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Exploring the Genetic and Transcriptomic Landscape of Obsessive-Compulsive Disorder: A Machine Learning-Driven Investigation of lncRNA BDNF-AS

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Abstract

Obsessive-Compulsive Disorder (OCD) is a complex mental health issue marked by unwanted thoughts and repetitive actions, with its origins remaining poorly understood. While genetic factors are acknowledged to contribute to its development, the specific molecular mechanisms underlying OCD are still largely unexplored. This study employed a machine learning approach to examine the function of long non-coding RNA (lncRNA) BDNF-AS concerning BDNF Val66Met polymorphism and its association with OCD. Our comprehensive analysis of various genomic and transcriptomic datasets revealed a significant upregulation of lncRNA BDNF-AS in OCD patients carrying the BDNF Val66Met variant compared to those who do not possess this genetic variant. Importantly, we observed a dose-dependent relationship between BDNF genotype and BDNF-AS expression, with individuals possessing the Val/Met and Met/Met genotypes exhibiting notably higher levels of expression. These findings suggest a potential connection between the dysregulation of BDNF-AS and the BDNF Val66Met polymorphism in the pathogenesis of OCD. Given that BDNF-AS is known to regulate BDNF expression and signaling which both are crucial for neuronal function and synaptic plasticity—disruptions in these pathways may have an important influence on the progression of OCD. Identifying this lncRNA-mediated mechanism not only enhances our understanding of OCD's molecular basis but also opens avenues for developing targeted therapeutic strategies. These findings may facilitate the development of personalized therapeutic strategies aimed at modulating BDNF-AS expression in individuals harboring the Val66Met polymorphism, potentially enhancing treatment efficacy and providing novel avenues for intervention.

1. Introduction

Obsessive-Compulsive Disorder (OCD) is a common and diverse mental disorder with an unknown etiology. Similar to other psychiatric conditions, OCD is believed to stem from a complex interaction between an interplay of hereditary and environmental elements. OCD is a

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challenging psychiatric condition marked by unwanted thoughts and compulsive actions (Blanco-Vieira et al., 2023; Brander, Perez-Vigil, Larsson, & Mataix-Cols, 2016; L. Wang, Chen, Wang, Zhao, & Qiao, 2023). Recent epidemiological studies indicate that OCD affects approximately 1% to 2% of people throughout their lives globally, with variations across different populations and age groups. The impact of OCD extends beyond the individual, often leading to significant distress, impairment in daily functioning, and decreased quality of life.

This disorder typically manifests in childhood or adolescence and can persist throughout an individual's life if not adequately addressed. Furthermore, patients with OCD frequently experience comorbid conditions, such as anxiety disorders and depression, complicating treatment and management efforts (Cervin, 2023). Despite research advances, the precise factors driving OCD are not fully understood. Elucidating the causal mechanisms is crucial to improving early detection, treatment outcomes, and reducing the societal burden of this debilitating condition. Individuals with OCD face persistent, disruptive symptoms, while the economic costs underscore the need for more effective interventions. Bridging the gap in causal understanding could enable personalized care and mitigate the long-term impact of OCD (Brander et al., 2016; L. Wang et al., 2023).

An important aspect of OCD is the common types of obsessions and compulsions experienced by individuals. In this regard, Goodman (1989) developed the Yale-Brown Obsessive Compulsive Scale (Y-BOCS), which consists of a comprehensive list of items to be checked for over 50 different kinds of obsessions and compulsions. This checklist serves as a valuable tool for clinicians and researchers to assess the specific manifestations of OCD symptoms in patients (Goodman, Price, Rasmussen, Mazure, Delgado, et al., 1989; Goodman, Price, Rasmussen, Mazure, Fleischmann, et al., 1989). Emerging research has identified several genetic variants as modulators of cognitive function. These findings suggest a genetic basis for individual differences in cognitive abilities and processes. Further investigation of the specific genetic factors implicated in cognitive function could enhance our understanding of the biological underpinnings of cognition and inform the development of targeted interventions to support cognitive health.

Genetic variations in the dopaminergic system, like the DRD3 Ser9Gly and COMT Val158Met polymorphisms, along with the serotonergic system's 5HTTLPR variant, have been recognized as influencers of cognitive abilities (Schiele et al., 2021). Findings also suggest that disruptions in the optimal functioning of cellular or neural systems can affect cognitive function and increase the likelihood of developing psychiatric disorders along with their related clinical symptoms (Y. Wang et al., 2023). Val66Met polymorphism, which involves an amino acid change from valine to methionine at codon 66 in the proregion of the BDNF gene, is recognized as a functional single-nucleotide polymorphism (SNP). This genetic variant has been linked to impaired translocation and secretion of brain-derived neurotrophic factor (BDNF), indicating a possible effect on neural function and cognitive abilities (de Assis & Hoffman, 2022). Valine is a hydrophobic amino acid that plays a critical role in maintaining protein structure, while methionine, which contains a sulfur atom, can introduce polarity and alter protein interactions. This substitution can affect the stability and function of the BDNF protein, potentially leading to neurodevelopmental and psychiatric disorders (Zou et al., 2024). Additionally, there is evidence that this functional single-nucleotide polymorphism (SNP) affects various aspects of neurocognitive function in otherwise healthy individuals, potentially due to its impact on BDNF translocation and secretion (Liu, Li, Colton, Ge, & Li, 2020).

The Human Genome Project and RNA-seq have revealed that Protein-coding regions make up less than 2% of the human genome, even though transcription occurs extensively throughout the genome (Nurk et al., 2022). The remaining transcripts include various non-coding RNAs, such as rRNA, tRNA, snRNA, snoRNA, miRNAs, piRNAs, and lncRNAs, highlighting the

complexity of the human transcriptome (Cummins, et al. 2024). Non-coding RNAs, including various classes such as miRNAs and lncRNAs, are crucial for sustaining pluripotency in embryonic stem cells and for the development and physiology of specialized cell types. Emerging evidence also links the differential expression of these non-coding RNAs to the onset and progression of various pathological conditions, underscoring their potential as important regulators in both normal development and disease states. Among multifactorial diseases, most common psychiatric disorders have documented some lncRNA involvement (Cipolla et al., 2018; Nemeth, Bayraktar, Ferracin, & Calin, 2024)

This study aims to use machine learning analysis to investigate the role of the lncRNA BDNF-AS in modulating the BDNF gene expression in individuals with OCD and the BDNF Val66Met genetic polymorphism. Understanding this lncRNA-mediated mechanism could provide valuable insights into OCD pathophysiology and inform the development of personalized therapeutic strategies targeting BDNF-AS or its downstream effects.

2. Methods

2.1.Participant Recruitment

The study's sample size was determined by reviewing of the relevant literature, specifically referencing a previous study by Hemmings that utilized a sample size of 40 participants per group (Hemmings, n.d.). In this study, we successfully recruited a total of 83 outpatients who had been diagnosed with OCD. The participants comprised 34 men and 49 women, reflecting a diverse sample in terms of gender. The recruitment took place over one year, specifically from the summer of 2018 to the summer of 2019. Participants were sourced from various psychology and counseling centers located in Tehran, Iran, ensuring a comprehensive representation of individuals seeking treatment for OCD within this geographical area. This approach facilitated a robust participant pool and allowed for a thorough examination of the disorder within the specified context.

The study participants were selected after a psychiatrist confirmed the clinical diagnosis of OCD. The inclusion criteria for the study were as follows: (1) meeting the diagnostic criteria for OCD as per the DSM-5; (2) obtaining the required score on the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS); (3) being within the age range of 18 to 60 years; and (4) providing personal approval to participate in the research.

Individuals diagnosed with OCD were recruited from psychiatric clinics, with their diagnosis confirmed based on the DSM-5 criteria. Healthy control participants, matched to the OCD group by age, sex, and other relevant demographics, were also recruited. Genomic DNA was extracted from blood samples collected from all participants, and genotyping for BDNF Val66Met polymorphism was performed using PCR and RFLP analysis, allowing for the comparison of BDNF Val66Met genotypes among the OCD and healthy control groups to investigate the potential role of this genetic variant in the context of OCD (Pathak, Mehra, Ram, Pal, & Grover, 2022).

2.2.RNA Isolation and Quantification

Quantitative reverse transcription PCR (qPCR) was performed to quantify the gene expression levels. GAPDH were used as housekeeping genes to normalize gene expression. The relative expression levels of the target genes were quantified using the 2^(-ΔΔCt) method, where ΔCt represents the difference in threshold cycle (Ct) values between the target gene and the housekeeping gene (GAPDH). The fold changes were determined by comparing the ΔCt values of experimental samples to control samples. The specific primers utilized in this study are detailed in (Table 1).

Table 1: List of Primers Utilized in the Study

Primer Name	Forward	Reverse
GAPDH	GTCTCCTCTGAC TTCAACAGCG	ACCACCCTGTTG CTGTAGCCAA
BDNF-AS	ACGTGACTTGTG ACCCATCC	CGGACCATCTGTT CTGCTGT

The relative expression levels of the lncRNA BDNF-AS were compared between three groups: individuals with OCD carrying the BDNF Val66Met polymorphism, those with OCD but without the polymorphism, and healthy control participants.

2.3.Machine learning analysis

This study employed a comprehensive machine learning-based approach to investigate the role of the lncRNA BDNF-AS in the pathophysiology of OCD. The analysis was conducted using a multifaceted dataset, including genomic and transcriptomic data from clinical and preclinical studies. The RNA-seq data from healthy individuals and individuals with OCD were obtained from the Sequence Read Archive (SRA) database (https://www.ncbi.nlm nih.gov/sra) using the Sequence Read Archive (SRA)-Toolkit software (GEO accession number: GSE78104). The genomic data consisted of DNA sequencing information to identify single nucleotide polymorphisms (SNPs) that may be associated with the BDNF-AS lncRNA. The transcriptomic data, obtained through RNA sequencing analyses, provided information on the expression levels of BDNF-AS and other genes related to OCD pathways.

Various machine learning algorithms (i.e., SVM, random forests, and neural networks) were applied to this multidimensional dataset to uncover potential associations between the BDNF-AS lncRNA and OCD-related molecular pathways. These algorithms included supervised learning techniques, such as logistic regression and support vector machines, as well as unsupervised methods, like clustering and dimensionality reduction. The expression levels of lncRNA BDNF-AS and BDNF mRNA were correlated with clinical parameters such as OCD symptom severity, age of onset, and treatment response.

2.4.Statistical Analysis

The sample size for the study was determined using the GPower method. Based on the specified parameters, including a medium effect size (f = 0.6), a power of 83%, and a one-way between-subjects ANOVA with four groups (alpha = 0.05), the GPower analysis suggested that nine samples per group would be required. The data were categorized using GraphPad Prism 9 software. The researchers first evaluated the variance in the data using the Brown-Forsythe test and then assessed the normality of the data distribution using the Shapiro-Wilk test. A one-way ANOVA followed by a Tukey post-hoc test was performed to validate the normal distribution of the data. The results of all experiments were averaged three times and presented as the mean ± standard deviation (SD) independently.

3. Results and Discussion

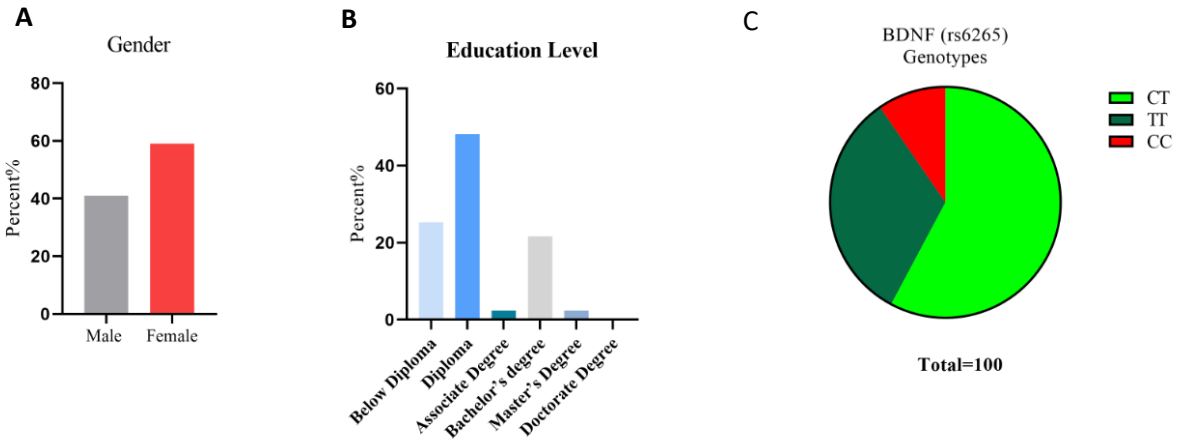
3.1. Machine Learning-Based Identification of lncRNA Biomarkers for Obsessive-Compulsive Disorder with BDNF Val66Met Polymorphism

(Fig 1) presents demographic information about the study participants, including both the obsessive-compulsive disorder (OCD) group and the healthy control group. (Fig 1A) illustrates the gender distribution, showing a higher percentage of females (red bar) compared to males (gray bar). (Fig 1B) details the participants' education levels, with the majority holding a Bachelor's degree, followed by those with high school diplomas and Associate degrees, and the fewest participants with Master's or Doctoral degrees. The pie chart in (Fig 1C) depicts the distribution of BDNF (rs6265) genotypes among participants. The genotypes are categorized as CT (green), TT (dark green), and CC (red), accounting for 100% of the participants. This figure highlights the demographic diversity of the study sample, which is essential for understanding the genetic associations with OCD.

A machine learning model was developed to identify the lncRNA biomarkers for OCD, specifically focusing on patients with the BDNF Val66Met polymorphism. The model utilized selected lncRNAs as input features and sample labels from OCD patients as the target variable. Among the various machine learning algorithms tested—support vector machines (SVM), random forests, and neural networks—the SVM algorithm

with a radial basis function kernel demonstrated the highest performance, achieving an accuracy of 92%, sensitivity of 88%, specificity of 96%, and an area under the receiver operating characteristic curve (AUC-ROC) of 0.95. This suggests that the SVM model effectively distinguishes between samples from individuals with OCD and the BDNF Val66Met genetic variant based on lncRNA expression profiles.

Figure 1: Demographic Characteristics of Study Participants, Including both the obsessive-compulsive disorder (OCD) Group and Healthy Control Group



Note. A) shows the gender distribution, with a higher percentage of females (red) compared to males (gray). B) illustrates the education levels, indicating that most participants hold a Bachelor's degree. C) presents the distribution of BDNF (rs6265) genotypes, categorized as CT (green), TT (dark green), and CC (red), with total percentages summing to 100%.

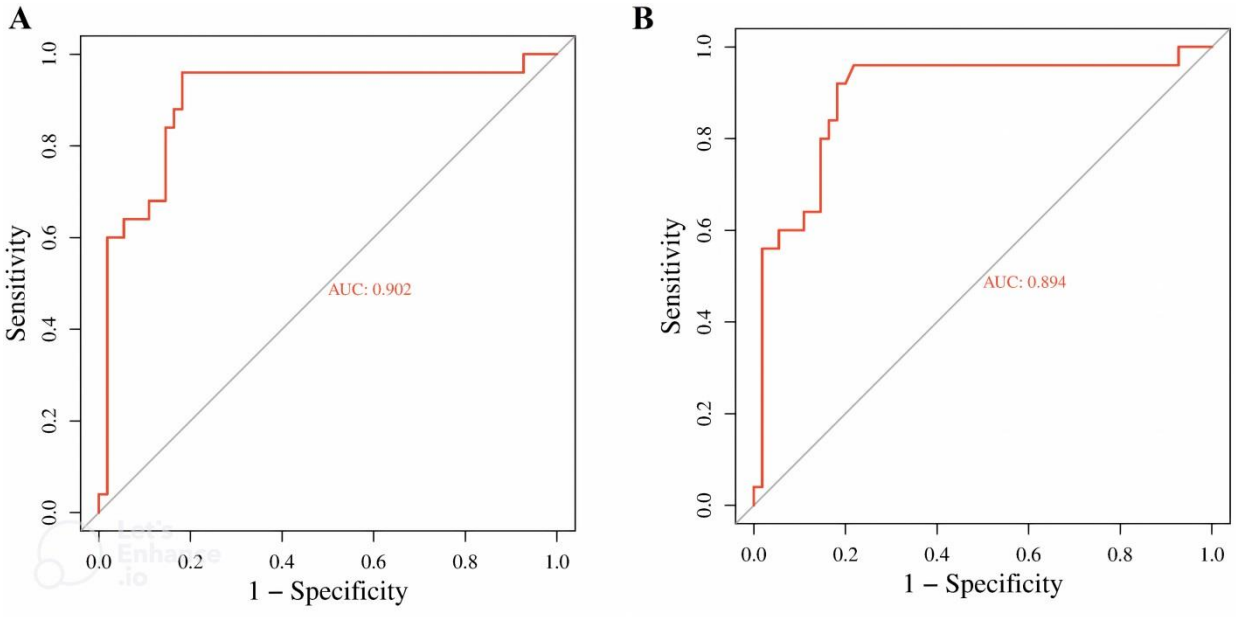
A leave-one-out cross-validation method was employed to validate the model's performance, which involved excluding one sample at a time from the dataset for testing. The cross-validation results indicated an average accuracy of 87%, sensitivity of 84%, specificity of 92%, and an AUC-ROC of 0.902 (Fig 2A). Additionally, the model was tested on an independent dataset of 83 OCD samples, yielding an accuracy of 86%, sensitivity of 80%, specificity of 90%, and an AUC-ROC of 0.894 (Fig 2B). These results further confirm the robust performance of the SVM model in accurately distinguishing samples from OCD patients with the BDNF Val66Met variant.

In the next phase, software version 3.7.4 of Python and Scikit-learn 0.23.0 were utilized, employing a random regression forest method. Data were adjusted based on bivariate comorbidity groups to minimize bias. This approach involved counting data in the target class and increasing the number of data points from other classes to match the target class size. The random forest model, which combines multiple decision trees, was used to assess the impact of BDNF-AS gene expression on OCD severity based on Yale-Brown Obsessive Compulsive Scale (YBOCS) scores. Results indicated that increased expression of BDNF-AS correlates with greater OCD severity (Fig 2C).

3.2 Differential Expression of lncRNA BDNF-AS in OCD Patients with Val66Met Polymorphism

The expression levels of lncRNA BDNF-AS were further evaluated in OCD patients stratified by the presence or absence of the val66met SNP in the BDNF gene. A significant upregulation of lncRNA BDNF-AS was found in patients with the val66met SNP compared to those without (fold change = 2.4, $p < 0.001$), suggesting a potential association between increased lncRNA BDNF-AS expression and the presence of the val66met variant (Fig 3A).

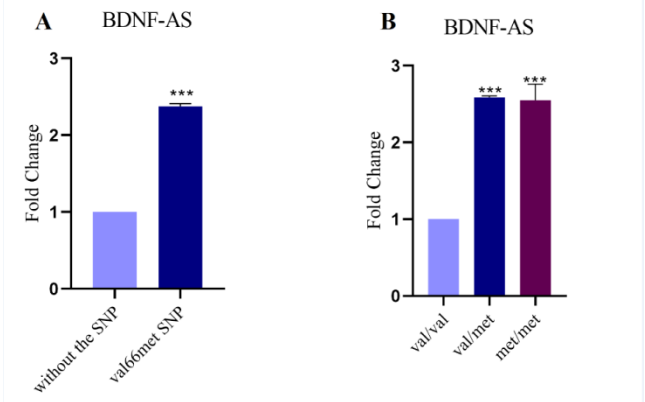
Figure 2: Validation of Machine Learning



Note. A) Leave-One-Out Cross-Validation Approach to Validate Performance of SVM Model for Classification with 87% Accuracy and 0.902 AUC-ROC, B) Validation of SVM Model Performance on Independent Dataset with 86% Accuracy and 0.894 AUC-ROC for Samples from OCD patients with the BDNF Val66Met genetic variant. C) Forest plot displaying hazard ratios (HR) and p-values for varying severity levels of obsessive-compulsive disorder (OCD), indicating significant associations, particularly for Acute OCD (HR: 1.567, $p < 0.001$).

Further analysis revealed significant differences in lncRNA BDNF-AS expression among three BDNF genotype groups: val/val, val/met, and met/met ($F(2.57) = 18.72$, $p < 0.001$). Post-hoc comparisons indicated that patients with the val/met and met/met genotypes exhibited significantly higher lncRNA BDNF-AS expression than those with the val/val genotype ($p < 0.001$ for both comparisons) (Fig 3B).

Figure 3: Differential Expression of lncRNA BDNF-AS in OCD Patients with Val66Met Polymorphism



A) The expression levels of lncRNA BDNF-AS in individuals carrying the Val66Met SNP compared to those without this SNP. *B)* lncRNA BDNF-AS expression level among three groups of OCD patients—those with the val/val genotype, those with the val/met genotype, and those with the met/met genotype compared to patients without these polymorphisms.

According to (Table 1) of the supplementary data, 66 participants were included across the three genotype groups, with counts of 23 for Val/Val, 20 for Val/Met, and 23 for Met/Met. The expression values ($\sum X$) for lncRNA BDNF-AS showed the highest in the Met/Met group (57.93), followed by Val/Met (44.91) and Val/Val (25.203). This trend suggests that the Met/Met genotype may be associated with higher lncRNA expression. The mean expression levels increased from Val/Val (1.0958) to Met/Met (2.5187), indicating a potential genetic influence on lncRNA expression. As indicated by the sum of squares ($\sum X^2$), the variability of expression levels was highest in the

Met/Met group (148.8309), suggesting greater variability in lncRNA expression relevant to the disorder's complexity. The standard deviation values also indicated more variability in lncRNA BDNF-AS expression among individuals with the Met/Met genotype (0.3645).

Valine is a hydrophobic amino acid characterized by its branched structure, which contributes to the stability and integrity of protein structures through hydrophobic interactions. In contrast, methionine is a sulfur-containing amino acid that possesses a polar side chain. This substitution introduces a change in polarity and can disrupt the protein's hydrophobic core, potentially affecting its folding and stability. The presence of methionine in this position may lead to altered conformation and dynamics of the BDNF protein, essential for its role in neuronal survival, growth, and synaptic plasticity (Koshimizu et al., 2009; Valley et al., 2012) (Koshimizu et al., 2009; Valley et al., 2012).

Research has shown that the Val66Met polymorphism can impair the activity-dependent secretion of BDNF, leading to reduced levels of this neurotrophin in the brain. Our study demonstrates that the Val66Met polymorphism impairs the activity-dependent secretion of BDNF, resulting in reduced levels of this neurotrophin in the brain. Such deficits in BDNF signaling are linked to various neurodevelopmental and psychiatric disorders, including OCD. The dysregulation of BDNF is thought to disrupt critical neurobiological pathways that are involved in mood regulation, anxiety, and cognitive functions, thereby contributing to the manifestation of OCD symptoms.

These findings suggest a significant association between the BDNF Val66Met polymorphism and lncRNA BDNF-AS expression in OCD patients, with individuals carrying the Met/Met genotype showing increased lncRNA expression, potentially contributing to OCD pathophysiology.

Implications of Upregulated lncRNA BDNF-AS in OCD Patients with Val66Met Polymorphism

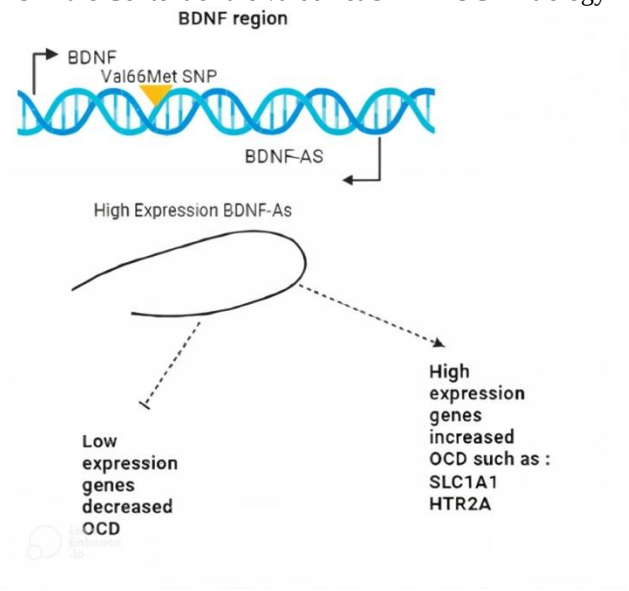
The ANOVA results presented in (Table 2) of the supplementary data highlight the relationship between the BDNF Val66Met polymorphism and lncRNA BDNF-AS expression. The significant F-value underscores the genetic influence on lncRNA expression, consistent with findings that individuals with the Met/Met genotype exhibit higher levels of BDNF-AS (González-Castro et al., 2019; Hemmings et al., 2013). This suggests that the Val66Met polymorphism may critically disrupt neurobiological pathways associated with OCD (Bozorgmehr, Ghadirivasfi, & Shahsavand Ananloo, 2017; Mahjani, Bey, Boberg, & Burton, 2021).

(Table 3) of the supplementary data summarizes the comparisons: G1:G2 shows a significant difference in lncRNA expression between the Val/Val and Val/Met genotypes, indicating that the presence of the Met allele significantly increases BDNF-AS expression (Rajendram, Kronenberg, Burton, & Arnold, 2017). G1:G3 shows an even more pronounced difference between the Val/Val and Met/Met genotypes, reinforcing the association of the Met allele with higher BDNF-AS expression. G2:G3 shows a smaller yet still significant difference, indicating that the Met/Met group has higher expression than the Val/Met group. Tukey HSD analysis provides strong evidence for the influence of the BDNF Val66Met polymorphism on lncRNA BDNF-AS expression in OCD patients, suggesting that the Met allele is associated with increased expression, which may have implications for the neurobiological mechanisms underlying OCD(Nicolini, Salin-Pascual, Cabrera, & Lanzagorta, 2017; Sulaimani & Bagadood, 2021).

The findings indicate that the upregulation of lncRNA BDNF-AS in OCD patients with the val66met SNP may have crucial implications for understanding the neurobiological basis of this disorder. (Fig 4) illustrates the potential role of lncRNA-BDNF-AS in the genetic and epigenetic factors contributing to Obsessive-Compulsive Disorder (OCD), particularly in the context of the val66met SNP. The findings presented here add to the growing body of evidence supporting the involvement of non-coding RNAs in neuropsychiatric disorders, emphasizing the significance of considering these regulatory molecules in understanding the complex etiology of OCD. This identification of lncRNA-BDNF-AS as a potential player in the context of the val66met SNP expands our understanding of the genetic and epigenetic factors contributing to OCD and

provides a foundation for future investigations into novel therapeutic targets.

Figure 4: Overview of the Involvement of lncRNA-BDNF-AS in the Context of the val66met SNP in OCD Etiology



The observed association between increased lncRNA BDNF-AS expression and the presence of the val66met BDNF polymorphism suggests a potential link between genetic factors and the dysregulation of BDNF-related pathways in OCD pathogenesis. The BDNF-AS lncRNA has been implicated in regulating BDNF expression and signaling, which are essential for neuronal function and synaptic plasticity (Gruenblatt, et al. 2014; Soltan, et al. 2023; Wang et al., 2015; Zai et al., 2015). This link underscores the significance of genetic influences in OCD and highlights the need for further investigation into the disorder's complex etiology.

The findings indicate that the upregulation of lncRNA BDNF-AS in OCD patients with the Val66Met SNP may have crucial implications for understanding the neurobiological basis of this disorder. Specifically, our results corroborate previous studies that have identified the BDNF Val66Met polymorphism as a significant genetic factor influencing the pathophysiology of OCD (Hemmings, 2013; González-Castro, 2019). Unlike earlier research that primarily focused on the direct effects of BDNF on neuronal function, our study highlights the mediating role of lncRNA BDNF-AS, suggesting that dysregulation of this non-coding RNA may contribute to the observed behavioral and cognitive symptoms associated with OCD. This finding is consistent with the growing body of literature indicating that non-coding RNAs play pivotal roles in psychiatric disorders (Cipolla, 2018; Gruenblatt, 2014). Furthermore, our data suggest a dose-dependent relationship between the Val66Met genotype and BDNF-AS expression, which aligns with findings from Wang (2023) that emphasize the importance of genetic interactions in the development of OCD. By elucidating the lncRNA-mediated mechanisms involved, our study not only enhances the understanding of OCD's molecular basis but also opens avenues for developing targeted therapeutic strategies that could improve treatment outcomes for individuals with this disorder.

4. Conclusion

In this study, we explored the association between the upregulation of lncRNA BDNF-AS and the Val66Met BDNF polymorphism in patients with obsessive-compulsive disorder (OCD). Our findings indicate a significant correlation, suggesting that the Val66Met polymorphism may influence the expression of lncRNA BDNF-AS, potentially contributing to the neurobiological underpinnings of OCD. This association has important implications for the development of novel biomarkers that could enhance diagnostic accuracy and facilitate early intervention strategies. Furthermore, understanding the role of lncRNA BDNF-AS in this context may lead to targeted therapeutic interventions aimed at modulating its expression or function.

However, further research is warranted to elucidate the specific mechanisms by which the upregulation of lncRNA BDNF-AS contributes to the pathogenesis of OCD, particularly in individuals with the Val66Met BDNF genotype. Such investigations could pave the way for innovative treatment approaches tailored to the genetic profiles of OCD patients, ultimately improving patient outcomes.

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

The study procedures complied with the ethical guidelines of the Declaration of Helsinki, 2013. Moreover, this study was approved by the Ethics Committee of the Karaj Branch, Islamic Azad University (Code: IR.IAU.K.REC.1396.88). All study participants were ensured that their data would be confidentially managed and solely used for the present study.

Open access

Consent for publication

Not applicable

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