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*Original article*

# Computational Analysis of the Structural and Functional Impact of the PRKN Gene Mutation (c.1130G>A) in Parkinson's Disease

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**Abstract**: Parkinson's disease (PD) is a neurodegenerative disorder associated with genetic mutations, including those in the PRKN gene, which encodes the E3 ubiquitin ligase parkin. This study employed computational approaches to investigate the pathogenic effect of the PRKN G>A mutation (position 1130) and identify potential therapeutic ligands. Comprehensive Database analysis (MalaCards, DisGeNET, OMIM) identified PRKN as a key PD-associated gene with p value ($P < 0.05$), pathogenicity analysis (MutationTaster, FATHMM, Polyphen-2) confirmed the mutation as deleterious, while protein stability (I- I-mutant, Mu Pro) revealed protein destabilization. Molecular docking simulations demonstrated N-aryl benzimidazole enhanced binding affinity for the mutant PRKN (−7.5 kcal/mol vs. −5.5 kcal/mol in wild-type), mediated by salt bridges (GLU, GLU) and hydrophobic contacts, contrasting kinetin showed no mutation-dependent affinity changes (-5.6 kcal/mol). Protein-protein interaction networks (STRING) further implicated mutant PRKN in disrupted mitochondrial regulation. These findings highlight N-aryl benzimidazole as a promising therapeutic candidate for mutation-specific PD treatment and provide a computational framework for targeting genetic subtypes of neurodegenerative diseases.

**Keywords:** Parkinson's disease, PRKN, MutationTaster, FATHMM, Polyphen-2, N-aryl, Molecular docking and Simulation

## 1. Introduction

Parkinson's disease neurodegenerative disorder affecting 2–3% of the population aged ≥ 45 years, resulting from progressive loss of ventral mesencephalic dopaminergic neurons, which causes dopamine deficiency and impairment of motor control [1]. The PD patient shows primary motor symptoms including bradykinesia, rigidity, and resting tremor. Moreover, PD patients display a broad spectrum of non-motor features such as cognitive deficits, autonomic dysfunctions, and mood disorders. While non-motor symptoms are more heavily focused on during advanced stages, they occur during all the stages of PD and have the potential to be early biomarkers of PD [2]. Due to the limited cure of the disease, Parkinson's disease focuses on managing symptoms through medications, surgery, or therapy, but options like levodopa often require adjustments over time as their effectiveness diminishes. Multiple genetic factors have been involved in the etiology of Parkinson's disease, including SNCA, LRRK2, PINK1, and PRKN. Among these, PRKN (also known as PARK2) plays a crucial role in early-onset Parkinson's, located on chromosome 6q25.2–q27. Bi-allelic pathogenic variants in the PRKN gene are the most frequent genetic cause of autosomal recessive juvenile parkinsonism (AR-JP), accounting for approximately 6.0–12.4% of early-onset Parkinson's disease (PD) cases with onset before age 50. The PRKN gene exhibits a high frequency of single-nucleotide variants (SNVs) and copy-number variations (CNVs), stemming from its location in a genomic region prone to rearrangements [3]. PRKN gene contains 12 exons, encodes par-

kin, an E3 ubiquitin ligase that is responsible for mitochondrial quality control and protein homeostasis. Parkin contains an N-terminal ubiquitin-like (UBL) domain at its start, two C-terminal RING finger domains (RING1 and RING2) at its end, connected by an IBR domain. Together, these form the distinctive RBR structure found only in complex organisms (eukaryotes) [4]. Through its ubiquitination activity, parkin targets damaged proteins for proteasomal degradation and mediates selective clearance of dysfunctional mitochondria through mitophagy. Loss-of-function mutations in PRKN and related genes like PINK1 disrupt these processes, accumulating defective mitochondria and neuronal cell death, and PD progression. Through sequential signaling, PINK1 phosphorylates ubiquitin and parkin at SER65, recruiting cytosolic Parkin to mitochondria. Activated parkin then ubiquitinates outer membrane proteins, generating phosphorylated ubiquitin chains that mark mitochondria for autophagic degradation [5]. The current study aims to conduct a molecular docking study for the PRKN gene with a specific ligand available in the database. By screening compounds capable of binding effectively to mutated parkin proteins, we hope to identify a possible treatment for genetically associated and early-onset cases of Parkinson's disease.

## 2. Materials And Methods

*Data Retrieval*

The current study was conducted with the retrieval of Parkinson's disease-associated genes using multiple databases. Initially, a comprehensive search for Parkinson's Disease was performed on the MalaCards database (https://www.malacards.org/). The multiple genes list was downloaded from the MalaCards database in CSV format. Parallelly, a similar search for Parkinson's Disease was conducted on the DisGeNET database, and the aligned genes were downloaded in Excel format (https://disgenet.com/). To compare both genes, both the MalaCards and DisGeNET gene lists were opened, and gene names were copied into the Venny 2.1 software for Venn diagram analysis (https://bioinfogp.cnb.csic.es/tools/venny/). Genes from the MalaCards were passed into List1, while genes from the DisGeNET dataset were passed into List2. The Venn diagram was generated to identify the similarity between two datasets. If no similarity were initially observed, new gene lists were downloaded and compared again until similarity between genes was identified. Now, overlapping genes were placed in the ShinyGO software to study their biochemical pathways (https://bioinformatics.sdstate.edu/go/). Put the similar genes obtained by Venn diagram tool. Download the data/multiple lists. From the analysis, the gene with the lowest P-value ($P < 0.05$) was selected because it is responsible for Parkinson's disease. The results show 2 genes for Parkinson's disease PINK 1and PRKN. The PRKN gene had a lowest P-value and a strong link to Parkinson's disease, so it was chosen for our further research. To understand PRKN better, it was searched in the OMIM (Online Mendelian Inheritance in Man) database (https://www.omim.org/). PRKN was finally selected as the principle gene of interest based on its strong association with mutations that can cause disease. use further tools to depict changes in 3D structure. Use all this data to form protein structure. The gnomAD were utilized to search gene based on the population variation in the different geographic regions (https://gnomad.broadinstitute.org/). Select variants in South Asia (SAS) by applying filters. Select variations not more than 1 or 2 nucleotides, select only 1 or 2 variants (M column).

*Pathogenicity analysis*

The mutation taster was utilized to check the pathogenicity of the selected gene (https://www.mutationtaster.org/). Find the specific mutation. Location 1130. Mutation G>A (select "A" as the mutated base). The results show mutated type as well as wild type. In FATHMM (https://fathmm.biocompute.org.uk/) FASTA format information of amino acid sequence along with it Ensemble transcript ID is given to server for predicting the impact of single –nucleotide variants(SNVs),which yields highly accurate predictions for SNVs across the entire human genome. SIFT(https://sift.bii.a-star.edu.sg/) predict that single nucleotide substitution in amino acid sequence cause higher probability to disrupt protein function. In SIFT paste the mutated sequence.>FASTA. remove the

stop codon.10mins will be required. In PolyPhen-2(http://genetics.bwh.harvard.edu/pph2/) the FASTA format information of amino acid sequence along with its position and substitution is given to server, which classify the input, an indicate a neutral effect or negative effect. All these server shows "NO SEQUENCE RECORD FOUND" which is indication of pathogenic nature of the SNPs mutation. If 3/3 tools confirm the mutated sequence to be disease causing, we will proceed further

*Protein Stability and structural analysis*

To predict protein stability, use I-Mutant (https://folding.biofold.org/i-mutant/i-mutant2.0.html) and MuPro (https://mupro.proteomics.ics.uci.edu/), inputting the wild-type protein sequence. For structure prediction use alphafold3 to build protein structure from mutant sequence(https://alphafoldserver.com/). Copy the sequence from mutation tester and use ChatGPT using the prompt "modify for alpha fold 3"

*Ligand Binding and Protein–Protein Interactions*

Ligand binding and protein-protein interaction were assessed using data from UniProt (https://www.uniprot.org/), PDB (https://www.rcsb.org/), and STRING database (https://string-db.org/) before docking. If ligand information was unavailable, literature sources and PubChem were consulted.

*Molecular Docking*

Molecular docking was performed to predict the binding energy and interaction energy between selected ligands and target protein which may serve as the basis for developing drugs against psychiatric diseases. The SDS files for identified ligands such as kinetin and N-aryl benzimidazole were sourced from PDB(https://www.rcsb.org/) and PubChem databases (https://pubchem.ncbi.nlm.nih.gov/). Pymol was used to convert the SDS file(ligand) and protein files into PDB format. For molecular docking simulations Autodock tools (https://vina.scripps.edu/) were employed, to convert both protein and ligand structures into PDBQT format. The ligand-binding pocket was parameterized in auto dock vina using a grid box positioned at the active site coordinates (x, y, z). Following molecular docking ten configurations were generated for each protein-ligand complex. The lowest binding energy (BE, kcal/mol) and root mean square deviation (RMSD) confirmation was considered as the most suitable docking pose. Pymol(https://www.pymol.org/) is then used to visualize and analyses the interaction between ligand and protein.

Table 1: Binding affinities of Kinetin and N-aryl benzimidazole with wild-type and mutant proteins, showing stronger binding of N-aryl benzimidazole to the mutant (-7.5 kcal/mol).

| Ligand | Protein type | Best binding energies (kcal/mol) |
|---|---|---|
| Kinetin | Wild type | -5.6 |
| Kinetin | Mutant | -5.6 |
| N-aryl benzimidazole | Wild type | -5.5 |
| N-aryl benzimidazole | Mutant | -7.5 |

## 3. Results

*Identification Of Parkinson's Disease-Associated Genes*

A comprehensive search for Parkinson's Disease-associated genes was conducted using the MalaCards and DisGeNET databases. Genes were extracted and compared using the Venny 2.1 tool to identify overlapping genes, followed by pathway enrichment analysis in Shiny GO, which revealed significant Parkinson's disease-relevant biological pathways. The PRKN gene showed a strong disease association having the lowest P value ($P < 0.05$) among intersecting genes. The finding showed 488 mutations in PRKN, 310 of which are missense, 124 are synonymous and 14 pLoF (Predicted loss of function) variants. Sequences in FASTA format were obtained from PDB. The protein sequence being evaluated is described in Table 2.

Table 2. Assessing the research protein composition

| Protein Information | PRKN |
|---|---|
| Recommended Name | Parkin RBR E3 Ubiquitin Protein Ligase |
| Amino Acids | 465 |
| Gene ID | 5071 |
| Ensemble ID | ENSG00000185345 |
| Cytogenetic band | 6q26 |
| Exon count | 13 |
| NCBI Nucleotide | NC_000006.12 |
| Base Pairs | 161347417..162727766 |
| Primary accession | O60260 |
| Specie | Homo Sapiens |
| | >NP_004553.2 E3 ubiquitin-protein ligase parkin isoform 1 [Homo sapiens] MIVFVRFNSSHGFPVEVDSDTSIFQLKEVVAKRQGVPADQLRVI FAGKELRNDWTVQNCDLDQQSIVHIVQRPWRKGQEMNATGG DDPRNAAGGCEREPQSLTRVDLSSSVLPGDSVGLAVILHTDSRK DSPPAGSPAGRSIYNSFYVYCKGPCQRVQPGKLRVQCSTCRQAT LTLTQGPSCWDDVLIPNRMSGECQSPHCPGTSAEFFFKCGAHPT SDKETSVALHLIATNSRNITCITCTDVRSPVLVFQCNSRHVICLDC FHLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNSLIKELHHFRILG EEQYNRYQQ |

*Pathogenicity Prediction of Selected Variant*

Mutation tester predicted the G>A mutation at position 1130 to be disease causing, to verify this prediction 3 computational tools were used:

*1) FATHMM:*

Mutation was entered using the proper Ensemble ID and amino acid sequence and it was predicted to be pathogenic indicating that mutation supports a link to Parkinson's disease.

| # | Protein ID | Substitution | Further information |
|---|---|---|---|
| 1 | PRKN | G1130A | No Sequence Record Found |
| 2 | MIVFVRFNSS | HGFPVEVDSD | No Sequence Record Found |
| 3 | LDQQSIVHIV | QRPWRKGQEM | No Sequence Record Found |
| 4 | VILHTDSRKD | SPPAGSPAGR | No Sequence Record Found |
| 5 | SCWDDVLIPN | RMSGECQSPH | No Sequence Record Found |
| 6 | CTDVRSPVLV | FQCNSRHVIC | No Sequence Record Found |
| 7 | LHHFRILGEE | QYNRYQQYGA | No Sequence Record Found |
| 8 | GFAFCRECKE | AYHEGEYSAV | No Sequence Record Found |
| 9 | CHVPVEKNGG | CMHMKCPQPQ | No Sequence Record Found |

*2) SHIFT*

SHIFT analysis predicted the G>A variant to be deleterious with a score of ≤0.05 indicating a higher chance of altered protein function due to mutation

Good SHIFT results = "Deleterious"

SHIFT Score ≤0.05 = Deleterious (damaging/ pathogenic)

SHIFT Score >0.05 = Tolerated (benign)

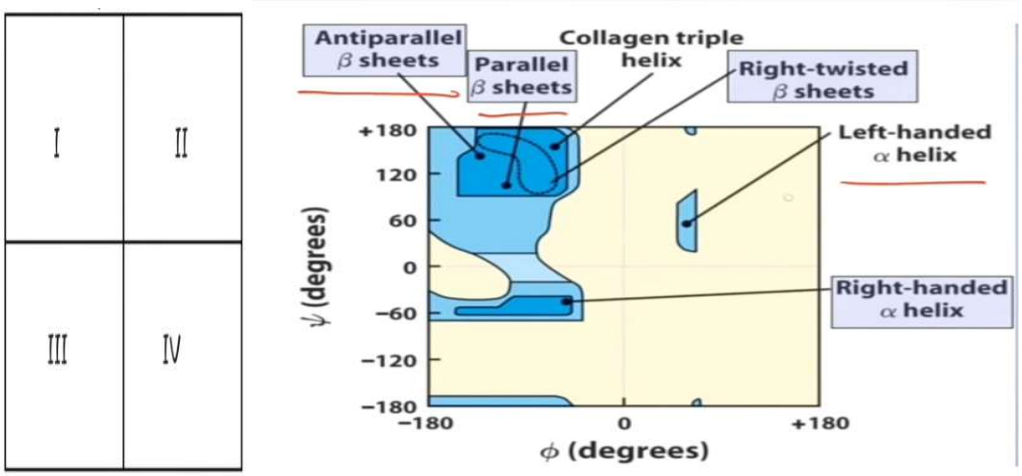
*3) PolyPhen-2*

PolyPhen- 2 shows no sequence records, which further suggests non-neutrality and supports pathogenicity

| **Tools** | **Prediction** | **Score** |
|---|---|---|
| MUTATION TESTER | Disease –causing | Prob :0.99 |
| FATHMM | Pathogenic(SNV) | -1.87 |
| SHIFT | Deleterious | ≤0.05 |
| PolyPhen- 2 | Probably damaging | 0.956 |

*Protein stability prediction*

Protein stability prediction was carried out using I-Mutant 2.0 and MuPro, the impact of the G>A point mutation on PRKN protein stability was assessed. Both tools predicted a decrease in protein stability. I-Mutant 2.0 showed a negative ΔΔG value, indicating that the mutation exerts a destabilizing effect, potentially disturbing proper protein folding and function.



***Wild type***

Table 2 presents the Ramachandran plot statistics for the wild-type protein structure. A total of 81 residues (71.1%) were located in the most favoured regions (A, B, C), indicating that the majority of the residues adopt ideal backbone dihedral angles. An additional 24 residues (21.2%) were found in the additional allowed regions (a, b, I, p), which are considered acceptable but not optimal. Six residues (5.3%) were observed in the generously allowed regions, reflecting less favorable conformations. Notably, only 2 residues (1.8%) were in the disallowed region, suggesting a generally well-modeled structure with minimal steric hindrance or unusual backbone conformations. These statistics indicate that the wild-type protein has a predominantly stable and reliable three-dimensional conformation.

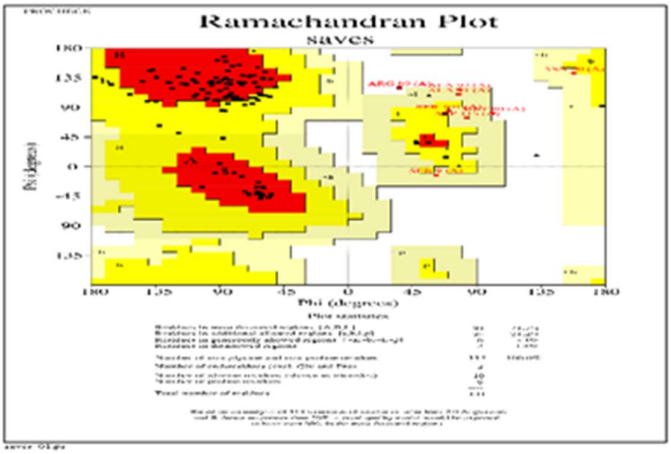



Table 2. Statistics of Ramachandran plot of Wild type

| Category | Description | Count | % |
|---|---|---|---|
| Most favored regions (A, B, C) | Ideal | 81 | 71.1% |
| Additional allowed regions (a, b, I, p) | Acceptable | 24 | 21.2% |
| Generously allowed regions | Less favorable | 6 | 5.3% |
| Disallowed region | Not good | 2 | 1.8% |

***Mutant Type***

Table 3 shows the Ramachandran plot statistics for the mutant-type protein structure. A total of 79 residues (69.9%) fall within the most favoured regions (A, B, L), indicating a high degree of structural stability. An additional 33 residues (29.2%) are found in the additional allowed regions (a, b, l, p), which are acceptable but represent less optimal conformations. Only 1 residue (0.9%) lies in the generously allowed region, which is generally less desirable for structural integrity. Importantly, no residues (0.0%) are present in the disallowed regions, indicating the absence of steric clashes or significant modeling errors. Overall, the mutant structure maintains a high-quality conformation with improved backbone geometry compared to the wild type.

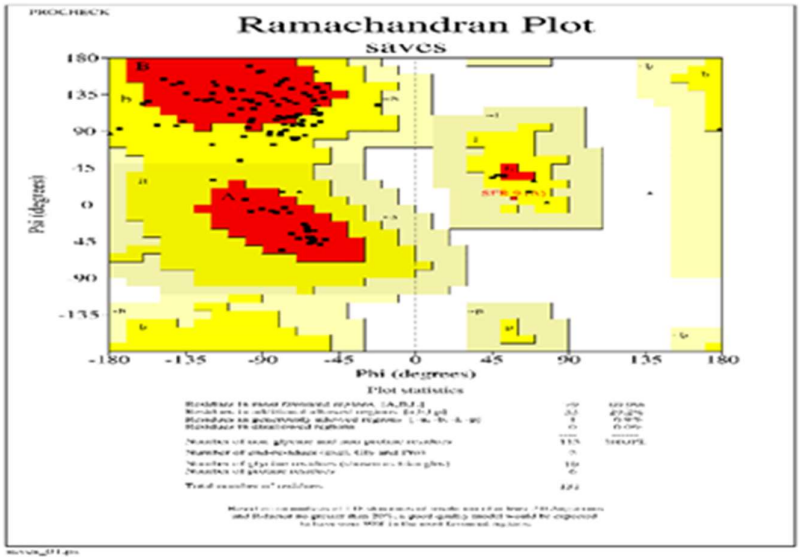



Table 3. Statistics of Ramachandran plot of Mutant type

| Category | Description | Count | % |
|---|---|---|---|
| Most favoured regions (A, B, L) | Ideal angles (very stable) | 79 | 69.9% |
| Additional allowed regions (a, b, l, p) | Acceptable but not ideal | 33 | 29.2% |
| Generously allowed regions | Should be avoided | 1 | 0.9% |
| Disallowed regions | Not good(errors) | 0 | 0.0% |

*Ramachandran plot*

The Ramachandran plot is a graphical representation used in structural biology to visualize the allowed and disallowed dihedral angles ϕ (phi) and ψ (psi) of amino acids residues in a protein structure. It is the way by which we could identify secondary structure of proteins depending upon rotation or degree of freedom of dihedral angles ϕ(N–C$\alpha$) and ψ(C$\alpha$–C). Secondary structure of protein could be $\alpha$ helix and β sheets or loop structure.

*Protein - Protein Interaction*

Functional association of the PRKN gene was analyzed using the STRING database, revealing its central role in neurological disorder pathways. As shown in Figure 2 the resulting protein-protein interaction network represents proteins as the circular node connected by edges indicating functional associations. Edge thickness depicts interaction confidence scores, while node size shows the number of interactions. The network illustrates PRKN's direct interaction with Parkinson's disease-related proteins such as PARK2, PINK1 and SNCA.

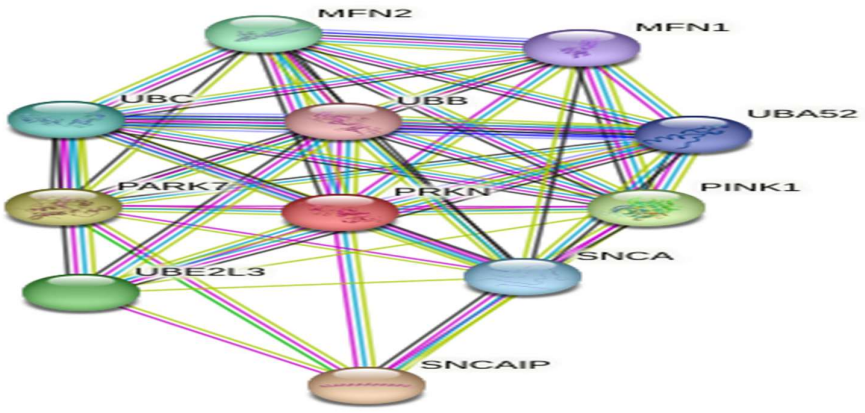



Figure2: Graphical view of the protein-protein interaction

Table 4. Protein –protein network stats by STRING analysis

| Protein-Protein Network Stats | PRKN |
|---|---|
| Number of nodes | 11 |
| Number of edges | 47 |
| Average node degree | 8.55 |
| Clustering coefficient | 0.894 |
| Expected number of edges | 20 |
| PP enrichment p-value | 3.64e-07 |

PRKN is an E3ubiquitin ligase parkin that forms a complex with UBEL2L3 or UBEL2L6, mediates Lys-63 linked polyubiquitination and associates with components like FBXW7 and CUL1 in SCF-like complexes. It interacts with mitochondrial regulators, including PINK1(damage sensor ), PARK7 (oxidative stress defence), CHPF(translocation), MFN2, FBXO7 (mitophagy)and ZNF746 (transcriptional repressor)which collectively regulate mitochondrial homeostasis.PRKN contains an N-terminal ubiquitin-like domain(proteasome recruitment), RING-type- 1 zinc finger domain(repress P53/TP53 transcription)and IBR domain.RBR family E3 ligases(like parkin) operate through a unique mechanism; they bind the E2 enzyme (UBE2L3) by using their first RING domain but require a cysteine residue of the secondary RING domain to facilitate the trans-thiolation reaction characteristic of HECT ligases.

## *Molecular Docking*

To analyse ligand-protein interactions, molecular docking was performed using AutoDock tools, docking the ligand kinetin and N-aryl benzimidazole with the PRKN protein. Different conformation was generated for each ligand characterized by binding affinity(-Kcal/mol). The docking results of ligands indicate that these binding affinities are related to their level of activity shown in table 5.

Table 5. docking score of the differenr poses of the ligand with protein

| Ligand | Protein type | Best binding energies (kcal/mol) |
|---|---|---|
| Kinetin | Wild type | -5.6 |
| Kinetin | Mutant | -5.6 |
| N-aryl benzimidazole | Wild type | -5.5 |
| N-aryl benzimidazole | Mutant | -7.5 |

*Analysis of Ligands with Wild - Type and Mutant Protein*

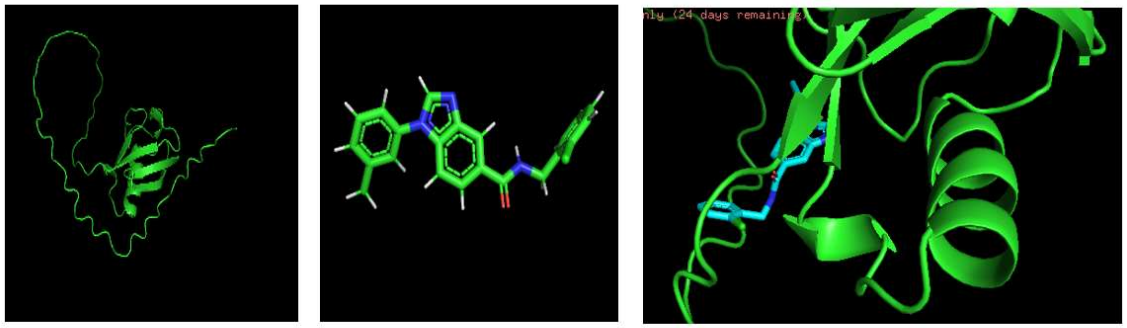
Comparative docking revealed that N - aryl benzimidazole showed stronger binding with the mutant protein (-7.5kcal/mol) as compared to the wild-type protein(-5.5kcal/mol), driven by two salt bridges (GLU, GLU) and expanded hydrophobic contacts, indicating compatibility with mutant protein while Kinetin exhibited no change in binding affinity (5.5kcal/mol in both wild and mutant type). Kinetin(mutant) formed an additional 5 H-bonds, but affinity could not be enhanced. In contrast, N – aryl benzimidazole had the same H-bond with more stable binding despite having a mutation. Comparative analysis of both ligands binding to wild type and mutant protein are shown in table 6.

Table 6. Comparative binding residue analysis of ligands with Wild type and Mutant PRKN

| Parameters | Kinetin (Wild-Type) | Kinetin (Mutant) | N-aryl benzimidazole (Wild-Type) | N-aryl benzimidazole (Mutant) |
|---|---|---|---|---|
| Docking score (-kcal/mol) | -5.6 | -5.6 | -5.5 | -7.5 |
| Intermolecular interactions | 2 H-bonds (LYS, ASN) | 7 H-bonds (4ARG, GLY, 2GLN) | 2 H-bonds (LYS, ASN) | 2 H-bonds (LYS, ASN) |
| Hydrophobic Contacts | ARG, TRP | TRP, GLN | ARG | GLU, ARG, GLN |
| Salt Bridges | - | - | - | GLU, GLU |

Based on these results, we conclude that Kinetin shows no change in binding affinity, indicating it may bind independently of mutation while N – aryl benzimidazole demonstrates enhanced binding with mutant protein, illustrating higher specificity or better fit post mutation. Figure 3. illustrates efficient docking interaction of PRKN and ligand (N – aryl benzimidazole), highlighting key stabilizing contacts. So, for drug design or inhibitor screening, N- Aryl benzimidazole could be therapeutic candidate for targeting the mutant protein.

Figure 3. Representation of docking interaction



PRKN N-aryl benzimidazole Protein- ligand interaction

(PRKN and N-aryl benzimidazole)

## 4. Discussion

This study successfully investigated the pathogenicity of the G>A point mutation at position 1130 in the PRKN gene and its role in early-onset Parkinson's disease using in silico tools. Using databases like MalaCards, DisGeNET, and pathway analysis, PRKN was identified as a key Parkinson's-associated gene. Pathogenicity prediction tools including MutationTaster, FATHMM, SHIFT, and PolyPhen-2 estimated the selected mutation to be disease causing. Protein stability analysis was assessed using I-Mutant 2.0 and MuPro both of which indicate that the mutation destabilizes the protein, potentially disrupting its folding and function. Using Ramachandran plot analysis showed slight diversions between wild-type and mutant PRKN, however no residues were found in the disallowed region for the mutant, indicating folding changes to be altered but remain tolerable mutant. STRING interaction analysis confirmed PRKN's central role in neuroprotective pathways, interacting with proteins like PINK1 and SNCA. Molecular docking disclosed that N-aryl benzimidazole binds more strongly to the mutant protein, suggesting its potential as a mutation-specific therapeutic agent while Kinetin's binding remained unchanged, Overall, the G>A mutation appears pathogenic, affecting PRKN stability and function, and N-aryl benzimidazole may offer targeted therapeutic benefits. This study primarily focuses on illustrating the G> A point mutation at position 1130 in the PRKN gene, which is strongly linked for the early onset of Parkinson's disease .Multiple databases( MalaCards, DisGeNET, OMIM, GnomAD), verified PRKN as the most significantly associated gene( $P< 0.05$) among others. Pathogenicity prediction tools,including (mutation tester, FATHMM, and PolyPhen-2) suggest that G>A mutation is disease-causing and deleterious ,likely disrupting normal protein function. Protein stability analysis was further carried out using i-mutant 2.0 and MuPro. Both tools predicted a decrease in protein stability,indicating that the mutation disturbs proper protein folding and function. Structural confirmation via Ramachandran plot showed slight diversions between wild-type and mutant PRKN,however no residues were found in the disallowed region for the mutant, indicating folding changes to be altered but remaining tolerable. Protein-protein interaction verification using String database, which confirm PRKN's functional connectivity, also highlighting its key role in mitochondrial regulation and neuroprotection. especially with most important PD-related proteins, such as PINK1 and SNCA. Autodock vina molecular docking stimulation revealed that from both ligands, Kinetin showed similar binding affinities for both wild-type and mutant (-5.6 kcal/mol), while N- aryl benzimidazole exhibited stronger binding with the mutant protein(-7.5 kcal/mol) as compared to wild-type(-5.5 kcal/mol). These results suggest N-aryl benzimidazole as a potential therapeutic candidate for targeting mutated PRKN in early-onset Parkinson's disease.

## 5. Conclusion

This study confirms that the G>A mutation at position 1130 in the PRKN gene is pathogenic and likely contributes to early-onset Parkinson's disease. It reduces protein stability and alters folding, while preserving tolerable structure. PRKN's key interactions with PINK1 and SNCA highlight its role in neuroprotection. Molecular docking suggests N-aryl benzimidazole as a promising therapeutic agent targeting the mutant PRKN protein.

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