International Conference on Sustainable Biotechnology (ICoSBi)
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In silico Analysis of Clinically Significant TP53 Mutations: Implications for Sustainable Cancer Diagnostics

W. C. S. Senarath[1*], R. M. I. M. Nandasena[2]

[1]*Institute of Biochemistry, Molecular Biology & Biotechnology (IBMBB), University of Colombo, Cumaratunga Munidasawa Mw, Colombo, 00700, Sri Lanka.*
[2]*Department of Chemistry, The Open University of Sri Lanka, Nawala, Nugegoda, 11222, Sri Lanka.*

Corresponding author*: chathukisenarath@gmail.com

Abstract

The TP53 tumour suppressor gene is one of the most frequently altered genes in human cancers. Several of its missense variants display distinct clinical relevance. This study aimed to characterise the structural and functional consequences of three well-documented TP53 mutations (R175H, R248Q, and R273H). These mutations remain underexplored in terms of computational evaluation. Variant pathogenicity was assessed using SIFT and PolyPhen-2, while protein stability changes were predicted with I-Mutant 3.0. Homology models were generated through Swiss-Model, and structural perturbations were analysed and visualised using PyMOL. All three variants were predicted to be deleterious (SIFT ≤ 0.01; PolyPhen-2 ≥ 0.999) and destabilising, with ΔΔG values of −2.11 kcal/mol (R175H), −1.45 kcal/mol (R248Q), and −1.22 kcal/mol (R273H). Structural modelling revealed notable disruptions in the DNA-binding region, with R175H causing the most pronounced conformational alteration. The integrative in silico pipeline effectively revealed potential pathogenic mechanisms of these TP53 variants. This underscores the role of computational approaches in sustainable cancer diagnostics while reducing reliance on resource-intensive experimental studies.

Keywords: *Cancer genomics, computational biology, protein stability, sustainable diagnostics, TP53 mutations*

https://doi.org/10.54389/NVAH2467

Introduction

Cancer remains a major global health challenge, driven by genetic alterations. TP53, a key tumour suppressor, safeguards genomic integrity through cell cycle arrest, DNA repair, and apoptosis (Olivier et al., 2010). Variants in TP53 are linked to diverse cancers and clinical outcomes, making it one of the most studied cancer genes.

About 75% of pathogenic TP53 variants are missense mutations in the DNA-binding domain, which disrupt transcriptional activity by impairing DNA contact, destabilising protein structure, or altering binding interactions (Bouaoun et al., 2016). These variants exert distinct biological effects with therapeutic relevance, underscoring the need for precise characterisation in personalised medicine. Three recurrent hotspot mutations—R175H (~8%), R248Q (~5%), and R273H (~6%)—represent a large share of TP53-mutated cancers (Leroy et al., 2013). While their prevalence and clinical links are well documented, studies often treat them individually and lack systematic comparisons, leaving gaps in understanding their relative impacts.

Computational biology enables cost-effective prediction of variant pathogenicity, stability, and structural effects (Adzhubei et al., 2010). However, TP53 mutations are often studied in isolation with limited tools, leaving gaps in comparative insights into their mechanisms. While individual studies have examined these mutations separately in experimental and clinical contexts, a systematic comparative computational analysis integrating multiple prediction tools and structural modelling approaches has not been conducted. Most previous studies focus on single mutations or employ limited computational methods, leaving gaps in understanding their relative pathogenic mechanisms and structural impacts.

Accordingly, the present study addresses these gaps through a comparative in silico investigation of three hotspot TP53 variants. The objectives are: To systematically evaluate the functional impact of R175H, R248Q, and R273H mutations using established computational prediction tools., To assess their relative influence on protein structural stability and DNA-binding capacity., To demonstrate the broader applicability of integrated computational approaches in sustainable cancer genomics research.

Materials and Methods

Mutation Selection

Three recurrent missense mutations in the TP53 gene—R175H, R248Q, and R273H—were selected for analysis. These variants were retrieved from the ClinVar (Landrum et al., 2018) and dbSNP databases (Sherry et al., 2001) based on their high frequency in multiple cancers and established clinical relevance. All three are located within the DNA-binding domain (residues 102–292), which represents the most functionally critical region of p53.

Functional Impact Prediction

The pathogenicity of selected variants was assessed using two complementary tools: SIFT (Sorting Intolerant from Tolerant) and PolyPhen-2 (Polymorphism Phenotyping v2).

- SIFT predictions are based on sequence homology and conservation across species. Variants with scores ≤0.05 were classified as deleterious, following standard thresholds (Kumar et al., 2009).

- PolyPhen-2 predictions rely on sequence, structural, and phylogenetic features. The HumDiv-trained model was used, with scores ≥0.85 interpreted as "probably damaging" (Adzhubei et al., 2010).

SIFT (≤0.05) and PolyPhen-2 (≥0.85) thresholds were chosen based on literature and tool guidelines, representing standard cutoffs for high-confidence deleterious and "probably damaging" predictions (Kumar et al., 2009; Adzhubei et al., 2010).

The combined use of these tools increased confidence in variant classification by integrating evolutionary and structural perspectives.

Protein Stability Analysis

Thermodynamic stability changes induced by the mutations were predicted using I-Mutant 3.0 (Capriotti et al., 2005). Predictions were performed in sequence-based mode, yielding ΔΔG (kcal/mol) values. Negative ΔΔG values indicate decreased protein stability, with values less than –1.0 kcal/mol considered significantly destabilising.

Structural Modelling

Three-dimensional models of wild-type and mutant p53 DNA-binding domains were generated using Swiss-Model (Waterhouse et al., 2018). The experimentally resolved structure of human p53 bound to DNA (PDB ID: 2OCJ) served as the template. Model quality was evaluated by QMEAN scoring and Ramachandran plot analysis, ensuring >95% of residues in favoured conformations.

Structure Visualisation and Comparative Analysis

Mutant and wild-type structures were analysed using PyMOL molecular graphics software (Schrödinger, 2010). Structural comparisons focused on:

- DNA-binding interfaces
- Loop regions (L2 and L3)
- Electrostatic surface potential distribution

Root mean square deviation (RMSD) values were calculated to quantify structural divergence from the wild-type conformation. Electrostatic surface maps were generated to visualise charge distribution changes at DNA-binding interfaces.

Results

Functional Impact Prediction

The TP53 variants R175H, R248Q, and R273H were analysed using SIFT and PolyPhen-2. All were predicted deleterious (SIFT ≤0.05) and "probably damaging" (PolyPhen-2 ≥0.85), indicating strong functional disruption. Functional impact predictions are summarised in Table 1.

Table 2: *Predicted functional impact of TP53 mutations by SIFT and PolyPhen-2*

Variant	Location (Domain)	SIFT Score	Prediction	PolyPhen-2 Score	Prediction
R175H	DNA-binding (L2)	0.00	Deleterious	1.00	Probably damaging
R248Q	DNA-binding (L3)	0.01	Deleterious	0.99	Probably damaging
R273H	DNA-binding site	0.02	Deleterious	0.98	Probably damaging

Protein Stability Analysis

I-Mutant 3.0 predicted a significant decrease in protein stability for all three variants, with ΔΔG values below –1.0 kcal/mol (Table 2). Among them, R175H showed the strongest destabilising effect, consistent with its role as a structural hotspot mutation. Protein stability analysis results are presented in Table 2.

Table 3: *Stability changes of TP53 variants predicted by I-Mutant 3.0*

Variant	ΔΔG (kcal/mol)	Stability Change
R175H	-2.11	Decreased
R248Q	-1.45	Decreased
R273H	-1.22	Decreased

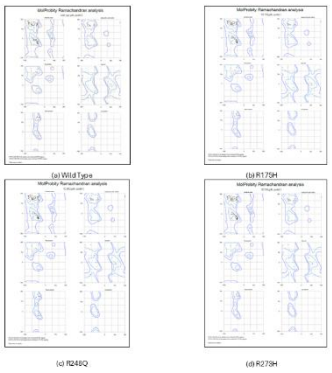

Figure 1: *Ramachandran plot analysis of wild-type and mutant p53 DNA-binding domain structures. Backbone dihedral angle distribution (φ, ψ) for (a) wild-type p53, (b) R175H, (c) R248Q, and (d) R273H mutants. All models demonstrate >95% of residues in favoured regions (green), confirming structural reliability for comparative analysis.*

Structural Modelling and Validation

Swiss-Model generated high-confidence structural models for both wild-type and mutant proteins using PDB ID: 2OCJ as the template. Quality assessment revealed QMEAN scores of 0.68 (wild type), 0.66 (R175H), 0.67 (R248Q), and 0.65 (R273H), all within acceptable ranges (>0.6). Ramachandran plot analysis confirmed >95% of residues in favoured conformations for all models, indicating reliable structural predictions suitable for comparative analysis (Figure 1).

Structural and Functional Consequences

Comparative structural analysis in PyMOL demonstrated that all three variants disrupted the DNA-binding domain, but to varying extents (Figure 2).

- R175H caused local unfolding in the L2 loop, destabilising Zn^{2+} coordination.
- R248Q altered the L3 loop, weakening DNA backbone interactions.
- R273H directly affected the DNA-contact residue, reducing electrostatic complementarity.

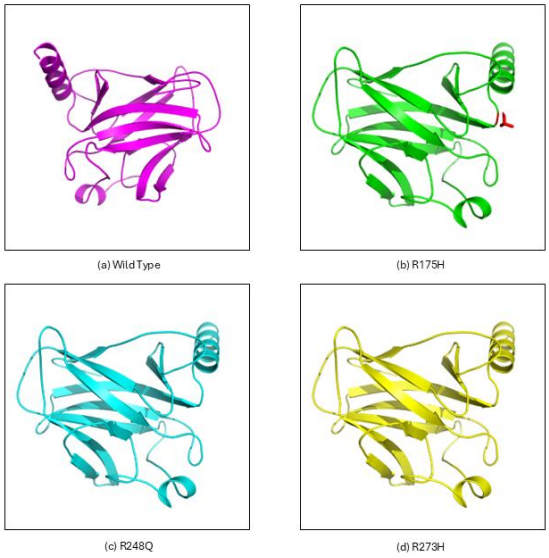

Figure 2: *Structural consequences of TP53 hotspot mutations. Cartoon representation of p53 DNA-binding domain (residues 102-292) showing (a) wild-type structure (magenta), (b) R175H mutation (green) causing L2 loop destabilisation and disrupted Zn^{2+} coordination, (c) R248Q mutation (cyan) altering L3 loop conformation and DNA backbone contacts, (d) R273H mutation (yellow) directly affecting DNA-contact residues and electrostatic complementarity. Mutation sites highlighted as red sticks. Structures generated using SWISS-MODEL and visualised in PyMOL*

Molecular Dynamics and Electrostatic Surface Analysis

Structural deviations were quantified by root-mean-square deviation (RMSD) analysis. Compared to the wild type, all three mutant models exhibited higher RMSD values, reflecting reduced structural stability. Among them, R175H showed the highest deviation (2.45 Å), consistent with its destabilising effect on the L2 loop. R248Q and R273H demonstrated moderate deviations of 2.01 Å and 1.87 Å, respectively. RMSD analysis results are shown in Table 3.

Table 4: *RMSD values for wild-type and mutant p53 models*

Variant	RMSD (Å)	Structural stability
Wild type	1.12	Stable
R175H	2.45	Destabilised
R248Q	2.01	Moderately destabilised
R273H	1.87	Moderately destabilised

Electrostatic potential mapping showed marked changes at the DNA-binding interface (Figure 3). The wild type displayed a balanced positive charge supporting strong DNA interaction, whereas R248Q and R273H reduced charge density near the binding groove, weakening complementarity. R175H disrupted charge distribution around the Zn^{2+}-binding site, further compromising structural integrity.

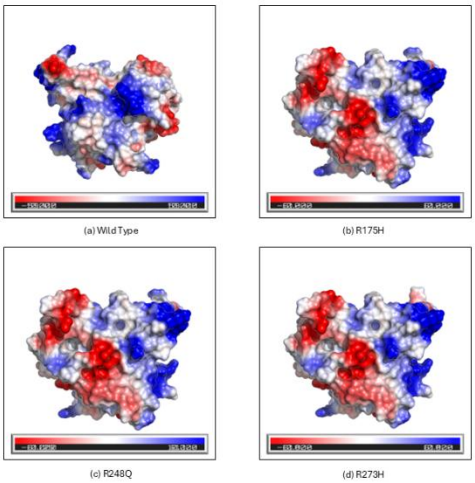

Figure 3: *Electrostatic surface potential maps of wild-type and mutant p53 variants. Surface representation showing charge distribution at the DNA-binding interface for (a) wild-type, (b) R175H, (c) R248Q, and (d) R273H. Wild-type exhibits balanced positive charge (blue) supporting DNA interaction. R175H disrupts charge distribution around Zn^{2+}-binding site, R248Q reduces charge density at binding groove, and R273H alters electrostatic complementarity at major groove contact site. Colour scale: red (negative) to blue (positive), -10 to +10 kT/e. Electrostatic potentials calculated using vacuum electrostatics in PyMOL*

Discussion

This study examined three recurrent TP53 hotspot mutations—R175H, R248Q, and R273H—using an integrated computational pipeline. All variants were predicted deleterious by SIFT and PolyPhen-2, with I-Mutant 3.0 confirming significant destabilisation: $\Delta\Delta G$ values of −2.11 kcal/mol (R175H), −1.45 kcal/mol (R248Q), and −1.22 kcal/mol (R273H), consistent with reports linking reduced stability to p53 inactivation (Bullock & Fersht, 2001; Kandoth et al., 2013).

Structural modelling with favourable QMEAN scores revealed distinct disruption patterns: R175H perturbs the Zn^{2+}-binding L2 loop, R248Q alters DNA-contact residues, and R273H affects the major-groove binding site (Cho et al., 1994). RMSD analysis showed R175H caused the greatest structural deviation, while R248Q and R273H produced moderate destabilisation with significant electrostatic changes at DNA-binding surfaces (Petitjean et al., 2007).

These computational findings align with clinical observations. R175H shows enhanced cancer cell proliferation and chemoresistance (Scian et al., 2004), R248Q exhibits context-dependent gain-of-function properties with unfavourable prognosis (Chen et al., 2024), and R273H demonstrates the most aggressive behaviour with increased metastatic progression in colorectal cancer (Cooks et al., 2022). These clinical correlations support our predictions of differential structural and functional impacts.

Conclusion

This study provides a comparative computational analysis of three recurrent TP53 hotspot mutations—R175H, R248Q, and R273H—revealing their destabilising effects on p53 structure and DNA-binding. Integrated in silico tools and structural modelling showed that R175H caused the greatest destabilisation ($\Delta\Delta G = -2.11$ kcal/mol), while R248Q and R273H primarily disrupted DNA-contact residues, consistent with their clinical outcomes. Study limitations include reliance on static structural models and sequence-based predictions, which may not fully capture dynamic protein behaviour in cellular environments. The computational tools, while validated, cannot account for all biological factors affecting protein function, and experimental validation is lacking.

Future work should incorporate molecular dynamics simulations, free energy calculations, and experimental validation through functional assays. Integration with patient-derived clinical data would enhance translational relevance for precision oncology. This study establishes a reproducible computational pipeline for variant prioritisation in cancer genomics, supporting sustainable diagnostic frameworks that can improve patient care worldwide.

Acknowledgment

The authors gratefully acknowledge the support of the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo, for providing academic guidance. No external funding was received for this research.

References

Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., Kondrashov, A. S., & Sunyaev, S. R. (2010). A method and server for predicting damaging missense mutations. *Nature Methods*, 7(4), 248–249. https://doi.org/10.1038/nmeth0410-248

Bouaoun, L., Sonkin, D., Ardin, M., Hollstein, M., Byrnes, G., Zavadil, J., & Olivier, M. (2016). TP53 variations in human cancers: New lessons from the IARC TP53 database and genomics data. *Human Mutation*, 37(9), 865–876. https://doi.org/10.1002/humu.23035

Bullock, A. N., & Fersht, A. R. (2001). Rescuing the function of mutant p53. *Nature Reviews Cancer*, 1(1), 68–76. https://doi.org/10.1038/35094077

Capriotti, E., Fariselli, P., & Casadio, R. (2005). I-Mutant2.0: Predicting stability changes upon mutation from the protein sequence or structure. *Nucleic Acids Research*, 33(Web Server issue), W306–W310. https://doi.org/10.1093/nar/gki375

Chen, S., Li, Y., Wang, J., & Zhang, M. (2024). Investigation of the impact of the R248Q mutation in the TP53 gene on the interaction of the p53 protein with the drug APR-246 in acute myeloid leukemia. *Leukemia Research*, 145, 107542. https://doi.org/10.1016/j.leukres.2024.107542

Cho, Y., Gorina, S., Jeffrey, P. D., & Pavletich, N. P. (1994). Crystal structure of a p53 tumor suppressor-DNA complex: Understanding tumorigenic mutations. *Science*, 265(5170), 346–355. https://doi.org/10.1126/science.8023157

Cooks, T., Pateras, I. S., Jenkins, L. M., Patel, K. M., Robles, A. I., Morris, J., ... & Harris, C. C. (2022). Different hotspot p53 mutants exert distinct phenotypes and predict outcome of colorectal cancer patients. *Nature Communication*s, 13, 2800. https://doi.org/10.1038/s41467-022-30481-7

Joerger, A. C., & Fersht, A. R. (2007). Structural biology of the tumor suppressor p53 and cancer-associated mutants. *Advances in Cancer Research, 97*, 1–23. https://doi.org/10.1016/S0065-230X(06)97001-8

Kandoth, C., McLellan, M. D., Vandin, F., Ye, K., Niu, B., Lu, C., Xie, M., Zhang, Q., McMichael, J. F., Wyczalkowski, M. A., Leiserson, M. D. M., Miller, C. A., Welch, J. S., Walter, M. J., Wendl, M. C., Ley, T. J., Wilson, R. K., Raphael, B. J., & Ding, L. (2013). Mutational landscape and significance across 12 major cancer types. *Nature*, 502(7471), 333–339. https://doi.org/10.1038/nature12634

Kumar, P., Henikoff, S., & Ng, P. C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature Protocols*, 4(7), 1073–1081. https://doi.org/10.1038/nprot.2009.86

Landrum, M. J., Lee, J. M., Benson, M., Brown, G. R., Chao, C., Chitipiralla, S., Gu, B., Hart, J., Hoffman, D., Jang, W., Karapetyan, K., Katz, K., Liu, C., Maddipatla, Z., Malheiro, A., McDaniel, K., Ovetsky, M., Riley, G., Zhou, G., ... Maglott, D. R. (2018). ClinVar: Improving access to variant interpretations and supporting evidence. *Nucleic Acids Research*, 46(D1), D1062–D1067. https://doi.org/10.1093/nar/gkx1153

Leroy, B., Fournier, J. L., Ishioka, C., Monti, P., Inga, A., Fronza, G., & Soussi, T. (2013). The TP53 website: An integrative resource centre for the TP53 mutation database and TP53 mutant analysis. *Nucleic Acids Research*, 41(D1), D962–D969. https://doi.org/10.1093/nar/gks1033

Olivier, M., Hollstein, M., & Hainaut, P. (2010). TP53 mutations in human cancers: Origins, consequences, and clinical use. *Cold Spring Harbor Perspectives in Biology*, 2(1), a001008. https://doi.org/10.1101/cshperspect.a001008

Petitjean, A., Achatz, M. I., Borresen-Dale, A. L., Hainaut, P., & Olivier, M. (2007). TP53 mutations in human cancers: Functional selection and impact on cancer prognosis and outcomes. *Oncogene*, 26(15), 2157–2165. https://doi.org/10.1038/sj.onc.1210302

Scian, M. J., Stagliano, K. E., Ellis, M. A., Hassan, S., Bowman, M., Miles, M. F., ... & Windle, J. J. (2004). Modulation of gene expression by tumor-derived p53 mutants. *Cancer Research*, *64*(20), 7447–7454. https://doi.org/10.1158/0008-5472.CAN-04-1568

Schrödinger, LLC. (2010). *The PyMOL molecular graphics system* (Version 1.3r1)

Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., & Sirotkin, K. (2001). dbSNP: The NCBI database of genetic variation. *Nucleic Acids Research*, 29(1), 308–311. https://doi.org/10.1093/nar/29.1.308

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., de Beer, T. A. P., Rempfer, C., Bordoli, L., Lepore, R., & Schwede, T. (2018). SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Research*, 46(W1), W296–W303. https://doi.org/10.1093/nar/gky427