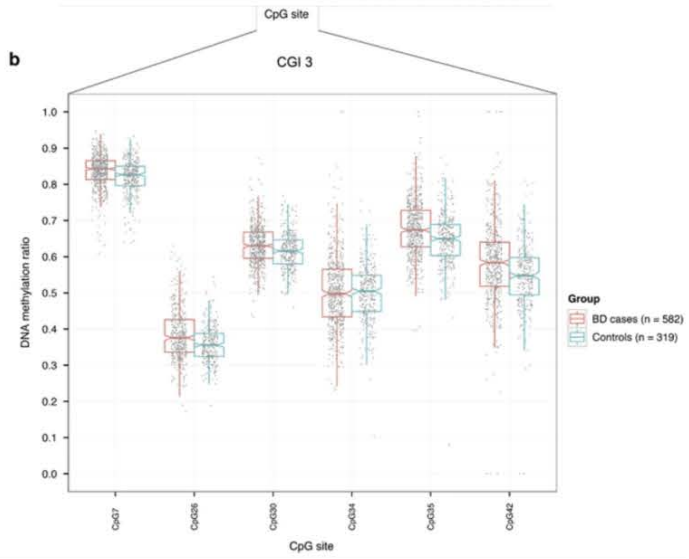


**Figure 7:** Above is the diagram of CGI 3 and the comparison between the methylation levels in bipolar disorder patients compared to the healthy controls

to a DNA molecule. As a result, methylation affects the functionality and, at times, even survival, of the individual (Holland, 2016). When a substantial amount of methyl groups are added, the resultant state is stated to be hypermethylated. In the hypermethylated state, the *CACNA1C* gene's function is impaired, causing an abnormal current of calcium ions generated in the CaV1.2 L-type voltage-gated calcium ion channel. In specific, hypermethylation occurs in the CpG islands, or, groups of repeating cytosine and guanine nucleotides linked with a phosphate. In an experiment by Starnawska *et al*. (2016), five CpG islands, abbreviated CGIs, were analyzed. While four of those islands were shown to be completely methylated or completely unmethylated, CGI 3 displayed intermediate levels of methylation. The experiment concluded that in the patients with bipolar disorder there was a highly significant increase in methylation compared to the healthy patients (Figure 7).

If tests were available to reveal the methylation status of the *CACNA1C* gene, perhaps it would inform patients if their offspring are predisposed to develop bipolar disorder and schizophrenia. Psychiatric genetic counseling is primarily about empowering the patient and the development of methylation testing will cater to this goal. There is power in knowledge and patients with a family history of mental illness can provide a sense of if they carry the hereditary components and if they will pass it to their offspring.

Implementation of methylation testing for genes like the *CACNA1C* gene, which is linked to mental illness in its methylated form, will allow for documentation of medical records. Thereby assisting medical professionals in making a diagnosis.

## X. Inheritance of Methylation

Methylation status can be impacted both by environmental and genetic factors. Environmental factors include diet, drugs, or undergoing trauma. In many psychiatric disorders, such as bipolar disorder and schizophrenia, stress or trauma is a large risk factor (Umeoka, 2021) as stress can cause long-term effects on the body. Commonly, the effect of stress is methylation of the promoter sites of genes that are expressed in the brain (Wein, 2010). The addition of methyl groups to cysteines of a promoter inhibits RNA polymerase from transcribing the gene, or in effect, silencing the gene. In an experiment with rats, pups who received a lower level of nurturing in their early phases of life had a higher level of methylation in exons encoding for the glucocorticoid receptor in the hippocampus. Contrastingly, the pups who received nurturing in the early phases of their life had a lower degree of gene methylation (Weaver *et al*., 2004) in the exons encoding for the glucocorticoid receptor in the hippocampus.

Although gene methylation does not alter one's DNA sequence, it does alter the gene's function. The addition of a methyl group on a gene is labeled as a "marker", or unique identifier, and can be inherited by gametes. This is due to DNA replication as well as independent assortment, the process by which genes separate independently from each other, contributing to genetic variation.

During DNA replication, the regions containing the additional methyl groups are replicated, resulting in the daughter chromosomes inheriting the methylated regions. During gametogenesis, the process by which gametes are produced, the chromosomes passed down to the gametes

contain the genes that have methylated regions. Therefore, the methylated status is maintained and manifested in the next generation (Kim & Costello, 2017). In the case of the *CACNA1C* gene, research indicates that in individuals with bipolar disorder and schizophrenia, chromosome 12 is transmitted in its hypermethylated form, serving as an indicator for these psychiatric disorders. The genetic inheritance of mutated or methylated genes to offspring is why family history is the strongest predictor of mental illness. One in three individuals with a mentally ill parent will develop the same illness (Sandstrom, 2019).

## XI. Transgenerational Epigenetics of Traumatic Stress: The Rwandan Genocide

Stress and trauma play a major role in epigenetics, as is seen in the offspring of women who survived the Rwandan Genocide of 1994, in which Hutu nationalists murdered around 800,000 people in an effort to eliminate the Tutsi ethnicity. The brutality of this genocide caused long-lasting trauma and stress in those who were exposed to this attempt at ethnic cleansing (History.com Editors, 2009).

Musanabaganwa *et al.* (2022) studied the offspring of women who were pregnant in Rwanda during the genocide and, thereby, were directly exposed to the tragedy. Subsequently, their

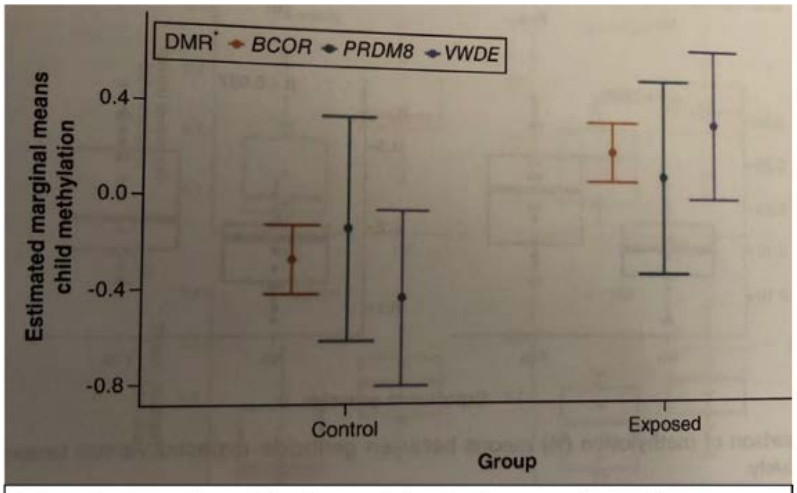


**Figure 8:** Comparison of the three methylated regions associated with *in utero* direct exposure to the Rwandan Genocide in offspring who were directly exposed vs offspring who were not. The methylation levels in the directly exposed offspring is significantly higher.

offspring were compared to offspring of women pregnant during the Rwandan Genocide, but who were not living in Rwanda. Leukocyte-derived DNA from mothers (exposed and nonexposed) and their offspring (exposed and nonexposed) identified 24 differentially methylated regions (DMRs). *In utero* genocide exposure was associated with cytosine residues in 3 of the 24 DMRs (Figure 8), with higher DNA methylation in exposed versus unexposed offspring. Thus, *in utero* genocide exposure was linked with methylation differences in maternal DMRs, with higher methylation in exposed offspring. Two of three DMRs showed correlation between brain and blood methylation within individuals, suggesting that peripherally derived signals of genocide exposure may be relevant to the brain. These results indicated that methylation due to trauma in a parent was transmitted to offspring (Musanabaganwa *et al.*, 2022).

In many psychiatric disorders, such as bipolar disorder and schizophrenia, a large role in the disorder is due to methylation of many genes. This includes the *CACNA1C* gene. The study of the Rwandan Genocide established that the transmission of methylation was due not only to genetics, but also to environmental parameters, *i.e*., trauma, which lead to transgenerational effects. The inheritance of epigenetic mechanisms from a pregnant woman to offspring, as seen in the Rwandan Genocide, allows insight into how environmental components can alter expression of DNA.

## XII. Single Nucleotide Polymorphisms (SNPs) and their Relation to Methylation

Molecular psychiatry is the study of biological pathways and mechanisms associated with mental illnesses and psychiatric disorders. This scientific method lacks consistency in its genetic components due to the complicated epigenetic nature of mental illness. However, with science evolving and more studies regarding risk genes for psychiatric disorders coming to completion, DNA methylation has become a consistent factor in molecular psychiatry. Specifically, DNA methylation in, or near, the promoter site of a gene which silences the expression of that gene (Starnawska and Demontis, 2021).

Scientists are able to locate genes associated with specific diseases as a result of SNP analyses, the main cause of genetic variability among individuals. A SNP is a change in one of the nucleotides within a sequence of DNA. On average, there are around four million SNPs per genome, which allows scientists to recognize reoccurring SNPs in people diagnosed with a common disorder. The specific sequence associated with the disease - complete with the SNPs - is termed a genetic marker (Medline Plus, 2020).

Typically, when SNPs are located close to a regulator region or a promoter site, they have an impact on the functioning of the gene. In turn, this allows the SNPs to have a greater connection to the manifestation of a disorder. In the case of the *CACNA1C*

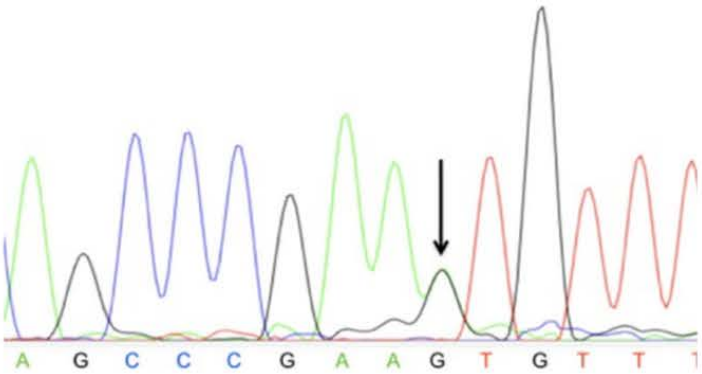


**Figure 9:** This is the DNA sequencing of the risk SNP rs1006737 as well as the sequence that surrounds it. The black arrow indicates where the SNP is located

gene, the SNPs associated with many psychiatric disorders are termed rs1006737 (Liu *et al*., 2020). When this biological marker was noted in individuals, it was shown to increase depression and anxiety scores, as well as paranoia and amygdala activity (Starnawska and Demontis, 2021). The amygdala is the part of the brain that processes fear and threatening stimuli (Dixon and Dweck, 2021). All of these traits are associated with the etiology of psychiatric disorders.

Rs1006737 (Figure 9) is a “risk SNP " that does not change the coding sequence of the *CACNA1C* gene. Despite this, since it is located in intron three, which affects important regulatory activity, rs1006737 does affect the structure and function of the *CACNA1C* gene. Additionally, recent research has indicated that the rs1006737 risk allele within the *CACNA1C* gene is associated with a statistically significant level of DNA methylation despite its location in a non-coding region of the genome. This is due to its location near a promoter site that regulates gene function (Starnawska and Demontis, 2021).

This risk allele and its relation to DNA methylation levels were implicated in both bipolar disorder and schizophrenia as a result of the impact the risk SNPs had on the functionality of the *CACNA1C* gene and the encoded L-type voltage-gated calcium ion channel (Bigos *et al*., 2010). The higher the degree of methylation, the more likely an individual is to be diagnosed with a psychiatric diagnosis (Liu, 2018). This newfound knowledge allows for future treatment of psychiatric disorders as well as grounds to create a testing option for hypermethylation within the *CACNA1C* gene, specifically by locating the risk SNP known as the rs1006737 allele.

## XIII. Future Direction

### Methylation Analysis

Methylation analysis is a tool that studies the patterns of methylation within a genome (Kurdyukov and Bullock, 2016). However, methylation analysis is not widely used and when used, is to locate the methylation of the *MTHFR* gene, responsible for neurotransmitter production. However, as limits of technology are continuously tested and the machine learning approach, through which computer analysis shifts with additional data, is utilized, methylation analysis continues to become more accurate. Specifically, as more data is added and more methylation patterns are noted in the genome, this new information will allow new genetic markers to be recognized (Sadikovic, 2018). The human genome was recently resequenced for epigenetic patterns, allowing scientists to recognize the points of methylation in the genome (Gershman *et al.,* 2022). This can assist with methylation analysis of the *CACNA1C* gene as well as other genes that impact psychiatric disorders.

With recent research indicating the effects of methylation on psychiatric disorders, a methylation analysis test for the *CACNA1C* gene that searches for the hypermethylation in intron three will further the future of psychiatric genetic counseling and the diagnosis of psychiatric disorders in general. It will allow a genetic marker to be established which is extremely helpful as psychiatric diagnoses are extremely difficult to make due to the uncertainty regarding the epigenetics surrounding each disorder. For genetic counselors, this methylation analysis of the *CACNA1C* gene will allow for a more accurate risk assessment of an offspring's likelihood to inherit a psychiatric disorder, such as bipolar disorder and schizophrenia. This is due to the high

hereditary values these disorders have, indicating that genetic components are the main factors in each disorder.

**Gene Therapy and CRISPR-Cas9**

Gene therapy is the process by which damaged or missing genes are replaced with normal, working copies. Although gene therapy is still in the experimental phases, the linkage between high methylation status in the *CACNA1C* gene and psychiatric disorders allows the thought that perhaps, with the assistance of gene therapy, a copy of the *CACNA1C* gene with the proper methylation levels can replace the highly methylated copy. This will result in a decreased likelihood of being diagnosed with a psychiatric disorder. Currently, gene therapy is being tested in disorders that have no known cures (Gelfand and Kaplitt, 2013). As psychiatric disorders have no cures, only treatment with medication, gene therapy is a tool that is essential in the progression of knowledge for these disorders and can be the cure that scientists are seeking.

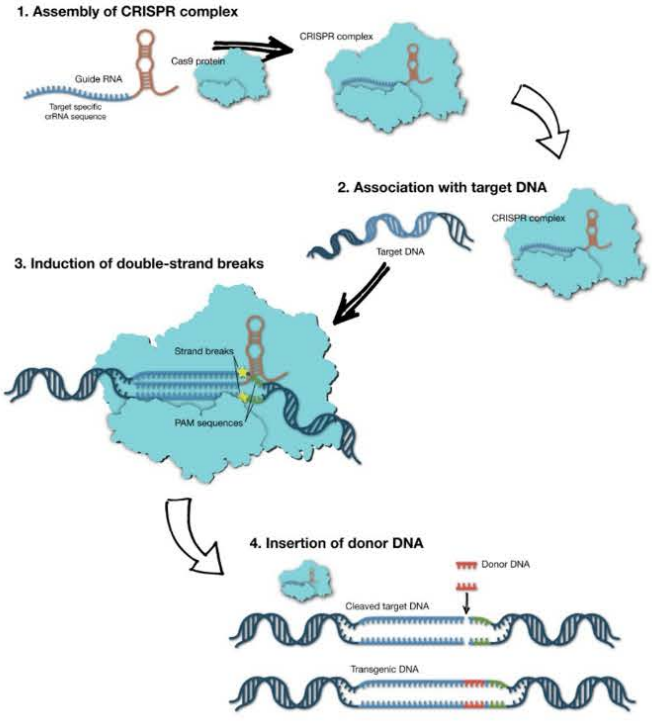


**Figure 10:** Explanation of the CRISPR- Cas9 system and how it inserts desired genes.

Gene therapy is a tricky process. As a result, it has raised some concerns specifically regarding its reliance on a viral delivery to inject DNA into the host cell.

There is concern that this can cause other issues within the genome, such as the cells triggering an immune response. However, within gene therapy, there is a technique called CRISPR-Cas9, or Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR- associated protein 9 that has shifted the concerns of gene therapy.

The CRISPR- Cas9 system captures pieces of DNA from invading viruses and uses them to create a copy to store in its DNA. These DNA copies are known as CRISPR arrays and allow recognition if viral DNA attempts to invade the host cell again. Cas9 is an endonuclease used to cut the target DNA. Researchers use the cell's DNA repair enzymes to replace the sequence with the desired sequence of DNA (Figure 10) (Nambiar *et al.,* 2022).

**Drugs to Reverse Methylation**

When discussing methylation of the *CACNA1C* gene, it refers to the covalent modification of a DNA molecule. In specific, it refers to the functional impairment of the encoded CaV1.2 L-type voltage-gated calcium ion channel, specifically the alpha subunit which makes up the structural component of the ion channel. As discussed earlier in the manuscript, the impairment of this L-type voltage-gated calcium ion channel leads to an abnormal flow of calcium ions into the cell and subsequently causes issues in neurological function.

However, there are epigenetic drugs that aim to reverse DNA methylation by a class of inhibitors called DNMT, DNA methyltransferases, inhibitors. DNA methyltransferases are the category of enzymes that catalyze DNA methylation. The use of DNMT inhibitors is shown to reverse

methylated DNA regions and prevent future methylation. By doing this, the impaired gene will be reactivated (Gurwitz & Karsli-Ceppioglu, 2016).

DNMT inhibitors are injected into a patient and get transported into the cell via hCNT1 (human concentrative nucleoside transporter-1). In the cell, it is converted to an active form. There are two forms of DNMT inhibitors, nucleoside inhibitors, and non-nucleoside inhibitors.

In nucleoside inhibitors, depending on if the DNMT inhibitor is a ribonucleoside or a deoxyribonucleoside, the DNMT inhibitor will get incorporated into the RNA or the DNA. Once integrated into the genome, the DNMT inhibitor works to disrupt the interaction between the DNA methyltransferases and the DNA region by covalently bonding to DNMTs. This results in the inability of the DNA methyltransferases to promote methylation (Figure 11). In non-nucleoside inhibitors, the DNMT inhibitor will covalently bond to the DNA methyltransferase, reactivating the inactivated gene.

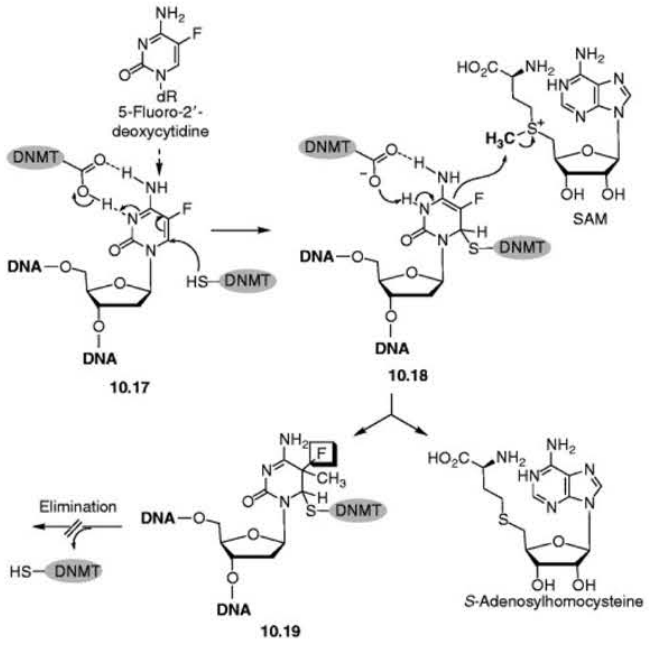


**Figure 11:** Depicted above is the mechanism of action used in a nucleoside DNMT inhibitor.

To prevent future methylation, the DNMT inhibitors also trigger a response to DNA damage. This leads to the degradation of any remaining DNA

methyltransferases embedded in the DNA (Gnyszka *et al.,* 2013).

If researchers were to specifically target the DNA methyltransferases located at the promoter region of the *CACNA1C* gene, it would result in the reactivation of the *CACNA1C* gene. Perhaps this would lessen the risk of one being diagnosed with a psychiatric disorder such as bipolar disorder or schizophrenia.

## XIV. Conclusion

In genetic counseling, an essential part of meeting with the patient is to ensure they understand the facts of the disorder and explain all available options. This gives the patient autonomy. As a future genetic counselor, giving a patient more choices such as undergoing a methylation analysis test, undergoing a gene therapy treatment, or taking a DNMT inhibitor drug will assist in the goal of empowerment and allowing the patient to make the choices best aligned with their values. Therefore, implementing methylation testing of the *CACNA1C* gene can be pivotal in evaluating the likelihood that a psychiatric disorder like bipolar disorder and schizophrenia will be inherited by the patient's offspring and in providing the next steps to lessen the offspring's risk.

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**References:**

Anna Starnawska, & Ditte Demontis. (2021). Role of DNA Methylation in Mediating Genetic Risk of Psychiatric Disorders. *Frontiers in Psychiatry*, *12*. https://doi.org/10.3389/fpsyt.2021.596821

Andrade, A., Brennecke, A., Mallat, S., Brown, J., Gomez-Rivadeneira, J., Czepiel, N., & Londrigan, L. (2019). Genetic Associations between Voltage-Gated Calcium Channels and Psychiatric Disorders. *International Journal of Molecular Sciences*, *20*(14), 3537, 1-9 https://doi.org/10.3390/ijms20143537

Austin, J. C. (2020). Evidence-Based Genetic Counseling for Psychiatric Disorders: A Road Map. *Cold Spring Harbor Perspectives in Medicine*, *10*(6), 1-13 https://doi.org/10.1101/cshperspect.a03660

Batinic B. (2019). Cognitive Models of Positive and Negative Symptoms of Schizophrenia and Implications for Treatment. *Psychiatria Danubina*, *31*(Suppl 2), 181–184.

Bigos, K. L., Mattay, V. S., Callicott, *et al.* (2010). Genetic variation in CACNA1C affects brain circuitries related to mental illness. *Archives of General Psychiatry*, *67*(9), 939.

Brooker, R.J., 2021, Genetics: Analysis & Principles, 7th edition, McGraw-Hill Book Company, NY, NY (ISBN 978-1-260-47302-5)

Canadian Mental Health Association. (2018). *What are positive and negative symptoms?* What are positive and negative symptoms? | Here to Help. Retrieved March 13, 2022, from https://www.heretohelp.bc.ca/q-and-a/what-are-positive-and-negative-symptoms

Chang, M., He, L., & Cai, L. (2018). An Overview of Genome-Wide Association Studies. *Methods in molecular biology*, *1754*, 97–108. https://doi.org/10.1007/978-1-4939-7717-8_6

Chudal, R., Gissler, M., Sucksdorff, D., *et al.* (2014). Parental age and the risk of bipolar disorders. *Bipolar disorders*, *16*(6), 624–632. https://doi.org/10.1111/bdi.12182

Clark, M.B., Wrzesinski, T., Garcia, A.B. *et al.* Long-read sequencing reveals the complex splicing profile of the psychiatric risk gene *CACNA1C* in human brain. *Mol Psychiatry* 25, 37–47 (2020). https://doi.org/10.1038/s41380-019-0583-1

Czamara, D., Eraslan, G., Page, C.M. *et al.* (2019) Integrated analysis of environmental and genetic influences on cord blood DNA methylation in new-borns. *Nat Commun* 10, 2548 . https://doi.org/10.1038/s41467-019-10461-0

Dailey, J. V., Pagnotto, M. A., Fontana-Bitton, S., & Brewster, S. J. (1995). Role of the genetic counselor: an overview. *The Journal of perinatal & neonatal nursing*, *9*(3), 32–44. https://doi.org/10.1097/00005237-199512000-00007

Dedic, N., Pöhlmann, M., Richter, J. *et al.* Cross-disorder risk gene *CACNA1C* differentially modulates susceptibility to psychiatric disorders during development and adulthood. *Mol Psychiatry* 23, 533–543 (2018). https://doi.org/10.1038/mp.2017.133

Dixon, M. L., & Dweck, C. S. (2021). The amygdala and the prefrontal cortex: The co-construction of intelligent decision-making. *Psychological Review*. https://doi.org/10.1037/rev0000339

Forsyth, J. K., & Lewis, D. A. (2017). Mapping the Consequences of Impaired Synaptic Plasticity in Schizophrenia through Development: An Integrative Model for Diverse Clinical Features. *Trends in cognitive sciences*, *21*(10), 760–778. https://doi.org/10.1016/j.tics.2017.06.006

Frizén, L. (2002, December). *Genetic Studies of Hypospadias.* 10.13140/RG.2.1.3526.2883.

Fusar-Poli, P., Howes, O., Bechdolf, A., & Borgwardt, S. (2012). Mapping vulnerability to bipolar disorder: a systematic review and meta-analysis of neuroimaging studies. *Journal of Psychiatry and Neuroscience*, *37*(3), 170. https://doi.org/10.1503/jpn.110061

Gelfand, Y., & Kaplitt, M. G. (2013). Gene Therapy for Psychiatric Disorders. *World Neurosurgery*, *80*(3–4), S32. https://doi.org/10.1016/j.wneu.2012.12.028

Genetic Alliance; The New York-Mid-Atlantic Consortium for Genetic and Newborn Screening Services. Understanding Genetics: A New York, Mid-Atlantic Guide for Patients and Health Professionals. Washington (DC): Genetic Alliance; 2009 Jul 8. Chapter 5, Genetic Counseling. Available from: https://www.ncbi.nlm.nih.gov/books/NBK115552/

Gejman, P. V., Sanders, A. R., & Duan, J. (2010). The role of genetics in the etiology of schizophrenia. *The Psychiatric Clinics of North America*, *33*(1), 35–66. https://doi.org/10.1016/j.psc.2009.12.003

Gershman, A., Sauria, M. E. G., Guitart, X., *et al.* (2022). Epigenetic patterns in a complete human genome. *Science (New York, N.Y.)*, *376*(6588), eabj5089. https://doi.org/10.1126/science.abj5089

Gnyszka, A., Jastrzebski, Z., & Flis, S. (2013). DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. *Anticancer Research*, *33*(8), 2989–2996.

Gurwitz, D., & Karsli-Ceppioglu, S. (2016). Epigenetic Mechanisms in Psychiatric Diseases and Epigenetic Therapy. *Drug Development Research*, 77(7), 407. https://doi.org/10.1002/ddr.21340

He, D., Fan, C., Qi, M. *et al.* (2021). Prioritization of schizophrenia risk genes from GWAS results by integrating multi-omics data. *Transl Psychiatry* 11, 175. https://doi.org/10.1038/s41398-021-01294-x

Harrison, P.J., Hall, N., Mould, A. *et al.* (2021). Cellular calcium in bipolar disorder: systematic review and meta-analysis. *Mol Psychiatry* 26, 4106–4116. https://doi.org/10.1038/s41380-019-0622-y

Hertzberg L, Katsel P, Roussos P, Haroutunian V, Domany E (2015). Integration of gene expression and GWAS results supports involvement of calcium signaling in schizophrenia. *Schizophr Res* 164:92–99.

Heyes S, Pratt WS, Rees E, Dahimene S, Ferron L, *et al*. (2015). Genetic disruption of voltage-gated calcium channels in psychiatric and neurological disorders. Prog Neurobiol. Nov;134:36-54. doi:10.1016/j.pneurobio.2015.09.002 Epub 2015 Sep 16. PMID: 26386135; PMCID: PMC4658333.

Hofmann, F., Flockerzi, V., Kahl, S., & Wegener, J. W. (2014). L-type CaV1.2 calcium channels: from in vitro findings to in vivo function. *Physiological Reviews*, *94*(1), 303–326. https://doi.org/10.1152/physrev.00016.2013

Holland, K. M. (2016). *DNA Methylation : Patterns, Functions and Roles in Disease*. Nova Science Publishers, Inc. (Hauppauge, NY)

History.com Editors. (2009, October 14). *Rwandan genocide*. History.com. Retrieved April 26, 2022, from https://www.history.com/topics/africa/rwandan-genocide

Imamura, A., Morimoto, Y., Ono, S. *et al.* Genetic and environmental factors of schizophrenia and autism spectrum disorder: insights from twin studies. *J Neural Transm* 127, 1501–1515 (2020). https://doi.org/10.1007/s00702-020-02188-w

Kerner B. (2014). Genetics of bipolar disorder. *The application of clinical genetics*, *7*, 33–42. https://doi.org/10.2147/TACG.S39297

Kim, M., & Costello, J. (2017, April 28). *DNA Methylation: An Epigenetic Mark of Cellular Memory*. Experimental & molecular medicine. Retrieved from https://pubmed.ncbi.nlm.nih.gov/28450738/

Kreutz, M. R., & Sala, C. (2012). *Synaptic plasticity. [electronic resource] : dynamics, development and disease*. Springer. Magdeburg, Germany.

Kornijcuk, V., Kim, D., Kim, G., & Jeong, D. S. (2020). Simplified calcium signaling cascade for synaptic plasticity. *Neural Networks*, *123*, 38–51. https://doi.org/10.1016/j.neunet.2019.11.022

Liu, C., Jiao, C., Wang, K., & Yuan, N. (2018). DNA Methylation and Psychiatric Disorders. *Progress in molecular biology and translational science*, *157*, 175–232. https://doi.org/10.1016/bs.pmbts.2018.01.006

Liu, Y. P., Wu, X., Xia, X., *et al.* (2020). The genome-wide supported CACNA1C gene polymorphisms and the risk of schizophrenia: an updated meta-analysis. *BMC medical genetics*, *21*(1), 159. https://doi.org/10.1186/s12881-020-01084-0

McGue, M., Gottesman, I. I., & Rao, D. C. (1983). The transmission of schizophrenia under a multifactorial threshold model. *American journal of human genetics*, *35*(6), 1161–1178.

McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A. (2013). The Heritability of Bipolar Affective Disorder and the Genetic Relationship to Unipolar Depression. *Arch Gen Psychiatry.*2003;60(5):497–502. doi:10.1001/archpsyc.60.5.497

Menculini, G., Balducci, P. M., Attademo, L., *et al.* (2020). Environmental Risk Factors for Bipolar Disorders and High-Risk States in Adolescence: A Systematic Review. *Medicina (Kaunas, Lithuania)*, *56*(12), 689. https://doi.org/10.3390/medicina56120689

Miller, B., Messias, E., Miettunen, J., *et al.* (2011). Meta-analysis of paternal age and schizophrenia risk in male versus female offspring. *Schizophrenia bulletin*, *37*(5), 1039–1047. https://doi.org/10.1093/schbul/sbq011

Moon, A. L., Haan, N., Wilkinson, L. S., *et al.* (2018). CACNA1C: Association With Psychiatric Disorders, Behavior, and Neurogenesis. *Schizophrenia Bulletin, 44*(5), 958–965. https://doi.org/10.1093/schbul/sby096

Musanabaganwa, C., Wani, A. H., Donglasan, J., Fatumo, S., Jansen, S., Mutabaruka, J., Rutembesa, E., Uwineza, A., Hermans, E. J., Roozendaal, B., Wildman, D. E., Mutesa, L., & Uddin, M. (2022). Leukocyte methylomic imprints of exposure to the genocide against the Tutsi in Rwanda: a pilot epigenome-wide analysis. *Epigenomics*, *14*(1), 11–25. https://doi.org/10.2217/epi-2021-0310

Nambiar, T. S., Baudrier, L., Billon, P., & Ciccia, A. (2022). CRISPR-based genome editing through the lens of DNA repair. *Molecular Cell*, *82*(2), 348–388. https://doi.org/10.1016/j.molcel.2021.12.026

Natassia Robinson, & Sarah E. Bergen. (2021). Environmental Risk Factors for Schizophrenia and Bipolar Disorder and Their Relationship to Genetic Risk: Current Knowledge and Future Directions. *Frontiers in Genetics*, *12*, 1-12. https://doi.org/10.3389/fgene.2021.686666

*NIMH » bipolar disorder - national institute of mental health*. (2017). Retrieved March 13, 2022, from https://www.nimh.nih.gov/health/statistics/bipolar-disorder

Porcelli, S., Lee, S. J., Han, C., *et al.* (2015). CACNA1C gene and schizophrenia: a case-control and pharmacogenetic study. *Psychiatric genetics*, *25*(4), 163–167. https://doi.org/10.1097/YPG.0000000000000092

Psychiatric GWAS Consortium Bipolar Disorder Working Group. (2011). Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near *ODZ4*. *Nat Genet* 43, 977–983 (2011). https://doi.org/10.1038/ng.943

Sadikovic, B. (2018, April 19). *The diagnostic power of DNA methylation*. The Pathologist. Retrieved February 20, 2022, from https://thepathologist.com/inside-the-lab/the-diagnostic-power-of-dna-methylation

Sandstrom, A., Sahiti, Q., Pavlova, B., & Uher, R. (2019). Offspring of parents with schizophrenia, bipolar disorder, and depression: a review of familial high-risk and molecular genetics studies. *Psychiatric genetics*, *29*(5), 160–169. https://doi.org/10.1097/YPG.0000000000000240

Sanfilippo, P. G., Hewitt, A. W., Hammond, C. J., & Mackey, D. A. (2010). The heritability of ocular traits. *Survey of ophthalmology*, *55*(6), 561–583. https://doi.org/10.1016/j.survophthal.2010.07.003

Schmitt, A., Malchow, B., Hasan, A., & Falkai, P. (2014). The impact of environmental factors in severe psychiatric disorders. *Frontiers in neuroscience*, *8*, 19. https://doi.org/10.3389/fnins.2014.00019

Kurdyukov S., & Bullock M. (2016). DNA Methylation Analysis: Choosing the Right Method. *Biology*, *5*(1), 3. https://doi.org/10.3390/biology5010003

Smoller, J. W., & Finn, C. T. (2003). Family, twin, and adoption studies of bipolar disorder. *American journal of medical genetics. Part C, Seminars in medical genetics*, *123C*(1), 48–58. https://doi.org/10.1002/ajmg.c.20013

Starnawska, A., Demontis, D., Pen, A., *et al.* (2016). CACNA1C hypermethylation is associated with bipolar disorder. *Translational Psychiatry*, *6*(6), e831. https://doi.org/10.1038/tp.2016.99

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003). Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Archives of general psychiatry*, *60*(12), 1187–1192. https://doi.org/10.1001/archpsyc.60.12.1187

Sutterland, A. L., Fond, G., Kuin, A., *et al.* (2015). Beyond the association. *Toxoplasma gondii* in schizophrenia, bipolar disorder, and addiction: systematic review and meta-analysis. *Acta Psychiatrica Scandinavica*, *132*(3), 161–179. https://doi.org/10.1111/acps.12423

Umeoka, E., van Leeuwen, J., Vinkers, C. H., & Joëls, M. (2021). The Role of Stress in Bipolar Disorder. *Current topics in behavioral neurosciences*, *48*, 21–39. https://doi.org/10.1007/7854_2020_151

*Understanding Your Diagnosis*. NAMI. (n.d.). Retrieved March 3, 2022, from https://www.nami.org/Your-Journey/Individuals-with-Mental-Illness/Understanding-Your-Diagnosis

U.S. National Library of Medicine. (2021, September 22). *Bipolar disorder*. MedlinePlus. Retrieved March 10, 2022, from https://medlineplus.gov/bipolardisorder.html

U.S. National Library of Medicine. (2020, August 18). *Cacna1c gene: Medlineplus genetics*. MedlinePlus. Retrieved February 20, 2022, from https://medlineplus.gov/genetics/gene/cacna1c/

U.S. National Library of Medicine. (2020, September 18). *What are single nucleotide polymorphisms (snps)?: Medlineplus Genetics*. MedlinePlus. Retrieved February 20, 2022, from https://medlineplus.gov/genetics/understanding/genomicresearch/snp/

Weaver, I. C., Cervoni, N., Champagne, F. A., *et al.* (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, *7*(8), 847–854. https://doi.org/10.1038/nn1276

Wein, H. (2010, September 27). *Stress Hormone Causes Epigenetic Changes*. National Institutes of Health. Retrieved March 24, 2022, from https://www.nih.gov/news-events/nih-research-matters/stress-hormone-causes-epigenetic-changes