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Computational Predictions of Functional Single Nucleotide Polymorphisms in the Human Nerve Growth Gene

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Abstract

Nerve Growth Factor (NGF) is a fundamental neurotrophin that plays a pivotal role in neuronal growth and survival, and is relevant to several diseases, including cancer. Single-nucleotide polymorphisms (SNPs), particularly non-synonymous SNPs (nsSNPs), can potentially alter protein function by changing its structure and stability. However, their impact on the NGF gene has been poorly characterized. We aimed to identify and characterize functionally impactful nsSNPs in NGF gene using computational tools. We retrieved nsSNPs from the NCBI dbSNP database and utilized various computational tools, including SIFT, PolyPhen-2, PredictSNP, PhD-SNP, PANTHER, and SNAP2, to predict their deleterious properties. I-Mutant 2.0, Project HOPE, Missense3D, and MutPred2 were utilized to assess the effects of mutations on protein stability and structure. The ConSurf server was used to determine the evolutionary conservation of high-risk residues. Besides, CScape, OncoVar, and canSAR Black databases predicted the oncogenic potential. Of the 268 nsSNPs analyzed, 36 were expected to be deleterious, with 32 contributing to NGF instability. Structural modeling showed drastic structural rearrangements of the critical residues, especially Q172R (rs1557933464) and Q172E (rs746593757), with enhanced flexibility and disruption of hydrogen bonding. ConSurf analysis revealed significant changes to the three-dimensional conformation of NGF, primarily in highly conserved regions, as determined by structural modeling using Phyre2 and SWISS-MODEL. We identified Q172E (rs746593757), E132K (rs772557857), V230A (rs767703003), and W142R (rs929155379) as putative oncogenic variants. All of these nsSNPs are within functional domains of the protein and may affect NGF-mediated signaling pathways. This study represents the first extensive in silico screening of deleterious nsSNPs within the NGF gene, suggesting their potential linkage to cancer. Our study highlights the utility of such computational predictions in prioritizing high-risk genetic variants; however, further experimental validation is needed to establish their role in disease pathogenesis and to assess their potential for therapeutic targeting.

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Introduction

Single-nucleotide polymorphisms (SNPs) are common variations in the human genome, constituting the predominant form of genetic polymorphism, with approximately 90% of all DNA polymorphisms being SNPs (Dabhi and Mistry 2014). These variations occur due to single-base mutations in DNA and are extensively cataloged in publicly available databases such as dbSNP, GWAS Central, and SwissVar. Non-synonymous SNPs (nsSNPs), also known as missense variants, are significant because they alter the amino acid sequence of translated proteins. nsSNPs are believed to significantly contribute to the functional diversity of coded proteins in human populations and have been associated with numerous diseases (Dakal *et al.*, 2017). They can impact protein function by altering protein solubility, destabilizing protein structure, and affecting gene regulation through changes in transcription and translation processes (Ajith and Subbiah, 2023).

Numerous studies have employed in silico tools to predict the structural and functional consequences of nsSNPs on various proteins, revealing associations with a wide range of diseases. For instance, investigations into ABCA1 polymorphisms have uncovered associations with familial hypoalphalipoproteinemia and Tangier disease, while studies on CYP27B1 polymorphisms have linked them to vitamin D deficiency (Marín-Martín *et al.*, 2014; Rotimi *et al.*, 2018). Similarly, research efforts have elucidated the roles of nsSNPs in mental disorders, congenital cataracts, rheumatoid arthritis, steroid-resistant nephrotic syndrome, and breast cancer (Akhtar *et al.*, 2021; Desai and Chauhan, 2016; Joshi *et al.*, 2015; Rajasekaran *et al.*, 2007; Zhang *et al.*, 2020).

Despite the extensive exploration of nsSNPs in various proteins, there has been no computational analysis of damaging nsSNPs in nerve growth factor (NGF) to date. NGF, as the first member of the neurotrophin family, plays a pivotal role in the development and maintenance of neurons in both the peripheral nervous system (PNS) and the central nervous system (CNS) (Aloe *et al.*, 1997). It is highly conserved nature across different species underscores its significance (Hallböök, 1999). NGF exerts its biological effects through binding to the tropomyosin kinase receptor A (TrkA), a typical tyrosine kinase receptor (Huang and Reichardt, 2003).

Several polymorphisms have been identified in the regulatory and exon regions of the NGF gene. For instance, the -198C>T polymorphism (rs11102930) in the promoter region has been associated with multiple sclerosis, childhood IgA neuropathy, and asthmatic disease (Akkad *et al.*, 2008; Hahn *et al.*, 2011; Szczepankiewicz *et al.*, 2012). This polymorphism alters NGF expression by modifying the binding sites for transcription factors such as vitamin D receptor (VDR) and specificity protein 1 (Sp1). Another relevant polymorphism is +273C>T (rs6330) in the 3rd exon, leading to the substitution of alanine with valine at position 35 of the peptide. This substitution modifies the tertiary structure of the protein, affecting signaling pathways and potentially influencing disease susceptibility, as observed in multiple sclerosis and Alzheimer's disease (Akkad *et al.*, 2008; Hahn *et al.*, 2011; Lester *et al.*, 2012; Nagata *et al.*, 2011; Szczepankiewicz *et al.*, 2012). In addition, a mutation in the NGF (−198C>T, Ala35Val) is associated with liver function in different histopathological profiles of patients with chronic viral liver disease in the Brazilian Amazon (Pereira *et al.*, 2020). Given the crucial role of NGF in various diseases and the potential impact of nsSNPs on its function, we aim to utilize computational tools to identify novel polymorphisms and assess their influence on the NGF protein structure, thereby providing insights into their role in disease susceptibility.

Materials and Methods

Retrieval of NGF nsSNPs

Information regarding SNPs, such as SNP ID, residue substitution, and worldwide minor allele frequency (MAF) (Bhagwat, 2010), was retrieved from the National Center for Biotechnology Information (NCBI) SNP database (https://www.ncbi.nlm.nih.gov/snp). The desired protein FASTA sequence was also downloaded from the protein database (https://www.ncbi.nlm.nih.gov/protein/). In the analysis, 268 human NGF nsSNPs were sorted and analyzed.

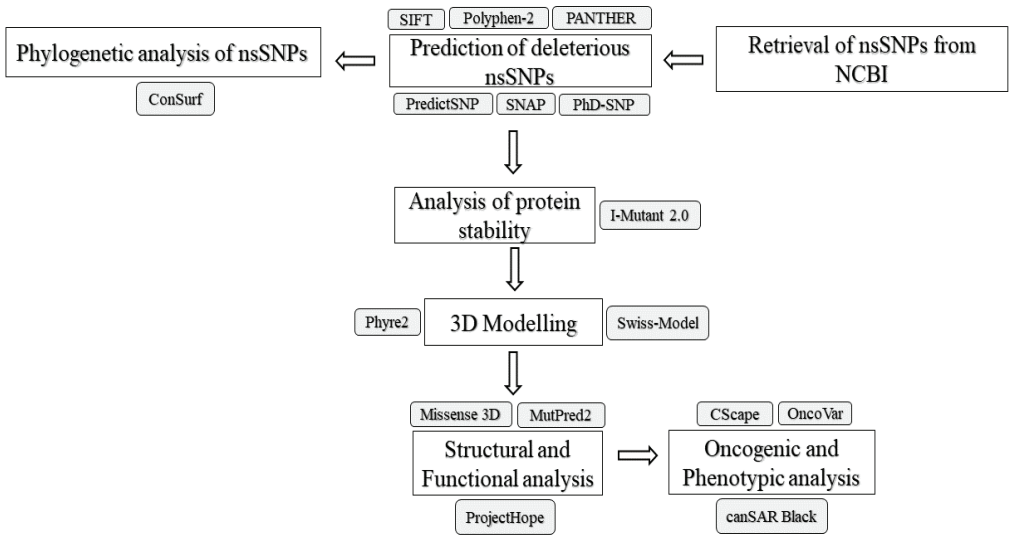

Figure 1. A flowchart depicting the study plan

Determination of the deleterious SNPs in the NGF gene

Six different computational tools: SIFT (Kumar *et al.*, 2009), Polyphen-2 (Adzhubei *et al.*, 2010), PrectSNP (Bendl *et al.*, 2014), PhD-SNP (Capriotti and Fariselli, 2017), PANTHER (Thomas *et al.*, 2006), and SNAP2 (Bromberg and Rost, 2007) web server were utilized. These algorithms predicted the functional effects of nsSNPs extracted from the SNP database. Alteration of an amino acid at a particular position with a probability <0.05 was recognized as deleterious, while alteration with a probability >0.05 was recognized as tolerated (Khokhlatchev *et al.*, 2002).

Analysis of the impact of nsSNPs on NGF stability

I-Mutant 2.0. (Capriotti *et al.*, 2005), a support vector machine-based predictor of changes in protein stability following single-point mutations in the protein structure was employed. Using the sequence information, the change in Gibbs free energy (ΔΔG) and its sign were predicted for the single-point mutations. The input data of 34 nsSNPs of NGF was provided in FASTA format.

Prediction of the impact of nsSNPs on protein 3D modelling

Two distinct online tools, including SWISS-MODEL (Waterhouse *et al.*, 2018) and Phyre2 (Kelley *et al.*, 2015), were used to build the 3D models of both the wild-type and mutant proteins. Both modeling tools developed a template based on the confidence score and sequence identity. Then, the wild-type and mutant protein structures were compared using the TM-align tool (Zhang and Skolnick, 2005), which measures the root mean square deviation (RMSD) and the template modeling-score (TM-score). The TM-score gives a result between 0 and 1, with 1 signifying a perfect structural match. On the other hand, higher RMSD implies a greater degree of dissimilarity between wild-type and mutant structures.

Prediction of the structural impact of nsSNPs on NGF protein

The HOPE (Venselaar *et al.*, 2010) web server was used to help understand the impact of nsSNPs on protein structure. HOPE is a user-friendly web-based server that examines how a point mutation in a protein sequence affects its structure. Additionally, Missense 3D (Ittisoponpisan *et al.*, 2019) and MutPred2 were employed to ensure the accuracy and rigor of the results. Missense 3D predicts the structural alterations induced by an amino acid change. MutPred2 is an online program that predicts a protein's pathogenicity based on modifications

to its amino acid composition. The output of MutPred2 includes a general score (g) indicating the probability that the amino acid substitution is pathogenic. This score is derived from the average of scores generated by all neural networks within MutPred2. Interpreted as a probability, a threshold of 0.50 for this score would suggest the likelihood of pathogenicity.

Structural alignment and visualization using chimera

To gain more insights into the structural effects of nsSNPs in NGF, structural alignment and visualization were performed using UCSF Chimera. Wild-type NGF and mutant NGF 3D models, which were previously created using Phyre2 and SWISS-MODEL, were imported into Chimera for superimposition and analysis of conformational deviation. Structures were aligned (based on backbone atoms; Cα, C, N) using the MatchMaker tool, and corresponding root-mean-square deviations (RMSD) were computed to represent corresponding structural deviations. Colors indicated regions with significant conformational shifts. The stability changes were evaluated through additional hydrogen bond and secondary structure analyses.

Phylogenetic analysis of SNPs in the NGF gene's conserved region

The ConSurf web-server (consurf.tau.ac.il/) was employed to identify evolutionarily conserved regions within NGF (Ashkenazy *et al.*, 2016). Following submission of the NGF FASTA sequence to ConSurf, homologs were aligned, and position-specific scores were computed utilizing an empirical Bayesian algorithm. Conservation scores were then used, along with a coloring scheme, to predict conserved regions. These regions were categorized into distinct scales comprising nine grades.

Prediction of the association between nsSNPs and cancer susceptibility

To determine the oncogenic potential of the selected point mutations, CScape (http://cscape.biocompute.org.uk/), OncoVar (https://oncovar.org/), and canSAR Black (https://cansarblack.icr.ac.uk/) were used. CScape depends on a statistical approach to identify cancer-driving mutations with a 91% balanced accuracy in coding regions of the genome (Rogers *et al.*, 2017). Predictions are given as p-values in the range of 0 to 1. Values above 0.5 are predicted to be oncogenic, while those below 0.5 are predicted to be neutral or benign. OncoVar is a platform to systematically prioritize the oncogenic ability of somatic mutations and cancer genes (Wang *et al.*, 2020). CanSAR Black is a multidisciplinary knowledge-based tool for translational cancer research (Bulusu *et al.*, 2014). The entire study design, including the various tools used, is shown in Figure 1.

Results and Discussions

Selected deleterious nsSNPs in the NGF gene

We retrieved NGF nsSNPs from the NCBI SNP database and obtained 268 missense variants. A detailed structural analysis was performed on the SNPs, utilizing six different in silico prediction tools to identify potentially harmful SNPs. Among these 36 nsSNPs (including E132K, V163M, Q172R, I223F, and Q172E) were predicted as deleterious mutations. A summary of these findings is presented in Table S1.

Validation of the deleterious effect of selected nsSNPs

Impact of nsSNPs on Protein Stability: Among the 36 mutants, 34, including E132K, V163M, Q172R, I223F, and Q172E, were found to destabilize the NGF protein, as analyzed by I-Mutant 2.0. In Table 1, the server-predicted free energy change (ΔΔG) values are reported along with their respective signs. Based on I-Mutant 2.0, a ΔΔG value of less than -0.5 kcal/mol is indicative of substantial protein destabilization caused by the corresponding mutation.

Impact of nsSNPs on Protein Structure: Each of the 34 deleterious nsSNPs was individually introduced into the native sequence of the template, resulting in the creation of 3D predictions for each mutant. Based on TM and RMSD scores, the TM-align tool determined the structural similarities between the wild-type and mutant models. The TM-scores of 11 nsSNPs were lower than those of 23 nsSNPs, and their RMSDs were higher than those of the others,

indicating that the differences between the wild-type and mutant models were more pronounced among the 34 mutant models.

Thus, we further investigated these 11 non-synonymous SNPs. In addition, the 3D structures of the 11 nsSNPs of the NGF protein mentioned earlier

Table 1. Impact of nsSNPs on protein stability

No	SNP ID	AA change	I-mutant	RI	DDG- free energy change value (Kcal/mol)
01	rs11466112	R221W	Decrease	6	-0.53
02	rs149823633	A149T	Decrease	1	-0.15
03	rs760753923	G188R	Decrease	9	-1.18
04	rs772557857	E132K	Decrease	7	-2.04
05	rs774878867	V163M	Decrease	8	-0.33
06	rs777582167	T177I	Decrease	4	-1.25
07	rs1326012011	T227M	Decrease	5	-0.01
08	rs1421686319	R130G	Decrease	7	-3.44
09	rs1432643322	R221L	Increase	4	0.26
10	rs1553234715	R224W	Decrease	7	-0.31
11	rs1557933464	Q172R	Decrease	5	-1.14
12	rs1653491943	L211P	Decrease	6	-1.70
13	rs2101018470	G191V	Decrease	8	-1.62
14	rs386518340	R221W	Decrease	6	-0.53
15	rs34542615	D226G	Decrease	5	-1.58
16	rs80277627	I223F	Decrease	9	-2.32
17	rs746593757	Q172E	Decrease	0	-0.72
18	rs755599709	V135A	Decrease	10	-2.67
19	rs758273427	V143A	Decrease	10	-2.40
20	rs767703003	V230A	Decrease	10	-2.59
21	rs773565185	V157M	Decrease	8	-0.59
22	rs777529073	T212S	Decrease	4	-0.23
23	rs929155379	W142R	Decrease	8	-2.36
24	rs1210460945	R190Q	Decrease	9	-2.28
25	rs1216943127	A210T	Decrease	3	-0.59
26	rs1319049263	A210V	Increase	4	0.21
27	rs1342067992	W197R	Decrease	9	-1.92
28	rs1384874552	R224L	Decrease	7	0.23
29	rs1474548348	C231W	Decrease	5	-0.27
30	rs1653489641	V230M	Decrease	8	-1.22
31	rs1653495717	F175V	Decrease	9	-3.15
32	rs1653500718	G154S	Decrease	7	-1.23
33	rs2101018564	E176A	Decrease	9	-2.09
34	rs2101018775	W142L	Decrease	6	-0.93

were analyzed using SWISS-MODEL. Significant differences were found in solvation and torsion values between the wild-type and mutant structures. In particular, the wild-type structure gave solvation and torsion values of -1.17 Å² and 0.14°, respectively, while those for the mutant structures differed. We used the Project HOPE Server to visualize the effect of point mutations on the structure of the NGF protein. Of these, E132K, V163M, Q172R, I223F, and V157M mutations were found to create larger residues relative to the wild type. E132K disrupted both the salt-bridge and hydrogen-bond interactions, V163M was buried within the core of the protein, and Q172R potentially introduced a flexible arginine residue that could disrupt the protein's rigidity. Functional analysis of V163M using Missense3D determined that it transformed the residue's buried/exposed property (where valine is buried, methionine is exposed). Q172R broke important hydrogen bonds, leading to clash alerts. On the other hand, mutations E132K, L211P, V143A, V230A, V157M, W142R, and W197R did not have structural damage. Additionally, based on amino acid composition alterations, MutPred2 was used to predict the pathogenicity of NGF. All 11 nsSNPs showed scores > 0.5, consistent with their pathogenic potential. Table 2 summarizes the deleterious effects of nsSNPs on the NGF protein's structure determined by three computational tools.

Table 2. Effects of deleterious nsSNPs on the NGF protein's structure

No	SNP ID	AA Change	Project Hope	Missense 3D	MutPred2 (g-value)
01	rs772557857	E132K	Affect size, charge. disrupt hydrogen bond and salt bridge.	No Structural damage detected	0.622
02	rs774878867	V163M	Affect size, buried in the core of protein.	Buried/exposed switch.	0.578
03	rs1557933464	Q172R	Affect size, charge, disrupt hydrogen bond, buried in the core of protein.	Clash. Buried H-bond breakage.	0.858
04	rs1653491943	L211P	Affect size.	No Structural damage detected	0.949
05	rs80277627	I223F	Affect size.	Clash	0.925
06	rs746593757	Q172E	Affect size, charge. lead to protein folding problems.	Buried charge introduced	0.823
07	rs758273427	V143A	Affects size. Probably damaging to the protein.	No Structural damage detected	0.566
08	rs767703003	V230A	Affects size. Cause a possible loss of external interactions.	No Structural damage detected	0.805
09	rs773565185	V157M	Affects size. Buried in the core of protein.	No Structural damage detected	0.804
10	rs929155379	W142R	Affects size, charge. Cause a possible loss of external interactions.	No Structural damage detected	0.938
11	rs1342067992	W197R	Affects size, charge. Loss of hydrophobic interactions.	No Structural damage detected	0.939

(g-value: a threshold of 0.50 for this score would suggest the likelihood of pathogenicity)

Chimera was used for structural superimposition and visual assessment to validate the structural changes exerted by nsSNPs. The mutations E132K, Q172E, V230A, and W142R induced significant conformational changes, particularly affecting key functional domains. Superimposed wild-type and mutant structures revealed significant backbone deviations, supporting the RMSD-based results. The results above strengthen the claim that identified deleterious nsSNPs have a considerable influence on the conformation and stability of the NGF protein.

Deleterious nsSNP conservancy in the NGF gene

ConSurf web server identified 34 high-risk nsSNP residues within the NGF protein. Among these residues, A149, T227, R224, G191, I223, V135, V143, V230, V157, W142, A210, and C231 were determined to be buried and structural, while residues E132, T177, Q172, T212, R190, F175, G194, and E176 were classified as exposed and functional. It was predicted that all these residues are located in the region of high conservancy, as depicted in Figure 2.

Association between deleterious nsSNPs and cancer susceptibility

To test oncogenicity, point mutations were first screened using CScape, which predicted E132K, L211P, Q172E, V230A, and W142R as oncogenic, with the highest confidence scores. These mutations were then analyzed by the OncoVar server, where several mutations of interest were identified in different cancer patient samples (Table 3).

Notably, some overlaps were observed with OncoVar in CanSAR Black results, whereas others revealed novel associations. It is worth mentioning

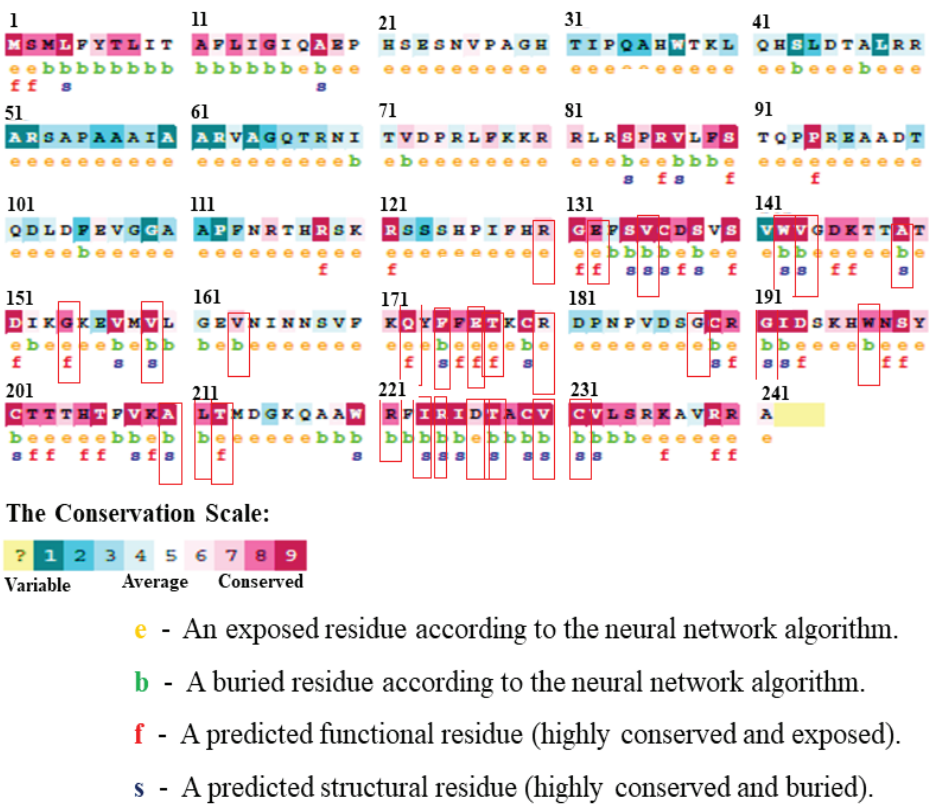

Figure 2. Evolutionary conservation and functional residue prediction by ConSurf

that multiple cancer types, such as PanCancer, Melanoma, Colorectal Cancer, Skin Cancer, Gastric Cancer, and Lung Adenocarcinoma, were each associated with two mutations. Interestingly, though L211P was predicted to be oncogenic by CScape with high confidence, it was not found to be associated with any cancer study.

Table 3. Association of Deleterious nsSNPs with Cancer.

No	AA Change	Cscape (p-values)	OncoVar	canSAR Black
01	E132K	0.646348	PanCancer, Melanoma	Colorectal Cancer, Melanoma
02	V163M	0.394060	No Cancer Association	No Cancer Association
03	Q172R	0.351064	No Cancer Association	No Cancer Association
04	L211P	0.647382	No Cancer Association	No Cancer Association
05	I223F		No Cancer Association	No Cancer Association
06	Q172E	0.734520	Melanoma	Skin Adenocarcinoma, Skin Cancer
07	V143A	--	No Cancer Association	No Cancer Association
08	V230A	0.569266	Gastric Cancer	Lung Adenocarcinoma
09	V157M		No Cancer Association	No Cancer Association
10	W142R	0.578322	Colorectal Cancer	Melanoma
11	W197R	--	No Cancer Association	No Cancer Association

(Red = Highly oncogenic, Yellow = oncogenic and Majenta = Non-oncogenic)

nsSNPs are a significant source of genetic variation that can influence protein function by altering amino acid sequences, leading to structural instability and functional impairments. Despite the critical roles of NGF in neurotrophic signaling and disease pathogenesis, the functional consequences of nsSNPs in the NGF gene have not been systematically investigated.

In the present study, we performed a comprehensive computational analysis of 268 nsSNPs within the NGF coding region. Out of these, 36 nsSNPs were predicted to be deleterious by consensus from six in silico tools (Kumar *et al.*, 2009; Adzhubei *et al.*, 2010; Bendl *et al.*, 2014; Capriotti and Fariselli, 2017; Thomas *et al.*, 2006; Bromberg and Rost, 2007). Further analysis using I-Mutant 2.0 revealed that 34 of these variants significantly reduced NGF protein stability, highlighting their potential to impair NGF function (Capriotti *et al.*, 2005). These destabilizing mutations were predominantly located in evolutionarily conserved regions, as identified by ConSurf analysis (Ashkenazy *et al.*, 2016), reinforcing their likely biological importance and potential involvement in disease mechanisms (Ibáñez *et al.*, 1990).

Structural comparison using Phyre2 and SWISS-MODEL revealed that 11 selected high-risk nsSNPs induced significant conformational deviations in NGF protein, with reduced TM-scores and elevated RMSD values compared to the wild type (Kelley *et al.*, 2015; Waterhouse *et al.*, 2018; Zhang and Skolnick, 2005). Mutations such as Q172R, Q172E, E132K, and V230A caused profound structural alterations. Project HOPE visualizations demonstrated the disruption of critical hydrogen bonding and salt-bridge interactions or the introduction of flexible or charged residues in structured protein regions (Venselaar *et al.*, 2010). Complementary predictions by Missense3D and MutPred2 confirmed the damaging potential of these mutations, with evidence of buried H-bond loss, buried charge introduction, or residue clash alerts (Ittisoponpisan *et al.*, 2019).

Additionally, visualization via UCSF Chimera clearly showed that several mutant structures (*e.g.,* E132K, Q172E, W142R, V230A) diverged significantly from the wild-type conformation, especially in regions crucial for NGF function. These observations suggest that the identified nsSNPs may compromise NGF-mediated signaling and protein-protein interactions.

Crucially, cancer association analysis using CScape, OncoVar, and canSAR Black further identified Q172E, E132K, V230A, and W142R as putative oncogenic mutations, linked to malignancies such as melanoma, colorectal cancer, gastric cancer, and

lung adenocarcinoma (Rogers *et al.*, 2017; Wang *et al.*, 2020; Bulusu *et al.*, 2014). While previous studies have reported NGF overexpression or dysregulation in several cancers (Truzzi *et al.*, 2008; Samario-Román *et al.*, 2023), our results provide novel insights into how specific genetic alterations in NGF may contribute to tumorigenesis.

This study is the first to offer an extensive in silico evaluation of NGF nsSNPs, integrating evolutionary, structural, and oncogenic assessments. Variants such as Q172E and E132K may act as critical modulators of NGF function and could serve as valuable targets for further experimental validation. These findings enhance our understanding of genetic variants in NGF, laying the foundation for future studies into their potential roles in disease susceptibility, progression, and therapeutic intervention.

Conclusions

This study presents the first comprehensive in silico analysis of nsSNPs in the NGF gene, identifying 36 potentially deleterious variants, with 34 predicted to destabilize the protein structure. Structural modeling revealed that key mutations, such as Q172E, E132K, V230A, and W142R, induce significant conformational changes and affect conserved functional domains. Notably, several of these nsSNPs showed oncogenic potential, linking them to cancers like melanoma and colorectal cancer. These findings highlight the importance of these variants in NGF function and suggest their relevance as potential biomarkers for disease susceptibility and therapeutic targeting, pending further experimental validation.

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Declaration

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. The authors also declare no conflict of interest.

Authors' Contributions

MMH: Writing– original draft, data curation, software, resources, formal analysis, visualization. AF: Writing – review and editing, conceptualization, supervision, project administration, methodology. MAH: Writing– original draft, data curation, software, resources, formal analysis, visualization. KSI: Writing – review and editing. AAAAJ: Writing – review and editing. TN: Writing – review and editing. MFK: Writing – review and editing, conceptualization, supervision, project administration, methodology.

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