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FUNCTIONAL ANALYSIS OF NEURODEGENERATIVE DISEASES-ASSOCIATED BDNF GENE'S POLYMORPHISMS: A BIOINFORMATIC APPROACH

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ABSTRACT

Brain-Derived Neurotrophic Factor (BDNF) is a crucial member of the neurotrophin family that plays an essential role in the development and maintenance of the nervous system. Altered BDNF expression in the blood and central nervous system has been associated with the pathogenesis of various neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, multiple sclerosis, and ischemic stroke. This study aimed to identify deleterious single-nucleotide polymorphisms (SNPs) and evaluate their effects on BDNF protein function and stability. Bioinformatics tools such as SNP and GO, PolyPhen-2, PANTHER, SIFT, PHD-SNP, Predict-SNP, SNAP2, I Mutant, MUpro, and ConSurf were used to detect pathogenic variants in BDNF. The 3D structure of the wild-type and mutant BDNF protein was predicted using I-TASSER, while gene-gene and protein-protein interactions were examined using Gene MANIA and STRING databases. Our analysis identified ten pathogenic missense SNPs within the BDNF coding region (rs866172975, rs771341699, rs1590217373, rs751698045, rs758638310, rs1330439007, rs77787410, rs1852795747, rs1590215885, rs780128716) that significantly reduced protein stability and altered its structural configuration. Furthermore, the gene interaction analysis highlighted the key role of BDNF in several biological pathways and its association with other genes. These findings highlight the potential contribution of pathogenic nsSNPs in BDNF-related disorders and provide new insights into the molecular mechanisms underlying the development of neurological diseases.

INTRODUCTION

Approximately 90% of genetic variations in the human genome are single-base pair changes, making single-nucleotide polymorphisms (SNPs) the most common type of DNA sequence alteration. The coding portion of the genome contains an estimated 500,000 SNPs (Collins et al., 1998). Among these, non-synonymous SNPs (nsSNPs), or missense variants, are significant because they alter amino acid residues and can thereby influence protein structure and function. Such changes may have either detrimental or beneficial effects, including destabilization of protein structure, disruption of gene regulation, or modifications in key features such as charge, geometry, hydrophobicity, dynamics, translation, and intra- or intermolecular interactions (Barroso et al., 1999). Brain-derived neurotrophic factor (BDNF), encoded by the BDNF gene, exemplifies the importance of functional genetic variation. BDNF is widely expressed in the central nervous system, where it plays crucial roles in neuroplasticity and influences diverse cognitive functions (Hing et al., 2018). Once produced in the CNS, BDNF is released into the extracellular space, where it binds to tropomyosin receptor kinase B (Trk-B) and the p75 neurotrophin receptor (p75NTR) to exert its effects (Thomas and Davies, 2005). Through these interactions, BDNF contributes to nervous system development by regulating neuronal differentiation, growth, and survival, as well as neurogenesis, synaptogenesis, and synaptic plasticity (Bath and Lee, 2010).

Recent studies highlight the central role of BDNF in the onset and progression of several brain disorders. Deficient BDNF signaling has been linked to major neurological and psychiatric conditions, including Alzheimer's disease, depression, schizophrenia, bipolar disorder, and anxiety disorders. These associations suggest that targeting BDNF pathways could provide new therapeutic opportunities for treating such disorders (Castrén and Hen, 2013). Beyond the nervous system, BDNF also plays a role in cardiovascular health. Altered BDNF signaling has been associated with coronary artery disease and is implicated in maintaining the physiological functions of the heart and vasculature (Kaess et al., 2015). In addition, BDNF influences the pathophysiology of diabetes mellitus (Eyileten et al., 2017), inflammatory conditions such as asthma (Prakash et al., 2014), and several types of cancer (Radin and Patel, 2017). Emerging evidence further demonstrates that BDNF is a critical regulator of energy balance and body weight. Neurons in the paraventricular hypothalamus that produce BDNF are essential for controlling food intake, energy expenditure, and thermoregulation, underscoring its role in eating behavior and metabolic homeostasis (Trinh et al., 2023).

In recent years, a variety of bioinformatics tools have been developed to identify functional SNPs and assess their potential adverse effects on candidate proteins (Yazar and Özbek, 2021). Likewise, several computational approaches are now capable of predicting structural alterations arising from single amino acid substitutions within proteins (Hassan et al., 2019). Building on these advancements, the present study comprehensively analyzed 117 nsSNPs in the BDNF protein to evaluate their pathogenic potential. High-risk variants were prioritized through multiple predictive approaches, and their influence on the structural and functional properties of BDNF was examined. Furthermore, molecular docking analyses were conducted to provide deeper insights into how these mutations affect protein conformation at both the secondary and tertiary levels.

Methodology

Data Collection

The ENSEMBL genome browser was used to examine the human BDNF gene**.** The BDNF protein sequence, identified by accession

number P23560, was obtained from the UniProt online repository. A total of 117 nsSnps were identified and retrieved from the Ensemble database.

nsSNPs Validation by Different Bioinformatics Tools

Several bioinformatics tools have been utilized to predict the harmful effects of nsSNPs on BDNF. The Sorting Intolerant from Tolerant (SIFT) tool was first employed to assess amino acid substitutions, classifying SNPs as tolerated (score ≤ 0.05) or damaging (score > 0.05) based on homology and sequence alignments (Sim et al., 2012). The PANTHER program, which analyzes evolutionary conservation scores, evaluates substitutions by categorizing proteins based on their molecular functions and interactions (Tang and Thomas, 2016). PolyPhen 2 predicted the impact of nsSNPs on protein sequences, structure, and phylogenetic properties, classifying variants as benign (near 0.0), possibly harmful, or damaging (up to 1.0) (Adzhubei et al., 2013). Predict SNP integrated multiple tools, including MAPP, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT, and SNAP2, to enhance the accuracy of disease-related mutation predictions. The SNAP2 classifier utilizes neural networks and in silico sequence data, and classifies nsSNPs as harmful or neutral.(Bromberg and Rost, 2007).PhD-SNP and SNPs & GO employed support vector machines (SVMs) for mutation analysis, while PhD-SNP used sequence-based features for variant interpretation.(Capriotti and Fariselli, 2017).While SNPs & GO predicted disease-associated mutations using SVMs(Capriotti et al., 2013)

Detecting the effect of nsSNPs on Protein Stability by I Mutant and MUpro

Predicting alterations in protein stability caused by single-residue substitutions is essential for understanding protein structures and creating new protein functions. To predict the point mutations in proteins and their impact, we used the SVM-based tools I-Mutant and MUpro, which utilized the protein structure and sequence data. We subjected the fata sequences and all missense variants of the BDNF gene to an I-Mutant to assess the , the stability of the protein was assessed at 25°C and pH 7 (Capriotti et al., 2005). MUpro, a server based on support vector machines (SVM), was used to predict variations in protein stability caused by mutations. Similarly, variations in the stability of proteins caused by various mutations were assessed using both structural and sequencing information, with an accuracy rate of 84% (Worth et al., 2011).

Analyzing nsSNPs Conserved Regions using ConSurf

The Consurf tool was used to evaluate the evolutionary conservation of the amino acids within the protein domain. This tool evaluates the evolutionary link between related sequences. The structural and functional significance of an amino acid or nucleotide location has a significant impact on its evolutionary rate. ConSurf gives conservation scores on a color-coded scale from 1 to 9, classifying the regions as variable, average, or highly conserved (Ashkenazy et al., 2010).

Structural Analysis of Proteins by Project HOPE Tool

The HOPE project is a comprehensive tool for identifying the functional and structural consequences of point mutations. It integrates data from various sources, including 3D protein coordinates obtained from the “WHATIF" web services, domain annotations from the UniProt database, and predictions from the DAS service. By combining these tools, Project HOPE investigated how mutations affect protein function and 3D structure through a systematic decision-making process. (Venselaar et al., 2010).

Proteins 3-D Modelling by I-TASSER

The online tool I-TASSER was used to construct the 3D models of the mutated protein, due to the unavailability of the crystal

structure of the BDNF protein. The I-TASSER tool is used for predicting protein structures, providing structure-based functional annotations, and utilizing structural templates sourced from the PDB to create the most suitable protein structure for the target, typically resulting in the top five models (Zhang et al., 2015). Furthermore, TM-align were employed to compare original and altered protein structures. This software calculates the TM-score (template modelling score) and RMSD (root mean square deviation), and helps in the alignment of the structures (Zhang and Skolnick, 2005). The TM score ranges between 0 and 1, where a score of 1 indicates perfect structural alignment. Conversely, a higher RMSD value indicates an increased difference between the original and altered proteins.

Prediction of Different PTM Sites in BDNF

GPS-MPS 3.0 (Wang et al., 2020), NetPhos 3.1(Ma et al., 2023), and UBpred were used to evaluate post-transcriptional modifications in the BDNF protein, such as methylation, phosphorylation, and ubiquitination. NetPhos 3.1 uses neural networks to predict phosphorylation at Serine, Threonine, and Tyrosine residues in proteins, with scores above 0.5, showing a high potential for phosphorylation. Similarly, higher scores in GPS 5.0 suggest an increased probability of phosphorylation. For identification of methylation sites, GPS-MSP 1.0 was used, while Ubpred was used to predict ubiquitination sites in the BDNF protein. UbPred detects lysine residues as ubiquitinated when their score exceeds a threshold of 0.62 (Radivojac et al., 2010).

Gene-Gene Interaction of BDNF by Gene MANIA

Gene MANIA is an in-silico tool which is used to study the interactions and associations between genes. It incorporates data from protein interactions, protein domains, similarities, co-expression, co-localization, and pathways to find gene linkages (Kamal et al., 2024). This tool was used to investigate the interaction between the BDNF gene and the effects of its nonsynonymous single-nucleotide polymorphisms (nsSNPs) on other genes (Zuberi et al., 2013).

Protein- Protein Interaction

STRING is an online database for predicting protein-protein interactions, including both functional and structural relationships. It provides insights into protein interaction networks, revealing the relations between key proteins and their top ten interacting partners. (Szklarczyk et al., 2023).

Molecular Docking

In this investigation, STRING was used to determine the interaction partners of the BDNF protein, with NGFR being closest binding partner. A high confidence threshold of 0.9 was applied to generate the interaction network. Subsequently, BDNF and NGFR proteins were subjected to molecular docking using the ClusPro web server with default parameters. Both wild-type BDNF protein and its mutant models were docked with NGFR. ClusPro generated ten distinct docking poses, each with modeling scores reflecting the binding energies of the docked complexes. (Kozakov et al., 2017).

RESULTS

Retrieval of SNPs

SNP data were obtained from the Ensembl Genome Browser, revealing that the BDNF gene contains 11,666 SNPs associated with the transcript ID ENST00000356660.9. These SNPs were categorised as shown in Figure 1, including 10,445 intronic SNPs, 768 SNPs in the 3' UTR, 120 SNPs in the 5'-UTR, 117 missense or non-synonymous SNPs (nsSNPs), and 79 synonymous SNPs. In addition, other SNPs were present in the dataset. In this study, only nsSNPs were selected for further analysis.

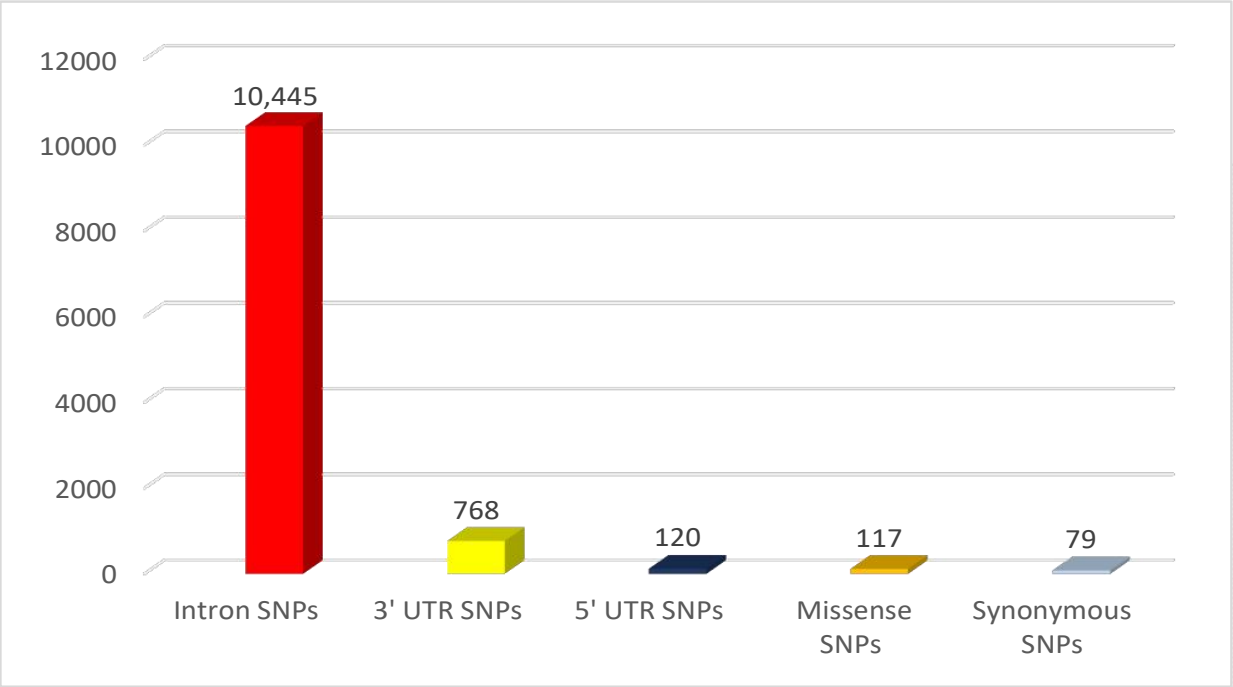


Figure 1: The bar graph represents the various SNPs detected during BDNF gene analysis.

Deleterious nsSNPs Prediction by Multiple Bioinformatics Tools

In this study, various bioinformatics methods were employed to detect important nsSNPs in the BDNF protein. From 117 missense nsSNPs, the SNPs and GO algorithm identified 20 harmful nsSNPs that may affect protein function. To assess the impact of these nsSNPs on the structure and function of the protein, further analysis was conducted using algorithms such as PolyPhen3, Panther, SIFT, PHD SNP, PredictSNP, and SNAP2. Initially, PANTHER predicted 66%, that is, the highest proportion of 78/ 117 nsSNPs was deleterious. The predict SNP identified was 50%, that is, 58 nsSNPs as affected. Similarly, PolyPhen2 predicted 46 %, that is, 54 of 117 nsSnps to be harmful, and SNAP2 identified 42% of Snps as disease-associated, that is, 52 of 117 nsSnps. SIFT identified 35% i.e. 41 / 117 nsSNPs with potential functional effects. Furthermore, the lowest proportion of harmful nsSNPs was identified by PHD SNP and SNPs & GO algorithms; they identified 30% and 15 % i.e. about 35 and 18 / 117 nsSNPs as of pathological nature. Seven different computational algorithms were employed in this bioinformatics study to identify high-risk nonsynonymous single-nucleotide polymorphisms (nsSNPs) associated with neurodegenerative susceptibility. From the algorithm's results, we unravelled 10 nsSNPs of a highly pathological nature and closely associated with disease susceptibility, as verified by multiple algorithms based on their predictive scores, as shown in Figure 2 and Table 1.

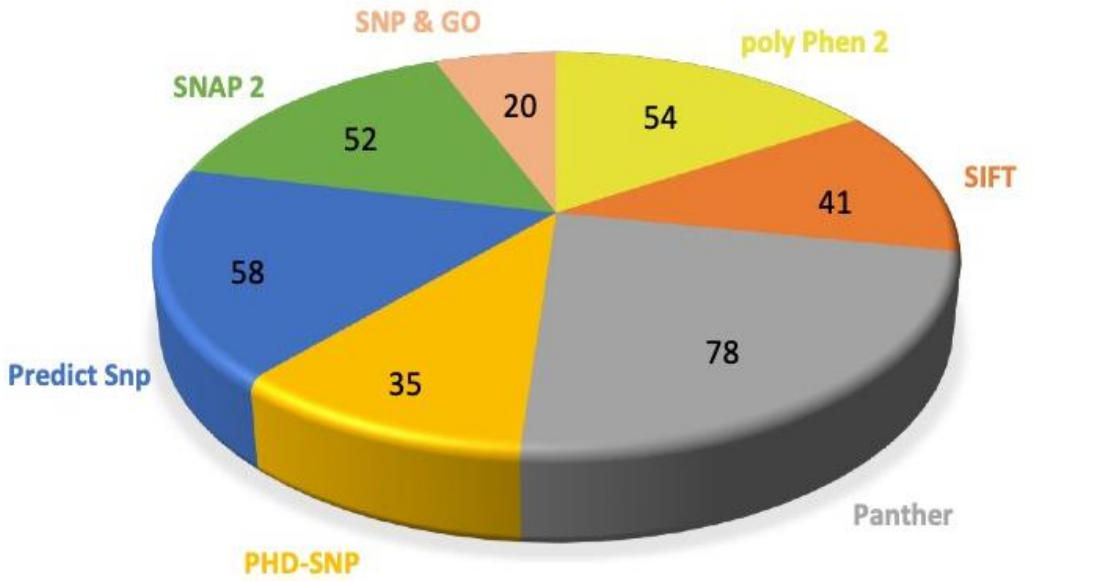


Figure 2: A pie chart illustrating the number of nsSNPs detected by multiple bioinformatics tools

Table 1: The most pathogenic variants of *BDNF* predicted by several computational tools

SNP ID	A. A variation	SNPs& GO	PolyPhen-2	Panther	SIFT	PhD-SNP	Predict SNP	SNAP 2
rs866172975	Ser11Leu	disease	Possibly damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious
rs771341699	Arg38trp	disease	Probably damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious
rs1590217373	Leu89Ser	disease	Possibly damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious
rs751698045	Glu111Lys	disease	Probably damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious
rs758638310	Asp118His	disease	Probably damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious
rs1330439007	Trp147Arg	disease	Probably damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious
rs77787410	Val170Phe	disease	Probably damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious
rs1852795747	Cys186Tyr	disease	Probably damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious
rs1590215885	Gly195Asp	disease	Probably damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious
rs780128716	Gly198Asp	disease	Probably damaging	Probably damaging	Not Tolerated	disease	Deleterious	Deleterious

Stability Analysis of BDNF Protein

To evaluate the stability of the altered BDNF protein, we utilized two online tools: I-Mutant 2.0 and MUPRO. These servers estimate the Gibbs free energy changes for genetic variants. The results indicated that 9 out of the 10 nsSNPs were predicted to reduce BDNF protein stability, as evidenced by negative DDG (ΔΔG) values for all the analyzed nsSNPs in I-Mutant 2.0. Furthermore,

MUPRO predicted that all 10 nsSNPs would negatively impact the functionality of BDNF protein, as detailed in Table 2.

Table 2: The effects of nsSNPs on the stability of BDNF amino acids based on their respective Gibbs free energy estimation

SNP ID	Amino Acid Variation	I-MUTANT 2.0 Server	DDG DDG<0, >0; Decrease, Increase Stability	MUPRO Server, Gibbs Free Energy Estimation
rs866172975	Ser11Leu	Decrease	-0.06	G = -0.31000074(Decrease stability)
rs771341699	Arg38trp	Decrease	-0.25	G = -0.35754624(Decrease stability)
rs1590217373	Leu89Ser	Decrease	-3.09	G = -1.8579979(Decrease stability)
rs751698045	Glu111Lys	Decrease	-0.03	G = -0.58255795(Decrease stability)
rs758638310	Asp118His	Decrease	-0.78	G = -0.59659855(Decrease stability)
rs1330439007	Trp147Arg	Decrease	-2.18	G = -1.1090315(Decrease stability)
rs77787410	Val170Phe	Decrease	-1.85	G = -1.4417744(Decrease stability)
rs1852795747	Cys186Tyr	Increase	1.38	G = -0.54506269(Decrease stability)
rs1590215885	Gly195Asp	Decrease	-1.52	G = -0.40410146(Decrease stability)
rs780128716	Gly198Asp	Decrease	-1.12	G = -0.44464835(Decrease stability)

Conservation Analysis through Consurf

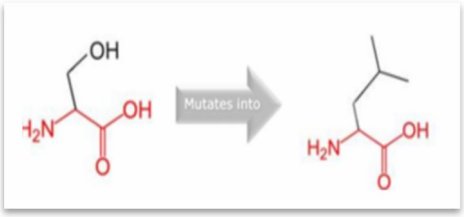
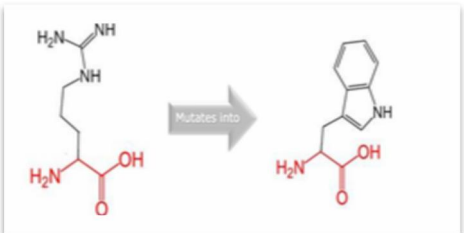
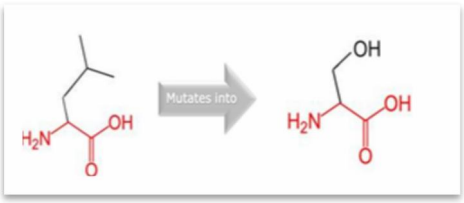
The Consurf web server was used to examine the ancestral conservation of the proteomic residues in the original BDNF protein. Considering ancestral preservation and solvent exposure, we identified functional domain residues affected by 10 high-risk nsSNPs in the BDNF protein. The residue S11L was predicted to be highly buried, whereas R38W was expected to be exposed and to be located in a conserved region. Residues L89S and E111K were also predicted to be exposed and were considered to have average characteristics. D118H, an exposed residue, was found in the variable region, whereas W147R was highly conserved and buried. V170F is a buried residue in an average region, and C186 is structurally buried and highly conserved. Lastly, G195D was an exposed residue, and G198D was functionally exposed and located in a conserved protein region as outlined in Figure 3.

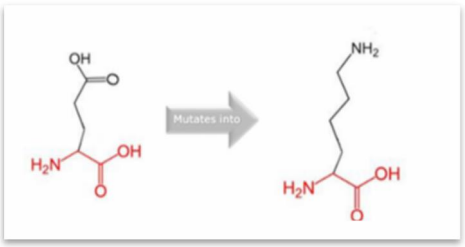
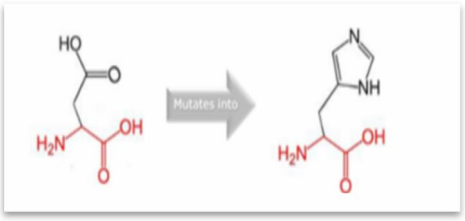
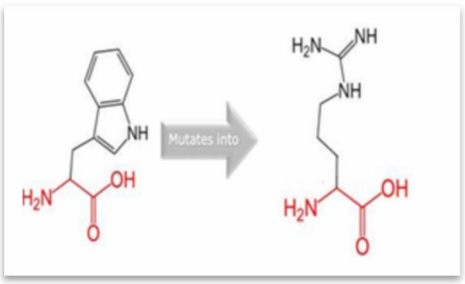
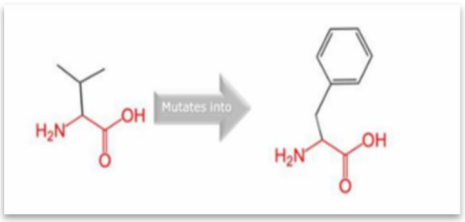
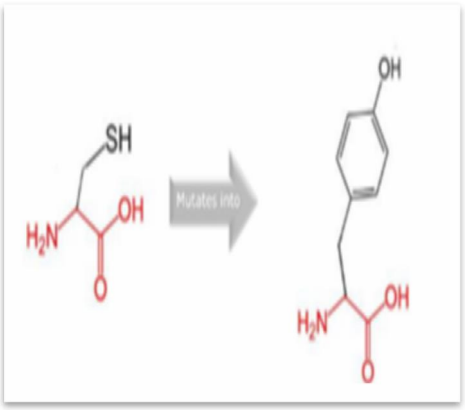
Figure 3: This figure shows the analysis of amino acid conservation using ConSurf web-based tool

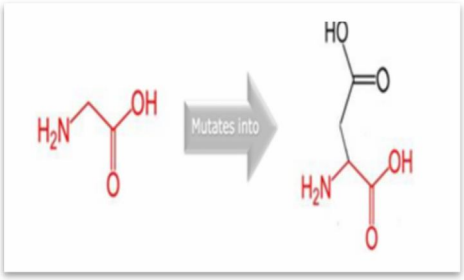
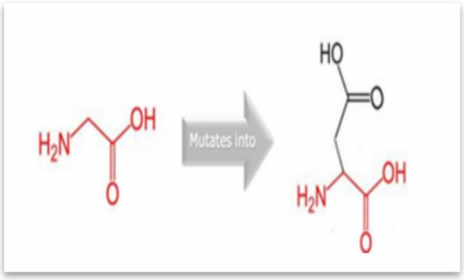
Structure Analysis by HOPE Server

In the present study, the HOPE online server was used to examine the influence of amino acid changes on the BDNF protein domain. The altered residues S11L, R38W, E111K, D118H, and V170F were larger and more hydrophobic than the original residues. In contrast, the altered residues, L89S and W147R, were smaller and less hydrophobic than their original counterparts. Additionally, the original residues, that is, C186Y, G195D, and G198D exhibited greater hydrophobicity than their respective mutant residues, which was also noted for their significance, as detailed in Table 3.

Table 3: This table outlines the influence of amino acid substitutions on the domain structure of BDNF Protein

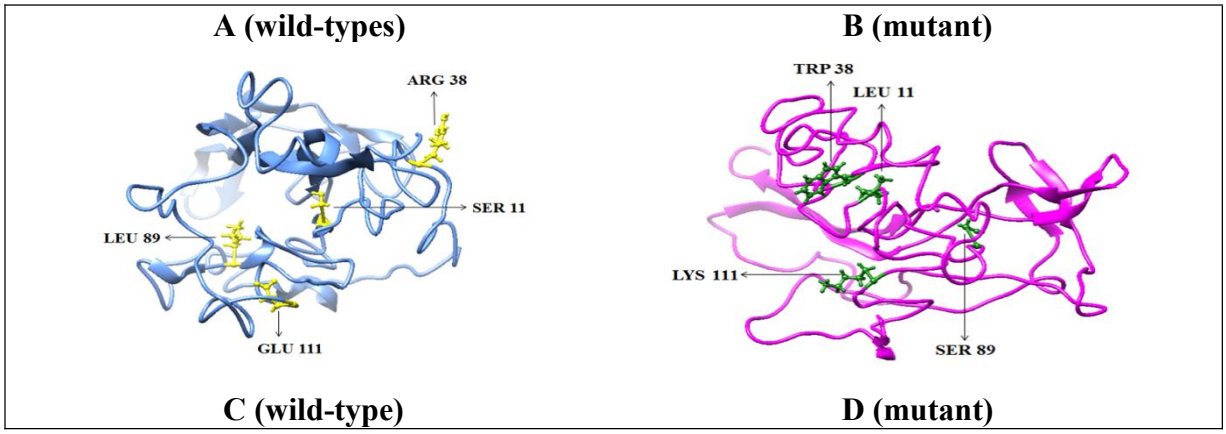
Residues	Structures	Properties
S11L		The altered amino acid is bulkier It possesses greater nonpolar activity relative to the original one
R38W		The altered residue is bigger than the original residue. The original residue had a positive charge The altered amino acids has no charge and more nonpolar compared to the original one.
L89S		The modified residue is tinier in size compared to the original type. The original residue is more nonpolar than the modified one.

E111K		The original amino acids carries a negative charge, while the altered one has a positive charge. The sizes of the original and altered amino acids vary, The altered residue being larger, possibly causing protrusions.
D118H		The original residue has a negative charge, whereas the altered one is neutral and larger in size. The increased size of the altered amino acids leads the formation of protrusions because of
W147R		The original residue has a neutral charge, while the altered carries a positive charge The original residues exhibit greater non-polarity compared to the altered one. The altered residue is smaller in size than the original.
V170F		The altered residue is bigger than the original one situated in the protein's core. Due to its larger size, the altered residue might not fit in the same position as the original.
C186Y		The original amino acid has greater non polarity compared to the altered one, which is larger in size and situated in a critical binding domain and interacts with amino acids in another key binding region. This mutation might interfere with the interaction between these two domains, possibly affecting the protein's functionality.

G195D		The original amino acids show higher non polarity compared to the mutated, which are larger in size and situated on the protein's surface. This mutation could interfere with interplay involving other molecules or regions of the protein.
G198D		The altered residues are bigger in size than the wild type. The original residue had a neutral charge, whereas the altered ones carry a negative charge. The original residue exhibits greater hydrophobicity compared to the altered ones.

3-D Modelling of wild-type and mutant protein

The 3D sequence of the BDNF protein was developed using the I-TASSER homology modelling service. Furthermore, variants were simulated to gauge the structural consequences of the high-risk nsSNPs. BDNF protein sequences, encompassing both the original and altered amino acids, were uploaded into I-TASSER to generate protein configurations. This technique is widely regarded as the most reliable and sophisticated method for predicting protein structure. The top five models were formulated for each BDNF protein and its variants by using this methodology. Subsequently, the resulting designs were evaluated via TM-Align to calculate RMSD and analyze the distinctions between originals and variants. The RMSD and TM-ALIGN scores for the original and variant proteins are shown in Table 4. Chimaera 1.11 was used to visualise protein structures and examine their molecular properties, as illustrated in Figure 4 and Table 4.



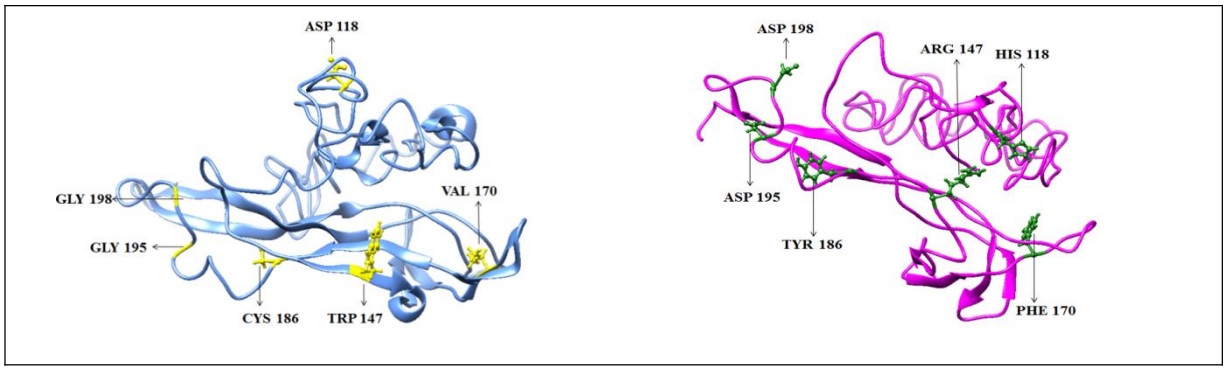


Figure 4: This figure illustrates the 3D structures of wild-type and mutant BDNF protein.

Table 4: The TM alignment and RMSD values for deleterious SNPs.

SNP ID	Amino acid change	TM Align	RMSD Score
rs866172975	Ser11Leu	0.54434	4.85
rs771341699	Arg38trp	0.32646	5.84
rs1590217373	Leu89Ser	0.66726	4.08
rs751698045	Glu111Lys	0.63881	4.54
rs758638310	Asp118His	0.70560	3.85
rs1330439007	Trp147Arg	0.57630	5.10
rs77787410	Val170Phe	0.52880	4.10
rs1852795747	Cys186Tyr	0.56325	4.27
rs1590215885	Gly195Asp	0.52767	4.85
rs780128716	Gly198Asp	0.50235	4.40

Post-Translational Modifications

Post-translational modifications (PTMs), such as protein-to-protein interactions and cell signalling, play a vital role in maintaining the structural functionality of proteins. This study explored the potential pathogenicity of high-risk nsSnps on PTMs impeding BDNF functionality via several computational methods to pinpoint potential PTM's located in various regions. The methylation of lysine residues within specific histones affects their binding to adjacent DNA, leading to modifications in gene expression. The GPS-MSP 3.0 server was used for this investigation. As per the findings from the GPS-MSP 3.0 server, it was determined that no methylation would occur at the BDNF sites. Protein phosphorylation acts as a regulatory mechanism in diverse biological processes, influencing protein structure through structural alterations, and modulating signal transduction pathways by activating or inhibiting specific proteins. This investigation employed NetPhos 3.1 and GPS 3.0, to forecast potential phosphorylation sites within the BDNF protein. The NetPhos 3.1 analysis identified 29 residues, comprising 14 Serine, 13 Threonine, and two Tyrosine residues, as potential phosphorylation sites. The GPS 3.0 server also predicted that 39% of serine-

specific sites, 41% of threonine-specific sites, and 20% of tyrosine residues within the BDNF protein are likely to be phosphorylated (Table 5).

Table 5: Phosphorylation sites in the BDNF gene as detected by GPS 3.0 and NETPhos 3.1 tools

Position	GPS 3.0		NETPHOS 3.1	
	Kinases	Scores	Kinases	Scores
S19	**AGC/DMPK/CRIK**	6.033	CKI	0.471
S53	**AGC/GRK/GRK/GRK1**	11.557	PKC	0.739
S61	**AGC/GRK/GRK/GRK1**	18.056	Unsp	0.613
S66	**AGC/DMPK/GEK/DMPK**	3.539	Unsp	0.989
S100	**AGC/RSK/RSKp70/RPS6KB1**	33.583	GSK3	0.464
S105	**TKL/RAF/KSR**	2.424	cdc2	0.454
S106	**AGC/NDR/NDR**	0.436	DNAPK	0.524
S131	**AGC/PKN**	133.912	Unsp	0.570
S138	**AGC**	5.744	unsp	0.997
S147	**STE/STE20/PAKB/PAK5**	5.404	Unsp	0.996
S151	**CMGC/CK2/CSNK2A2**	0.001	Unsp	0.855
S153	**CAMK/RAD53/DUN1**	2.058	cdc2	0.497
S168	**AGC/DMPK/CRIK**	1.067	PKA	0.629
S181	**AGC/GRK/GRK/GRK4**	15.705	unsp	0.955
S214	**AGC/GRK/GRK/GRK7**	1.959	Unsp	0.841
S221	**AGC/GRK/BARK/GRK3**	15.52	PKC	0.772
S230	**AGC/GRK/BARK/GRK3**	18.191	Unsp	0.948
S244	**AGC/GRK/GRK/GRK1**	18.988	Unsp	0.593
T10	**STE/STE20/MSN**	4.627	PKA	0.569
T15	**STE/STE20/MSN**	4.627	GSK3	0.457
T47	**CMGC/CLK/CLK2**	0.007	GSK3	0.458
T50	**STE/STE20/MST/STK3**	83.069	Unsp	0.744
T65	**AGC/DMPK**	15.086	Unsp	0.623
T70	**CAMK/PIM/PIM3**	8.46	CKI	0.554
T157	**CAMK/CAMK1/PNCK**	2.974	PKC	0.487
T171	**AGC/DMPK/GEK**	35.005	CaM-II	0.455
T173	**STE/STE20/MST**	23.76	PKC	0.572
T192	**STE/STE20/MST**	22.274	Unsp	0.691
T200	**AGC/GRK/GRK/GRK6**	96.826	CaM-II	0.452
T218	**STE/STE20/FRAY**	88.447	PKC	0.608
T219	**AGC/GRK/BARK/GRK3**	14.018	DNAPK	0.584
T227	**AGC/Akt**	9.8	Unsp	0.810
T243	**AGC/Akt/AKT1**	12.171	Unsp	0.982
T248	**AGC/GRK/BARK/GRK3**	13.528	GSK3	0.452
T250	**AGC/GRK/BARK/GRK3**	16.719	PKC	0.755
Y20	**TK/PDGFR/CSF1R**	1.45E-4	INSR	0.390
Y42	**TK/Ack**	1.31E-6	INSR	0.454
Y98	**TK/Jak/JAK2**	9.024	unsp	0.786
Y121	**TK/Tie**	2.182	INSR	0.375
Y124	**TK/Src/SrcB/BLK**	2.006	unsp	0.484
Y188	**TK/Met/MST1R**	0.001	INSR	0.441
Y190	**TK/Src/SrcA/FGR**	7.142	EGFR	0.367
Y199	**TK/InsR**	14.375	INSR	0.408

Y222	**TK/PDGFR/CSF1R**	2.72E-4	Unsp	0.531

UBIQUATINATION

UbPred was utilized for the prediction of ubiquitination of BDNF. UbPred detects lysine residues as ubiquitinated when the value is equal to or above the 0.62 threshold. Ubiquitinations of lysine residues as predicted by UbPred are presented in Table 6.

Table 6: Ubiquitination scores for BDNF protein and their thresholds as predicted by UbPred software.

PEPTIDE	POSITION	SCORE	THRESHOLD
CMKAAPMKEANIRGQ	30	0.83	0.3
LESVNGPKAGSRGLT	58	0.99	0.3
EWVTAADKKTAVDMS	161	2.00	0.3
WVTAADKKTAVDMSG	162	1.68	0.3
GTVTVLEKVPVSKGQ	177	0.82	0.3
LEKVPVSKGQLKQYF	182	1.11	0.3
PVSKGQLKQYFYETK	186	1.08	0.3
EGCRGIDKRHWNSQC	209	0.83	0.3
ALTMDSKKRIGWRFI	232	0.42	0.3
CVCTLTIKRGR****	252	3.51	0.3

Gene-Gene Interaction

BDNF physically interacts with POU4F3, NTF4, NTF3, SORCS2, HAP1, SORT1, and NTRK2. It was co-expressed with NTF4, MMP3, and NGF. BDNF is co-localized with NTF4, ZNF274, and other proteins. It also has gene interaction with POU4F3. In pathways, BDNF interacts with ADAM17, MAP2K5, CASP6, ZNF274, SORT1, NTRK2, MMP7, GIPC1, DYNLT1, EHD4, PLG, and MMP3. BDNF shares a protein domain with NTF4, NTF3, and NGF respectively as out lined in figure 5.

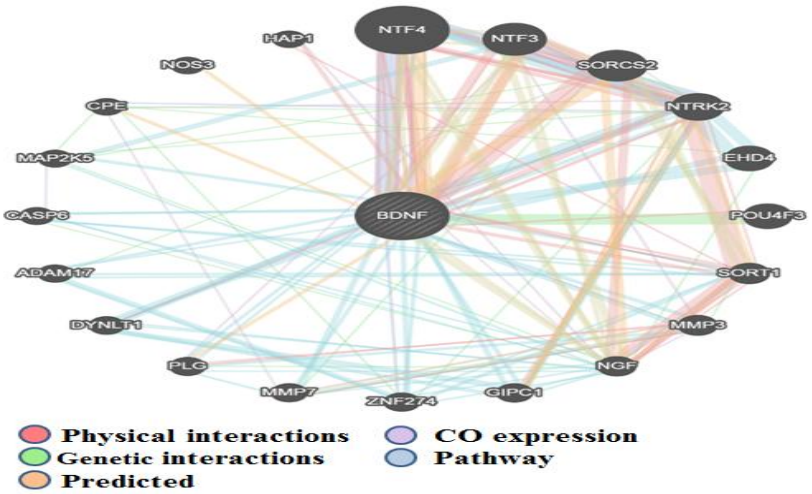


Figure 5: Gene-gene interaction network by GeneMania

Protein-Protein interactions

The STRING repository was employed to pinpoint the protein–protein network linked with BDNF. The analysis revealed a densely interlinked network comprising 11 nodes and 46 edges. The mean node angle was computed to be 8.36 °, along with a PPI enrichment value of 1.53e-12
and clustering coefficient of 0.922, as outlined in figure 6.

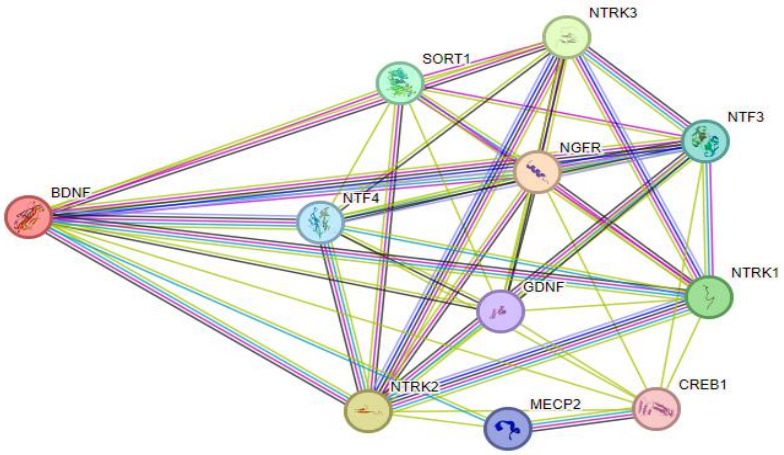


Figure 6: Protein-Protein interaction network by using the STRING tool

Docking Analysis

The original BDNF protein and its various forms were aligned with the closely associated NGFR protein, revealing significant distinctions between the interaction sites of the original type. The interference statistics for both the original and modified proteins are described in Figure 7 and Table 7.

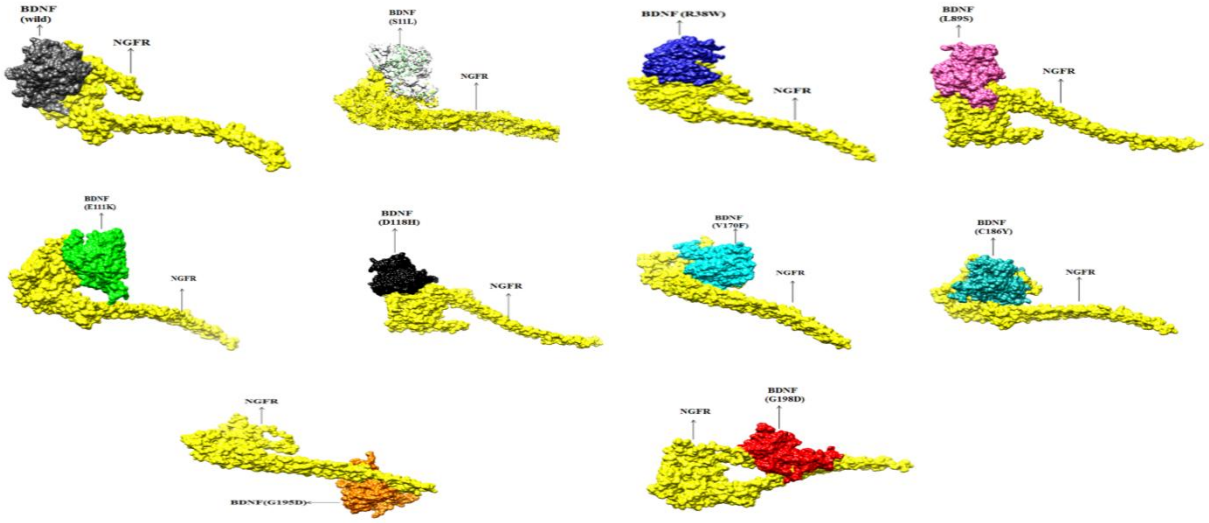


Figure 7: BDNF protein and its variants alignment with the closely associated NGFR protein

Table 7: Protein-to-protein molecular Docking of BDNF with the closely interacting NGFR protein

Docked protein	No. of interface residues	Interface area (Å²)	No. of salt bridges	No. of disulphide bonds	No. of hydrogen bonds	No. of non-bonded contacts
BDNF WILD NGFR	41 47	12084 12084	2	-	16	226
Ser11leu NGFR	39 38	1854 1904	4	-	32	280
Arg38trp NGFR	27 30	1525 1511	-	-	13	177
Leu89Ser NGFR	33 36	1488 1367	7	-	22	204
Glu111Lys NGFR	30 29	1725 1796	-	-	16	155
Asp118His NGFR	23 22	1225 1305	2	-	9	139
Trp147Arg NGFR	32 25	1205 1421	3	-	8	150
Val170Phe NGFR	51 56	2733 2656	6	-	40	441
Cys186Tyr NGFR	45 51	2358 2343	4	-	26	300
Gly195Asp	29	1416	2	-	17	231

NGFR	28	1434				
Gly198Asp **NGFR**	40 40	1902 1772	2	-	7	308

DISCUSSION

Non-synonymous single-nucleotide polymorphisms (nsSNPs) are among the most common genomic mutations associated with complex neurological diseases. SNPs located in coding regions of the genome are mainly harmful to a protein's structural and functional integrity, drawing considerable attention in current research. Modern medical research focuses on investigating the detrimental effects of nsSNPs, which have been implicated in various complex disorders.

In our study, we evaluated the functional impact of these nsSNPs by predicting whether they were neutral or disease-causing using a set of computational tools to analyze the available sequence and structural data. A thorough analysis of nsSNPs in BDNF was conducted using multiple computational techniques, enabling us to predict the functional consequences of these mutations with greater accuracy. To improve the precision of our predictions, we utilized tools from several categories, including those based on sequence, homology, consensus, and structural analysis. This comprehensive approach aims to enhance confidence in identifying potentially harmful nsSNPs. Initially, the SNPs were annotated using seven bioinformatics tools, including SNP and GO, Panther, SIFT, PHD-SNP, Snap2, Predict SNP, and PolyPhen, which distinguish disease-associated nsSNPs from neutral ones. Ten non-synonymous SNPs (Ser11Leu, Arg38Trp, Leu89Ser, Glu111Lys, Asp118His, Trp147Arg, Val170Phe, Cys186Tyr, Gly195A and Gly198Asp) were predicted to be significantly deleterious. For further validation, we selected the most detrimental nsSNPs and verified them using additional tools, such as the Ensembl genome browser 96, CADD, REVEL, Mutation Assessor, and Metal-R. CADD, REVEL, and Metal-R consistently identified these ten nsSNPs as harmful and damaging, while the Mutation Assessor flagged five of these SNPs as high-risk. The CADD scores for the nsSNPs were as follows: 24 for S11L, 31 for R38W, and 32 for G195D; 24 for L89S, 33 for E111K, 27 for W147R, and 27 for V170F; 32 for D118H, 33 for C186Y, and 33 for G198D. (A CADD score of 30 ranks the SNP among the top 0.1% of the most damaging SNPs in the human genome, while a score of 20 places it within the top 1%.). Previous studies have highlighted the importance of evaluating protein stability in understanding its functional performance, as a protein's structural integrity directly influences its function.

Alterations in protein stability can result in misfolding, degradation, abnormal accumulation, and, ultimately, loss of function. Among the ten identified protein variants, nine showed a reduction in protein stability according to the I-Mutant tool, and all exhibited decreased robustness based on Mupro software analysis. To confirm the accuracy of these predictions, we validated the results using the CupSat server, and the outcomes were consistent with those of I-Mutant and Mupro. We also employed ConSurf to evaluate the conserved regions of the BDNF protein, predicting the conservation status of each amino acid as conserved, buried, or functionally significant. Previous studies have demonstrated that highly conserved amino acids are crucial for maintaining proper protein functions. It is well established that the nsSNPs located in these conserved regions are often more harmful. Accordingly, our analysis focused on ten selected nsSNPs situated in these highly conserved areas. Our findings revealed that the amino acids at the

variant sites Ser11Leu, Arg38Trp, Trp147Arg, Cys186Tyr, Gly195Asp, and Gly198Asp were highly conserved, emphasizing their functional importance and sequence value. Moreover, mutation analysis using the HOPE server highlighted the effects of these amino acid substitutions on contact points and structural alignment. To generate the protein structures, we employed I-TASSER to provide FASTA sequences. The tool automatically selected the appropriate templates and performed protein modelling using 2nbiA and 2co9 templates, which exhibited 85% coverage and 83% identity. To assess the quality of the modeled protein structures, we calculated RAMPAGE values through Ramachandran plot analysis using the RAMPAGE server. Wild-type BDNF protein exhibited a RAMPAGE value of 81.2% for favored and allowed residues, with 18.8% classified as outliers. For the mutated sequences S11L, R38W, and L89S, the favored and allowed residues were 83.8%, 80.7%, and 85.4%, respectively, with outliers at 16.2%, 19.3%, and 14.6%. Similarly, the mutant residues E111K, W147R, and V170F showed favorable and allowed values of 84.8%, 81.7%, and 84.4%, respectively, with outliers at 15.2%, 18.3%, and 13.6%. Additionally, for D118H, C186Y, G195D, and G198D, the favored and allowed residues were 82.8%, 80.3%, and 86.3%, with outliers at 17.2%, 19.7%, and 13.7%, respectively. It is important to note that protein structures are regarded as high quality when their RAMPAGE values exceed 80. All four substitutions showed significantly elevated root mean square deviation (RMSD) values, suggesting that these nsSNPs have a profound negative impact on BDNF protein. Similarly, the nsSNP rs771341699 (R38W) displayed the highest RMSD value of 5.84 Å and a TM score of 0.3264, indicating a significant impact on the protein structure. In contrast, rs758638310 (D118H) exhibited the lowest RMSD value of 3.85 Å and a TM score of 0.7056, as shown in Table 4, suggesting a comparatively smaller effect on the protein structure. RMSD values greater than 2 Å indicated substantial structural differences between the mutant and wild-type forms. All the most damaging mutant structures had RMSD values exceeding 2 Å, implying that these nsSNPs may severely affect BDNF protein function. Post-translational modifications (PTMs) play a critical role in mediating essential activities, such as protein-protein interactions (PPIs) and cell signaling. Additionally, we analyzed alternative nsSNPs at seven locations predicted to undergo phosphorylation using GPS3.0 and NetPhos3.1, as summarized in Table 5. Furthermore, six nsSNPs were found at four potential ubiquitination sites, as determined using BDM-PUB. Our docking analysis suggests that these variants could influence the functionality of BDNF. Docking studies were performed using ClusPro software. These results indicated that mutations significantly affected the interaction between BDNF and NGFR proteins. For example, for the aligned PDB sequences of both proteins, the binding energy of the native BDNF-NGFR complex was -1280.0 kcal/mol. However, when evaluating the mutant sequences with NGFR, a notable reduction in the binding energy was observed. The interaction energies for various mutations were -1447.9 for Ser11Leu, -1379.0 for Arg38Trp, -1273.2 for Leu89Ser, -1390.3 for Glu111Lys, -1456.4 for Asp118His, -1408.9 for Trp147Arg, -1354.4 for Val170Phe, -1189.7 for Cys186Tyr, and -1452.2 both Gly195Asp and Gly198Asp. This difference in docking energies, due to mutations, disrupts the optimal function of the protein. Additionally, surface area analysis revealed that the wild-type aligned complex demonstrated greater stability than the mutant model. Based on these findings, we can predict that the mutations interfere with protein-protein interactions between BDNF

and NGFR, potentially impairing their functionality.

CONCLUSION

Altered expression of the BDNF gene plays a crucial role in the development of various neurological conditions, including anxiety, depression, and neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's, and cerebellar ataxia. In our investigation of 117 nsSNPs, ten NSPS (rs866172975, rs771341699, rs1590217373, rs751698045, rs758638310, rs1330439007, rs77787410, rs1852795747, rs1590215885 and rs780128716) were identified as highly pathogenic and harmful within the BDNF protein. At the same time, the remaining variants were predicted to be neutral or of low impact. Structural analysis of these variants revealed significant disruptions to the protein's structure and activity, indicating that these mutations likely impair the protein's proper functioning by altering its original 3D structure. These molecular changes are highly significant and require experimental validation and population-based studies to check for association of these SNPs with various neurological disorders.

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Concept and Design: AA, NK**; Data Retrieval and Analysis:** NH, WG, with inputs from NK, AA; **Manuscript drafting:** NH, WG, with inputs from NK, AA, MI**; Editing and Revision**: NH, WG, AA, MI, NK

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