

Predisposition of Serotonin and Dopamine Transporter Genes among Jail Inmates with Suicide Ideation



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Predisposition of Serotonin and Dopamine Transporter Genes among Jail Inmates with Suicide Ideation



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Faiza Shabbir

DEDICATED

To

My Father

Ch. Shabbir Ahmed Tahir

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LIST OF ABBREVIATIONS

Abbreviation	Description
5-HT	5-Hydroxytryptamine
5-HT1A	Serotonin 1A Receptor
5-HTTLPR	5-HTT-Linked Polymorphic Region
Aces	Adverse Childhood Experiences
AD	Aggressive Disorder
ADHD	Attention-Deficit / Hyperactivity Disorder
ADHS	Attention Deficit Hyperactivity Disorder
ANOVA	One-Way Analysis Of Variance
APA	American Psychiatric Association
BDI	Beck Depression Inventory
BDNF	Brain-Derived Neurotrophic Factor
BNDF	Brain Derived Neurotrophic Factor
BP	Bipolar Disorder
Camp	Cyclic Adenosine Monophosphate
CBC	Complete Blood Count
CI	Confidence Interval
CNS	Central Nervous System
COMIT	Catechol-O-Methyltransferase
CREB1	cAMP Responsive Element Binding Protein 1
DA	Dopamine
Daergic	Dopaminergic
DAT	Dopamine Transporter
DNA	Deoxyribonucleic Acid
DRD2	Dopamine Receptor Gene
Drs	Dopaminergic Receptors
Eaats	Excitatory Amino-Acid Transporters

EDTA	Ethylenediaminetetraacidic Acid
Elas	Early Life Adversities
GFAP	Glial Fibrillary Acidic Protein
Gpcrs	G-Protein Coupled Receptors
GSEA	Gene Set Enrichment Analysis
GSK3	Glycogen Synthase Kinase3
GWAS	Genome Wide Association Studies
HPA	Hypothalamic-Pituitary-Adrenal
IEC	Institutional Ethical Committee
IL6	Interleukin 6
IPLEX	(Integrated Primer Extension) And Matrix Assisted Laser Desorption
L-DOPA	Levodopa
MAD	Major Aggressive Disorder
MAF	Major Allele Frequency
MAO	Monoamine Oxidase
MAOA	Monoamine Oxidase A
MAOB	Monoamine Oxidase B
MAPK	Mitogen Activated Protein Kinase
MDD	Major Depressive Disorder
Nac	Nucleus Accumbens
NF-Kb	Nuclear Factor Kappa B
NMDA	N-Methyl-D-Aspartate
NSSI	Non-Suicidal Self-Injury
OD	Odds Ratio
P13K	Phosphatidylinositol 3-Kinase
PAD	Persistent Aggressive Disorder
PCR	Polymerase Chain Reaction
PD	Parkinson Disease
PKA	Protein Kinase A

PNS	Peripheral Nervous System
SAD	Seasonal Affective Disorder
SDS	Social Defeat Stress
SDS	Sodium Dodecyl Sulphate
SI	Suicide Ideation
SLC	Serotonin Transport Gene
SN	Substantia Nigra
Snps	Single Nucleotide Polymorphism
Ssris	Selective Serotonin Reuptake Inhibition
TAE	Tris-Acetate-EDTA
TBE	Tris-Borate-EDTA
TPH2	Tryptophan Hydroxylase 2
UCSC	University Of California, Santa Cruz
UTR	Un-Translated Regions
VMAT2	Vesicular Monoamine Transporter 2
VNTR	Variable Number Tandem Repeat
WHO	World Health Organization

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ABSTRACT

Suicide ideation is a recognized foundation to suicidal behavior in imprisoned populations. These are thoughts about suicide which induces the development of suicidal behavior and finally leads towards suicidal attempts and suicide. Different contributors including genes, life adversities and psychopathologies and the easy access of the methods of suicide are the causes of suicide ideation. These contributors have different synergistic effects on the beginning of suicidal behavior in individuals. The main aim of the research was to investigate the genetic factors responsible for suicide ideation among jail inmates and to find out the distinct haplotypes of different polymorphism in serotonin, dopamine and their metabolizing enzyme genes as risk factors for trajectories of suicide ideation.

Genetic analysis focused on polymorphisms in genes related to serotonin and dopamine pathways. Significant associations were found between specific polymorphisms (rs6347, rs1801412, rs6318, rs1800532, rs1799913) and suicide ideation. Notably, some genotypes indicated higher suicide scores, underscoring the interplay of genetic predisposition and environmental factors.

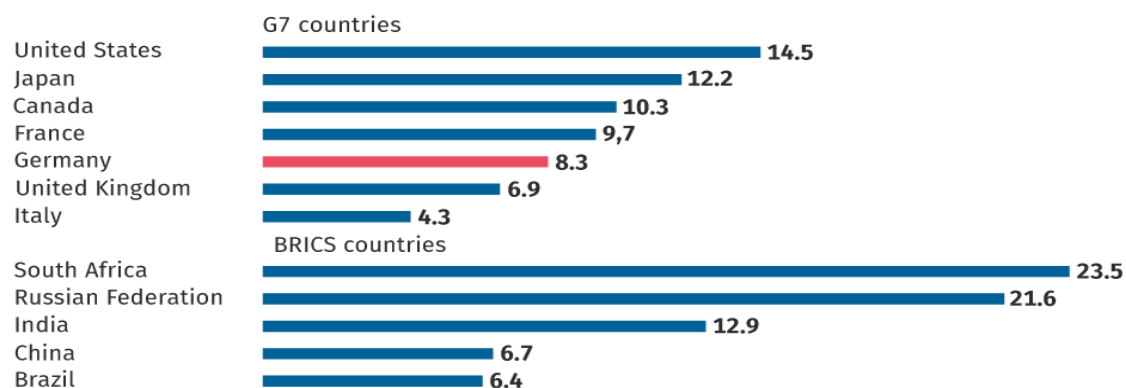
TPH1 and TPH2 gene variants, particularly the A/A genotype of rs1799913, were linked to higher suicide scores, aligning with findings in depressive disorder studies. Additionally, BDNF, CREB1, and COMT gene variants, notably the A/A genotype of rs4680, correlated with elevated suicide scores. HTR2B and HTR2C gene polymorphisms revealed the association of rs6437000 (A/A) and rs1801412 (G/G) with heightened suicide scores. Furthermore, significant disparities were found in the allele frequencies of rs6347 and rs1799913, with the A allele of rs6347 being strongly associated with depressive disorder in different studies. These findings underscore the complex genetic underpinnings of suicide ideation. These findings shed light on the intricate relationship between genetics, socioeconomic status, and suicide ideation, emphasizing the need for targeted interventions and support systems for vulnerable populations.

INTRODUCTION

Suicide is an intricate and multifaceted occurrence, involving a purposeful engagement in self-harming actions that lead to death. (Studart *et al.*, 2019). It is a difficult and devastating issue that has become a major public health concern globally. Being a major health challenge, it incorporates mental health conditions, cultural and social factors, genetic and biological factors, ranking as the 17th primary contributor to global mortality (Thapalia *et al.*, 2018). Suicide spans a wide spectrum of stages, encompassing different points along a continuum. This spectrum includes Suicidal Thoughts, Non-Suicidal Self-Injury (NSSI) with low intent, Suicide Attempts involving greater intent and medical danger, and ultimately, purposeful Self-Inflicted Fatality (Moseley *et al.*, 2022).

The World Health Organization (WHO) has considered suicide as a leading factor in worldwide mortality, with an estimation of 700,000 deaths every year. According to WHO, the actual figures of suicides are higher than the reported cases, with males being more effected (13.7 per 100,000) than females (4.6 per 100,000) (Ilic & Ilic, 2022). Like many other countries, it is also underreported in Pakistan, so there is a need to implement operative approaches to get the real data, so that this problem can be solved promptly (Shagufta *et al.*, 2015).

Suicide rate 2019 per 100,000 persons



Source: WHO

© Statistisches Bundesamt (Destatis), 2023

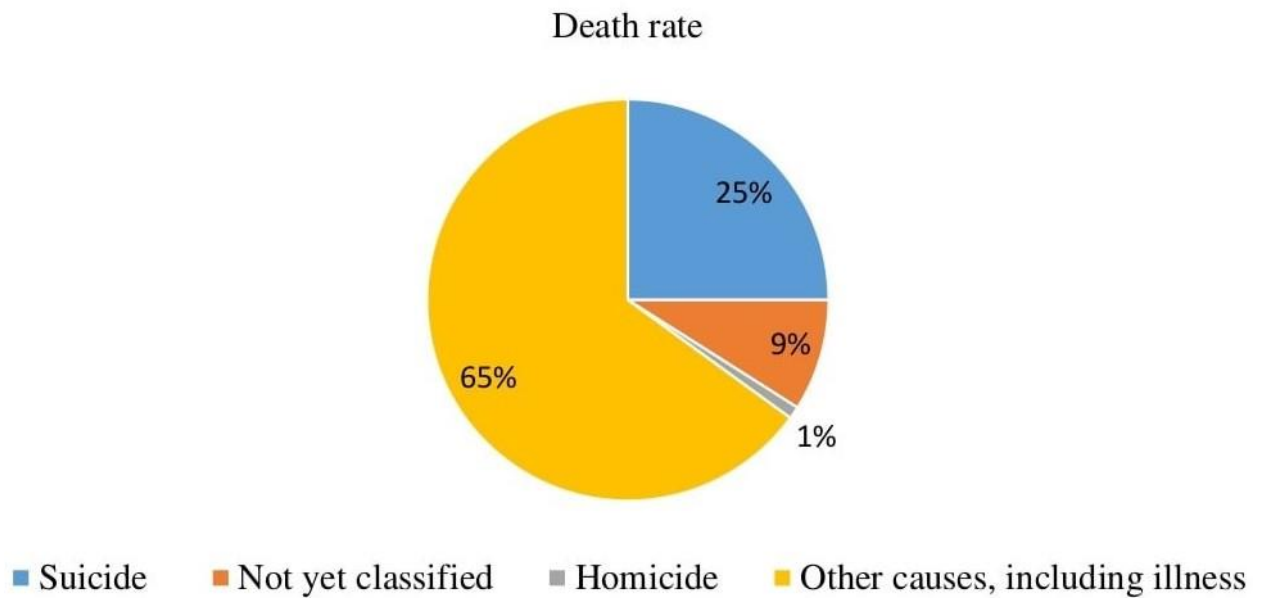
STATIS
Statistisches Bundesamt

Figure 1.1 Suicide rate 2019 (adapted from https://www.destatis.de/EN/Themes/Countries-Regions/International-Statistics/Graphic/Suizidrate.png?__blob=normal&v=9)

Suicide ideation reflects psychological distress through thoughts of self-harm, while antisocial delinquencies involve behaviors deviating from societal norms. Recent research has noted a noteworthy overlap between these behaviors in some individuals, suggesting potential shared underlying mechanisms (Noam *et al.*, 2015). The phenomenon of suicide ideation is a complex multilayered subject that spans psychology, criminology, genetics, and public health. These behavioral tendencies have drawn significant scholarly attention due to their profound societal implications and potential for harmful consequences (Patrick, 2022). The tricky relationship between suicide ideation (thinking about self-harm or death) and delinquent acts (deviant behaviors like aggression and rule-breaking) offers a fertile ground for interdisciplinary exploration (Barak, 2009).

Suicide Ideation among prisoners can be up to ten times greater than general population and depends on several factors like country, region and particular prison (Zhong *et al.*, 2021). So, implementation of effective prevention and intervention strategies like substance abuse treatment, suicide risk assessments and comprehensive mental screening is necessary for the prisoners experiencing suicidal ideation (Daniel, 2006). In Europe, one third of all custodial deaths are caused by suicide. According to the data of United States, it is one of the major causes of death reported in prisoners, while it is four times higher in Australia than the general community (Pratt *et al.*, 2006; Sattar, 2001; Kariminia *et al.*, 2012).

The epidemiology of Prisoners in Pakistan is not well documented, and very limited data is available on the prison population. The word prison brief reports that as of August 2021, the prison population rate in Pakistan was 47 per 100,000 populations (Khan *et al.*, 2008), which is lower than global average of 144 per 100,000 populations (Herbert *et al.*, 2012). However, Pakistani prisons face numerous challenges, including poor conditions, overcrowding, and limited access to mental and healthcare services leading to mental illness, and data is very limited. But suicide is not uncommon and in recent years several cases of suicide have been reported, in fact actual number of suicides in Pakistan is higher, as many cases go unreported (Anwar *et al.*, 2017).



Fazel S, Hayes AJ, Bartellas K, Clerici M, Trestman R. Mental health of prisoners: prevalence, adverse outcomes, and interventions. *The Lancet Psychiatry*. 2016;3(9):871-81.

Figure 1.2 Breakdown of death in Prisons

Suicide can be due to the response of social behaviors like violence, abuse and mental illness. External deadly events affect the identities and memories, so, it is concluded that such traumatic experiences lead to the psychological states which results in desire for suicide and death (JJ Mann, 2003). In some cases, these mental health problems are genetically inherited but, in many cases, these are due to disturbing experiences, caused by sexual, domestic, emotional and physical abuse. Suicidal ideation is a social behavior that is induced by external forces. Once, they are effected enough they become into mental health concerns leading to suicide. (Mehdi *et al.*, 2021).

Almost from 2000 years, aggression has been considered as chronic mental illness causing suicide ideation. It shows persistent clinical symptoms affecting all behavioral aspects such as conation, affection and cognition (Beck *et al.*, 2014). In 2015, WHO declared that aggression has 8-12% prevalence and had affected more than 4.4% people worldwide (Belujon and Grace, 2017). Many published reports have stated that aggression is neither a gender specific nor an age-related disorder. But its waves strikes less frequently in younger boys than in younger girls(Hira *et al.*, 2019).

Aggression is categorized into various types because of mood and all types have a negative

effect on routine life. American Psychiatric Association (APA) categorized mood disorders into on the basis of its extent:

- i. Aggressive Disorders (AD); which include Major Aggressive Disorder (MAD) and Dysthymic Disorder,
- ii. Bipolar Disorders (BP).
- iii. Less common types of Aggression are Persistent Aggressive Disorders (PAD), Seasonal Affective Disorders (SAD), Psychotic Aggression and Prepartum or Postpartum Aggression (Beck *et al.*, 2014; Jennifer Casarella, 2020).

Agitation is the prominent aspect of aggression, while recognizable traits and prevalent signs of aggression might encompass shifts in emotional state resulting in emotions such as sorrow, isolation, indifference, self-reproach, contemplation of suicide, or a wish to break free or cease existing (Beck & Alford, 2009). Modifications in behavior and shifts in cognitive reactions and functioning encompass reduced appetite (anorexia), changes in sleep patterns (insomnia), and disillusionment. Additionally, there is a physical aspect involving diminished self-worth, lowered ability to function, feelings of fatigue, and a lack of capacity to derive enjoyment from life (Beck *et al.*, 2014; Venty *et al.*, 2018).

Suicidal behavior includes contributions from genes, life adversities, and psychopathology (Lloyd *et al.*, 2015). Other notable contributions influencing suicide include social factors and the accessibility to the methods of suicide (Turecki *et al.*, 2019). Genetic predisposition to suicide ideation is the idea of an individual's risk for developing thoughts of suicide, influenced by their genetic makeup. According to studies, along with environmental factors, genetic factors also cause 40-60% of variation in suicide risk (Mirza *et al.*, 2022).

The present understanding of suicide is that subjects with a genetic predisposition are particularly vulnerable to stressful environmental inputs (Jimenez *et al.*, 2018). The biological basis of suicide has been investigated in several studies to better understand its precipitating factors (Orsolini *et al.*, 2020). There have been several biological hypotheses. Recent studies have explored the genetic impact on suicidal behavior through Genome-wide association studies (Tam *et al.*, 2019). However, many GWAS suggested associations that did not reach genome-wide significance, pointing out that there are multiple genomic targets that need further investigations

in suicidal behavior (Gonda *et al.*, 2021). A recent study identified by several genes involved in neural functions such as neurodevelopment (cellular assembly, function, and organization), cell death, survival mechanisms, and immunological/inflammatory mechanisms (Fatemi *et al.*, 2018) has been found to link with genetic predisposition and suicide ideation.

The use of GWAS has been limited to the investigation of common genetic variants. However, more recent studies have explored the influence of environmental input on gene function (Thomas, 2010). The associations of early life adversities (ELAs) and suicide have suggested interaction with genetic liability leading to the stress diathesis model of suicide (Smart *et al.*, 2015). Studies on functional genomics have consistently identified brain regions involved in psychiatric disorders and suicide ideation including hippocampus, amygdala, anterior cingulate cortex and the prefrontal cortex (Schmaal *et al.*, 2010).

Furthermore, the emerging realm of epigenetics provides a nuanced lens through which to view the interplay between genetic factors and behavioral outcomes is possible. Genetic predisposition and environmental stimuli have influence on epigenetic modifications, which pertains to modifications in gene expression that occur without making changes in DNA sequence. Epigenetic markers, including DNA methylation and histone modifications, can act as intermediaries, illustrating how genetic susceptibilities interact with life experiences to mold an individual's vulnerability to suicide ideation (Ludwig & Dwivedi, 2006).

Studying postmortem brain tissue from individuals who died by suicide, along with conducting imaging studies on those who survived suicide attempts, has identified a correlation between these brain regions and observed changes in both their functional and structural characteristics (Lloyd *et al.*, 2015). According to the neurological basis of suicide, neurotransmitter systems within the brain regions are also involved in suicidality (Roy *et al.*, 2017). Considering it from a neurochemical perspective, the serotonin neurotransmitter system and the hypothalamic-pituitary-adrenal (HPA) axis are thought to have a crucial involvement in this scenario (Bao & Swabb, 2019). Importantly, irregularities within the frontal, temporal, and parietal cortices appear to have a substantial contribution to the manifestation of suicidal behavior. (Jollant, 2016).

Consistent findings from twin, family, and adoption studies have indicated substantial heritability estimates for both suicide ideation and antisocial behaviors, underscoring a significant

genetic influence. Furthermore, recent advancements in molecular genetics have facilitated the identification of specific gene variations linked to these behaviors, particularly those associated with neurotransmitter systems and neural pathways involved in mood regulation, impulsivity, and decision-making (Fowles, 2011).

In addition to genes directly related to neurotransmission, variations in genes associated with neural plasticity, emotion regulation, and stress response may also contribute to the link between suicide ideations. Epigenetic mechanisms, which modulate gene expression in response to environmental factors, further contribute to the intricacy of this interplay, potentially mediating the effects of early-life experiences (Maccari *et al.*, 2014).

Risk Factors involved in the prevalence of Suicide Ideation

In contrast to various medical conditions that rely on specific diagnostic tests, suicidal ideation is identified based on the persistence and regularity of the symptoms mentioned earlier. The prevalence of suicide involves the following risk factors.

- i. Biological aspects: These encompass hormonal and neurobiological systems, which include the functioning of neurotransmitters. Neurons release neurotransmitters, which are chemical messengers that transmit nerve signals by stimulating neighboring neurons and muscle cells. Key neurotransmitters involved in mood, such as serotonin, noradrenaline, and dopamine, play a significant role in the contemplation of suicide (Lee & Kim 2018; Liu *et al.*, 2018).
- ii. Genetic elements: Suicidal thoughts have a hereditary component, although the precise mechanisms and specific genes contributing to the prevalence of these thoughts remain unclear. Numerous studies have verified that, in addition to hormones and neurotransmitters as vulnerable factors for suicidal ideation, several genes—acting independently or exerting their influence through linked interactions—are substantial risk factors for the prevalence of suicidal thoughts (Mirkovic *et al.*, 2006). Family and twin studies centered on suicidal ideation also provide support for genetic risk factors related to such ideation. It is also important to note that everyone might possess distinct sets of genetic and environmental factors that impact their mental well-being and behavior, potentially leading to a diagnosis of suicidal ideation. (Council, 2009;

Dobson and Dozois, 2011; Hankin *et al.*, 2015).

With the advancement of genetics, the exploration of gene-environment interactions becomes increasingly significant. Gene-environment interplay encompasses the intricate relationship between genetic predispositions and environmental elements, working together to influence the development of distinct behaviors. The core objective of this research is to comprehend the interaction between genetic components and environmental factors, molding the course of suicidal thoughts and antisocial delinquency (Miller *et al.*, 2022). Recent studies in epigenetic research have underscored the impact of early-life adversity on shaping epigenetic patterns that may contribute to subsequent psychopathological conditions. Adverse childhood experiences, like maltreatment or trauma, can imprint enduring epigenetic signatures on genes implicated in stress response, emotional regulation, and impulsivity. This imprinting process could serve as a molecular conduit linking genetic predispositions to the emergence of suicide ideation and antisocial behaviors (Mill & Petronis, 2007).

- iii. Environmental factors emerge as the most influential contenders in the genesis of suicidal thoughts. The precise mechanism through which environmental factors contribute to the emergence of these thoughts is currently being investigated. Certain studies suggest that these factors may disrupt the typical functioning of the nervous system, thereby fostering the emergence of thoughts of suicide (Córdova-Palomera *et al.*, 2016; Tornador *et al.*, 2016). Predominant among these environmental factors are age, gender, absence of positive environments, stress resulting from chronic illnesses, socioeconomic disadvantages, and acute life events such as violence or prolonged neglect, and substance misuse. When combined with genetic influences and individual vulnerabilities, these environmental factors present themselves as the most promising risk factors in the development of suicidal ideation. (Council, 2009; Kwong *et al.*, 2019).
- iv. Personal vulnerabilities: Various personality traits, in conjunction with genetic and environmental influences, can contribute to the emergence of suicidal thoughts. Among these traits, a primary one is having low self-esteem and self-criticism, coupled with an overwhelming response to unfavorable situations, anxiety, pessimism, fragile social

connections, excessive rumination, and disrupted cognitive functioning (Council, 2009). It's essential to recognize that the risk factors associated with suicidal ideation, whether stemming from personal susceptibilities, environmental circumstances, or biological factors, aren't fixed throughout an individual's life. Instead, they can transform with experiences and different life phases (Butler, 2018), potentially impacting the psychological processes and serotonin (5-HT) neurotransmitter function. When 5-HT function and processing deviate from the norm, it can disrupt the equilibrium of dopamine levels in synaptic gaps and presynaptic neurons. This disruption has been associated with the development of various psychological disorders, such as aggression, major depressive disorder, anxiety, and suicidal tendencies (Zhang 2010; Zhong *et al.*, 2010). Studies suggest that neurotoxic amphetamines and DA-Quinones (DAQ) have a direct impact on TPH2, leading to the inhibition of TPH2 activity in the brain by dopamine (Makoto *et al.*, 1994; Kuhn & Arthur, 1998; Teixeira-Gomes *et al.*, 2015). Furthermore, genetic variations in the TPH gene contribute to a decrease in dopamine levels.

In the synthesis of 5-Hydroxytryptamine (5-HT), a specific enzyme called Tryptophan hydroxylase-2 (TPH2) is the sole participant (Tara & Christian, 2020). The TPH genes have been identified as potential candidates linked to suicidal behavior (López-Narváez *et al.*, 2015). Disrupted enzymatic activity due to gene polymorphism of TPH2 within the brain leads to variations in serotonin (5HT) levels, which in turn influences the release of dopamine in brain tissues. Polymorphisms in TPH occur at multiple sites, including "rs4570625, rs7305115, rs1799913, and rs1800532" (Laksono *et al.*, 2019), and these have a significant correlation with neuropsychiatric disorders like aggression, ADHD, and suicidal behavior have been linked to these conditions (Sheehan *et al.*, 2005; Tao *et al.*, 2018). Notably, the TPH2 SNP rs7305115, which features A/G allele polymorphism, is significantly associated with psychological difficulties, especially suicidal ideation and behaviors, in individuals diagnosed with major depressive disorder (MDD) (Zhang *et al.*, 2010).

In the development of suicide ideation, numerous genes have been found to have a significant effect. Many genes regulate neurotransmitters like serotonin and dopamine, and these are also found to be related to stress and inflammation (Mariani *et al.*, 2021). It's important to

highlight that although genetic factors contribute to suicidal thoughts, they are not the sole determining factor (Smith *et al.*, 2012). The interplay between genetic and environmental influences is often intricate. It is vital to recognize that seeking assistance and finding support are critical steps in mitigating the risk of suicide and enhancing mental well-being, irrespective of any genetic predisposition (Hofer & Savell, 2021).

Advancements in genetic research have led to the identification of specific polymorphisms and gene variations associated with neurotransmitter systems, neural pathways, and behavioral traits pertinent to suicide ideation. An illustrative example is the serotonin transporter gene (5HTT), a key player in serotonin regulation (Suchankova Karlsson, 2010). Certain allelic variations in the 5HTT gene have been linked to modified serotonin transport and availability, potentially influencing mood regulation, impulsivity, and aggression – all pivotal factors in both suicide ideation (Lotrich *et al.*, 2001).

Furthermore, the dopaminergic system, renowned for its role in reward processing, motivation, and reinforcement, has garnered significant attention in the investigation of these behaviors. Variants in genes encoding constituents of the dopamine pathway, encompassing dopamine receptors and transporters, have been associated with heightened impulsivity, sensation-seeking, and aggression – traits that could contribute to suicide ideation (Archer *et al.*, 2012).

Suicide ideation, characterized by persistent thoughts about ending one's life, is a complex and multifaceted phenomenon that emerges from the intricate interplay of various biological, psychological, and environmental factors. Among the biological factors, the role of neurotransmitters, particularly dopamine and serotonin, has garnered significant attention in understanding the neurobiological basis of suicide ideation. Dopamine is a neurotransmitter that plays a crucial role in regulating mood, reward, motivation, and pleasure. Imbalances in the dopamine pathway have been associated with mood disorders and suicidal behavior. Research suggests that altered dopamine receptor sensitivity and dysfunction in dopaminergic circuits may contribute to a person's vulnerability to suicide ideation. High levels of dopamine activity in certain brain regions have been linked to impulsivity and aggression, both of which are risk factors for suicidal thoughts and behaviors. Imbalances in the dopamine pathway may contribute to the emotional dysregulation observed in individuals with suicide ideation.

Serotonin is another neurotransmitter that influences mood, emotions, and behavior.

Dysregulation of the serotonin system has long been implicated in depression and other mood disorders, which are often connected with suicide ideation. Low levels of serotonin are associated with feelings of hopelessness, sadness, and impulsivity – factors that contribute to the development of suicidal thoughts. Serotonin also regulates the brain's stress response, and disruptions in this regulation may contribute to increased vulnerability to suicide ideation during times of distress.

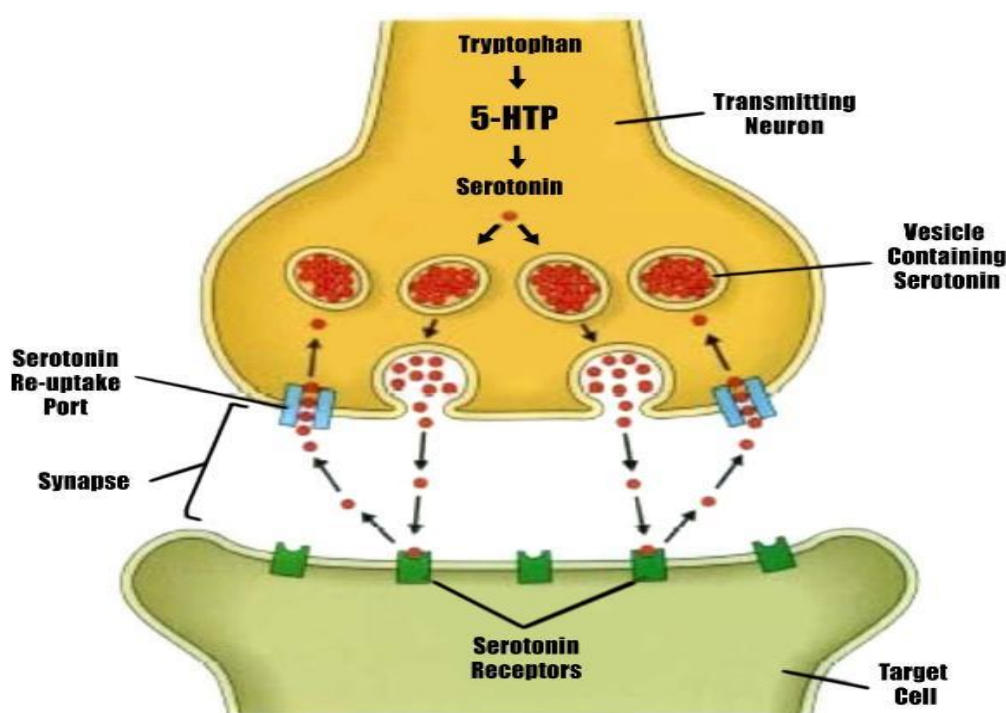


Figure 1.3 Serotonin Pathway Neurotransmitter System

(https://www.researchgate.net/figure/illustrates-the-signaling-from-the-presynaptic-serotonergic-neuron-to-the_fig3_228903634)

Dopamine and serotonin pathways are not isolated systems; they interact with each other and with other neurochemical pathways in complex ways. Altered functioning in either or both pathways can disrupt the delicate balance that maintains mental well-being. Dysregulation of these neurotransmitter systems may contribute to cognitive distortions, emotional instability, and impulsive behaviors that are often seen in individuals with suicide ideation.

The dopamine and serotonin pathways play significant roles in shaping the neurobiological foundation of suicide ideation. While imbalances in these pathways have been associated with increased vulnerability to suicidal thoughts, they are part of a larger puzzle that encompasses diverse factors. Ongoing research in this area contributes to the understanding of the intricate mechanisms underlying suicide ideation, offering potential insights into the development of effective prevention and intervention strategies.

According to research findings, certain genetic variations in the SLC6A4 and SLC6A3 genes, responsible for regulating serotonin and dopamine transporters, may be associated with an elevated susceptibility to suicidal thoughts (Mandelli & Serretti, 2013). In the case of higher risk of aggression and suicide specific variants of SLC6A4 gene have been seen to have a link with the decrease of serotonin reuptake. Additionally, the medications having the levels of serotonin and dopamine are found to have an impact on suicide ideation (Murphy *et al.*, 2008). In relation to suicidal thoughts, the efficacy of selective serotonin reuptake inhibitors (SSRIs) is found to reduce the risk of suicide in specific populations, which underscores the importance of serotonin regulation (Kenna *et al.*, 2012). Nonetheless, it is crucial to acknowledge that an increased risk of suicide ideation is contributed by neurotransmitter regulation of transporter genes. Suicide ideation typically involves an intricate interplay of genetic and environmental factors (Oliván *et al.*, 2021). Seeking appropriate treatment and support is paramount in significantly reducing the risk of suicide.

The genes encoding the components of neurotransmitter systems, like the serotonergic and dopaminergic pathways are of the particular interest (Murphy *et al.*, 2013). So, the individual's predisposition to suicide ideation and antisocial behaviors are affected by the variations in the genes that have been implicated in altering neurotransmitter levels and receptor functioning. (5HTT), the serotonin transporter gene has been found to be linked with both suicide risk and aggression, emphasizing its role in the interplay between these behavioral tendencies (Devor *et al.*, 2017). Investigations have identified specific genetic variations, known as polymorphisms, in genes related to the serotonin and dopamine neurotransmitter systems that may be associated with suicide ideation (Clayden *et al.*, 2012). Notably, a research study published in the Journal of Affective Disorders in 2019 observed a connection between a particular variation in the SLC6A4 gene and heightened suicidal ideation among Chinese individuals diagnosed with major depressive

disorder.

Across diverse populations, similar associations have been found between polymorphisms in the SLC6A4 gene and suicide ideation across diverse populations (Fanelli & Serretti, 2019). Additionally, a study published in *Neuropsychobiology* in 2018 demonstrated that variations in the DRD2 gene, responsible for encoding the dopamine D2 receptor, an association was found between The utilization of selective serotonin reuptake inhibitors (SSRIs) has been associated with an increased susceptibility to suicidal ideation among individuals diagnosed with major depressive disorder (De Berardis *et al.*, 2021). Genetic factors alone do not solely cause suicide ideation or suicidal behavior. Suicide ideation is an intricate phenomenon influenced by a fusion of environmental, genetic and social factors. To understand the role of genetics in suicide ideation, there is a need for comprehensive research (Orsolini *et al.*, 2020).

Research findings have indicated that genetic factors may enhance the risk of suicide ideation, although the exact genes and mechanisms remain incompletely understood. Variants in genes associated with the serotonin and dopamine neurotransmitter systems have been linked to an increased propensity for suicidal thoughts across diverse populations (Wang *et al.*, 2021). Nevertheless, it is crucial to recognize that suicide ideation is a multifaceted matter influenced by various environmental and social factors, alongside genetic factors. Consequently, additional research is imperative to acquire a more comprehensive understanding of the precise role genetics plays in suicide ideation (Winsor & Mueller, 2020).

Gene set enrichment analyses (GSEA) and pathway analyses offer insights into the collective impact of multiple genes within functional clusters. These analyses can unveil gene networks linked to stress response, emotional regulation, and neural plasticity, providing insights into the amalgamation of genetic influences contributing to the interplay towards suicide ideation (Sanacora *et al.*, 2022). Unraveling gene-environment interactions can offer insights into pivotal questions, such as why individuals sharing similar genetic predispositions may manifest different behavioral outcomes or how specific environmental contexts might magnify genetic susceptibilities (Shayeb, 2016). This specific Gene-Environment interaction also underscores the potential for preventive interventions that target both genetic vulnerabilities and environmental risks.

Early interventions that focus on altering environmental influences could serve to mitigate

the impact of genetic predispositions. Similarly, genetic information can guide customized interventions by identifying individuals who might be particularly receptive to specific preventive strategies, thus amplifying the precision and efficacy of interventions (Halldorsdottir & Binder, 2017).

The complex interplay between suicide ideation and antisocial delinquencies, influenced by genetics, warrants investigation. Integrating genetic perspectives across psychology, criminology and public health offers promise in understanding the mechanisms connecting these behaviors. By bridging gaps in current knowledge and shedding light on genetic susceptibility and behavior, this study aims to enhance our grasp of human behavior's multifaceted nature and guide strategies to mitigate negative outcomes (Richters, 2021). Then these study's investigations will contribute to the broader discourse surrounding personalized medicine and mental health. The era of precision psychiatry acknowledges the distinctive genetic and biological composition of each individual, paving the way for treatments tailored to an individual's distinct requirements (Demkow & Wolanczyk, 2017). Through the identification of genetic markers, epigenetic alterations, and gene-environment interactions associated with these behaviors, the study aligns with the tenets of personalized medicine, potentially reshaping our approach to preventing and treating these conditions.

This holistic viewpoint also carries implications for the conceptualization of mental health disorders. Rather than perceiving suicide ideation and antisocial delinquencies in isolation, this study embraces the concept of a spectrum of behaviors intertwined with interconnected genetic, neurobiological, and environmental foundations (Whitcomb, 2013). This approach emphasizes the necessity to transcend categorical diagnoses and instead focus on comprehending the underlying mechanisms steering these behaviors. This shift mirrors contemporary trends in psychiatry that emphasize dimensional approaches and Trans diagnostic frameworks (Gabbard, 2014).

The study's outcomes hold potential to shape public health strategies geared towards preventing suicide and curtailing antisocial behaviors. By pinpointing genetic markers, epigenetic patterns, and interactive factors that contribute to the genesis of these behaviors, policymakers and healthcare practitioners can design targeted interventions (Tang & Ho, 2007).

The relationship between genetic markers and suicide is complex and potentially influenced by other biological, environmental, and psychological factors. The presence of these variants does

not necessarily mean an individual will experience suicidal ideation – it only signifies a higher risk. Moreover, the study's discoveries carry implications for the development of biomarkers that could function as indicators of risk or vulnerability. Early identification of individuals at heightened risk could enable prompt interventions, potentially curbing the escalation of these behaviors and enhancing long-term outcomes (Tang & Crane, 2006).

The importance of this study extends to the realm of genetics, where an expanding body of research underscores the significance of genetic factors and gene variations in shaping human behavior and vulnerability to various psychological conditions. By incorporating genetic dimensions into the exploration of suicide ideation and antisocial delinquencies, the aim is to untangle the intricate genetic framework that could underlie the co-occurrence of these behaviors (McDade et al., 2017).

Furthermore, the knowledge extracted from this research holds the potential to steer policy efforts and precautionary approaches on both personal and communal levels. By uncovering common genetic vulnerabilities and clarifying the intricate neurological routes that connect these conducts, decision-makers and medical experts could enhance their ability to formulate preemptive schemes and assistance frameworks that adeptly address the diverse requirements of individuals who are susceptible to these issues (Dick et al., 2018).

Aim and Objectives:

The aim of this study is to investigate the genetic predisposition of serotonin and dopamine genes and their role in developing suicide ideation in jail intimates with the following objectives:

1. To investigate the association of different polymorphisms in the genes SLC6A3 and DRD2, HTR2B, HTR2C, TPH2, TPH1, BDNF, COMT and CREB1 in suicide ideation
2. To find out the haplotypes of different polymorphisms of serotonin, dopamine and their metabolizing enzyme genes as risk factors of suicide ideation, identify potential biomarkers related to serotonin and dopamine transporter functions that could serve as indicators for assessing the risk of suicide ideation among incarcerated individuals.

3. Investigating gender-based differences in the prevalence of the identified polymorphisms and haplotypes associated with suicide ideation.

LITERATURE REVIEW

Suicide involves the intentional act of ending one's own life, representing a profoundly distressing matter that has evolved into a significant worldwide public health issue. Despite its prevalence and impact, there is a shortage of reliable and representative data on the burden of suicide (Joshi *et al.*, 2022). It carries profound social, emotional, and economic consequences. It is estimated that approximately 700,000 individuals lose their lives to suicide annually, elevating it to the status of the foremost cause of deaths among individuals (Velasco, 2022). Approximately 1.5% of all deaths in Europe alone are caused by suicide (Mathers *et al.*, 2009). Furthermore, suicide rates are often underreported and misrepresented due to various factors such as poor registration systems, misclassification by hospitals, and the stigma attached to suicide (Pandey *et al.*, 2019). So, this represents a constrained comprehension of the actual weight of suicide and the fundamental elements that contribute to it.

As an intentional act involving self-inflicted death, suicide has captured substantial scholarly attention due to its profound societal ramifications and the potential for extensive individual and collective consequences (Staples & Widger, 2012).

The consequences of suicide are not confined to the individual level but reverberate throughout society. Apart from the emotional distress felt by families and communities following a suicide, there are broader societal implications. These include economic burdens, healthcare costs, and social disruptions that underscore the need for a comprehensive public health approach to suicide prevention (Knox *et al.*, 2004).

Suicidal behavior constitutes a spectrum of self-injurious behaviors exhibiting varying degrees of severity, encompassing ideation about suicide, suicide attempts, and ultimately, completed suicide (Silverman, 2016). It is a significant public health concern that profoundly affects individuals, families, and communities. The impact of suicide extends far beyond the individual who dies by suicide (Hjelmeland, 2013).

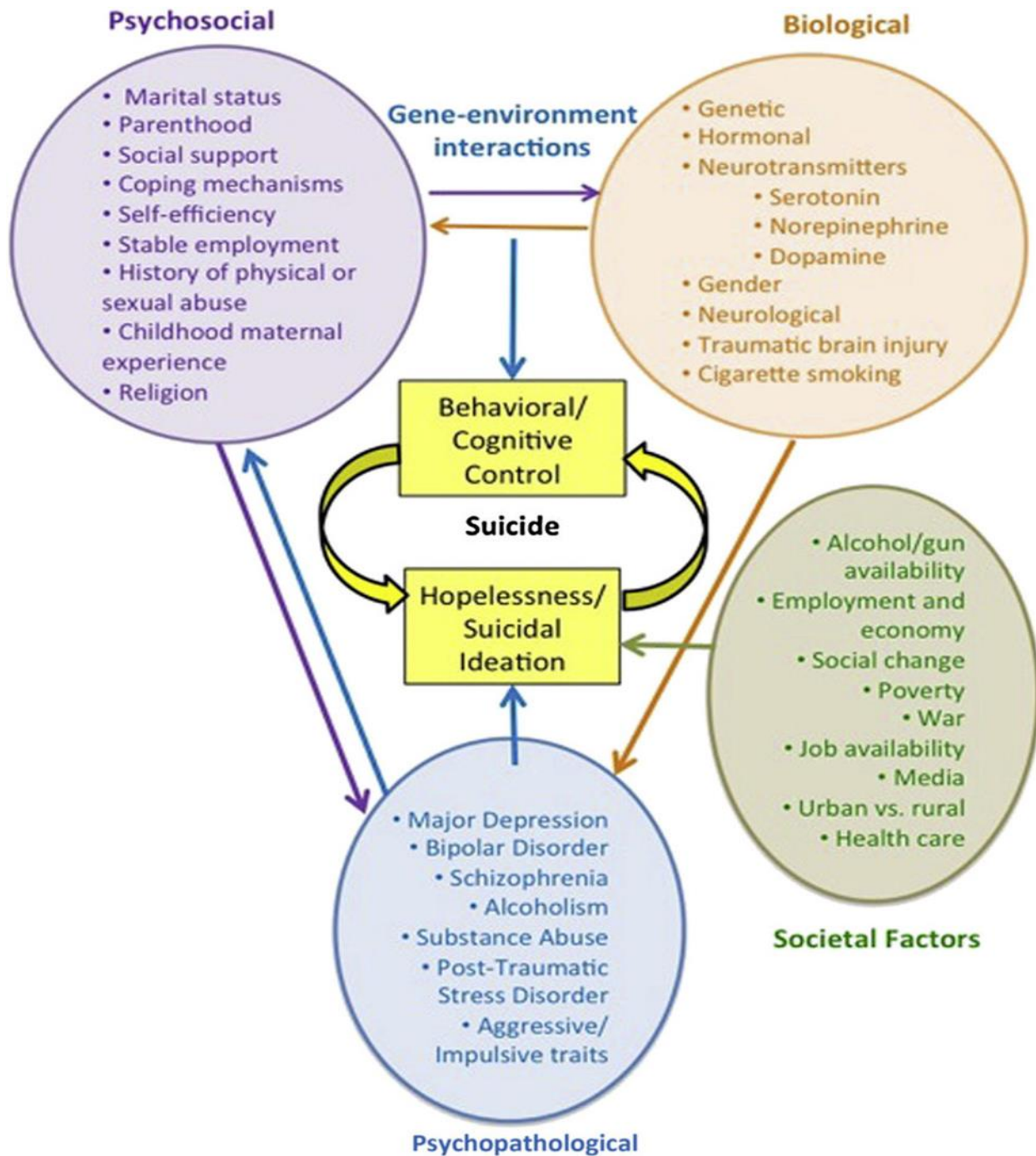


Figure 2.1 Pathophysiological model of suicide Boldrini, Maura & Mann, J. (2015). Depression and Suicide. 10.1016/B978-0-12-398270-4.00043-4.

2.1 Suicide Ideation

Suicide ideation, a complex and deeply concerning phenomenon, involves contemplating one's own death and has significant implications for individuals (Miklin *et al.*, 2019). Suicide ideation involves a diverse array of thoughts, emotions, and experiences connected to thoughts of considering or contemplating suicide. It is not a standardized experience and can differ greatly in terms of intensity, duration, and specificity (Bryan *et al.*, 2015). While some individuals may have fleeting thoughts about suicide, others may endure persistent and intrusive thoughts, possibly even formulating specific plans (Sveticic & Leo, 2012). The causes of suicide ideation are complex, stemming from a combination of psychological, social, environmental, and biological factors (Chehil & Kutcher, 2012). It spans from fleeting reflections on death to more concrete considerations of self-harm, ranging from passive thoughts to active planning (Klonsky *et al.*, 2016).

2.2 Suicide ideation in Prisoners

Suicidal ideation (SI), also referred to as suicidal thoughts or ideas, encompasses a wide spectrum of reflections, desires, and fixations related to death and thoughts of ending one's life. Prisoners constitute a group with notably elevated risk, and while studies on the suicidal tendencies of incarcerated individuals have gained substantial attention in recent years, there remain several aspects of investigation that have yet to be thoroughly explored. Drawing from data collected across 12 high-income nations, it has been revealed that suicide rates among male inmates are notably elevated, ranging from three to eight times higher compared to individuals not in incarceration within the general population. This disparity results in rates exceeding 100 suicides per 100,000 incarcerated individuals (Favril *et al.*, 2017). The suicide rate in England and Wales stands at 40 per 100,000 individuals, marking a rate four times higher than that observed in the wider population. It has gained public concern and media attention, although little research has been done for actual cause or nature (Liebling, 2002).

2.3 Types of Suicide Ideation

Various forms of suicide ideation can be identified based on the nature and characteristics of the thoughts and emotions individuals experience. Although these classifications are not all-

encompassing, they offer an overview of the different types of suicide ideation.

Suicide ideation has two types i.e., passive suicide ideation in which a person has thoughts to commit suicide but has no plan to carry it out. Another is active suicide ideation, in which thoughts are developed into a plan to carry it out (Whiteman *et al.*, 2019). The differentiation between passive and active ideation facilitates an informed approach to risk assessment, aiding clinicians in directing appropriate resources and tailored interventions (Harmer *et al.*, 2020). Passive ideation serves as an avenue for early intervention, potentially mitigating the progression of distress, while active ideation necessitates immediate attention to curtail the potential for escalation (Cramer *et al.*, 2020).

1. **Passive Suicide Ideation.** This form of suicide ideation is characterized by general thoughts or desires for death, lacking a specific plan or intent. Individuals may express a wish to no longer exist or envision being in a better state if they were no longer alive (Vehling *et al.*, 2021).
2. **Active Suicide Ideation.** This form of suicide ideation encompasses thoughts, intentions, or plans related to self-harm or taking one's own life. This may involve developing detailed plans, including specific methods or timelines (Fox *et al.*, 2020).

2.4 Suicidal Urges and Impulses

Suicidal ideation involves experiencing intense and intrusive urges or impulses to self-harm or end one's life. Individuals may feel compelled to act on these impulses even if they don't have a fully developed plan (Veale *et al.*, 2009).

1. **Suicidal Fantasies.** Suicidal ideation entails vivid and persistent thoughts, daydreams, or fantasies revolving around self-inflicted harm or suicide. Individuals may mentally explore intricate scenarios or envision a desired outcome through self-destructive actions (Álvarez Riera Escandón, 2018).
2. **Suicidal Preoccupation.** Suicidal ideation is marked by constant and intrusive preoccupation with death, dying, or themes related to suicide. Individuals may devote substantial time to ruminating on suicidal thoughts or researching self-harm methods (Williams *et al.*, 2017)

3. **Suicidal Ambivalence.** Suicidal ideation encompasses conflicting thoughts and emotions regarding suicide. Individuals may experience a desire for relief from emotional pain, while simultaneously fearing the consequences of self-harm, leading to inner turmoil (McAllister, 2003).

2.5 Aggression

Aggression has long captivated the attention of scholars across diverse fields, including psychology, sociology, and neuroscience. It encompasses an array of behaviors, each motivated by a distinct blend of factors and contributing to complex human interactions (Decety, 2018). Aggression encompasses a diverse array of behaviors, actions, or expressions aimed at inflicting harm, physical or psychological—upon oneself, others, or the environment. While it may manifest as overt physical violence, aggression can also be communicated through verbal assaults, social rejection, or even subtler forms like passive-aggressive behavior (Rippon, 2000).

The taxonomy of aggression is a product of its underlying motives, expressions, and context, leading to several distinct categories. This classification not only enhances comprehension but also unveils a comprehensive framework for appreciating the manifold dimensions of aggression:

- i. **Physical Aggression:** This category encapsulates direct physical harm or violence directed towards individuals or objects. It encompasses actions like striking, shoving, or physical intimidation, often arising in competitive or confrontational settings and reflecting dominance-oriented motivations (Hearn & Hall, 2022).
- ii. **Verbal Aggression:** Verbal aggression entails the use of hostile language, insults, threats, or derogatory language to inflict psychological injury. It may manifest in interpersonal clashes, instances of cyberbullying, or other forms of communication aimed at diminishing or degrading the subject (Deschamps & McNutt, 2016).
- iii. **Relational Aggression:** Relational aggression revolves around undermining social relationships and connections. Actions like rumor-spreading, gossiping, social exclusion, or manipulation of social dynamics are hallmarks of this type, aiming to erode an individual's social standing (Kircher, 2011).

- iv. **Hostile Aggression:** Driven by anger, hostile aggression emerges impulsively, seeking to harm others without clear objectives. It is often precipitated by emotional triggers and escalates swiftly in heated situations (Deffenbacher, 2011).
- v. **Instrumental Aggression:** In contrast, instrumental aggression is purposeful and calculated. It involves aggression as a strategic tool to achieve specific goals, such as acquiring resources or asserting dominance (Ramirez & Andreu, 2006).
- vi. **Reactive Aggression:** Reactive aggression springs forth as a response to perceived threats, provocations, or frustrations. It emerges impulsively, driven by emotional reactions to situations where an individual perceives their well-being or interests to be under siege (Ireland & Vecchi, 2009).
- vii. **Proactive Aggression:** Proactive aggression, by contrast, is premeditated and goal oriented. It involves planning and executing aggressive actions to attain desired outcomes, often devoid of immediate provocation (Tapscott, 2014).

2.6 Adverse effects of anti-social behavior and suicide ideation

Anti-social behavior and suicide ideation are two significant public health concerns that share underlying psychological and social factors contributing to their adverse effects. Individuals engaging in anti-social behavior may experience reduced empathy and increased aggression, potentially leading to the development of personality disorders (Durkee *et al.*, 2011).

Suicide ideation takes a toll on individuals' mental well-being, often linked to conditions like depression and anxiety, causing emotional distress, hopelessness, and feelings of worthlessness. Suicide ideation not only affects the individual but also extends its repercussions to family members and the broader society, leading to emotional trauma, increased healthcare costs, and reduced productivity (Lawn *et al.*, 2020).

Early detection and screening are vital for effective intervention against both anti-social behavior and suicide ideation. Increased access to mental health support, including counseling and therapy, is essential for individuals struggling with these issues, diminishing the stigma related to mental health can foster a willingness to seek help for mental health concerns (Wasserman, 2012).

2.7 Epidemiology of suicide Ideation

This scholarly review offers a comprehensive exploration of the epidemiology of suicide

and antisocial behavior. These two matters of public health carry substantial effects on both individuals and society, demanding a comprehensive investigation into their occurrence, factors that contribute to them, and related aspects. Every year, around 700,000 individuals lose their lives to suicide, establishing it as a significant factor in worldwide mortality. (Bolton *et al.*, 2008). Risk elements for suicide encompass mental health ailments, a background of prior suicide endeavors, availability of lethal methods, and familial history of suicide, social isolation, and exposure to suicidal acts by others.

Additionally, antisocial behavior, characterized by actions contravening societal norms and neglecting others' rights, is more prevalent among males during the transition from adolescence to early adulthood (Torres *et al.*, 2006). Understanding the epidemiology of these behaviors is essential for developing targeted prevention and intervention strategies to reduce their impact on individuals and society by collaborative efforts among policymakers, healthcare professionals, and communities (Beautrais, 2000).

Gaining insights into the extent and consequences of suicide ideation, which involves contemplating self-inflicted death, is essential in comprehending the prevalence and epidemiology of this distressing issue (Mukherjee & Kumar, 2017). It is crucial to have a thorough understanding of how often and in what manner suicide ideation occurs among diverse populations and in various contexts (Patterson & Holden, 2012). Such knowledge enables the development of targeted prevention strategies and the provision of appropriate assistance to individuals at risk. While data may differ across studies and demographic categories, research provides valuable details regarding the occurrence and epidemiological trends associated with suicide ideation (Carpenter *et al.*, 2000).

Accurately assessing the prevalence of suicide ideation poses challenges due to its sensitive nature. Nevertheless, research indicates that suicidal thoughts are not uncommon, with a significant proportion of individuals experiencing them at certain junctures in their lives (Oquendo *et al.*, 2003). Global estimates show variations in prevalence rates based on factors such as age, gender, socioeconomic status, and cultural context (Biswas *et al.*, 2020).

2.8 Environmental Risk Factors

Within the scope of risk evaluation and preventive strategies, the environment assumes a central role in influencing individuals' susceptibility to unfavorable outcomes. These external

circumstances notably influence diverse facets of human conduct, encompassing mental well-being, delinquent conduct, and the likelihood of experiencing suicidal ideation (Zastrow *et al.*, 2019).

2.8.1 Socioeconomic Factors

The prevalence of suicide ideation can be influenced by socioeconomic factors, such as income, education level, and employment status. Those experiencing economic hardships, unemployment, or social isolation may be at a heightened risk of developing suicidal thoughts (Langhinrichsen-Rohling, Friend, Powell, & Behavior, 2009).

2.8.2 Family Dynamics and Challenges

- i. **Disrupted Family Dynamics:** Dysfunctional family interactions, marked by inadequate communication, conflicts, and insufficient emotional support, can substantially heighten individuals' proneness to suicidal ideation and delinquent conduct. The absence of a nurturing family environment might contribute to isolation and the adoption of maladaptive coping mechanisms (Liebling, 2012).
- ii. **Parent-Child Attachment:** Insecure or disrupted attachment relationships between parents and children have been associated with an increased susceptibility to experiencing suicidal ideation and involvement in antisocial behaviors. Unstable attachment can hinder the development of emotional regulation skills and healthy interpersonal relationships (Park & Schepp, 2015).

2.8.3 Influence of Peers

- i. **Peer Rejection:** Social isolation and peer rejection during pivotal developmental stages can exacerbate the risk of both suicidal ideation and antisocial behaviors (Parker & Asher, 1987). The absence of positive peer interactions may lead to feelings of loneliness and the embrace of deviant coping strategies (Nangle *et al.*, 2003).
- ii. **Peer Pressure:** Succumbing to negative peer norms and pressures can sway individuals towards suicidal ideation and delinquency. The need for belonging and the fear of exclusion might prompt individuals to adopt behaviors that align with the group, even if they are maladaptive (Roebuck, 2014).

2.8.4 Educational Settings

- i. **Academic Struggles:** Widespread challenges in academics, combined with the pressure of academic performance, have been linked to an increased likelihood of experiencing suicidal ideation and engaging in delinquent behaviors. Persistent academic challenges may lead to lowered self-esteem and feelings of hopelessness (Tam *et al.*, 2007).
- ii. **Bullying and Victimization:** Encountering bullying and victimization within the school environment can contribute to the emergence of both suicidal ideation and antisocial behaviors (Klomek *et al.*, 2009). The emotional toll of bullying can worsen existing vulnerabilities and lead to the adoption of maladaptive coping mechanisms (Troop-Gorden, 2017).

2.8.5 Socioeconomic Disparities

- i. **Economic Hardships/Unemployment:** Growing up in economically disadvantaged environments is associated with an elevated susceptibility to suicidal ideation and engagement in antisocial behaviors. The stress of financial adversity may contribute to mental health challenges and limited access to resources. Financial instability can be stressors contributing to suicide ideation, which can lead to feelings of hopelessness and a perceived lack of control over one's life (Ogundele, 2018).
- ii. **Neighborhood Crime:** Residing in neighborhoods characterized by high crime rates and instability can heighten the risk of suicidal ideation and involvement in delinquent activities. Exposure to violence and crime can shape individuals' perceptions of their environment and personal safety (Lambert *et al.*, 2010).

2.8.6 Media and Technology

Media Exposure: Excessive exposure to media content depicting violence, self-harm, and suicidal behaviors can play a role in fostering the emergence of suicidal ideation and antisocial tendencies. Media influences can shape attitudes and behaviors through the process of social learning, media reports, can profoundly impact an individual's mental state. Witnessing such acts may normalize suicidal behaviors and increase the likelihood of contemplating suicide as a solution to personal problems (Villani, 2001).

2.8.7 Exposure to Suicidal Behavior

- i. **Social Isolation and Lack of Social Support:** Feeling socially isolated and experiencing loneliness are major environmental risk factors for suicide ideation. Individuals lacking meaningful social connections may encounter intensified emotional distress and a sense of hopelessness, thereby elevating the probability of experiencing suicidal thoughts. The absence of support systems can exacerbate feelings of isolation (Quadt *et al.*, 2020).
- ii. **Access to Lethal Means:** Convenient availability of lethal methods, like firearms and medications, drugs, substantially amplifies the risk of suicidal ideation culminating in lethal attempts. Limiting access to these means can serve as a preventive measure, potentially saving lives in moments of crisis. Restricting access to these means can act as a preventive measure (Bunney *et al.*, 2002).
- iii. **Presence of Suicide Clusters:** Suicide clusters in specific communities or social groups can be a powerful environmental risk factor. The imitation of suicidal behaviors within these clusters can lead to a contagious effect, resulting in an increase in suicide ideation among vulnerable individuals (Cheng *et al.*, 2014).
- iv. **Stigma Surrounding Mental Health:** Stigmatization of mental health issues and discussions about suicide can discourage individuals from seeking help and support. Social stigma may cause individuals to internalize their struggles and avoid reaching out for assistance, exacerbating feelings of isolation and hopelessness (Grady *et al.*, 2019).

Cultural and Religious Factors: Cultural norms and religious beliefs significantly influence how suicide and mental health are perceived. Societies with stigmatizing attitudes toward mental health or religious beliefs that view suicide as sinful or morally wrong may discourage individuals from seeking help, further contributing to suicide ideation (Dardas & Simmons, 2015).

2.9 Psychological Risk Factors

Mental Health Disorders: Conditions like depression, anxiety, and personality disorders consistently elevate the risk of suicide ideation and antisocial delinquencies. The interplay of distorted cognition, negative affect, and impaired coping mechanisms may heighten vulnerability to these behaviors (Siever, 2008).

Impulsivity and Emotional Dysregulation: Heightened impulsivity and emotional dysregulation are contributing factors to both suicide ideation and antisocial behaviors. Difficulties in managing emotions and controlling impulsive reactions may drive individuals towards maladaptive coping strategies (Okado & Bierman, 2015).

Feelings of Hopelessness and Desperation: A sense of hopelessness combined with a lack of perceived alternatives can fuel both suicide ideation and engagement in antisocial activities. The perception that life lack's purpose or positive prospects may increase the inclination towards these behaviors (VandeWalle, 2003).

Mood disorders: Such as depression, bipolar disorder, anxiety disorders stand out as notable risk factors for suicidal ideation. These conditions often lead to emotional distress, hopelessness, and feelings of isolation (Dillon *et al.*, 2018).

History of Trauma and Adverse Childhood Experiences (ACEs): Encountering traumatic incidents or adverse experiences during childhood, such as abuse or exposure to violence, could raise the chances of developing thoughts of suicide because of lingering emotional wounds and unresolved trauma (Brockie *et al.*, 2015).

Early Behavioral Problems: Early behavioral problems, like oppositional and aggressive behavior, can predict the onset of antisocial delinquencies later in life. Failure to address these behaviors in childhood can exacerbate the risk (Wasserman, 2003).

2.9.1 Interactions and Overlapping Factors

The causes of suicide ideation and antisocial delinquencies often interact and overlap in intricate ways:

- i. **Gene-Environment Interaction:** Genetic predisposition interacts with environmental influences to modulate the risk for both behaviors. Individuals with a genetic vulnerability may exhibit heightened susceptibility when exposed to adverse life circumstances (Meyer-Lindenberg & Tost, 2012).
- ii. **Role of Mediating Psychopathology:** Psychological factors, such as impulsivity and emotional dysregulation, can act as mediators between biological and social determinants. These psychological traits can amplify the effects of genetic and environmental influences

(Turecki *et al.*, 2019).

- iii. **Implications for Prevention and Intervention.** The nuanced understanding of the complex interplay of causative factors has significant implications for prevention and intervention strategies:
- iv. **Holistic Approaches:** Comprehensive interventions addressing biological, psychological, and social dimensions hold promise for mitigating suicide ideation and antisocial delinquencies. Comprehensive programs that target multiple facets can provide a more effective approach (Corner *et al.*, 2004).
- v. **Timely Identification and Screening:** Early identification of risk factors, especially during adolescence, can facilitate targeted interventions. Screening for mental health disorders, impulsivity, and adverse childhood experiences can enable early intervention (Perez *et al.*, 2016).
- vi. **Psychological Interventions:** Psychotherapeutic modalities, such as cognitive-behavioral therapy and dialectical behavior therapy, can address maladaptive cognitive patterns, emotional dysregulation, and impulsivity, thereby reducing the risk of both suicide ideation and antisocial behaviors (Shafti *et al.*, 2021).

2.10 Biological Risk Factors

Neurochemical Imbalances: Alterations in neurotransmitter systems, particularly serotonin and dopamine, have been associated with both suicide ideation and antisocial behaviors. Disruptions in these neurochemical pathways may impact mood stability, impulsivity, and emotional regulation, contributing to the emergence of these behaviors (Beauchaine *et al.*, 2011).

- i. **Serotonin System:** The serotonin neurotransmitter system significantly impacts mood regulation and emotional processing. Low serotonin levels have consistently been associated with suicidal behavior. Dysregulation of serotonin receptors and transporters may contribute to mood alterations and impulsive behavior, leading to suicidal tendencies (Arias *et al.*, 2021).
- ii. **Dopamine Systems:** Similar to suicide, dopamine neurotransmitter systems also influence antisocial behavior. Abnormalities in dopamine levels may contribute to impulsivity, aggression, and a lack of behavioral inhibition (Pavlov, 2012).

Genetic Predisposition: Genetic factors play a role in predisposing individuals to suicide ideation and antisocial delinquencies. Twin and family studies have indicated a heritable component for both behaviors, suggesting a genetic foundation for their occurrence (Lynskey *et al.*, 2010).

Neurobiological Dysfunctions: Changes in the configuration and operation of brain areas tasked with handling emotions, making decisions, and managing impulses—such as the prefrontal cortex and amygdala—might play a role in the tendency toward both thoughts of suicide and behaviors characterized by antisocial tendencies. (Simic *et al.*, 2021).

- i. **Prefrontal Cortex and Limbic System:** This encompasses the prefrontal cortex and the limbic system, encompassing structures like the amygdala and hippocampus, play vital roles in emotional regulation, decision making, planning and impulse control and social behavior. Dysfunction in these regions is associated with deficits in empathy, suicidal inclinations, moral decision-making, and impulse control in individuals with antisocial tendencies (Banfield *et al.*, 2004).
- ii. **HPA Axis Dysregulation:** The malfunctioning of the hypothalamic-pituitary-adrenal (HPA) axis, which governs the stress response, is linked to an increased vulnerability to participate in suicidal actions. Prolonged stress and alterations in the HPA axis could impact the management of emotions and mood (Pompili *et al.*, 2010).
- iii. **Amygdala:** The amygdala, involved in emotional processing and response to social stimuli, may be dysregulated in individuals with antisocial behavior. This can lead to heightened emotional reactivity and reduced emotional empathy, characteristic features of antisocial behavior (Jones *et al.*, 2009).

Shared Neural Mechanisms: Interestingly, some neural mechanisms are shared between suicide and antisocial behavior. Dysregulation in the serotonin system, prefrontal cortex, and the limbic system is implicated in both behaviors, suggesting potential overlapping neurobiological pathways (Schmaal *et al.*, 2020).

Other factors

- i. **Age.** Suicide ideation can impact individuals of all age groups, ranging from children and adolescents to adults and older adults. However, prevalence rates tend to be higher in specific age ranges. For instance, adolescents and young adults frequently show elevated rates of suicide ideation compared to other age groups (Hatch & Dohrenwend, 2007)

- ii. **Gender.** Consistent research findings reveal that females tend to report a higher prevalence of suicide ideation compared to males. However, it is crucial to acknowledge that while suicide ideation rates may vary between genders, completed suicide rates are typically higher among males, which underscores the gender paradox in suicide (Chan *et al.*, 2008).

2.11 Genetics of suicide

The emergence and endurance of thoughts about suicide can be impacted by a range of risk elements. These include aspects like a background of previous suicide endeavors, a family history associated with suicide, encounters with traumatic events or abuse, the presence of accessible lethal means, and insufficient social backing (Chehil & Kutcher, 2012). The present understanding of suicide is that subjects with a genetic predisposition are particularly vulnerable to stressful environmental inputs (Jimenez *et al.*, 2018). The biological basis of suicide has been investigated in several studies to better understand its precipitating factors (Orsolini *et al.*, 2020). There have been several biological hypotheses. Recent studies have explored the genetic impact on suicidal behavior through Genome-wide association studies (Tam *et al.*, 2019).

However, many GWAS suggested associations that did not reach genome-wide significance, pointing out that there are multiple genomic targets that need further investigations in suicidal behavior (Gonda *et al.*, 2021). A recent study by identified several genes involved in neural functions such as neurodevelopment (cellular assembly, function, and organization), cell death, survival mechanisms, and immunological/inflammatory mechanisms (Fatemi *et al.*, 2018). The use of GWAS has been limited to the investigation of common genetic variants. However, more recent studies have explored the influence of environmental input on gene function (Thomas, 2010).

The associations of early life adversities (ELAs) and suicide have suggested interaction with genetic liability leading to the stress diathesis model of suicide (Smart *et al.*, 2015). Consistent exploration in the field of functional genomics has revealed brain areas associated with psychiatric disorders and suicide, including the prefrontal cortex, anterior cingulate cortex, amygdala, and hippocampus (Schmaal *et al.*, 2010). These areas have displayed both structural and functional changes in investigations involving postmortem tissue from completed suicides and imaging studies of suicide attempts (Lloyd *et al.*, 2015).

At the level of neuroanatomy, neuroimaging research demonstrates alterations in various brain regions associated with susceptibility to suicidal behavior. Notably, dysfunctions within the frontal, temporal, and parietal cortices emerge as pivotal factors in suicidal behavior (Jollant, 2016).

Genetic predisposition studies have become increasingly important in understanding the role of specific genes in various health conditions. One area of interest is the genetic predisposition of serotonin and dopamine transporter genes in suicide ideation (Serotonergic genes and suicidality - PubMed). Association studies have revealed that specific alleles of genes related to serotonin and dopamine transporters are linked to an augmented susceptibility to suicidal behavior (Blum *et al.*, 2014). For example, the serotonin transporter gene (5-HTT) has undergone comprehensive investigation in connection with suicide ideation.

Studies have revealed that the short allelic version of the serotonin transporter gene-linked polymorphic region (5-HTTLPR) relates to a heightened occurrence of suicide attempts and behaviors. It's thought that this variant causes a decrease in the availability and function of the serotonin transporter protein compared to the longer allele. This decrease in protein availability and function might play a role in hindering serotonin neurotransmission, which is associated with mood regulation and emotional processing (Cicchetti, 2010).

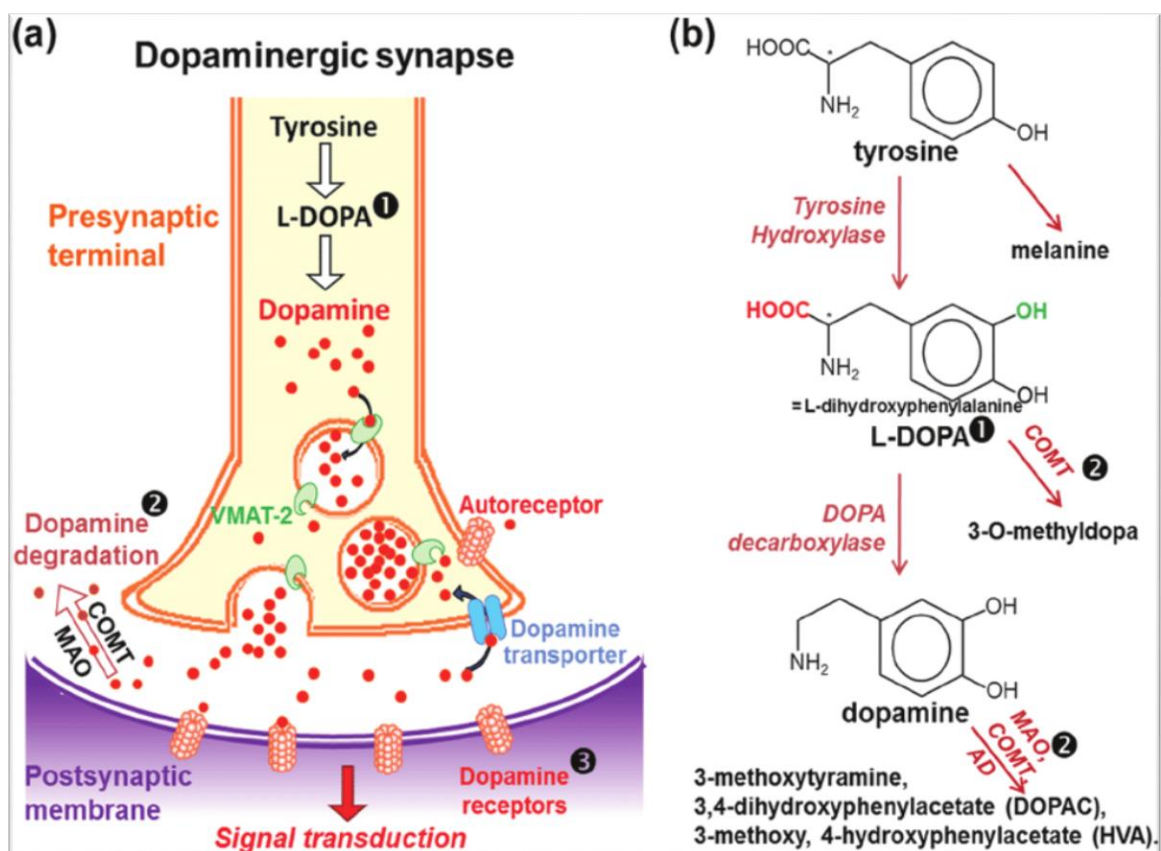


Figure 2.2 Dopaminergic Synapse. Jones, Daryl & Moussaoud, Simon & McLean, Pamela. (2014). Targeting heat shock proteins to modulate α -synuclein toxicity. *Therapeutic advances in neurological disorders*. 7. 33-51. 10.1177/1756285613493469.

Similarly, inquiries have delved into the role of the dopamine transporter gene in relation to thoughts of suicide. Research has indicated that differences in the dopamine transporter gene could contribute to an increased susceptibility to engaging in suicidal behavior. As an example, a study identified specific alleles of the dopamine transporter gene as being linked to a higher likelihood of attempting suicide (Salatino-Oliveira *et al.*, 2018).

Several studies have identified specific alleles of serotonin and dopamine transporter genes that are associated with suicidal ideation.

- i. **Serotonin Transporter Gene (5-HTT):** The serotonin transporter gene-linked polymorphic region (5-HTTLPR) has a shorter allelic variant (s-allele) that has been associated with an increased susceptibility to suicidal behavior. Individuals with one or

two copies of the s-allele display a stronger propensity for suicidal tendencies and actions when compared to those carrying the longer variant (l-allele) (Manucks *et al.*, 2004).

- ii. **Dopamine Transporter Gene (DAT1 or SLC6A3):** Genetic variations, notably those tied to a variable number tandem repeat (VNTR) polymorphism situated in the 3' untranslated region (UTR) of this gene, have been connected to a heightened vulnerability to suicidal tendencies. Specific research indicates that the 9-repeat (9R) allele of the 40-base pair (bp) VNTR might be associated with an increased inclination toward contemplating suicide (Bogdan *et al.*, 2013).

Numerous research investigations have pinpointed variations of the serotonin and dopamine transporter genes that could potentially be linked to an elevated risk of suicide. The shorter "s" allelic variant within the serotonin transporter gene-linked polymorphic region (5-HTTLPR) has been correlated with a heightened susceptibility to suicidal behavior. Individuals carrying one or two instances of this "s" allele have demonstrated a greater inclination toward both contemplating and attempting suicide, in comparison to those carrying the longer "l" allele (Kenna *et al.*, 2012). Distinct alleles, particularly those tied to a variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region (3'UTR) of this gene, have shown connections with an increased vulnerability to suicide. Multiple studies propose that the 9-repeat (9R) allele of the 40 base-pair (bp) VNTR might be linked to an elevated probability of experiencing thoughts of suicide and engaging in suicidal behaviors.

Note, however, that these are statistical associations and shouldn't be used to predict individual behaviors. The link between these specific genes and suicide is complex and potentially influenced by other biological, environmental, and psychological factors. The presence of these variants doesn't guarantee that an individual will experience suicidal ideation or behavior; it only indicates a potentially increased risk (Brookes *et al.*, 2007).

The serotonin transporter gene-linked polymorphic region (5-HTTLPR) has a short allelic variant (S-allele) associated with reduced expression and availability of the serotonin transporter compared to the long allele (L-allele). This can potentially lead to a decrease in the reuptake of serotonin (5-HT) from the synaptic cleft into the presynaptic neuron, resulting in lower levels of serotonin within the cell. Since the serotonin transporter plays a vital role in the serotonin system, which is crucial for regulating mood and processing emotions, any malfunction in this transporter

could disrupt these functions. This reduced reuptake and potential disruption of the serotonin system have been connected to various mood disorders, such as depression and anxiety. Furthermore, it is also associated with an elevated risk of suicidal ideation and behavior in specific populations.

It is worth mentioning that these effects can be shaped by diverse environmental elements and additional genetic factors, rendering them more intricate than a straightforward one-to-one correlation (McCaffery *et al*, 2003). Apart from the serotonin and dopamine transporter genes, other genes have been linked to suicide ideation and behavior. These can include:

Tryptophan Hydroxylase (TPH): The two isoforms of this gene (TPH1 and TPH2) are integral to serotonin synthesis and have exhibited connections with suicidal ideation across various studies. Distinct variations in TPH2, which is predominantly expressed in the brain, may specifically impact susceptibility to suicidal behavior (Hernandez-Hernandez *et al.*, 2019).

The Brain-Derived Neurotrophic Factor (BDNF) gene, specifically the Val66Met polymorphism, has been linked to the domain of suicidal behavior due to genetic variations. BDNF plays a pivotal role in supporting neuronal survival, growth, and functioning. Alterations in BDNF levels have been linked to mood disorders and the occurrence of suicide (Szarowicz *et al.*, 2022).

The breakdown of catecholamines, encompassing dopamine and noradrenaline, is significantly influenced by the COMT gene. Variations in the COMT gene, particularly the Val158Met polymorphism, could potentially impact the risk of suicide by influencing these neurotransmitter systems. It's important to acknowledge, however, that the connection between these genetic factors and suicidal ideation is intricate. The presence of these genetic variations doesn't automatically signify that an individual will encounter suicidal thoughts or behaviors. Environmental factors and individual genetic backgrounds must also be considered. The diminished functionality of the serotonin transporter can have multiple implications (Currier & Mann, 2008).

2.11.1 Genome-Wide Association Studies (GWAS) interpretation of Suicide

The investigation of genetic complexities within the context of intricate human traits has been significantly advanced through Genome-Wide Association Studies (GWAS). This segment delves into the importance of GWAS within the context of understanding suicide ideation and

criminal behavior, elucidating how these studies offer a comprehensive view of the genetic landscape, potentially identifying specific genetic markers and pathways associated with these phenomena (Aragam, 2011).

2.11.2 GWAS in Suicide Ideation Research

GWAS entails the systematic analysis of an extensive array of genetic variants spanning an individual's entire genome. This comprehensive approach facilitates the identification of genetic variations significantly linked to specific traits, behaviors, or disorders. By scrutinizing large cohorts, GWAS has the potential to unveil common genetic markers linked to suicide ideation and criminal behavior (Plassais *et al.*, 2019).

Detection of Risk Loci: GWAS has played a pivotal role in uncovering potential genetic loci connected to suicide ideation. These loci might encompass genes related to neurotransmitter regulation, neural development, and stress response. Identifying risk loci contributes to a deeper grasp of the biological mechanisms underlying suicide ideation (Hagemann *et al.*, 2005).

Polygenic Risk Scores: GWAS findings can be harnessed to formulate polygenic risk scores, amalgamating multiple genetic variants to predict an individual's vulnerability to suicide ideation. These scores offer a personalized approach to assessing risk, enhancing our ability to identify individuals at heightened susceptibility (Eley *et al.*, 2004).

2.11.3 GWAS in Criminal Behavior Research

Exploration of Genetic Influences: GWAS has provided insights into the genetic framework of criminal behavior, shedding light on potential candidate genes and pathways associated with antisocial tendencies. By revealing genetic variations, GWAS contributes to a deeper understanding of the biological factors contributing to criminal behavior.

Revelation of Overlapping Genetic Signals: In criminal behavior research, GWAS have unveiled shared genetic signals between criminal tendencies and related traits such as aggression and impulsivity. This suggests the existence of shared genetic vulnerabilities across these behaviors (Waltes *et al.*, 2016).

The inclination toward suicidal thoughts and engagement in criminal activities are

characteristics shaped by the interplay of both genetic and environmental elements. GWAS encounters challenges in disentangling the complex interaction between these elements. GWAS necessitates sizable sample sizes to achieve statistical robustness. The replication of findings in independent cohorts is essential to validate the identified genetic associations (Alemany Sierra, 2013).

2.12 Serotonin pathway and neurotransmitter system

- i. **Serotonin neurotransmitter system.** Within the intricate tapestry of neurochemical systems that influence human behavior, the serotonin neurotransmitter system emerges as a pivotal actor. This section delves into the profound significance of the serotonin system in the context of suicide ideation, elucidating its contributions to mood regulation, emotional processing, and the genesis of suicidal thoughts. Serotonin, also referred to as 5-hydroxytryptamine (5-HT), functions as a neurotransmitter predominantly synthesized within brainstem raphe nuclei. It plays a central role in inter-neuronal communication, facilitating various physiological processes. The serotonin system exerts a profound influence on mood regulation, contributing to emotional equilibrium and well-being. Disruptions in serotonin levels have been linked to mood disorders, including aggression and anxiety (Bryan & Rudd, 2010).
- ii. **Serotonin's Implications in Suicide Ideation.** The theory suggesting a lack of serotonin suggests that changes in the availability of serotonin play a crucial part in the fundamental mechanisms behind thoughts and actions related to suicide. Diminished serotonin levels are linked to traits such as impulsivity, aggression, and emotional dysregulation, commonly observed in individuals with suicide ideation. Scientific investigations have unveiled impaired serotonergic transmission in individuals who have died by suicide, evidenced by decreased levels of serotonin metabolites in post-mortem brain analyses. These findings underscore the connection between perturbed serotonergic function and the emergence of suicide ideation (Takahashi *et al.*, 2012).

2.12.1 Serotonin Receptors and Genetic Variability

Serotonin exerts its effects through a spectrum of receptor subtypes, each exerting distinct functions and exhibiting specific distribution patterns. Variations within these receptor subtypes

can modulate an individual's susceptibility to suicide ideation. Genetic variances in genes encoding serotonin receptors and transporters have been explored in the context of suicide ideation. Polymorphisms within the serotonin transporter gene (SLC6A4) and serotonin receptor genes (e.g., HTR1A, HTR2A) have been associated with heightened vulnerability to suicidal behaviors (Bortolato *et al.*, 2013).

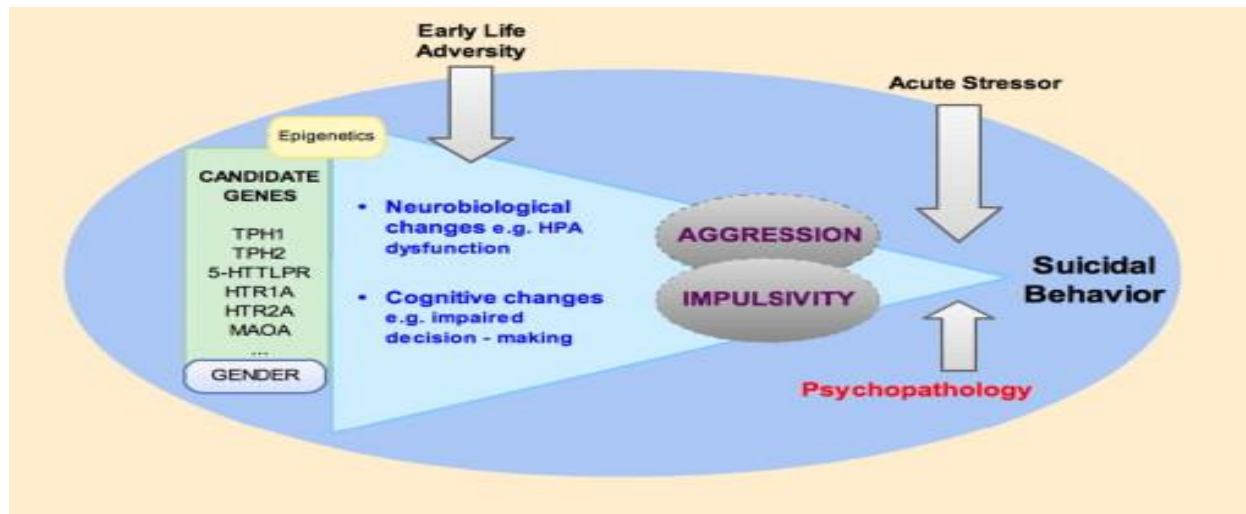


Figure 2.3 Serotonergic genes associated with suicidal behavior. Niki Antypa, Alessandro Serretti, Dan Rujescu, Serotonergic genes and suicide: A systematic review, *European Neuropsychopharmacology*, Volume 23, Issue 10, 2013.

Within the framework of neurochemical systems influencing human behavior, the serotonin neurotransmitter pathway emerges as a pivotal orchestrator. This subsection embarks on a journey into the dynamics of the serotonin pathway, casting light on its profound implications concerning suicide ideation. Serotonin, synthesized from the amino acid tryptophan via enzymatic reactions primarily occurring within brainstem raphe nuclei, finds itself packaged into vesicles and subsequently released into the synaptic cleft, where it engages with postsynaptic. Serotonin wields its influence by binding to specific receptor subtypes, particularly the 5-HT_{1A} and 5-HT_{2A} receptors, which are strategically distributed in brain areas like the prefrontal cortex and limbic system, shaping emotional processing and mood regulation (Chandley & Ordway, 2012).

2.12.2 Serotonin Pathway's Involvement in Suicide Ideation

Emotional Terrain: The serotonin pathway intricately influences emotional processing, shaping neural circuits governing mood regulation. Perturbations within this pathway hold the

potential to disrupt emotional harmony, potentially fostering the emergence of suicidal thoughts (McDevitt et al., 2021). Genetic Variability at Play: Genetic polymorphisms within key components of the serotonin pathway, such as tryptophan hydroxylase (TPH) and the serotonin transporter (SLC6A4), have been linked to changes in serotonin levels and an increased vulnerability to suicidal ideation (Bellivier et al., 2011).

2.12.3 Neurotransmitter Disarray and Its Link to Suicide Ideation

The notion of a serotonin deficiency finds resurgence, positing that disturbances within the serotonin pathway, leading to decreased serotonin availability, could contribute to mood dysregulation and impulsive behaviors, potentially amplifying the risk of suicidal ideation. The serotonin pathway interlaces with other neurotransmitter systems, such as dopamine and norepinephrine, influencing emotional responses and cognitive processes. Malfunctions in these interplays may intensify predisposition to suicide ideation (Du & Pang, 2015). The focus here is on the serotonergic transporter gene (5HTT), which assumes a crucial role in the regulation and neurotransmission of serotonin. By exploring how genetic variations within the 5HTT gene might contribute to the emergence of suicidal ideation, we aim to illuminate the complex interplay between genetics, neurobiology, and the vulnerability to suicidal thoughts (Kenna *et al.*, 2012).

2.12.4 The Serotonergic Transporter Gene (5HTT)

The gene responsible for the serotonergic transporter, alternatively named 5HTT or SLC6A4, encodes a protein accountable for retrieving serotonin from the synaptic cleft and returning it to the presynaptic neurons, a process crucial for sustaining serotonin levels and managing neurotransmission. Thorough analysis has been conducted on the 5HTT gene; particularly concentrating on a specific genetic variation termed the serotonin-transporter-linked polymorphic region (5-HTTLPR). This polymorphism leads to different forms of alleles that influence the serotonin transporter's expression and operation (Sadkowski *et al.*, 2013).

The 5HTT gene is notably intriguing as a potential candidate gene that influences suicidal ideation, owing to its central role in regulating serotonin, which subsequently impacts mood, impulsivity, and emotional control. The connection between 5HTT polymorphisms and suicidal ideation extends beyond genetics. Gene-environment interactions, particularly exposure to stress and life events are believed to modulate the impact of 5HTT variations on the inclination toward

suicidal ideation (Courtet *et al.*, 2005).

2.12.5 Genetic Loci of Serotonergic Receptors: Significance and Role

Genetic loci related to serotonergic receptors hold substantial importance as key genomic sites where variations can exert influence over the expression and functionality of these receptors. Serotonergic receptors, including notable subtypes like 5-HT_{1A} and 5-HT_{2A}, wield pivotal roles in mediating the effects of serotonin within the brain's intricate network. The spotlight has been cast upon polymorphic variations present within the genetic loci of serotonergic receptors due to their potential to impact receptor density, affinity, and subsequent signaling pathways. These allelic variants are believed to play a role in reshaping processes associated with serotonin function (Holmes, 2008)

2.12.6 Modulating Serotonergic Enzymes for Suicide Ideation

Serotonergic enzymes, such as tryptophan hydroxylase (TPH) and monoamine oxidase (MAO), assume a crucial role in both the synthesis and breakdown of serotonin. The activity of these enzymes directly influences the availability of serotonin, a neurotransmitter with critical implications for emotional processing and mood modulation. The modulation of serotonergic enzymes is subject to the collective impact of genetic and environmental factors. Genetic variations in the genes encoding these enzymes can impact their function, while external influences such as stress can also contribute to alterations in enzyme activity (Sabir *et al.*, 2008).

Fluctuations in serotonin levels have been associated with mood disorders and emotional instability, both of which are pivotal factors in the genesis of suicide ideation. Changes in the activity of serotonergic enzymes can disturb the delicate equilibrium of serotonin neurotransmission. Altered serotonin levels can exert an influence on emotional states, potentially contributing to the emergence of suicidal thoughts, particularly in individuals who are predisposed (Pompili *et al.*, 2010).

2.13 Dopamine system and Suicide Ideation

By closely examining the potential link between dopamine neurotransmission and the emergence of suicidal thoughts, this exploration aims to unravel the dynamics between neurobiology and susceptibility to suicide ideation.

2.13.1 The Dopamine System: Importance and Mechanisms

Within the brain's complex network of neurotransmitters, the dopamine system holds a central role. It profoundly influences processes such as reward, motivation, pleasure, and emotional regulation. Dopamine is synthesized and released by neurons, binding to specific receptors to modulate a range of cognitive and emotional functions. The realm of dopaminergic signaling encompasses diverse pathways, including the mesolimbic and mesocortical pathways. These pathways contribute to the regulation of emotional responses, while also playing a critical role in the manifestation of several neuropsychiatric conditions (Baskerville & Douglas, 2010).

2.13.2 Dopamine Synthesis

It is produced within the cytosol of dopaminergic neurons in the nervous system and within the digestive system, specifically in organs like the spleen and pancreas. It arises from the amino acid tyrosine and serves important functions in both the central nervous system (CNS) and peripheral nervous system (PNS). The substantia nigra (SN) is linked to the dorsal striatum's caudate and putamen nuclei through the primary dopaminergic (DAergic) nigrostriatal pathway (Daeron, 2022). In the liver, phenylalanine is metabolized by phenylalanine hydroxylase, leading to the creation of tyrosine. This tyrosine is subsequently transported to the brain via an active transport mechanism. Within the brain, tyrosine goes through a series of reactions to transform into dopamine. Tyrosine hydroxylase adds hydroxyl groups to tyrosine, converting it into levodopa (L-DOPA), an intermediary product that is rapidly transformed into dopamine by the enzyme dopa decarboxylase, also known as aromatic amino acid decarboxylase, situated in the cytoplasm (Du *et al.*, 2022).

2.13.3 Dopamine Function

Dopamine operates through its interaction with DAergic receptors (DRs) located on the surfaces of cells. It oversees a range of functions, including movement, appetite, emotions, rewards, sleep, attention, cognitive and learning behaviors, brain adaptability, and the creation of new neurons. Dopamine has earned the label of an "anti-stress molecule" and holds a significant role in the motivational process (Baumgarten *et al.*, 2022). Mood is also under the influence of dopamine, as disruptions in dopamine function can lead to anhedonia, a state of joylessness

characterized by the loss of interest and pleasure in activities. Anhedonia serves as the principal symptom of major depressive disorder (MDD) and is closely tied to thoughts of suicide, regardless of aggressive tendencies (Keedwell, 2008).

Research has demonstrated that the dopaminergic (DAergic) gene system is connected to both substance addiction and mental disorders, given dopamine's pivotal role in shaping reward-related behaviors. Consequently, understanding the contributions of genes related to the dopaminergic (DAergic) system, encompassing receptors, transporters, and metabolic enzymes for dopamine, has been a focal point for many years (Baik, 2013). Various clinical conditions, such as Schizophrenia, Parkinson's disease, Tourette's syndrome, and hyperprolactinemia, have been associated with disruptions in the dopaminergic gene system. Dopamine receptors, operating in an antagonist or agonist manner, have been established as tools for addressing nervous, psychiatric, and ocular disorders (Wang et al., 2019).

2.13.4 DAT-1 Polymorphism

A variable number of tandem repeat (VNTR) polymorphism spanning 40 base pairs, ranging from 3 to 13 repeats, is in the 3'-untranslated region (3'-UTR) of the DAT-1 gene. The most common alleles in the population are those containing either 9 or 10 repeats of the VNTR sequence. Research indicates that the 9-repeat allele of the VNTR is associated with higher DAT activity in the brain compared to the 10-repeat allele, although there is a debate about this (VanNess, 2006). The length of the VNTR polymorphism has a connection with the regulation of DAT at the transcriptional level and is linked to various neuropsychiatric conditions, including depression.

Dopamine signaling and equilibrium are managed by dopamine transporters (DAT-1), which cease dopamine signaling by reabsorbing dopamine back into the presynaptic neuron and clearing the synapse. When DAT functioning is flawed or disrupted, it leads to an uneven level of dopamine in the neurons and is closely linked to a range of neurological and psychological disorders, including depression, bipolar disorder (BP), schizophrenia, Parkinson's disease (PD), and attention deficit hyperactivity disorder (ADHD) (Dreher et al., 2009).

Research indicates that alongside neurotransmitter imbalances, depressive disorders are also linked to gene polymorphisms and the inhibition or irregularity of modulatory enzymes related

to neurotransmitters, including tryptophan hydroxylase (TPH), monoamine oxidase A (MAOA), and monoamine oxidase B (MAOB). These enzymes participate in the breakdown of dopamine, serotonin, and norepinephrine (Gurvits, 2000).

2.13.5 The Dopamine System and its Relation to Suicidal Ideation

This consideration is rooted in the dopamine system's involvement in mood regulation, reward processing, and impulsivity, all of which are relevant to suicide ideation. Central to mood and reward-related processes, dopamine wields a crucial role. Perturbations in dopamine levels or receptor activity have the potential to disrupt emotional equilibrium, thus potentially facilitating the onset of suicidal thoughts. Impulsivity constitutes a cornerstone of suicide ideation. The connection between dopamine's impact on impulsive behavior and potential alterations in dopaminergic pathways suggests a plausible link between dysregulated dopamine systems and impulsive tendencies underlying suicidal ideation (Porter, 2022).

Dopamine, classified as a catecholamine neurotransmitter, is a chemical released by neurons in the brain, with its highest concentration located in the basal ganglia. It holds a significant role as one of the primary components for proper brain function. Its influence extends to both motor and non-motor functions, encompassing a wide array of psychological and physiological processes. These processes include but are not limited to reward, motivation, cognition, emotion, neuroendocrine secretion, as well as feelings of well-being and pleasure. Within the brain, dopaminergic neurons are concentrated in cognitive regions like the substantia nigra (SN), nucleus accumbens (NAc), and dorsal striatum. This leads to the release of dopamine in pathways known as the mesolimbic and mesostriatal pathways. The interplay of various neurotransmitters, such as serotonin and opioids, with dopamine contributes to the sensation of well-being (Nieullon, 2002). Changes in the levels of these neurotransmitters can lead to neuropsychiatric disorders, including thoughts of suicide and other mental illnesses.

Literature reviews indicate a strong correlation between mood disorders and dopamine levels. Reduced dopamine levels are associated with hypoactivity, which can be linked to mood disorders, while elevated dopamine levels are tied to conditions like mania, characterized by feelings of euphoria, delusion, hyperactivity, and increased motivation and focus. Dopamine signaling pathways, which involve proteins like the dopamine transporter (DAT), play a crucial role in regulating the process. These pathways are responsible for the reabsorption of unbound

dopamine molecules into the presynaptic neuron through dopamine transporter proteins (Ashok *et al.*, 2017).

2.13.6 Dopaminergic genetic loci in suicide ideation

In humans, the genes responsible for dopamine receptors (both D1-like and D2-like) are found on separate chromosomes. Specifically, the gene encoding the D1 receptor protein is located on chromosome 5's long arm at position 5q35.1, while the gene responsible for the D5 receptor protein is situated on chromosome 4's short arm at position 4p15.1-16.1 (Tumova, 2003). The D1 receptor protein consists of 446 amino acids, whereas the D5 receptor protein is composed of 477 amino acids. In the realm of D2-like receptors, the gene that encodes the D2 receptor protein is positioned on the long arm of chromosome 11 at location 11q22-23. The D3 receptor protein is the product of a gene located on chromosome 3's long arm at position 3q13.3, and the gene accountable for the D4 receptor protein is also found on chromosome 11's short arm at a specific site (Baldessarini, 2013).

Molecular examination reveals that the gene sequence for D1-like receptors lacks intronic regions; conversely, the genes for D2-like receptors contain varying numbers of introns, resulting in the formation of different variants of D2-like receptor proteins with varying amino acid counts. Specifically, D2-like receptor genes carry 6, 5, and 3 introns for D2, D3, and D4 receptors (Hearn *et al.*, 2002).

2.13.7 Dopamine transporter gene

The human dopamine transporter is alternatively referred to as the dopamine active transporter (DAT-1) or SLC6A3. SLC6A3 is categorized as a protein-coding gene belonging to the solute carrier family of 6 members 3. This gene encodes a presynaptic monoamine transporter protein that relies on sodium and chloride, featuring 12 transmembrane domains. The DAT-1 protein, encoded by the SLC6A3 gene, is positioned on chromosome 5 at the 5p15.3 region, spanning about 64kbp and containing 15 coding exons. Despite being a significant neurotransmitter, dopamine can act as an endogenous neurotoxic substance when its levels are unregulated in both extracellular and intracellular (cytosol) spaces. The dopamine transporter (DAT-1) and vesicular monoamine transporter 2 (VMAT2) are essential in controlling dopamine levels (Rudnick *et al.*, 2014).

DAT-1 is responsible for maintaining dopamine levels by efficiently reabsorbing dopamine from the synaptic cleft back into the cytosol of dopaminergic neurons (presynaptic neurons). This function plays a crucial role in maintaining the equilibrium of dopamine levels in the central nervous system (CNS), while VMAT2 sequesters cytosolic dopamine. When DAT-1 or VMAT2 is dysregulated, the concentration of free dopamine molecules increases, disrupting normal cellular functions. Research indicates that the accumulation of dopamine in the cytosol triggers the generation of reactive oxygen species (ROS), leading to oxidative stress and neurotoxicity. This process also prompts the nonenzymatic conversion of dopamine into quinones, which in turn modify protein structures (Chen et al., 2010). Reduced expression or absence of DAT-1 leads to higher and prolonged dopamine levels in the synaptic cleft, resulting in hyperactivity. Conversely, diminished dopamine levels in presynaptic terminals lead to a decline in tyrosine hydroxylase and the downregulation of dopamine receptor expression, specifically D1 and D2 receptors. On the other hand, an overexpression of the DAT gene leads to excessive reuptake of dopamine back into the presynaptic neuron. Consequently, lower levels of extracellular DAT in the synaptic cleft lead to hypoactive functioning (Klein et al., 2019).

2.13.8 Dopamine receptor genes

Dopamine receptors are members of a larger group of 7-transmembrane G-protein coupled receptors (GPCRs). They are divided into five types - D1, D2, D3, D4, and D5 - based on their composition, roles, biochemical and physiological impacts in both the central nervous system (CNS) and peripheral nervous system (PNS), as well as their interactions with various cell surface receptors found on different neurons. D1 and D5 receptors share a similar structure and trigger the activity of adenylyl cyclase. This leads to an increase in the production of cyclic adenosine monophosphate (cAMP), resulting in comparable drug responsiveness. Due to these similarities, D1 and D5 receptors are grouped together as D1-like receptors. In contrast, D2, D3, and D4 receptors show structural resemblance and are classified as D2-like receptors. The operational characteristics of D2-like receptors differ from those of D1-like receptors; their activation reduces the synthesis of cAMP (Zhuang et al., 2021).

D1-like and D2-like receptors are characterized Distinguishing factors such as distinct binding affinities for various agonists and antagonists, diverse effector responses, and varying

distribution patterns in the central nervous system (CNS) are utilized to classify these receptors. The concentration of these receptors within the human nervous system displays significant variability. Notably, D1 receptors hold the highest prevalence, followed by D2 receptors as the second most abundant (Vallone *et al.*, 2000).

Genetic loci related to dopaminergic pathways hold a pivotal role as specific sites within the genome where variations can exert influence over the expression and functioning of genes implicated in dopamine synthesis, transport, and receptor interaction. Dopamine, a pivotal neurotransmitter, assumes a fundamental role in a multitude of cognitive, emotional, and motivational processes. Polymorphisms within dopaminergic genetic loci have garnered considerable attention due to their potential to impact factors such as dopamine receptor density, the function of dopamine transporters, and downstream signaling. These allelic variations hold the potential to shape dopaminergic-related processes and have been associated with diverse neuropsychiatric conditions (Bossers *et al.*, 2009).

Suicidal ideation is an intricate outcome arising from a fusion of genetic predisposition, environmental influences, and underlying neurobiological mechanisms. Genetic loci within dopaminergic pathways emerge as potential contributors to influencing susceptibility to suicidal thoughts due to their central role in mood regulation, reward mechanisms, and emotional processing (Belsky & IJzendoorn, 2017).

The impact of genetic variations within dopaminergic loci on suicidal ideation may be significantly shaped by interactions between genetic factors and the environment. Stressful life events and psychosocial circumstances can potentially interact with genetic predispositions, thus influencing the emergence of suicidal thoughts (Araya *et al.*, 2009).

Dopaminergic pathways substantially contribute to mood regulation and the processing of rewards. Disruptions in dopamine receptor densities or alterations in transporter functions, stemming from genetic variations, can potentially disrupt emotional equilibrium, thus potentially contributing to the emergence of suicidal ideation. Genetic variations within dopaminergic loci have the potential to disrupt mechanisms related to reward processing. This disruption could potentially lead to anhedonia and reduced motivation, phenomena often observed in individuals with inclinations toward suicidal thoughts (Brummett *et al.*, 2008). At the core of the dopamine system, the dopamine transporter gene (DAT) plays a pivotal role. This gene is responsible for

encoding the dopamine transporter protein, a crucial participant in the reuptake mechanisms that govern dopamine neurotransmission. As a result, it exerts influence over mood, reward processing, and emotional modulation. Within the dopamine transporter gene, genetic polymorphisms can lead to diverse variations in transporter function. These variations, in turn, can impact the rate of dopamine clearance and availability, potentially affecting emotional states (Cartier *et al.*, 2015).

2.13.9 The Dopamine Transporter Gene and its Connection to Suicidal Ideation

Given the dopamine transporter gene's involvement in dopamine reuptake, it becomes a prospective influencer of mood regulation. Genetic variations that influence transporter function have the potential to disrupt the delicate balance of dopamine neurotransmission, potentially contributing to emotional disturbances and the emergence of suicidal ideation. The intricate relationship between genetic variations within the dopamine transporter gene and suicidal ideation may be further modulated by gene-environment interactions. Stressors and environmental factors may intersect with genetic predispositions, potentially influencing the susceptibility to developing suicidal thoughts (Savitz *et al.*, 2006).

Disturbances in dopamine transporter function can potentially affect emotional states and equilibrium. Disruptions in dopamine reuptake may contribute to mood disorders and fluctuations in emotional stability, which are pertinent to the manifestation of suicidal ideation. Influence on Reward Processing and Impulsivity: The dopamine transporter gene's genetic variations might have implications for reward processing and impulsivity, both of which are intricately linked to suicidal ideation. Perturbations in dopamine clearance could potentially perturb the brain's reward circuitry, potentially contributing to impulsive behaviors (Femenia *et al.*, 2012).

The spotlight falls on dopamine receptor genes within the intricate realm of neurotransmission. These genes house the code for dopamine receptors, pivotal players in translating dopamine's effects on mood regulation, reward processing, and emotional modulation. The dance of genetic variations within dopamine receptor genes can lead to diverse structural and functional changes in receptors. These variations have the potential to sway receptor affinity, alter signaling pathways, and ultimately shape dopamine-mediated processes (Ikemoto, 2010). Dopamine receptor genes and mood regulation render them potential influencers of emotional equilibrium. The genetic tapestry woven within these genes might disrupt dopamine's pathways, contributing to mood disorders and potential predisposition to suicidal thoughts.

The relationship between genetic variations within dopamine receptor genes and suicidal ideation is a dynamic one, potentially influenced by the symphony between genetics and the environment. External stressors and contextual elements could orchestrate intricate interactions with genetic predispositions, molding the landscape for the emergence of suicidal thoughts (Garland *et al.*, 2010).

Within the intricate tapestry of neurotransmission, dopamine degrading enzymes occupy a pivotal position. These enzymes, responsible for the breakdown of dopamine, play a critical role in regulating dopamine levels, thereby exerting influence over mood, emotional processing, and reward mechanisms. Genetic polymorphisms within genes coding for dopamine degrading enzymes can lead to variations in enzymatic activity. These variations can impact the rate of dopamine breakdown, potentially affecting emotional states and contributing to neuropsychiatric conditions (Kim, 2023).

Dopamine degrading enzymes and mood modulation positions them as potential influencers of emotional balance. Genetic variations influencing enzymatic activity may disturb the delicate equilibrium of dopamine levels, contributing to mood instability and susceptibility to suicidal thoughts. The relationship between genetic variations within dopamine degrading enzyme genes and suicidal ideation may be further shaped by interactions between genetics and the environment. External stressors and environmental factors may intersect with genetic predispositions, potentially shaping the propensity for developing suicidal thoughts (Steimer, 2022).

2.14 Genetic Implications for Suicide Ideation

- i. **Heritability Studies:** Extensive twin and family studies have provided compelling evidence of a genetic component contributing to suicide ideation (Brent *et al.*, 2002). These studies reveal that Individuals with a familial history of suicidal ideation are at an increased risk, indicating an inherited predisposition (Mann *et al.*, 2009).
- ii. **Candidate Genes:** Potential candidate genes are found to be associated with suicide ideation, encompassing those involved in neurotransmitter regulation, neuroplasticity, and stress response. Variations in these genes may lead to altered neurobiological pathways, heightening vulnerability (Brent & Melhem, 2008). **COMT** (Catechol-O-

Methyltransferase) is responsible for the breakdown of neurotransmitters such as dopamine. Researchers have examined different versions of the COMT gene in connection with impulsive behaviors, which might play a part in behaviors that lead to thoughts of suicide. **TPH2** (Tryptophan Hydroxylase 2) takes part in the creation of serotonin. Variations in the TPH2 gene have been explored due to their potential involvement in mood disorders and impulsive behavior, both of which are linked to the risk of suicide. **MAOA** (Monoamine Oxidase A) contributes to the breakdown of neurotransmitters like serotonin and dopamine. Specific variations within the MAOA gene have been investigated in terms of their association with aggressive behavior and impulsivity, which might contribute to inclinations toward suicide. The **CRH** (Corticotropin-Releasing Hormone) gene has genetic differences that have been linked to disorders related to stress and irregular mood regulation. These factors collectively contribute to the risk of suicide.

- iii. **Twin and Adoption Studies:** Twin and adoption studies have substantiated the heritability of criminal behavior, indicating that genetic factors contribute to variations in an individual's propensity for criminal tendencies. These studies shed light on the extent to which genetics plays a role (McGue & Bouchard, 1998).

2.15 Other signaling pathways.

Cytokines, including IL-6 and TNF α , have a potential role to the elevated risk for suicide ideation. Limited research has explored the connections between cytokine mRNA expression and depression as well as suicidal ideation and behavior (Gananca *et al.*, 2016).

GSK3 exists in two isoforms (α and β) within the brain and is broadly distributed, with its activity being suppressed through phosphorylation (as shown in Figure 3). Lithium directly impedes the catalytic function of GSK3 while simultaneously enhancing its phosphorylation at therapeutic dosages. This impact is orchestrated by several kinases, with Akt being particularly noteworthy. The process through which lithium triggers phosphorylation of GSK3 involves disrupting an Akt/ β -arrestin/protein phosphatase 2A (PP2A) complex (Avrahami *et al.*, 2013).

Research has pointed out anomalies in signaling mechanisms linked to receptors, encompassing pathways like phosphoinositide and adenylyl cyclase. Other disturbed biological systems associated with suicide include the hypothalamic-pituitary-adrenal axis, neurotrophins,

and neurotrophin receptors. Recent investigations have also spotlighted irregularities in neuroimmune functions associated with suicide (Pandey, 2013).

LH (learned helplessness) procedure serves as a diathesis, followed by SDS (social defeat stress) to induce various endophenotypes linked to suicide, like hopelessness, irritability, impulsivity, aggression, and anhedonia. Additionally, proteomic analyses indicate that the key pathways mediating suicide-related endophenotypes involve PKA and GABA receptor pathways (Teng *et al.*, 2022).

Neurotrophic factors attaching to Trk receptors initiate critical signaling routes, encompassing phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and phospholipase C- γ (PLC- γ), which hold utmost importance in ensuring proper synaptic performance and cellular viability. When NGF binds with p75NTR, it could trigger nuclear factor kappa B (NF- κ B) or c-Jun N-terminal kinase, prompting apoptosis and neurodegeneration due to excessive activation. Achieving equilibrium among these pathways via accurate receptor interaction is indispensable for effective synaptic operation and the management of stress. Reduced BDNF expression is associated with the genesis of depression, and the administration of antidepressants might enhance the production of BDNF. Broadly speaking, modifications in the BDNF signaling pathway constitute significant biological risk factors in the origin and advancement of suicidal behaviors (Mehterov *et al.*, 2022).

2.15.1 Role of distal factors in suicidal behavior

Highlighted by post-mortem analyses, alterations in the expression of specific biomarkers within brain tissue from individuals with Major Depressive Disorder (MDD) are evident. For instance, changes in the levels of GFAP (glial fibrillary acidic protein), GS (glutamine synthetase), and glial-specific excitatory amino-acid transporters (EAATs) have been documented among those with MDD. These modifications indicate a possible dysfunction in the activity of glial cells (Wang *et al.*, 2017).

The accumulation of glutamate can trigger the activation of N-methyl-D-aspartate (NMDA) receptors, resulting in the suppression of the BDNF (brain-derived neurotrophic factor) pathway. This suppression can have implications for the regulation of mood and the processing of emotions, contributing to the underlying mechanisms of depression and potentially even behaviors linked to

suicide (Autry & Monteggia, 2012). Studies have also revealed a decrease in the expression of GFAP mRNA and protein, along with a reduced density of GFAP-immunoreactive (IR) astrocytes within the locus coeruleus (LC) in individuals diagnosed with MDD.

This finding suggests a potential disruption in astrocyte function within this brain region, which is implicated in stress responses and emotional regulation. Additionally, different astrocyte-associated genes have been found to be dysregulated in MDD within astrocytes that were isolated from the LC (Nagy, 2017). These findings highlight the intricate relationship between glial cells, neurotransmitter systems like glutamate, and neurotrophic factors like BDNF in the context of mood disorders and suicidal behavior. Distal factors such as these can contribute to the overall susceptibility to suicidal tendencies, acting in conjunction with other genetic, environmental, and psychological factors (Lutz *et al.*, 2017)

Material and Methods

The objective of the research is to investigate the predisposition of serotonin and dopamine transporter genes among jail inmates with suicide ideation. To achieve this objective, the IPLEX genotyping technique (Agena Biosciences) was employed. This advanced method allows for the analysis of genetic variations in these transporter genes by identifying specific genetic markers that might be associated with suicide ideation. The IPLEX approach offers a cutting-edge solution to scrutinize the genetic landscape and uncover potential associations with mental health outcomes including suicidal thoughts.

3.1 Ethical Approval

Prior to enrolling in the study, each prospective participant received a comprehensive explanation of the research's purpose, methodologies, and potential risks. This was a pivotal aspect of the process, as it empowered participants to make informed decisions. Written informed consent was obtained from everyone, signifying their willingness to engage in the study. This consent process was conducted to adhere to ethical standards.

To facilitate a clear understanding among participants, the information and consent forms were made available in Urdu, catering to those who might encounter difficulties comprehending English. A range of options were presented to participants, allowing them to express their consent through means such as signing their name or providing a thumb impression. The questionnaire, along with the research protocol, underwent a rigorous review by the Ethical Review Committee (ERC) of the International Islamic University. This committee's approval underscored the adherence to ethical guidelines and the study's alignment with responsible research practices.

3.2 Participant Selection and Enrollment

3.2.1 Study subjects

The study population for this research comprised of male and female prisoners from six (06) jails in Punjab province of Pakistan namely Central Jail Gujranwala, District Jail Lahore, District Jail Faisalabad, District Jail Gujrat, District Jail Sargodha, and District Jail Shahpur. The recruitment of these participants adhered to a meticulous procedure, ensuring their voluntary

participation and informed consent.

The questionnaire itself encompassed a diverse range of information, crucial for comprehending the socio-demographic and environmental factors influencing the study population. Socio-demographic details, such as age, educational background, occupation, and marital status of the prisoners, were recorded. Additionally, factors pertaining to the prisoners' parental marital history, socioeconomic status, familial and personal history of suicide, and the type of prisoners they were categorized as, were captured through specially designed questionnaire (Annex I).

Recognizing the importance of environmental factors in shaping mental health, the questionnaire examined areas such as quality of life, hobbies, and experiences of domestic violence, societal neglect, and parental relations. These facets were explored in the context of their contribution to psychiatric conditions, including depression and aggression. Moreover, the questionnaire facilitated the collection of data on childhood history of depression, aggressive tendencies, and other pertinent activities that could have influenced the participants' mental well-being.

3.2.2 Methodology for Evaluating Suicidal Ideation Phenotype

To gauge the status of suicidal ideation within the study participants, the "Beck Inventory Scale for Suicide Ideation," originally developed by Beck and colleagues in 1996, was employed as a structured assessment tool. This scale has undergone certain modifications for the purposes of this study, as outlined in Annexure II.

The "Beck Inventory Scale for Suicide Ideation" serves as a standardized and well-validated instrument designed to quantify the degree of suicidal ideation experienced by individuals. Suicidal ideation encompasses thoughts, contemplations, or considerations related to the act of suicide. Within the context of this research, the scale was utilized to systematically assess and quantify the extent of participants' suicidal thoughts.

Each study participant was presented with a series of questions strategically crafted to discern the presence and intensity of their suicidal ideation. These questions were formulated based on established psychological principles and informed by the existing body of research in the realm of suicide ideation assessment. This approach ensured that the questions were both sensitive and

pertinent to capturing the traces of suicidal thoughts.

Participants were instructed to respond to these questions, allowing the calculation of a suicide ideation score for everyone. This score serves as a quantitative representation of the severity or intensity of their suicidal ideation. Higher scores on the scale indicate a more pronounced level of suicidal ideation, whereas lower scores indicate a comparatively diminished degree of such thoughts.

The incorporation of the Beck Inventory Scale into the research design bolsters the methodological rigor of the study, as it enables a comprehensive analysis of the intricate domain of suicide ideation. The utilization of a standardized assessment tool like this scale holds several benefits. It fosters uniformity and consistency in data collection, ensuring that responses are comparable across participants. This, in turn, facilitates the meaningful interpretation of findings and enhances the reliability of the study's conclusions.

Participants were presented with the scale's statements and instructed to mark the appropriate circle on the rating scale. The scale statements were assigned numerical values ranging from 0 to 4, corresponding to the categories: not at all, not, somehow, moderately, much, and very much, respectively. These scores were recorded, culminating in the computation of a total score. Additionally, specific score levels were designated to signify the clear absence, absence, somewhat presence, presence, and severe presence of suicidal thoughts.

By implementing the Beck Inventory Scale for Suicide Ideation, this study ensures a systematic and rigorous approach to evaluating participants' suicidal ideation.

3.2.3 Data Collection Process

The present research investigation encompassed a comprehensive cohort of participants, consisting of a total of 694 prisoners and 422 individuals serving as controls. The participants were recruited to ensure that a representative sample was obtained for the study's objectives. The participants were divided into two distinct groups: prisoners and controls, allowing for a comparative analysis to be conducted. The data of controls was also collected after obtaining informed consent and explanation of the purpose of the research.

3.2.4 Collection of blood sample

The blood samples of the prisoners and controls were collected for the purpose of this study. First the tourniquet was applied on the arm, and then the alcohol swab was used to clean the venipuncture site, so that proper hygiene should be obtained. 5ml blood samples were collected from each participant. To ensure both the integrity of the samples and the comfort of the participants, these blood draws were carried out by skilled phlebotomists. The blood sample was collected from antecubital vein. The blood samples were carefully transferred into specialized vials that contained ethylenediaminetetraacetic acid (EDTA), a well-established anticoagulant. The presence of EDTA served a crucial role in preventing any unwanted clotting or coagulation of the blood, ensuring that the samples remained suitable for subsequent analyses. To facilitate thorough mixing, the vials containing the blood samples were gently placed on rolling devices, allowing for consistent and uniform distribution of the anticoagulant throughout the blood.

After the mixing process, the EDTA vials containing the blood samples were maintained at a temperature of 4°C to ensure that the samples remained viable for both complete blood count (CBC) analyses and the subsequent DNA extraction process.

3.2.5 Preparation of Genomic DNA Samples

The process of preparing genomic DNA from the reserved leukocytes within EDTA vacutainers was carried out using a modified version of the traditional phenol-chloroform method (MWER *et al.* 1988). This DNA extraction technique, based on the phenol-chloroform method, was employed to isolate the genetic material from the cellular components, thus enabling subsequent analyses. The following protocol outlines the steps undertaken for DNA extraction from the subject samples:

1. **Sample Collection and Preparation:** The procedure commenced with the collection of leukocytes, a type of white blood cells, which were reserved in vacutainers containing EDTA, an anticoagulant. The EDTA served to prevent coagulation and maintain the integrity of the blood sample.
2. **Phenol-Chloroform Extraction:** The primary technique utilized for DNA extraction was the phenol-chloroform method. This method capitalizes on the distinct properties of phenol and chloroform to selectively partition DNA from cellular and proteinaceous components.

After the DNA extraction process, the mixture containing DNA in the aqueous phase is separated from the organic phase through centrifugation. This separation step helps to isolate the DNA for further analysis.

3. **Sample Lysis and Precipitation:** The process involved breaking down cellular membranes and releasing DNA from the cellular environment. Phenol and chloroform were employed to facilitate the separation of the DNA from cellular debris. The DNA was then precipitated from the solution using alcohol, which causes the DNA to aggregate.
4. **Final DNA Product:** The culmination of this process resulted in the isolation of genomic DNA from the leukocytes. This genomic DNA served as the foundation for subsequent analyses, such as genotyping, sequencing, or other molecular investigations.

3.2.5.1 Extraction Using Phenol-Chloroform Technique

3.2.5.2 Day 1

To prepare blood samples for downstream applications, a 2ml Eppendorf tube played a central role. To initiate this procedure, 400µl of the blood sample was combined with a larger volume of TE buffer, specifically 1200µl. This TE buffer, recognized as a washing buffer, was chosen to be three times the volume of the blood sample. This precise ratio ensured optimal washing conditions.

The Eppendorf tubes containing the blood-TE buffer mixture underwent an initial incubation at room temperature. This step served to facilitate the initial stages of the process. Subsequent to this incubation, centrifugation was carried out at a speed of 13,000 revolutions per minute (rpm), employing a temperature of 4°C for a duration of 10 minutes. This centrifugation maneuver effectively separated the components of the mixture, leading to the formation of a distinct pellet at the bottom of the tube. The supernatant, or the liquid portion above the pellet, was carefully discarded at this point.

To further process the pellet, a method known as pulse vortexing was applied. This technique involved subjecting the pellet to short and controlled bursts of vortexing, ensuring the pellet's structure was disrupted and effectively broken. This step was crucial for the subsequent washing phase.

The washing process was performed with diligence, involving the repeated addition of TE buffer, incubation, and centrifugation cycles. This washing step was iterated 2 to 3 times until the pellet achieved an off-white or pale pink coloration. The shift in color was indicative of successful washing, suggesting that contaminants and extraneous materials had been effectively removed from the pellet.

Following the completion of the washing phase, a series of additional components were introduced to the Eppendorf tubes. Specifically, 20µl of Sodium Dodecyl Sulfate (SDS) was added at a concentration of 5 µl for every 100µl of the initial blood sample. Alongside this addition, 300µl of a solution containing 3M sodium acetate, equivalent to 75µl for every 100µl of the blood sample, was introduced. 10µl of proteinase K (at a concentration of 10mg/ml) was also used to facilitate enzymatic degradation of proteins. These components, when combined, worked harmoniously to disrupt cellular structures and break down proteins.

With these additions in place, the Eppendorf tubes underwent thorough mixing through a short spin, followed by an incubation period. This incubation was executed at a temperature of 55°C, utilizing a shaking water bath for enhanced and consistent mixing. This extended incubation allowed for the enzymatic reactions to proceed, effectively breaking down cellular components and liberating the DNA.

3.2.5.3 Day 2

Eppendorf tubes were carefully removed from the water bath and subjected to a thorough mixing process. An equivalent volume of a mixture containing phenol and chloroform-isoamyl alcohol was added to each tube, with the ratio being 300 µl of phenol combined with 300 µl of chloroform-isoamyl alcohol for every 400 µl of blood sample. These components were mixed vigorously for duration of 10 minutes. The ensuing mixture underwent centrifugation at a speed of 13000 revolutions per minute (rpm) for duration of 25-30 minutes. Post-centrifugation, the upper aqueous layer, containing the desired DNA, was cautiously transferred into new Eppendorf tubes, which were appropriately labeled. The transfer was facilitated by employing cut-tips.

In these freshly labeled tubes housing the aqueous layer, an equivalent volume of chilled isopropanol, or alternatively, twice the volume of chilled absolute alcohol, was introduced. This addition caused the DNA to precipitate, resulting in the appearance of distinct white DNA threads.

Subsequently, the tubes were incubated at an ultra-low temperature of -80°C for a period of 30 minutes. Following this incubation, another round of centrifugation at 13000 rpm, lasting 15-20 minutes, was conducted, leading to the removal of the supernatant.

To washing and further purification, 400-500 μl of 70% ethanol was added to the DNA pellet, which was gently mixed to dislodge the pellet. Subsequent centrifugation at 13000 rpm for 10-15 minutes effectively separated the ethanol and supernatant from the DNA pellet. This step was crucial in eliminating residual contaminants from the DNA sample. To finalize the purification process, the DNA pellet was thoroughly dried. The Eppendorf tubes were placed at an inclined position, allowing the DNA pellet to dry overnight at a temperature of either 37°C or room temperature. The utmost care was taken to ensure complete dryness of the pellet.

Following the drying process, a low TE buffer, measuring 60 μl , was added to the Eppendorf tubes, aligning with the position where DNA precipitation occurred. This addition was gently but meticulously mixed into the tube's contents. To neutralize any remaining DNases, a heat shock mechanism was employed at a temperature of 55°C for a period of 30 minutes. This step effectively inactivated any residual enzymes that could potentially degrade the DNA. The samples were then allowed to incubate at a temperature of 4°C for a continuous duration of 24 hours. This prolonged incubation period ensured thorough and consistent DNA mixing within the sample. Prior to transferring the samples to a storage temperature of -80°C , a quantitative assessment of the DNA was conducted using the NanoDrop technique.

3.2.5.4 Assessment of Genomic DNA Preparations: Quantitative Analysis

3.2.5.4.1 Measurement of Genomic DNA Concentration Using Nano Drop

To quantify DNA, the Nano Drop 2000c spectrophotometer (manufactured by Thermo Scientific Nano Drop Products) was employed as a vital analytical tool. This quantification process adhered to the instructions outlined in the user manual, ensuring accuracy and consistency in the results obtained.

The quantification procedure commenced by thoroughly cleaning the upper and lower optical surfaces of the spectrophotometer. This cleaning step involved pipetting 4-5 μl of clean deionized water onto the lower optical surface. The lever arm was then securely closed, causing

the upper pedestal to encounter the deionized water. Subsequently, the lever arm was lifted, and both optical surfaces were meticulously wiped clean using a lint-free wipe. This meticulous cleaning process ensured that the optical surfaces were devoid of any potential contaminants that could interfere with the subsequent measurements. The Nano Drop software, designed to interface with the spectrophotometer, was initiated for the quantification process. Within this software, the "Nucleic Acid" application was selected, tailoring the system's settings for the specific requirements of quantifying DNA samples.

To establish a baseline reading, a "blank" measurement was conducted. This involved dispensing 1µl of TE buffer (the buffer initially used for DNA suspension) onto the lower optical surface of the instrument. This buffer serves as a reference for the instrument's readings. After this step, the "Blank" setting was selected within the Nucleic Acid application. Once the blank measurement was complete, both optical surfaces were again carefully cleaned with a lint-free wipe. Next, a 1µl sample of the nucleic acid to be quantified was deposited onto the lower optical surface. The lever arm was closed, and the "Measure" option was selected within the application software.

The spectrophotometer's software then automatically processed the data, generating calculated values for the nucleic acid concentration and its corresponding purity ratios. The analysis was based on the principles of absorbance at specific wavelengths.

It's important to note that only nucleic acid samples exhibiting a 260/280 ratio ranging from 1.8 to 2.0 were deemed suitable for further analysis. This ratio range is indicative of the purity of the DNA sample, suggesting minimal contamination by substances such as phenol or protein. In instances where the samples fell outside this specified range, it was concluded that the samples might be compromised and contaminated. Consequently, these samples were excluded from the analysis and were subject to further optimization or reprocessing.

3.3 SNP Selection and Assay Design Strategy

Two key genetic variations, or common Single Nucleotide Polymorphisms (SNPs), originating from significant genes, were deliberately chosen to feature in this study. These genetic variations hail from two prominent gene systems: the dopaminergic gene system and Tryptophan hydroxylase 2 (TPH2). The selection of these specific SNPs was based on a thorough exploration of data derived from a multitude of literature databases, constituting a comprehensive and

systematic approach.

This selection process included a comprehensive review of the dbSNP database, a renowned and widely used repository of genetic variations (Sherry *et al.*, 2001). Within this database, the criteria for inclusion involved the identification of SNP variants that exhibited a minor allele frequency (MAF) of at least 5%. This stringent MAF threshold ensures that the selected SNPs are sufficiently common in the population to warrant meaningful investigation.

The guiding principle behind this selection strategy was to pinpoint SNPs that held substantial relevance in the context of depression research. These chosen SNPs had been previously highlighted and discussed in various studies due to their potential association with depression. By leveraging the collective insights of the scientific community as expressed in published research, the study aimed to concentrate on genetic variations that had demonstrated a plausible link to depression.

Through this process two genetic polymorphisms were identified and isolated that fulfilled these stringent criteria. These SNPs, originating from genes implicated in the dopaminergic pathway and Tryptophan hydroxylase 2 (TPH2), were strategically chosen as the focal points of the investigation.

3.4 Primers' Design and Validation

The primers employed in this research have been illustrated in table 3.3. The sequences of these primers, which play a pivotal role in the study, underwent a rigorous validation process to ensure their accuracy and suitability for the experimental procedures.

Table 3.1 Primer Sequences Employed in the Current Investigation

Sr. #	PRIMER	PRIMER SEQUENCE	Length(bp)	References
1	TPH2-rs7305115-IF-A	5' ATGGCTCAGATCCCCCTCTACACCACA 3'	26	Self-designed
2	TPH2-rs7305115-IR-G	5' GGGCTTTAATGTAGGTACTCACGGTGCC 3'	28	
3	TPH2-rs7305115 OF	5' CTGGATACCTGAGCCACGAGACTTTC 3'	27	
4	TPH2-rs7305115 OR	5' AGGAGTCTGATCCTTCAGTGAGCCCTTT 3'	28	
5	DRD2 rs1800497 F	5' CCGTCGACGGCTGGCCAAGTTGTCTA 3'	26	(Chiang <i>et al.</i> , 2020)
6	DRD2 rs1800497 R	5' CCGTCGACCCTTCCTGAGTGTCATCA 3'	26	
7	DRD2-rs1799978 F	5' CAACCATATCTGTAATGGCTGATCC 3'	25	(X. c. Zhang <i>et al.</i> , 2019)
8	DRD2-rs1799978 R	5' CTTCTAAGTGGCGAGGAGGCTAC 3'	23	

9	HTR2C-rs6318 F	5' GGGCTCACAGAAATATATCAC 3'	21	(Myakishev <i>et al.</i> , 2001)
10	HTR2C-rs6318 R	5' TGCACCTAATTGGCCTATTGGTTT 3'	24	
11	TPH1-rs1799913 F	5' ATTGGATTTCGATTGATTG 3'	20	(Zaboli <i>et al.</i> , 2006)
12	TPH1-rs1799913 R	5' GGCAAACTAGGTTTCAGC 3'	18	
13	TPHI-rs1800532 F	5' GTTTTCCATCCGTCCTGTG 3'	20	(Alam <i>et al.</i> , 2021)
14	TPHI-rs1800532 R	5' CTGTTTCCCCACTGGAATA 3'	20	
15	COMT-rs4680 F	5' TCGTGGACGCCGTGATTCAGG 3'	21	(He <i>et al.</i> , 2020)
16	COMT-rs4680 R	5' AGGTCTGACAACGGGTCAGGC 3'	21	
17	COMTrs6269 F	5' CAACAGCCTGAGTCCGTGTC 3'	20	(Qian <i>et al.</i> , 2017)
18	COMTrs6269 R	5' TCCAGCCGATAAGGCACAGG 3'	20	
19	SLC6A3rs6347 F	5' TTCATCATCTACCCGGAAGCC 3'	21	(Pinsonneault <i>et al.</i> , 2011)
20	SLC6A3rs6347 R	5' GAAGAAGACCACGGCCAG 3'	19	
21	BDNFr6265 F	5' CCTACAGTTCCACCAGGTGAGAAGAGTG 3'	28	(Sheikh <i>et al.</i> , 2010)
22	BDNFr6265 R	5' TCATGGACATGTTTGCAGCATCTAGGTA 3'	28	
23	BDNFr12273363 F	5' GGAAATCTCGGGAAATAGGC 3'	20	(Hing <i>et al.</i> , 2012)
24	BDNFr12273363 R	5' GACCCATCTCAGGTCTCCAG 3'	20	
25	CREB1rs2253206 F	5' GTGCTGTTGCTAGGGAGAGG 3'	20	(Wang <i>et al.</i> , 2023)
26	CREB1rs2253206 R	5' GGCATTTACACATGCCCTTC 3'	20	

The validation of these primer sequences was conducted through the utilization of an in-silico PCR tool, available within the framework of the UCSC genome browser (<https://genome.ucsc.edu/>). This tool operates within a virtual environment, simulating PCR reactions based on the provided primer sequences and a reference genome virtually assessing the specificity and efficiency of the primers in terms of their potential to amplify the target DNA sequences within the genome.

This in-silico PCR process represents an important quality control step, aimed at confirming that the selected primer sequences are indeed aligned with the intended target regions of the genome. The tool's simulation capabilities allow for the prediction of potential amplification outcomes, shedding light on any potential non-specific binding or off-target effects that could interfere with the experimental accuracy.

Incorporating this validation step adds a layer of reliability to the research by ensuring that the primers' sequences are consistent with the desired genomic regions. This approach ultimately contributes to the overall credibility of the experimental outcomes and supports the accuracy of the subsequent PCR-based procedures that rely on these primers.

3.4.1 Preparation of Primer Stock and Working Solutions

Upon their reception, each of the primers underwent a careful preparation process, characterized by attention to detail and preservation of their integrity. This process encompassed several strategic steps that ensured the optimal condition and functionality of the primers. Initially, to facilitate the manipulation of the primers, they were gently centrifuged at a low speed. This delicate spinning action was employed to dislodge any residual material that might have accumulated on the surface of the primer vials or caps. This step aimed to ensure that the primers were thoroughly ready for further handling.

To establish a master stock solution of the primers, with a concentration of 100 μM , a formula known as the FGRS formula was applied. This formula integrated the quantity of lyophilized primer (expressed as moles) with an appropriate volume of molecular grade H_2O (expressed as μl). The resulting master stock, now containing the primers at the desired concentration, was prepared for subsequent use.

For the purpose of resuspending and diluting the primers, a 1X TE buffer (composed of Tris-HCl at pH 8.0) was selected as the solvent. The TE buffer served as an ideal medium, offering the necessary pH and ionic conditions to maintain the stability and functionality of the primers. Once the primers were mixed with the TE buffer, this primer-suspended solution was allowed to stand at room temperature for a period of 10 minutes. This step, termed incubation, facilitated the complete dissolution and uniform distribution of the primers within the TE buffer.

Subsequently, to create working solutions of the primers, dilutions were performed. A 1:10 dilution ratio was chosen, ensuring that the working primers were appropriately diluted for subsequent experimental applications. This dilution step played a crucial role in guaranteeing that the primers were utilized in the optimal concentration range for PCR reactions. To maintain the longevity and functionality of the primers, a precautionary approach was taken to avoid repeated freeze-thaw cycles. To achieve this, the primers were subdivided into smaller aliquots, each containing the necessary quantity for a specific number of experiments. These aliquots were then carefully stored at a temperature of -20°C , which prevented unnecessary exposure to fluctuating temperatures and freeze-thaw cycles that could compromise the primer's integrity over time.

3.4.2 PCR Amplification

The process of amplifying the desired PCR product was executed with precision, utilizing appropriate measures and specialized equipment. To facilitate this amplification, PCR strips with 8 wells and attached bubble caps were employed. These PCR strips, procured from Biologix in the USA, were specifically designed to accommodate the PCR reactions with optimal efficiency and reliability. Each of these PCR strips, with a total volume capacity of 0.02 ml, was utilized for the purpose of containing the reaction mixture. The reaction mixture, consisting of essential components required for the PCR process, was carefully prepared and standardized to a volume of 15 µl for each well.

The core component of the reaction mixture was the DreamTaq Green 2X PCR Master Mix sourced from Thermo Scientific in the USA (Catalog No. K1082). This Master Mix was chosen due to its compatibility with the study's requirements. The composition of this Master Mix is detailed in table 3.2, and its constituents were carefully thawed on ice just prior to their utilization. This precautionary step ensured that the components were in optimal condition for the PCR process.

Table 3.2: Constituents of Dream Taq Green PCR Master Mix (2X) (ThermoScientific, USA (#K1082))

Dream Taq Green PCR Master Mix 2X	
Ingredient	Concentration
DreamTaq DNA polymerase	0.05 U* / µL
Dream Taq Green buffer	2X
MgCl ₂	4Mm
dATP, dCTP, dGTP, dTTP	4mM each

*One unit of Taq polymerase is defined as the amount of enzyme incorporate 10nmol of dNTPs into acid-precipitated material in 30 minutes at +65°C.

Table 3.3: PCR mix preparation

	Volume (µl) for one reaction	Final Concentration
Master Mix	1.84	2x
Forward Primer (25µM)	0.08	0.2µM

Reverse Primer (25µM)	0.08	0.2µM
Water	7	-
DNA	1	-
Total	10	

Throughout the experimental process, stringent quality control measures were adhered. For the PCR reactions, plastic consumables used were of the highest quality, and they were certified to be devoid of any contaminants that could potentially interfere with the PCR reaction. This included ensuring that these consumables were free from DNase, RNase, and Human DNA. Such meticulous attention to the quality of consumables guaranteed the integrity of the experimental outcomes. The actual PCR reactions were performed utilizing an ATC201 thermal cycler, a specialized instrument designed to precisely control temperature conditions throughout the PCR process. This thermal cycler, manufactured by Nyx Technik in the USA, played a pivotal role in ensuring that the necessary temperature cycles for denaturation, annealing, and extension were executed with accuracy and consistency.

3.5 Amplification of Polymorphic Marker within DAT-1 Gene

The study of genotyping involved the analysis of a 40bp Variable Number Tandem Repeat (VNTR) polymorphism within the 3' untranslated region of the DAT-1 gene, building upon a methodology previously established by Vandenberg and colleagues (Vandenberg *et al.*, 1992), with certain adaptations to suit the current study. This approach involved a careful sequence of steps to ensure accurate and reliable results.

To initiate the genotyping process, a quantity of 100 ng of genomic DNA was selected for amplification. This DNA sample was subjected to PCR (Polymerase Chain Reaction) amplification using a pair of primers, namely DAT-1-F and DAT-1-R as mentioned in table 3.5, each present at a concentration of 100 pmol. The entire reaction was prepared within a volume of 15 µl. This mixture incorporated the genomic DNA, the two primers, and necessary reaction components. Optimal PCR conditions, specific to this study, were determined in the research laboratory and are comprehensively detailed in table 3.4. These conditions were carefully fine-tuned to ensure efficient and specific amplification of the target DNA sequence.

Table 3.4: PCR Conditions for Amplification of DAT-1 DNA Fragment

Thermal cycling profile <i>DAT-1</i> polymorphism	
Step 1 (Lid temperature was at 95°C)	
Initial denaturation at 95°C	3 minutes
Steps 2 (35 cycles)	
Denaturation at 94°C	35 seconds
Annealing at 56°C	45 seconds
Extension at 72°C	40 seconds
Step 3	
Final extension at 72°C	5 minutes
Step 4 (Held at 4°C till samples were removed from thermal cycler)	

3.6 Genotyping of SNPs within TPH System Genes

3.6.1 Genotyping of TPH2 (rs73051115, G/A) Variant

The amplification process targeting the TPH2 (G/A) variant was executed utilizing a tetra-primer ARMS PCR-based methodology, as elaborated by Nazree and colleagues in their work (Ye *et al.*, 2001).

**Figure 3.1** The gel image with normal sample and heterozygous sample

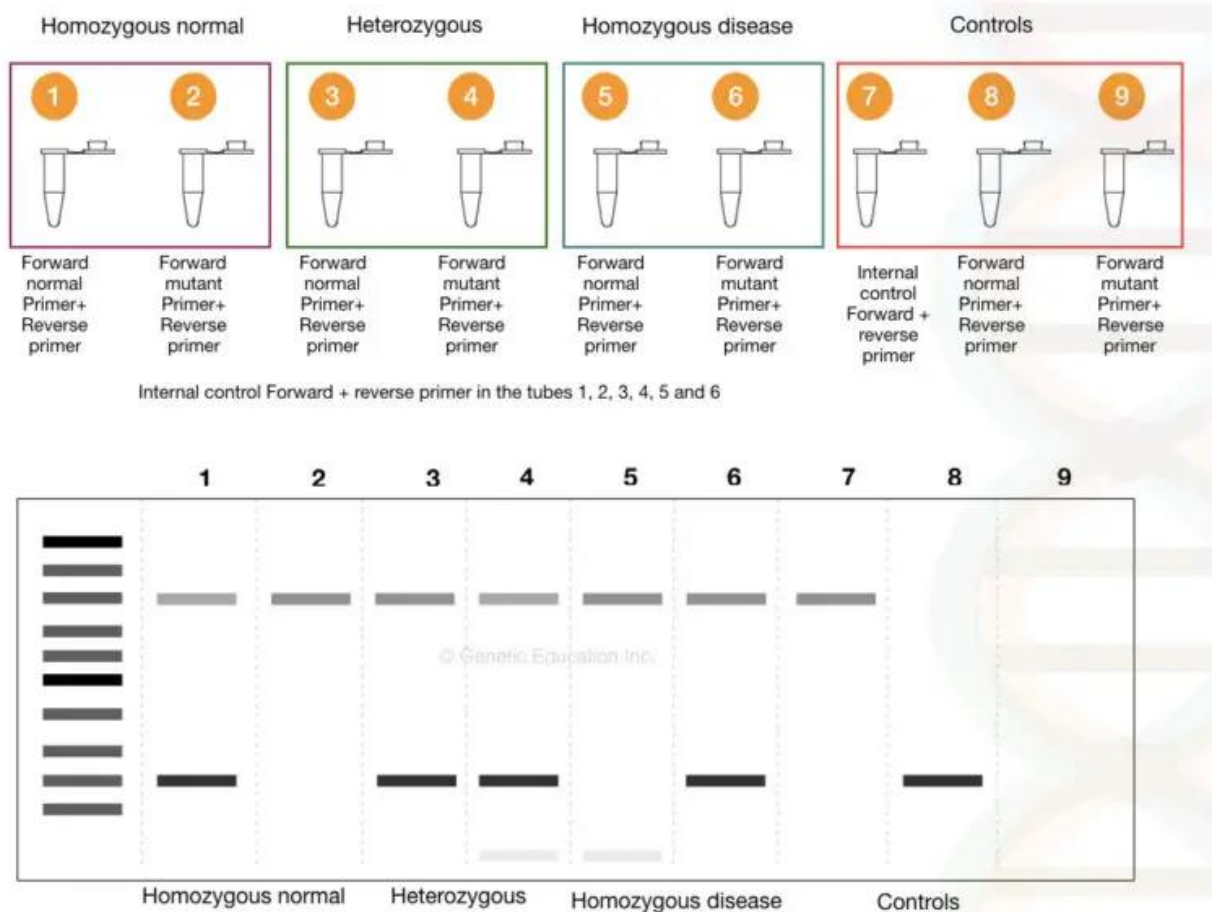


Figure 3.2 The whole process of ARMS-PCR, reaction preparation and results analysis. Image from <https://geneticeducation.co.in/arms-or-allele-specific-pcr-principle-procedure-protocol-applications-and-limitations/>

This innovative approach involved the utilization of two sets of self-designed primers: two allele-specific primers and two common primers. These primers were named TPH2-rs7305115-IF-A, rs7305115-IR-G, rs7305115-OF, and rs7305115-OR. A comprehensive depiction of the employed primers can be found in table 3.3. Each of these primers, with a concentration of 100 pmol, was combined with 100 ng of genomic DNA. This mixture was incorporated into a total reaction volume of 15 μ l, supplemented with 2X Master Mix (Dream Taq, ThermoFisher) to facilitate the PCR amplification process. The specific procedural steps involved in the amplification are illustrated in table 3.5.

Table 3.5: PCR Steps for Amplification of TPH2 Polymorphism

Thermal cycling profile <i>TPH1</i> polymorphism	
Step 1 (Lid temperature was at 95°C)	
Initial denaturation at 95°C	3 minutes
Steps 2 (35 cycles)	
Denaturation at 94°C	35 seconds
Annealing at 62°C	40 seconds
Extension at 72°C	30 seconds
Step 3	
Final extension at 72°C	5 minutes
Step 4	
(Held at 4°C till samples were removed from thermal cycler)	

3.6.2 Genotyping of Missense Variants (rs4680 G/T, rs6269 Val/Meth) within COMT Gene

The genotyping process for the specific genetic variant under investigation was conducted in accordance with a previously established protocol (Lavigne *et al.*, 1997). To carry out this genotyping, a pair of primers, namely COMT-F and COMT-R as indicated in table 3.3, were employed. The experiment was conducted using a total reaction volume of 25 µl. This reaction mixture comprised 200 ng of genomic DNA, 100 pmol of each of the two primers, and 2X PCR Master Mix (Dream Taq, Thermoscientific, USA), all meticulously combined within 200 µl capped strips PCR tubes.

The specific conditions for the PCR amplification were determined as illustrated in table 3.6.

Table 3.6 PCR Steps for Amplification of COMT gene Polymorphism

Thermal cycling profile <i>COMT</i> polymorphism	
Step 1 (Lid temperature was at 95°C)	
Initial denaturation at 95°C	3 minutes
Steps 2 (35 cycles)	
Denaturation at 94°C	35 seconds
Annealing at 62°C	40 seconds

Extension at 72°C	30 seconds
Step 3	
Final extension at 72°C	5 minutes
Step 4 (Held at 4°C till samples were removed from thermal cycler)	

3.6.3 Genotyping of (rs1801412/T SNP) HTR2C Gene Polymorphism

To investigate this specific polymorphism, DNA amplification was conducted using a primer set (5-HTR-F and 5-HTR-R) created for this purpose, as outlined in table 3.1. For each sample, two primers were used at a concentration of 100 pmol, and 200 ng of genomic DNA was combined with 2x master mix (Green Taq, Thermoscientific, USA). The PCR amplification was executed within a total reaction volume of 50 µl, utilizing 200 µl capped strips tubes.

Table 3.7: PCR Steps for Amplification of HTR2C gene Polymorphism

Thermal cycling profile HTR2C polymorphism	
Step 1 (Lid temperature was at 95°C)	
Initial denaturation at 95°C	3 minutes
Steps 2 (35 cycles)	
Denaturation at 94°C	35 seconds
Annealing at 62°C	35 seconds
Extension at 72°C	30 seconds
Step 3	
Final extension at 72°C	5 minutes
Step 4 (Held at 4°C till samples were removed from thermal cycler)	

3.6.4 Genotyping of CREB1 Gene Polymorphisms (rs2253206/rs7594560)

The examination of CREB1 in its upstream region was conducted using the methodology outlined by Sabol et al. (1998). The process involved PCR amplification employing specific primers. Each reaction consisted of 200 µg of DNA mixed with 2X mix (Green Taq, Thermoscientific) in 200 µl reaction PCR tubes, resulting in a final reaction volume of 25 µl.

Table 3.8: PCR Steps for Amplification of CREB1 gene Polymorphism

Thermal cycling profile <i>CREB1</i> polymorphism	
Step 1 (Lid temperature was at 95°C)	
Initial denaturation at 95°C	3 minutes
Steps 2 (35 cycles)	
Denaturation at 94°C	35 seconds
Annealing at 62°C	30 seconds
Extension at 72°C	40 seconds
Step 3	
Final extension at 72°C	5 minutes
Step 4 (Held at 4°C till samples were removed from thermal cycler)	

3.7 POST PCR: SAP Reaction

Unincorporated dNTPs were dephosphorylated by shrimp alkaline phosphatase (SAP), which cleaved the phosphate groups from the five termini. To eliminate any remaining nonincorporated dNTPs from amplification products, SAP treatment was applied. This process was carried out using a post-PCR 96-tip SpectroPREP Multimek robot. Two microliters of a SAP cocktail were dispensed by the Multimek into every well of the 384-well post-PCR reaction plate. The plate was taken out of the robot and centrifuged once the SAP cocktail had been added. To inactivate the SAP enzyme, treated plates were first placed in an incubator set at 37°C for 50 minutes, and then they were immediately placed in an incubator set at 85°C for an additional 20 minutes. An alternative was to use a 384-well block thermal cycler for incubation.

The following components were used:

- Sequenom's 1x SAP buffer
- 1.7 U/μl SAP (sequenom; stored at 0°C)
- Shrimp alkaline phosphatase
- 384-well PCR plates containing PCR products
- 1.5 ml microcentrifuge tubes or conical polypropylene centrifuge tubes

- ABGene plates with 96 wells (AB-0800)
- Beckman-Coulter 96-tip post-PCR Multimek robotic pipettor with stacker
- Plate sealers with adhesive
- MicroAmp (Applied Biosystems)
- Post-PCR tabletop centrifuge with microtiter plate carriers
- ABI or Hybaid heat cycler with 384-well blocks
- Precision incubators (VWR) at 37°C and 85°C

The components of the SAP reaction were mixed in quantities and the sequence indicated in T, depending on the quantity of plates and assays to be conducted, in 1.5 ml microcentrifuge tubes or in 15 – 50 ml conical polypropylene centrifuge tubes. The appropriate volume of SAP reaction cocktail was aliquoted per well of a 96-well ABGene plate based on the number of plates to be run. The 384-well reaction plates were transferred to carriers and then loaded onto the Multimek SpectroPREP robot's stackers for post-PCR analysis. The SAP cocktail-containing 96-well ABGene plate was placed onto the Multimek in Position 1. 2 µl of SAP cocktail were added to each well by selecting the SAP program from the StakNet Control Program. After dispensing 2 µl of the cocktail, the robot combined the 6 µl of post-PCR product that was previously present in every well with the 2 µl of additional SAP cocktail, resulting in a uniform mixture. The reaction plates were carefully taken out of the carriers and removed from the stacker. Using MicroAmp sealers, the plates were carefully sealed. The used ABGene plate was removed and discarded. The plates were vortexed using a plate vortexer.

SAP Reaction Cocktail consisted of:

- Water
- 10× SAP buffer
- SAP (1.7 U/µl)

A total of 1.53 microliter volumes of the reactants were used.

A post-PCR tabletop centrifuge equipped with microtiter plate carriers was used to centrifuge the plates for 1 minute at $425 \times g$ at room temperature to push solutions to the bottom

of the wells. After 50 minutes of incubation in a 37°C Precision incubator, the plates were quickly moved to an 85°C incubator and incubated for an additional 20 minutes. The plates were taken out of the incubator at 85°C and allowed to cool to room temperature. Once the primer extension procedure was ready to be processed, they were centrifuged as in step 6 and stored at 4°C. The plates were inserted into the 384-well block made by Hybaid or ABI. The following software was run: One cycle: forty minutes at 37°C, one cycle: ten minutes at 85°C, and the last phase was an indefinite 4°C. They were centrifuged as in step 6 and stored at 4°C until the primer extension procedure was ready to be processed.

RESULTS

4.1 Genotyping of SNPs using iPLEX

During that stage, mass-modified ddNTPs were incorporated into SNP-specific oligos (primers) in an allele-specific manner, constituting the primer extension reaction. To ensure the reliability of the results and control possible sources of contamination, certain precautions were taken. It was recommended that extension primers undergo HPLC purification and MALDI-TOF validation.

Additionally, in MALDI-TOF mass spectrometry, an inverse relationship between detection intensity and analyte mass existed. This necessitated the use of different primer concentrations within the same extension primer mix, with lighter primers being less concentrated. To address this, varying primer working concentrations were employed for different primers. To facilitate this adjustment, extension primers within each plex were categorized into four groups based on their molecular masses. For example, in a multiplex reaction with 35 assays, primers were divided into groups of 9 (lowest mass group), 9, 9, and 8 (highest mass group) primers according to their unextended masses.

The primer concentrations were then tailored based on the specific assays used in the genotyping process. To prepare the iPLEX reaction, the following steps were followed:

For each plex, primers were mixed and diluted to the required working concentrations. The components of the iPLEX reaction were combined according to the specifications. Subsequently, 2 ml of the primer extension mix was added to each well of the 384-well plate. The microplate was sealed, and then it was centrifuged for 1 minute at 425×g at room temperature. Finally, the plate was placed into an appropriate thermal cycler for further processing. The presence of salts from previous reactions led to an increased background noise in the mass spectrometry analysis. Therefore, iPLEX reaction products were treated with a cationic resin (Clean resin) to remove salts. All the holes of a 384-dimple plate were filled with the resin (each hole took 6 mg). The plastic scraper was used to spread the resin evenly throughout the dimple plate and to remove

excess resin. The 384-well plate with the iPLEX reaction products was placed upside down onto the dimple plate. The plates were flipped over one another so that the dimple plate was now on top of the 384-well plate. The dimple plate was tapped to make the resin fall into the wells with the iPLEX reaction products. 16 ml of ultrapure water was added to each well of the 384-well plate. The plate was rotated for 10 minutes along its main axis on a plate rotator. The 384-well plate was then centrifuged for 5 minutes at $\sim 1,950\times g$.

The remaining steps of the protocol involved using the MassARRAY® specific workstation and MassARRAY® software. This step described the transfer of the extended/desalted iPLEX reaction products onto a SpectroChip® from a 384-well plate. The SpectroChip® is a pad for the analysis of DNA samples by MALDI-TOF mass spectrometry, supplied in a 384-well format and prespotted with a specially formulated MALDI matrix. The MassARRAY® Nanodispenser station for spotting a 384-microtiter plate iPLEX reaction onto a SpectroChip® was used in this section. A small volume (25 nl) of the reaction was dispensed onto the matrix spots on the SpectroChip using the following steps in the software that navigated the MassARRAY® Nanodispenser station: Before beginning, it was ensured that the tank reservoirs (distilled water tank and the 50% ethanol tank) were filled, and the waste tank was empty. The preconditioning of the spotting pins was performed. On the nanodispensing software, the Pin conditioning tab was selected (/Operation/Status/Home Machine/Pin Conditioning). The sonicator was filled with 100% ethanol. Pin conditioning of the main head (24 pins) was started and run for 30 minutes. The pin conditioning was repeated, but now for the single head (one pin). The sonicator was drained by selecting the Drain Sonicator button. The Fill sonicator button was selected to refill the sonicator reservoir with 50% ethanol. For dispensing quality check, the 384-well plate and an old SpectroChip (a test Chip) were placed on the deck of the MassARRAY® Nanodispenser. Under Load Method, System was chosen, the file type was changed from *.tmf to *.vmf, and the file Volume384.vmf, present in the "Volume" folder, was selected. The volume check was run to adjust the dispense speed. In the Run Setup tab, the file iPLEX was loaded, and the dispense speed was adjusted to the one that provided the best results in the volume check. For final dispensing check, the test Chip was replaced with a new one. The Status tab was selected and start was clicked. A few nanoliters (approximately 15 nl) of the samples were transferred onto the SpectroCHIP. 70 nl of calibrant was added to the calibrant reservoir on the MassARRAY® Nanodispenser. In the Run

Setup tab, the file Calibrant dispense was loaded, and the Start button in the Status tab was selected. The masses of the products resulting from the experimental steps were analyzed in real-time using the MassARRAY® mass spectrometer. Figure 3.1 shows the standard protocol of SNP genotyping using Sequenom's iPLEX® Gold and MassARRAY®.

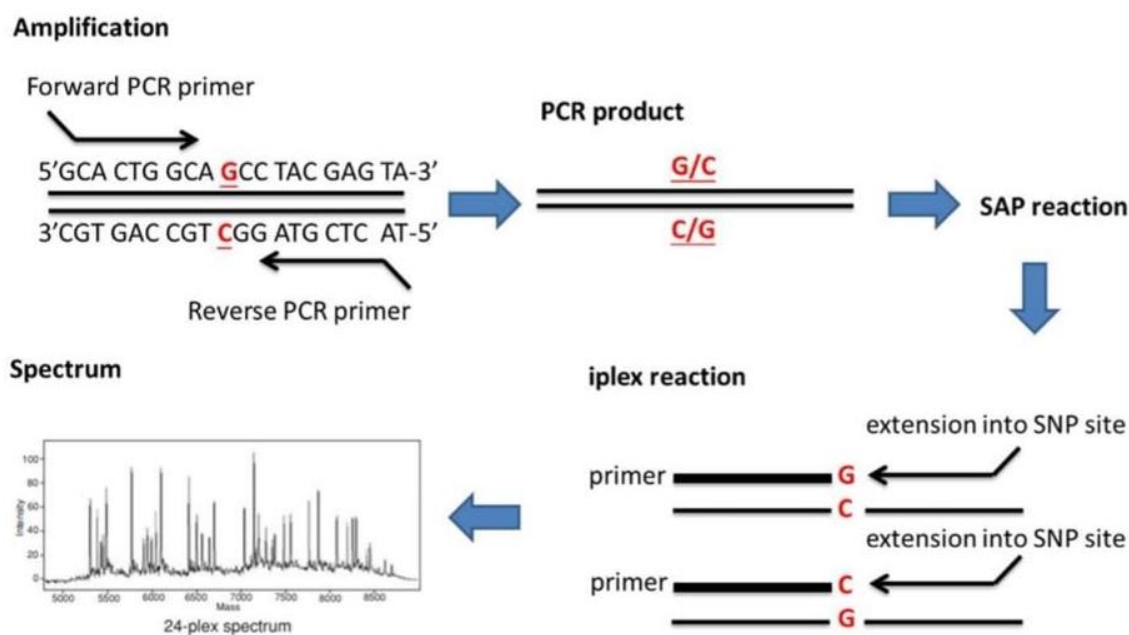


Figure 4.1 Experiment steps in genotyping using MassARRAY system

<https://www.nature.com/articles/s41598-023-33149-4>

4.2 Statistical Methods and Analysis Procedures

The basic characteristics of the participants were encoded and subjected to analysis using SPSS Statistical Package for the Social Sciences, version 28.0.0 for the Windows operating system, as well as GraphPad Prism 9. A significance level of $p < 0.05$ was used as the threshold for establishing statistical significance, unless stated otherwise. Allele and genotype frequencies were presented as numerical values (percentages), which were obtained through direct counting. These frequencies were then compared between various groups using the Chi-square test. The compliance of genotype frequencies with the Hardy-Weinberg equilibrium was evaluated using the Chi-square test.

To determine associations, odds ratios (OR) along with their corresponding 95% confidence intervals (CI) and related p-values were calculated using the SNPStats program developed by Solé

et al. (2006). This allowed for quantifying the likelihood ratios between different groups. Additionally, the collective frequencies of genotypes for all analyzed genes were computed and subsequently compared using the same SNPStats program.

The potential relationship between genotypes and scores on the Beck Depression Inventory (BDI) was explored using a non-parametric one-way analysis of variance (ANOVA) test. This analysis aimed to ascertain whether there were any statistically significant variations in BDI scores among different genotype groups.

4.3 Demographical characteristics of study participants

Demographic data indicated that mean age of 1,111 study participants was 31.793 ± 11.096 ranges from 11-80 years. Mean age of study participants from case group were 35.565 ± 11.534 and from control group were 25.192 ± 7.55 . Educational background level was divided into 7 levels ranging from middle group to doctorate. It was observed that there is a significant difference in educational level of both groups. All the study participants from control group were literate while 31% from case group only have educational level up to middle school. Socioeconomic status and Marital status of two groups also indicated a significant difference (table 4.1).

Table 4.1: Demographic characteristics of study participants

Variables	Cases (n=691)	Controls (n=420)	p-value
Age	35.565±11.534	25.192±7.55	
Educational Background			
Illiterate	33(4.7%)	-	<0.0001
Middle	188(27.2%)	25(5.9%)	
Secondary School	-	45(10.7%)	
High School	296(42.8%)	137(32.6%)	
Graduation	119(17.2%)	78(18.5%)	
Post-Graduation	58(8.3%)	92(21.9%)	
Doctorate	-	45(10.7%)	
Socioeconomic Status			
Low	60(8.6%)	11(2.6%)	<0.0001
Lower Middle	128(18.5%)	94(22.3%)	

Upper Middle	499(72.2%)	290(69.0%)	
High	7(1.0%)	27(6.4%)	
Marital Status			
Single	284(41.0%)	314(81.1%)	<0.0001
Married	377(54.5%)	106(25.2%)	
Widowed	28(4.05%)	2(0.4%)	

4.4 Suicidal score assessment of study participants

Suicide ideation score were calculated on the basis of “Beck Inventory scale for suicide ideation” as described in methodology. Responses from both case and control groups were analyzed and summarized in table 4.2. It was observed that participants from case group showed relatively high scores as compared to control group and a significant difference was detected in responses of both groups. Total suicide scores among both groups are represented in figure 4.1.

Table 4.2 Participant’s responses to Suicide scale

Parameters	Cases (n=691)	Controls (n=420)	p-value
Afraid to Die			
Yes	423(61%)	170(40%)	<0.0001
No	268(39%)	250(60%)	
Thought of killing			
Yes	386(55%)	95(22%)	<0.0001
No	305(45%)	325(78%)	
Plans for future			
Yes	411(59%)	176(41%)	<0.0001
No	280(41%)	244(59%)	
Hurting yourself in anger			
Yes	388(56%)	38(9%)	<0.0001
No	303(44%)	382(91%)	
Problems with friends and family			

Yes	387(56%)	71(16%)	<0.0001
No	304(44%)	349(84%)	
Control on anger			
Yes	418(60%)	156(37%)	<0.0001
No	273(40%)	264(63%)	
Fatigue or loss of energy			
Yes	358(51%)	104(24%)	<0.0001
No	333(49%)	316(76%)	
Dispute with police			
Yes	152(21%)	8(2%)	<0.0001
No	539(79%)	412(98%)	
Recurrent thoughts of death			
Yes	361(52%)	75(17%)	<0.0001
No	330(48%)	345(83%)	
low self esteem			
Yes	425(61%)	85(20%)	<0.0001
No	266(39%)	335(80%)	
Loss of interest in life			
Yes	344(49%)	53(12%)	<0.0001
No	347(51%)	367(88%)	
Difficulties in daily activities			
Yes	378(54%)	57(13%)	<0.0001
No	313(46%)	363(87%)	
Like to sit alone			
Yes	370(53%)	58(13%)	<0.0001
No	321(47%)	362(87%)	
Attempted Suicide			
Yes	161(23%)	4(0.9%)	<0.0001
No	530(77%)	416(99.1%)	

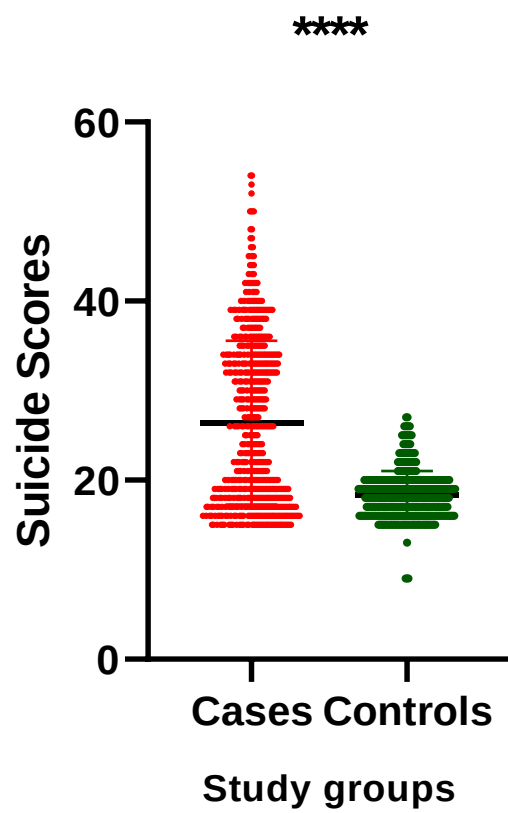


Figure 4.2 Suicide scores among study participants

4.5 Suicide score among different age groups

Suicide scores were calculated and compared in different age groups as indicated in table 4.3. All the study participants from case and control groups were divided into two age groups (<30 years and >30 years). It was observed that suicide score was higher in both sub-groups of cases as compared to control groups as indicated in figure 4.2 and figure 4.3.

Table 4.3 Suicide score compared with age groups of study participants

Age (Y)	Number of Participants (n)	Suicide Score (Mean±S.D)
17.0	8	19.375±3.2043
18.0	37	19.676±3.6290
19.0	39	19.154±4.8965
20.0	44	18.409±2.9280
21.0	33	19.152±4.8420
22.0	59	20.712±6.1084
23.0	48	23.104±9.2856
24.0	48	24.354±10.6221
25.0	59	22.814±7.7958
26.0	33	26.848±10.1831
27.0	50	22.080±7.9278
28.0	57	22.333±8.0497
29.0	30	23.700±7.6885
30.0	57	25.158±8.6660
31.0	35	23.343±7.2027
32.0	48	25.708±9.7522
33.0	31	24.742±7.7845
34.0	25	23.880±9.9596
35.0	42	25.500±9.2003
36.0	32	22.813±8.0420
37.0	13	24.462±9.1980
38.0	26	23.885±8.3920
39.0	7	19.286±3.9881
40.0	37	27.432±9.1698
41.0	10	20.200±7.1771
42.0	14	30.500±11.0853
43.0	8	27.250±9.0198
44.0	5	31.600±10.9225

45.0	22	27.182±8.7376
46.0	5	26.200±7.9498
47.0	6	29.667±14.0380
48.0	16	21.625±7.5971
49.0	3	31.000±10.5830
50.0	33	24.303±7.7922
51.0	3	23.667±7.3711
52.0	8	24.125±6.6427
53.0	5	19.400±4.0988
55.0	6	23.167±9.5167
56.0	4	23.250±7.5884
57.0	1	15.000±0.00
58.0	5	26.000±8.7750
59.0	4	22.500±9.2556
60.0	15	26.400±8.5924
61.0	6	18.167±2.2286
62.0	5	27.400±9.8641
63.0	3	18.667±6.3509
64.0	3	27.667±11.5036
65.0	4	27.000±9.8319
66.0	1	16.000±0.00
70.0	2	28.500±7.7782
71.0	1	27.000±0.00
80.0	1	21.000±0.00
Total	1111	23.364±8.3598

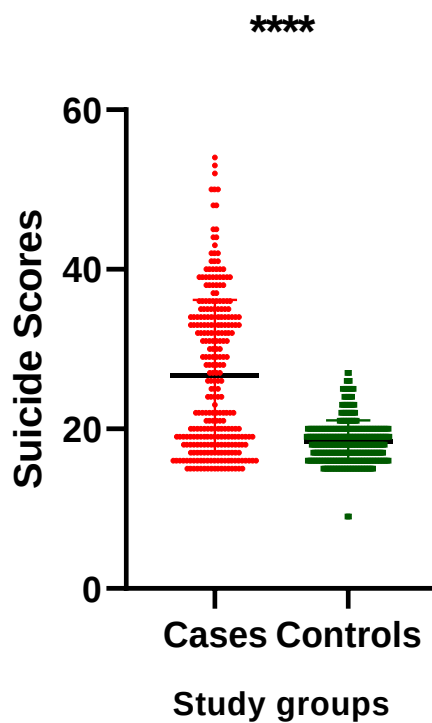


Figure 4.3 Suicide score of participants from age group <30 years

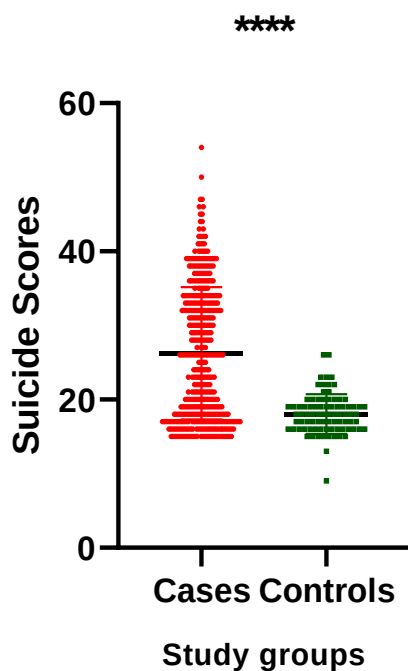


Figure 4.4 Suicide score of participants from age group >30 years

4.6 Suicide score among different casts of study participants

Mean suicide scores of participants belonging to different casts were also compared. It was observed that Abbasi showed highest (38) suicide score followed by Faqir (34) and Dogar (30) while lowest suicide score was observed in Memon (16) as indicated in table 4.4.

Table 4.4 Suicide score among different casts of the study participants

Caste	Number of Participants (n)	Suicide (Mean±S.D)
Abbasi	2	37.500±2.1213
Ansari	22	22.455±9.5704
Arian	91	20.571±6.3301
Awan	79	26.139±8.6153
Baloach	18	23.222±7.5814
Deendar	3	22.000±10.4403
Dogar	2	30.000±14.1421
Faqir	4	34.500±7.1880
Gujjar	26	23.769±9.6594
Jaat	275	24.695±8.7442
Kamboh	7	24.000±8.6410
Kashmiri	31	22.645±8.0645
Khattak	3	23.000±9.6437
Makhdoom	2	24.500±6.3640
Malik	58	23.345±8.3866
Masih	2	18.500±4.9497
Mayo	12	17.667±3.3934
Memon	2	16.000±1.4142
Mochi	17	24.529±7.7952
Mughal	43	22.256±6.2758
Muslim Sheikh	14	23.571±10.3010
Niazi	34	28.176±9.1070
Pathan	77	24.273±9.0839
Qazi	2	21.500±.7071
Qureshi	10	20.500±8.1411
Rajpoot	163	21.448±8.1242
Rehmani	15	22.400±7.4046
Sheikh	37	20.541±5.6302
Syed	41	23.268±7.3076

Tarkhan	3	26.000±11.2694
Zameendar	12	19.750±4.3719
Total	1111	23.360±8.3570

4.7 Suicide score and Socioeconomic status of study participants

The participants were divided into four groups based on their socioeconomic status. Suicide scores were compared in these groups of study participants. It was observed that the socioeconomic status has no impact on suicidal thoughts in both case and control group as represented in figure 4.5 and figure 4.6.

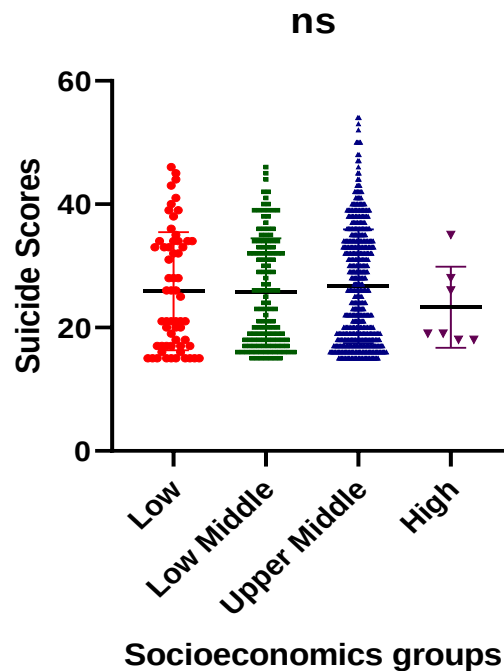


Figure 4.5 The Socioeconomic status and suicide score of participants from case group

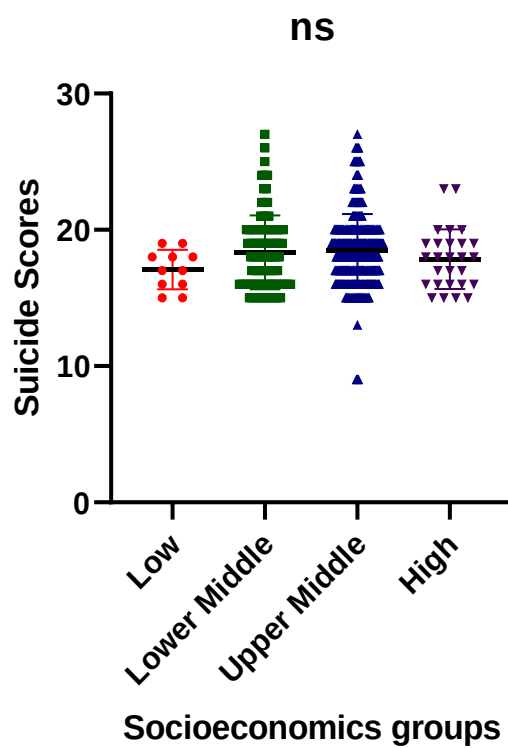


Figure 4.6 The Socioeconomic status and suicide score of participants from control group

4.8 Dopaminergic, serotonin receptor, tryptophan hydroxylase, monoaminergic transmitter system and neural growth factor and differentiation gene polymorphisms in case and control groups

The allele and genotype frequency of two polymorphism of each SLC6A3 and DRD2, HTR2B, HTR2C, TPH2, TPH1, BDNF, COMT and CREB1 was calculated in both study groups. Statistically significant difference was observed in allele and genotype frequency of rs6347, rs1801412, rs6318, rs1800532 and rs1799913 gene polymorphism in case and control groups. Allele frequency of alleles of selected SNPs is represented in figure 4.7.

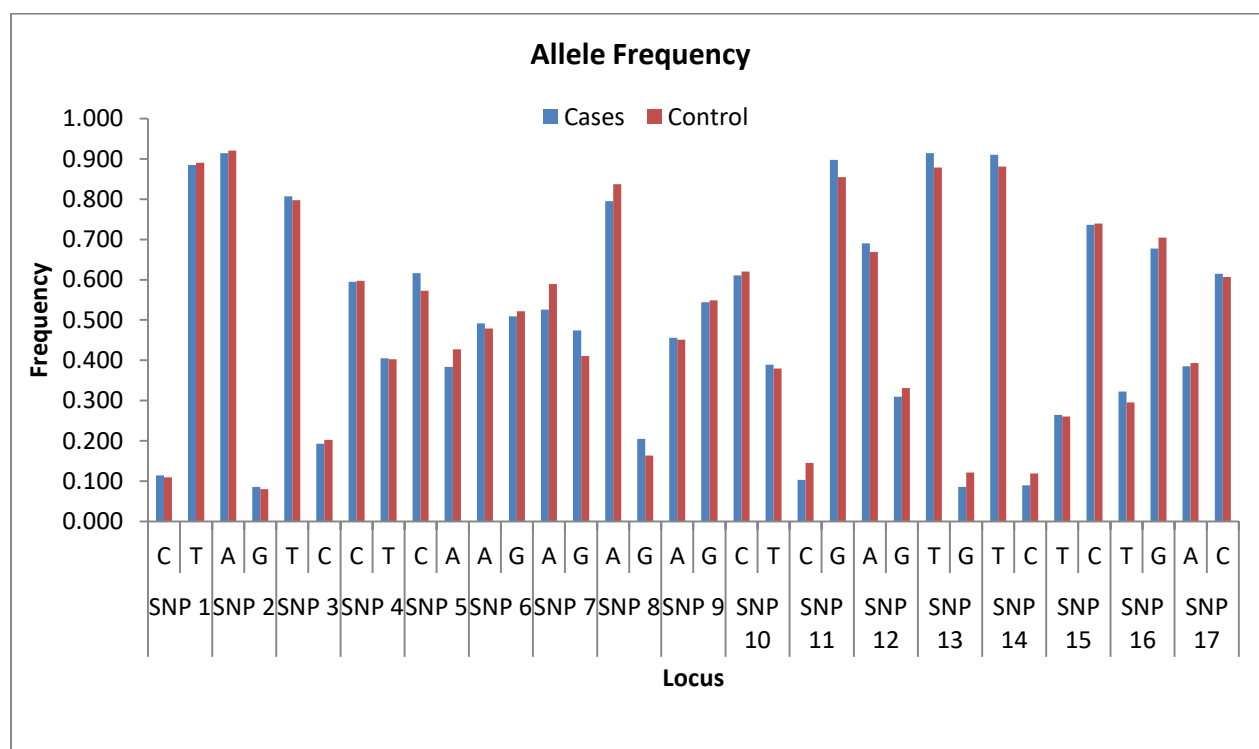


Figure 4.7 Allele Frequencies of SNPs in cases and controls

Table 4.5 Allele and genotype frequency of SLC6A3 and DRD2 gene (Dopaminergic system) Polymorphisms

Gene	Polymorphism	Genotype/Allele	Cases n (f)	Controls n (f)	p-value
SLC6A3	rs6347	A/A	219(0.317)	166(0.395)	0.021
		A/G	289(0.418)	163(0.388)	
		G/G	183(0.265)	91(0.217)	
		A	727(0.526)	495(0.589)	0.003
		G	655(0.474)	345(0.411)	
	rs464049	C/C	261(0.378)	157(0.374)	0.511
		C/T	322(0.466)	207(0.493)	
		T/T	108(0.156)	56(0.133)	
		C	844(0.611)	521(0.620)	0.654
		T	538(0.389)	319(0.380)	
DRD2	rs1800497	T/T	376(0.544)	227(0.540)	0.756
		T/C	265(0.384)	167(0.398)	
		C/C	50(0.072)	26(0.062)	
		T	1017(0.736)	621(0.739)	0.860
		C	365(0.264)	219(0.261)	
	rs1799978	A/A	576(0.834)	358(0.852)	0.184
		A/G	112(0.162)	57(0.136)	
		G/G	3(0.004)	5(0.012)	
		A	1264(0.915)	773(0.920)	0.641
		G	118(0.085)	67(0.080)	

Table 4.6 Allele and genotype frequency of HTR2B and HTR2C gene (Serotonin system) Polymorphisms

Gene	Polymorphism	Genotype/Allele	Cases n (f)	Controls n (f)	p-value
HTR2B	rs1549339	C/C	255(0.369)	157(0.374)	0.987
		C/T	312(0.452)	188(0.448)	
		T/T	124(0.179)	75(0.179)	
		C	822(0.595)	502(0.598)	0.891
		T	560(0.405)	338(0.402)	
	rs6437000	A/A	111(0.161)	71(0.169)	0.927
		A/C	310(0.449)	188(0.448)	
		C/C	270(0.391)	161(0.383)	
		A	532(0.385)	330(0.393)	0.710
		C	850(0.615)	510(0.607)	
HTR2C	rs6318	C/C	71(0.103)	61(0.145)	0.033
		C/G	517(0.748)	291(0.692)	
		G/G	103(0.149)	68(0.161)	
		C	142(0.103)	122(0.145)	0.002
		G	1240(0.897)	718(0.855)	
	rs1801412	T/T	632(0.915)	369(0.879)	0.051
		T/G	49(0.070)	44(0.104)	
		G/G	10(0.014)	7(0.016)	
		T	1264(0.915)	738(0.879)	0.005
		G	118(0.085)	102(0.121)	

Table 4.7 Allele and genotype frequency of TPH1 and TPH2 gene (Tryptophan hydroxylase system) Polymorphisms

Gene	Polymorphism	Genotype/Allele	Cases n (f)	Controls n (f)	p-value
TPH1	rs1799913	C/C	261(0.378)	130(0.310)	0.068
		C/A	330(0.478)	221(0.526)	
		A/A	100(0.145)	69(0.164)	
		C	852(0.616)	481(0.573)	0.040
		A	530(0.384)	359(0.427)	
	rs1800532	A/A	437(0.632)	290(0.690)	0.024
		A/G	225(0.326)	123(0.293)	
		G/G	29(0.042)	7(0.017)	
		A	1099(0.795)	703(0.837)	0.014
		G	283(0.205)	137(0.163)	
TPH2	rs7305115	A/A	177(0.256)	88(0.210)	0.072
		A/G	325(0.470)	226(0.538)	
		G/G	189(0.274)	106(0.252)	
		A	679(0.491)	402(0.479)	0.560
		G	703(0.509)	438(0.521)	
	rs4570625	T/T	74(0.107)	38(0.090)	0.403
		T/G	298(0.431)	172(0.410)	
		G/G	319(0.462)	210(0.500)	
		T	446(0.323)	248(0.295)	0.175
		G	936(0.677)	592(0.705)	

Table 4.8 Allele and genotype frequency of BDNF (Brain-derived Neurotrophic Factor), CREB1(cAMP Response Element Binding Protein) and COMT (Catechol-O-Methyltransferase) gene polymorphism

Gene	Polymorphism	Genotype/Allele	Cases n (f)	Controls n (f)	p-value
BDNF	rs12273363	C/C	546(0.790)	333(0.793)	0.700
		C/T	131(0.190)	82(0.195)	
		T/T	13(0.019)	5(0.012)	
		C	1223(0.885)	748(0.890)	0.693
		T	158(0.114)	92(0.110)	
CREB1	rs7594560	T/T	444(0.643)	270(0.643)	0.243
		T/C	227(0.329)	130(0.310)	
		C/C	20(0.029)	20(0.048)	
		T	1115(0.807)	670(0.798)	0.597
		C	267(0.193)	170(0.202)	
COMT	rs4680	A/A	152(0.220)	86(0.205)	0.758
		A/G	326(0.472)	207(0.493)	
		G/G	213(0.308)	127(0.302)	
		A	630(0.456)	379(0.451)	0.830
		G	752(0.544)	461(0.549)	

4.9 Validation of gene and allele frequency of polymorphism by Hardy-Weinberg Equilibrium

Allele and genotype frequency of all the studied SNPs in case and control groups was validated by applying Hardy-Weinberg equilibrium as indicated in table 4.9 and table 4.10 respectively.

Table 4.9 Chi-Square Tests for Hardy-Weinberg Equilibrium for Cases

Polymorphism	χ^2	p-value	Significance
rs12273363	10.090	0.018	*
rs1799978	0.985	0.321	ns
rs7594560	1.998	0.157	ns
rs1549339	2.768	0.096	ns
rs1799913	0.069	0.793	ns
rs7305115	2.409	0.121	ns
rs6347	17.968	0.000	***
rs1800532	0.000	0.995	ns
rs4680	1.661	0.197	ns
rs464049	0.276	0.600	ns
rs6318	691.000	0.000	***
rs2276307	0.002	0.961	ns
rs1801412	691.000	0.000	***
rs11606194	0.448	0.503	ns
rs1800497	0.124	0.725	ns
rs4570625	0.125	0.723	ns
rs6437000	1.911	0.167	ns

Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

Table 4.10 Chi-Square Tests for Hardy-Weinberg Equilibrium for Controls

Polymorphism	χ^2	<i>p</i>-value	Significance
rs12273363	0.000	0.985	ns
rs1799978	2.395	0.122	ns
rs7594560	0.715	0.398	ns
rs1549339	2.016	0.156	ns
rs1799913	2.366	0.124	ns
rs7305115	2.566	0.109	ns
rs6347	16.506	0.000	***
rs1800532	2.224	0.136	ns
rs4680	0.010	0.922	ns
rs464049	0.897	0.344	ns
rs6318	420.000	0.000	***
rs2276307	0.000	1.000	ns
rs1801412	420.000	0.000	***
rs11606194	0.196	0.658	ns
rs1800497	0.416	0.519	ns
rs4570625	0.106	0.744	ns
rs6437000	1.598	0.206	ns

Key: ns=not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

4.10 Association of SLC6A3 and DRD2 gene polymorphism with suicide score

Association of SLC6A3 and DRD2 gene polymorphism was observed with mean suicide score among study participants. It was observed that recessive genotype G/G of rs6347 and heterozygous mutant T/C of rs1800497 indicated a higher suicide score as compared to other genotypes as indicated in table 4.11.

Table 4.11 Suicide score and Dopaminergic system gene polymorphism

Gene	Polymorphism	Genotype	Suicide Score (Mean±S.E)	p-value
SLC6A3	rs6347	A/A	23.453±0.76	0.021
		A/G	23.427±0.86	
		G/G	25.23±1.68	
	rs464049	C/C	23.544±0.84	0.511
		C/T	23.739±0.83	
		T/T	22.343±1.09	
DRD2	rs1800497	T/T	23.224±0.79	0.042
		T/C	24.143±0.87	
		C/C	23.388±1.35	
	rs1799978	A/A	23.567±0.67	0.184
		A/G	23.032±0.89	
		G/G	23.500±3.14	

4.11 Association of HTR2B and HTR2C Genes Polymorphism with suicide score

Association of HTR2B and HTR2C gene polymorphism was observed with mean suicide score among study participants. It was observed that dominant genotype A/A of rs6437000 and recessive G/G of rs1801412 indicated higher suicide score as compared to other genotypes as indicated in table 4.12.

Table 4.12 Suicide score and Serotonin system gene polymorphism

Gene	Polymorphism	Genotype	Suicide Score (Mean±S.E)	p-value
HTR2B	rs1549339	C/C	24.152±0.87	0.987
		C/T	22.808±0.80	
		T/T	24.665±1.03	
	rs6437000	A/A	25.237±1.10	0.047
		A/C	22.685±0.80	
		C/C	24.400±0.86	
HTR2C	rs6318	C/C	21.953±1.23	0.082
		C/G	23.925±0.72	
		G/G	22.685±0.80	
	rs1801412	T/T	22.820±1.17	0.051
		T/G	23.492±0.64	
		G/G	24.665±1.03	

4.12 Association of TPH1 and TPH2 gene polymorphism with suicide score

Association of TPH1 and TPH2 gene polymorphism was observed with mean suicide score among study participants. It was observed that dominant genotype C/C of rs1799913 indicated a higher suicide score as compared to other genotypes as indicated in table 4.13.

Table 4.13 Suicide score and Tryptophan hydroxylase system gene polymorphism

Gene	Polymorphism	Genotype	Suicide Score (Mean±S.E)	p-value
TPH1	rs1799913	C/C	23.862±1.08	0.028
		C/A	24.110±0.78	
		A/A	24.700±0.87	
	rs1800532	A/A	18.00±8.19	0.074
		A/G	22.625±2.50	
		G/G	23.757±0.94	
TPH2	rs7305115	A/A	23.189±0.94	0.082
		A/G	23.726±0.86	
		G/G	23.257±0.92	
	rs4570625	T/T	23.510±0.78	0.063
		T/G	23.735±0.87	
		G/G	23.914±1.25	

4.13 Association of BDNF, CREB1, COMT gene polymorphism with suicide score

Association of BDNF, CREB1 and COMT gene polymorphism was observed with mean suicide score among study participants. It was observed that dominant genotype A/A of rs4680 indicated a higher suicide score as compared to other genotypes as indicated in table 4.14.

Table 4.14 Suicide score and BDNF, CREB1, COMT gene polymorphism

Gene	Polymorphism	Genotype	Suicide Score (Mean±S.E)	p-value
BDNF	rs12273363	C/C	23.110±0.78	0.421
		C/T	23.93±0.99	
		T/T	23.443±0.66	
CREB1	rs7594560	T/T	23.564±1.63	0.243
		T/C	23.191±0.85	
		C/C	23.889±0.79	
COMT	rs4680	A/A	24.100±1.01	0.041
		A/G	23.44±0.77	
		G/G	23.346±1.01	

DISCUSSIONS

Suicidal ideation and suicidal behavior are initial steps that ultimately lead to suicide. Suicide accounts for a major proportion of death toll all around the globe. Suicide incidences have been increasing in both developed and developing countries. As per the World Health Organization (WHO), over 700,000 individuals lose their lives to suicide annually, with 7% of these cases documented in countries categorized as having low to middle incomes. Suicide stands as the fourth primary contributor to mortality in the age group of 15 to 29. The contemplation of suicide serves as a pivotal predictive factor, resulting in physical harm, psychological strain, hospital stays, legal ramifications, and placing a financial strain on healthcare systems (Klonsky *et al.*, 2016). Common causes of suicide ideations are constant depression, stress, and regretful conscience, lack of resources, malnutrition, poor economic condition, unwanted circumstances and bad behavior of people. These factors are more prevalent in prison convicts which make them a risk group for suicide.

Prisons are resource-stricken places all around the world. Prisoners are considered as people who do not deserve a good life or services. The quality of life in prisons is worst in developing countries. It was reported previously that quality of life in prisons leads to suicide behind bars (Combalbert *et al.*, 2018). In Pakistan, living conditions in prisons are not good. Lack of facilities and ill behavior of surrounding people make the situation worse. Prison itself imparts a negative impact on human psychology and people who are punished with prolonged confinement become more prone to develop anxiety and aggression. This constant aggression and depression ultimately lead to suicide attempts among prisoners.

Environmental and psychological factors are not the only factors causing suicide ideation, gene also play their crucial role in this regard. To date, many studies have been conducted to describe the genetic underpinnings of depressive disorder (Shadrina *et al.*, 2018). It was reported that genetic factors are 30-40% responsible for developing aggression while non-genetic factor are 60-70% involves in aggression development (Hasler, 2010). Many neurotransmitters like dopamine, serotonin, norepinephrine are known for their role in pathophysiology of aggression (Khanna *et al.*, 2019). As there are numerous factors involved in causing depression and suicide ideation in people particularly prisoners, it is necessary to evaluate these factors as to minimize

the incidences of suicide in low-middle income country like Pakistan. Considering this alarming condition, the major aim of presented work was to evaluate the genetic, psychological and environmental factors associated to suicide ideation in jail convicts of Pakistan.

In the present study, a total of 1,111 study participants were recruited and divided into two major groups: cases and controls. Cases (n=691) were jail convicts from different jails of Punjab while controls (n=420) were people from the general population. All the study participants were requested to sign a consent form which stated that participants are taking part in study voluntarily. Demographic details and suicide ideation score were noted on a pre-designed Performa. Names of participants were replaced with pseudonyms to ensure anonymity. Blood samples from both groups were collected and processed for genetic analysis.

The age range of study participants was 17-80 years. Mean age for case and control group was 35.565 ± 11.534 and 25.192 ± 7.55 respectively. Educational level of study participants indicated that among cases 42% have passed high school followed by middle school (27%), graduation (17%) and post-graduation (8.3%). About 5% of case group participants were illiterate. Among 420 controls, 32% have passed high school, followed by post-graduation (22%), graduation (18%), secondary school (11%), doctorate (11%) and middle school (5%). All the control group participants were literate and there was significant difference between the educational groups among cases and controls. A previous study reported the similar results indicating that jail population have low educational level as compared to general population (Ewert and Wildhagen, 2011).

The socioeconomic status plays a key role in social crime incidence as suicide ideation. People belonging to low-middle income groups face many socioeconomic difficulties, which leads to suicide ideation, and they sometime develop criminal attitudes. The presented study indicated that 72% of cases groups belong to upper middle followed by lower middle (18%), low (8.6%) and high (1%) while 69% participants from control group belongs to upper middle followed by lower middle (22%), high (6%) and low (2%). There was a statistically significant difference in the socioeconomic status of study groups. About 81% of control group participants were single. In case-group 54% participants were married followed by 41% single.

Suicide ideation score was calculated based on “Beck Inventory scale for suicide ideation”. Responses from both case and control groups were analyzed. It was observed that participants

from case group showed relatively high scores as compared to control group and a significant difference was detected in responses of both groups. Another study conducted in China however showed different results indicating that the suicide ideation score was same in jail inmates as in college students (J. Zhang *et al.*, 2010). The difference between presented study and previous study can be justified from different genetic and environmental factors in China and Pakistan.

Suicide scores were calculated and compared in different age groups. All the study participants from case and control groups were divided into two age groups (<30 years and >30 years). It was observed that suicide score was higher in both sub-groups of cases as compared to control groups. The reason behind higher suicide ideation score among all age-groups of cases could be environmental factors such as isolation and loneliness as reported by a previous study by McClelland and co-researchers which indicate that prisoners form age group <30 and >80 are more prone to suicide ideation due to feeling of loneliness (McClelland *et al.*, 2020). Accumulations of risk alleles in families that continue to transfer in offspring by limiting the gene pool are responsible for expression of many mutations. Mean suicide scores of participants belonging to different casts were also compared. It was observed that Abbasi showed highest (38) suicide score followed by Faqir (34) and Dogar (30) while lowest suicide score was observed in Memon (16).

The allele and genotype frequency of two polymorphisms of each SLC6A3 and DRD2, HTR2B, HTR2C, TPH2, TPH1, BDNF, COMT and CREB1 was calculated in both study groups. Statistically significant difference was observed in allele and genotype frequency of rs6347, rs1801412, rs6318, rs1800532 and rs1799913 gene polymorphism in case and control groups. Another study indicated the strong association of A allele of rs6347 with depressive disorder (Lee *et al.*, 2021). However, a non-significant association between rs6318 and suicide ideation was reported by TB González-Castro and co-researchers (González-Castro *et al.*, 2017). The difference between findings can be due to different genetic make-up of study groups of both regions.

Association of SLC6A3 and DRD2 gene polymorphism was observed with mean suicide score among study participants. It was observed that recessive genotype G/G of rs6347 and heterozygous mutant T/C of rs1800497 indicated higher suicide score as compared to other genotypes. It was observed that A allele was more dominant in rs6347 in case-group, but the higher suicide score was observed in G/G group which highlights the role of environmental factors on suicide ideation. Moreover, a previous study indicates that role of rs1800497 in suicide ideation

(Hill *et al.*, 2020). Association of HTR2B and HTR2C gene polymorphism was observed with mean suicide score among study participants. It was observed that dominant genotype A/A of rs6437000 and recessive G/G of rs1801412 indicated higher suicide score as compared to other genotypes. Another study indicated the association of minor allele of rs1801412 with schizophrenia (Pozhidaev *et al.*, 2020). Association of TPH1 and TPH2 gene polymorphism was observed with mean suicide score among study participants. It was observed that dominant genotype A/A of rs1799913 indicated higher suicide score as compared to other genotypes. The results of presented study were consistent with another study which reported role of A allele of rs1799913 in depressive disorder (Lee *et al.*, 2018). Association of BDNF, CREB1 and COMT gene polymorphism was observed with mean suicide score among study participants. It was observed that dominant genotype A/A of rs4680 indicated a higher suicide score as compared to other genotypes. While another study conducted in Korea reported no association of rs4680 polymorphism with depression or suicide ideation (Chiesa *et al.*, 2014).

It was observed that psychological state, genetic make-up and environmental factors play key roles in the pathophysiology of depression and suicide ideation. Further studies are required to evaluate the other key factors that are involved in causing depression in prisoners which is the high-risk group for suicide ideation in our society.

Conclusion: The study concludes that specific polymorphism in serotonin and dopamine-related genes are associated with increased suicide ideation among prisoners. Notably, genotypes such as A/A of rs1799913 (TPH1/TPH2) and A/A of rs4680 (COMT) are linked to higher suicide scores. The research emphasizes the complex interaction between genetic predisposition and environmental factors in influencing suicidal thoughts. These findings underscores the need for targeted interventions to address mental health issues in incarcerated populations, considering both genetic and socioeconomic factors.

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