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In silico Analysis of nsSNPs of Human *BDNF* Gene Using Bioinformatic Tools

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ABSTRACT *Brain Derived Neurotrophic Factor* (*BDNF*) plays a significant role in the synaptic plasticity and memory processes. Its contribution to the pathogenesis of cognitive symptoms in pathological situations has a direct impact on memory function in both healthy and diseased animals. For this purpose, computational tools (Provean with mutant score cut-off -2.5 and PolyPhen 2 score ranging from 0 to 1) have been used in this study to evaluate the deleterious or damaging effect of non-synonymous SNPs (nsSNPs) that are harmful to the structure and function of the *BDNF* gene. Results of the present study found a total of 50 highly deleterious SNPs by both the tools which could be disease susceptible.

INTRODUCTION

Neurotrophic factors are growth factors that induce the chemicals, which control the process of neurogenesis. Neurotrophic factors are found in brain and periphery which include growth factors for nerves, brain-derived neurotrophic factor, neurotrophin-3 (Maisonpierre et al. 1990) and neurotrophin-4/5 (Hallbook et al. 1991; Ip et al. 1992) belonging to neurotrophic family**.** Being the second member of the neurotrophic family, *Brain-derived neurotrophic factor (BDNF)* gene encodes proteins that are vital for the growth, maintenance, and survival of neurons. It is responsible for the production of BDNF protein, which further regulates the neuron survival, development, mood modulation, synapse formation, balance in neurotransmitters, and neuroprotection (Sakhawat et al. 2023). *BDNF* was discovered and isolated from pig brain by Yves-Alain Barde and Hans Thoenen in 1982 (Barde et al. 1982). In brain, BDNF is active in hippocampus, cortex and cerebellum. It stimulates the process of neurogenesis, that is, growth of new neurons from neural stem cells (Zigova et al. 1998). It plays an important role in neuronal plasticity which is essential for learning and memory. Moreover, it is also considered important energy homeostasis. It is expressed in some parts of the brain as well as in kidney, saliva, motor neurons and skeletal muscle. It has a similar structure to NGF.

BDNF gene encoded the 247-amino acid secretory protein known as BDNF, is located on chromosome 11q14.1 (Bateman et al. 2017). BDNF, a precursor protein (pro-BDNF) is synthesized in an endoplasmic reticulum. Molecular weight of this precursor protein is 32-35 kDa. After that, BDNF moves through the Golgi apparatus and trans-Golgi network. In the presence of carboxypeptidase E (CPE), pro-BDNF is sorted into vesicles and with the help of postsynaptic dendrites transported into activity dependent secretion. Then, pro-BDNF changes into mature BDNF with the help of protein convertase enzyme because the convertase enzyme cleaves the terminal domain of pro-BDNF. Mature BDNF is biologically active and the molecular weight of mature BDNF is 13kDa (Bothwell 1995).

There are multiple stimulations that may alter gene expression of *BDNF* in pathological and physiological states (Lindholm et al. 1994). The stimulation of light enhances the BDNF mRNA in visual cortex (Castren et al. 1992) and osmotic stimulation also leads to increase in BDNF mRNA in the hypothalamus (Castren et al. 1995; Dias et al. 2003). Moreover, physical exercise also increases NGF and BDNF mRNA in the hippocampus (Neeper et al. 1995).

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BDNF is responsible for promoting different functions by interacting with different cells via the TrkB receptor, as it controls differentiation, survival and functioning of neurons by interacting with the TrkB receptor that is present on neurons. It also initiates the haematopoiesis by interacting with the TrkB receptor on haematopoietic precursor cells (HPCs). Angiogenesis also depends on BDNF in interaction with TrkB that is expressed in endothelial cells (ECs).

As *BDNF* gene has a vital role in development of CNS and for neuronal plasticity, it is commonly implicated in psychiatric disorders and neurodegenerative disorders due to the dysregulation of BDNF (Greenberg et al. 2009; Autry and Monteggia 2012). Although many disorders such as anxiety disorders, major depression, Alzheimer's disease and Parkinson's disease are associated with the Val66Met mutation or rs6265, the most common SNP in the *BDNF* gene (Nguyen et al. 2023). It is a point mutation that takes place in the coding sequence of *BDNF* gene in which guanine is replaced by adenine at position 196, leading to amino acid switch-exchange of valine to methionine at codon 66 (Sheikh et al. 2010). *BDNF* gene contains several SNPs such as rs66866077, rs7103411, rs2203877, rs540912088 and many more.

Neuropsychiatric disorders are characterised by cognitive, behavioural and psychological problems due to several endocrine and metabolic disorders and due to pathological and structural conditions in grey matter. Neurodegenerative diseases are also triggered by the degradation of specific brain regions, such as neurons (Nguyen et al. 2023). Additionally, it has been projected that over half of the people in middle- and high-income nations will experience at least one psychiatric illness during their lifetime. According to Trautmann et al. (2016), the economic cost of mental disorders is comparable to that of cardiovascular diseases and even higher than that of cancer, diabetes, and chronic respiratory diseases. The estimated cost of these diseases between 2011 and 2030 is USD 16.3 trillion dollars.

The recent high throughput techniques are used to investigate the inheritance of a disease on the basis of genetics. In this way, these techniques are able to yield a large number of pathogenic sequence alterations (Tennessen et al. 2012; Purcell et al. 2014). Reliable strategies have allowed the researchers to choose which variations are vital. Investigators always focus on nonsynonymous single nucleotide variants (nsSNVs), which are damaging in nature as compared to synonymous variants (Hindorff et al. 2009; Kiezun et al. 2012; MacArthur et al. 2012). nsSNPs are present in coding regions of DNA and lead to variations in the phenotypes between the individuals. Several *BDNF* gene polymorphisms present in the upstream regulatory region are reported to be associated with different diseases like type 2 diabetes, obesity, bipolar disorder, and Parkinson's Disease in different populations. Among these, a few important ones are rs6265, rs925946, rs4074134, rs492346, rs2030324. But knowledge about the clinical relevance for many of the *BDNF* SNPs is still limited. The present study explored the understanding of the association between the genetic variations and phenotypic effect using *in silico* tools such as Provean and PolyPhen-2 tools.

Objective

In the present study, screening of nsSNPs was done to identify the most deleterious or damaging nsSNPs in the human *BDNF* gene, thereby predicting functional partners of BDNF protein using bioinformatic tools, which provide a better understanding of their properties for therapeutic targets.

METHODOLOGY

All the information and sequences of *BDNF* gene were retrieved from OMIM (OMIM ID: 113505), UniProt (UniProt ID: P23560) and PubMed databases.

Retrieving nsSNPs

From the National Centre for Biotechnology Information (NCBI) dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP), the information of SNPs of *BDNF* gene including SNP ID, protein accession number, location and residue alteration was retrieved. The three types of SNPs found in the coding area are synonymous, non-synonymous, and frame-shift. While non-

synonymous SNPs alter the protein sequence, synonymous SNPs have no effect on the protein sequence. Hence, non-synonymous SNPs have been retrieved.

Retrieving Sequence

A bioinformatic tool Uniprot (www.uniprot.org) was used to retrieve the FASTA sequence of *BDNF* gene that codes for the BDNF protein.

Identifying the Most Deleterious SNPs

Two distinct bioinformatics tools that is Provean (Protein Variation Effect Analyser) (https://provean.jcvi.org/) and PolyPhen 2 (Polymorphism Phenotyping v2) (https://genetics.bwh.harvard.edu/pph2/) were used to identify the most deleterious SNPs collected from dbSNP database.

The Provean Web Server gives the pre-computed prediction for huge sets of genome wide nucleotide or amino acid variants for both human and mouse. A free online computational tool called PROVEAN can determine whether changing an amino acid would affect a protein's biological function. In order to find nonsynonymous or indel variations that are anticipated to be functionally significant, PROVEAN is helpful for filtering sequence variants.

For prediction of missense mutation, FASTA sequence of the gene of which the SNPs were to be predicted and the position of variant at which substitution takes place were filled in the tool. PROVEAN is a web server that uses sequence homology to predict the functional effect of an amino acid substitution. Results are given as "deleterious" or "neutral", according to scores retrieved and the cutoff value of PROVEAN is set at -2.5. Substitutions of amino acids that exceed the cut off value were considered as deleterious.

Using simple physical and comparative considerations, PolyPhen-2 predicts the potential effects of an amino acid change on the structure and function of a human protein based on sequence homology and knowledge of 3D structures. Enter a protein identifier in the "protein or SNP identifier" text box. Place the "protein sequence in FASTA format" in a different text box of your choosing, and then type the protein sequence's substitution location in the Position text box. Next, pick the right boxes for the replacement residue AA2 and the wild-type (query sequence) amino acid residue AA1. The results are classified as "benign" for score 0.0, "possibly damaging", "probably damaging", or "unknown". Prediction was based on the number of features comprising sequence, phylogenetic and structural information characterising the substitution. PolyPhen-2 score was ranging from 0.0 (Benign) to 1.0 (Damaging) and prediction outcome could be one of Probably Damaging, Possibly Damaging and Benign (Adzhubei et al. 2013).

RESULTS

SNP Annotation

The *BDNF* gene is located on the 11th chromosome short arm in region p$^{13-14}$. The gene locus of human *BDNF* comprises 11 exons and distinguishing promoters. It encodes for BDNF protein, which is a secretory growth factor supporting the survival of neurons and promoting the synaptogenesis and differentiation of new neurons (Park and Poo 2013). SNPs can be defined as the variation in the DNA sequence, which takes place when a single nucleotide present in the genome varies between paired chromosomes in an individual. SNPs may occur within either coding sequence or non-coding sequence. SNPs in the coding region are categorised into two types, that is, Synonymous and Non-synonymous SNPs. Nonsynonymous SNPs can change the sequence of amino acids in protein.

SNP Annotation

dbSNP database showed a total of 24,143 SNPs from which 23,072 were intronic variants, 940 non-coding transcript variants, 127 synonymous variants and 241 were missense SNPs (nsSNPs). Only 241 nsSNPs from the BDNF gene were used for this study.

Identification Of Deleterious nsSNPs Using Provean

Out of 241 nsSNPs, a total of 52 were predicted to be deleterious SNPs whereas 189 were found to be neutral according to the default

threshold score given by Provean, that is, -2.5 (Table 1). The predicted deleterious nsSNPs include rs66866077, rs1048221, rs66866078, rs766646640, rs769259549, rs780128716, rs1253363411, rs1286344063, rs1330439007, rs1564939224, rs1590215885, rs1048220, rs751074725, rs765994816, rs1302619105, rs1333565866 and rs1302619105.

Table 1: Comparison of number of nsSNPs predicted to be neutral and deleterious with Provean and Polyphen 2

Deleterious status	*Provean*	*Polyphen 2*
Neutral	189	60
Possibly damaging	0	42
Damaging	52	139

Identification Of Deleterious nsSNPs Using PolyPhen-2

Out of 241 nsSNPs, 60 were predicted to be benign, whereas 42 as possibly damaging and 139 were predicted as probably damaging with mutant score near or equal to 1.000 (Table 1). PolyPhen-2 uses empirically derived rules to predict the nsSNP as probably damaging, that is, it is with high confidence supposed to affect protein function or structure, possibly damaging, that is, it is supposed to affect protein function or structure and benign, most likely lacking any phenotypic effect. The most damaging (with mutant score 1.000) predicted nsSNPs include rs8192466, rs955482595, rs1048221, rs373221153, rs374758100, rs540912088, rs751074725, rs752449726, rs754061487, rs754270781, rs755151584, rs758638310, rs761800998, rs765994816, rs766646640, rs769259549, rs769727156, rs771341699, rs773773442, rs774229943, rs774985498, rs776885796, rs780128716, rs780865517, rs868827437, rs1045077557, rs1049779568, rs1175628249, rs1181485648, rs1184013173, rs1205815689, rs1211387366, rs1253363411, rs1286344063, rs1302619105, rs1330439007, rs1333565866, rs1339220115, rs1349681470, rs1415210991, rs1474254687, rs1564941169, rs1564961314, rs1590215885, rs78675155, rs1852776284, rs1852800024, rs1852805392, rs1852851466, rs1855761032, rs1855795619, rs1859863113, rs1859865525 and rs1852788290.

The list of most deleterious and damaging SNPs of *BDNF* gene which were common by using both the tools were selected as enlisted (Table 2).

Table 2: Highly deleterious nsSNPs

S.	*No.*	*PROVEAN mutant value*	*Polyphen2 score*
1	rs66866077	-5.510	0.999
2	rs1048220	-4.595	0.999
3	rs1048221	-4.234	1.000
4	rs201468555	-2.521	0.907
5	rs751074725	-4.757	1.000
6	rs754061487	-3.489	1.000
7	rs755151584	-2.751	1.000
8	rs758638310	-2.833	1.000
9	rs759156741	-3.141	0.628
10	rs761800998	-3.190	1.000
11	rs765357564	-2.663	0.998
12	rs765994816	-5.246	1.000
13	rs766646640	-4.482	1.000
14	rs767394378	-3.145	0.973
15	rs769259549	-4.282	1.000
16	rs774985498	-2.850	1.000
17	rs780128716	-6.431	1.000
18	rs868827437	-3.913	1.000
19	rs1163567412	-3.026	0.990
20	rs1184013173	-3.669	1.000
21	rs1200786887	-2.506	0.941
22	rs1253363411	-5.105	1.000
23	rs1255286595	-3.664	0.996
24	rs1286344063	-4.460	1.000
25	rs1302619105	-7.728	1.000
26	rs1322699219	-2.638	0.999
27	rs1330439007	-12.909	1.000
28	rs1333565866	-4.541	1.000
29	rs1339220115	-2.840	1.000
30	rs1349681470	-2.870	1.000
31	rs1356064775	-2.749	0.991
32	rs1415210991	-3.535	1.000
33	rs1430193004	-3.118	0.998
34	rs1459327095	-3.649	0.999
35	rs1480364113	-2.649	0.999
36	rs1564941169	-1.321	1.000
37	rs1590215885	-5.966	1.000
38	rs1590217373	-3.000	0.891
39	rs1852795747	-10.210	0.992
40	rs1852776284	-3.544	1.000
41	rs1852785504	-3.144	1.000
42	rs1852791032	-3.516	0.994
43	rs1852797020	-3.721	0.997
44	rs1852800024	-2.828	1.000
45	rs1852801336	-3.567	0.966
46	rs1852831233	-2.596	0.999
47	rs1855795619	-3.161	1.000
48	rs1859617916	-3.084	0.999
49	rs1859865525	-2.554	1.000
50	rs1852788290	-3.327	1.000

Gene-Gene Interaction

Further, STRING, a resource method for retrieving interacting proteins, was used to predict the interactions of respective genes or proteins with other proteins. The string-db.org web server can be found at https://string-db.org/. (Szklarczyk et al. 2011). The results predicted

from the STRING identified high functional association of BDNF protein with NGFR (tumour necrosis factor receptor), SORT1(Sortilin), NTRK2 (BDNF/NT-3 growth factors receptor) (Fig. 1).

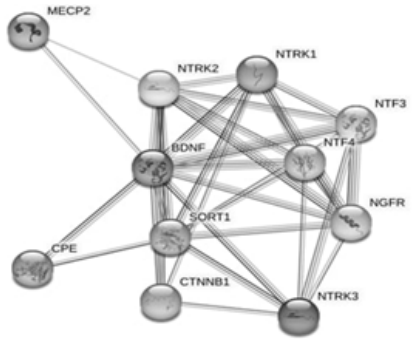

Figure 1: Gene-Gene interaction of BDNF by STRING showing major interaction with NGFR, SORT1 and NTRK2

DISCUSSION

Understanding the impact of specific gene or protein mutations on physiological functioning leading to any disease or disorder is a critical area of genetic research. SNPs are essential for understanding the genetic basis of human diseases. On the other hand, finding working SNPs remains a major challenge. There are many diseases and disorders that are associated with BDNF, especially neurodegenerative and psychiatric disorders as mentioned by Cuzik et al. (2015) and Murray et al. (2018). A genome-wide association study by Poll and Grzywacz (2017) has not yet discovered any conclusive link between *BDNF* gene variation and psychiatric illnesses but this might be due to the mounting evidence that psychiatric disorders are complicated diseases caused by a number of common mutations in several vulnerability genes, each of which has a negligible overall impact. The *Brain-Derived Neurotrophic Factor (BDNF)* gene acts on the central nervous system and peripheral nervous system and plays a vital role in promoting the survival of neurons and their growth, maturation and maintenance. *BDNF* codes for a protein that is important for learning and memory. An *in silico* study done by De Oliveira et al. (2019) used bioinformatic tools such as SIFT, PolyPhen-2 and found V66M mutation as deleterious that lead to many disorders including psychiatric disorders. When the level of BDNF is decreased, many neurodegenerative disorders are developed such as Parkinson's disease (Scalzo et al. 2010), Huntington's disease (Mughal et al. 2011) and Schizophrenia (Green et al. 2011). Shan et al. (2024) recently confirmed the linkage of V66M mutation with the reduced BDNF function, which ultimately affected the neural plasticity and mood. The study found that when BDNF activity is decreased, the brain may have a harder time adjusting to stimuli, which suggests that people with the V66M mutation may be more vulnerable to the negative effects of stress. It is now clear that this mutation is linked to a higher prevalence of mood disorders like sadness and anxiety. Furthermore, for those with the V66M mutation, stress might exacerbate these symptoms. The FoldX prediction indicated that the mutation had no effect on BDNF stability (ddG = -0.04 kcal/mol), however the I-Mutant prediction indicated that the V66M mutation decreases protein stability (ddG = -0.49 kcal/mol). The discordance shown by Capriotti et al. (2005) and Schymkowitz et al. (2005) in these outcomes could perhaps stem from the distinct methodologies employed by the FoldX and I-Mutant algorithms for forecasting and categorising mutations. Sakhawat et al. (2023) also prospected the inhibitory effect of many compounds including vitamin D and quercetin on human BDNF V66M variant. Moreover, the molecular docking and simulation results of Quercetin showed that it has more potent inhibitory binding effect on *BDNF* variant (V66M), which can be more acceptable as anti-stress agent. Quercetin was also subjected for *in silico* analysis and favourable results were observed for pharmacokinetic and toxicological profiles. The PolyPhen-2 was utilised by Shan et al. (2024) to evaluate the functional consequences of the Val66Met mutation. With a score of 0.822, PolyPhen-2 determined that the mutation would probably be harmful. Consequently, the likelihood that the mutation will impact the protein's functionality is increased. PolyPhen-2's 0.93 specificity and 0.84 sensitivity ratings for the prediction provide more proof of its exceptional accuracy in identifying potentially harmful changes.

It is important to notice that 50 novel nsSNPs were found in the current study that might affect the protein structure and function using both the tools. To the best of the researchers' knowledge, more than 100 variants of the *BDNF* gene were already known to be associated with many diseases and these findings were not the same as observed in the present study. Already known SNPs associating with diseases include rs10835210, which is responsible for schizophrenia (Zhang et al. 2016), rs11030101 for depression (Zeilinger et al. 2009) and bipolar disorder (Pae 2012), rs11030103 for depression and suicide (Licinio et al. 2009), rs11030104 for panic attacks (Huang et al. 2007), bipolar disorder and Alzheimer's disease, rs11030107 for bipolar disorder (Sears et al. 2011), rs12273539 for major depressive disorder (Hing et al. 2012) and bipolar disorder, rs12273539 for depression and suicide, rs16917237 for bipolar disorder (Yinli et al. 2013), rs2049045 for bipolar disorder, rs28722151 for depression and suicide, rs41282918 for depression and suicide, rs6265 for major depressive disorder, Alzheimer's disease, Parkinson's disease and depression (Guo et al. 2018), rs7103873 for depression (Liu et al. 2014), rs8192466 for schizophrenia, rs962369 for depression and suicide (Mirkovic et al. 2016), and rs988748 for bipolar disorder and depression (Szczepankiewicz 2013) were observed in the literature but there was still a paucity of literature to confirm all the possible variants or SNPs.

The relevance of the *BDNF* variations identified in this study has to be confirmed because they have never been reported in relation to *in silico* analysis. Through laboratory experiments, these findings might aid researchers in understanding how *BDNF* SNPs contribute to illness vulnerability.

CONCLUSION

The present paper suggests that in the *BDNF* gene, out of 241 SNPs, 50 SNPs were found to be highly deleterious using Provean and PolyPhen-2. As a result, these nsSNPs can be strongly considered as key candidates in causing diseases associated with BDNF dysfunction, which will aid in drug discovery and development.

RECOMMENDATIONS

To unravel the potential variants leading to various diseases, it becomes pertinent to conduct large-scale genetic studies or population based genetic studies. So, the prediction of deleterious SNPs is still required using *in silico* analysis, which is important to sort out before planning a larger population study. Further studies on different bioinformatic tools are suggested to be done to validate the current results. Moreover, to investigate the effects of these polymorphisms on protein structure and function, more experimentation is required.

AVAILABILITY OF DATA AND MATERIAL

The datasets and bioinformatic tools used in the current study are available online.

CONFLICTS OF INTEREST

None

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None

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