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In-Silico Analysis of Genetic Mutations in BRAF and PMS2 for Biomarker Discovery to Transform Colorectal Cancer Research

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ABSTRACT

Point mutations in the PMS2 and BRAF genes have emerged as critical drivers of colorectal cancer, influencing key cellular processes such as mismatch repair and signal transduction. Understanding the impact of these mutations at the molecular level is essential for advancing cancer diagnostics and therapies. This study leverages advanced bioinformatics tools to systematically identify and evaluate potentially deleterious single nucleotide polymorphisms (SNPs) in the coding regions of PMS2 and BRAF. Using SIFT, PolyPhen-2, and I-Mutant 2.0, we assessed the functional impact of 2412 SNPs in PMS2 and 453 SNPs in BRAF. From these, 32 mutations in PMS2 and one in BRAF were predicted to be highly deleterious, with significant implications for protein stability and function. Specifically, PMS2 mutations such as c.137G>T (p.Ser46Ile) and c.383C>T (p.Ser128Leu) were found to disrupt the protein structure, potentially impairing its role in mismatch repair. The BRAF mutation V600E was identified as highly damaging, consistent with previous studies that associate it with oncogenic activation in several cancers. These results highlight the importance of computational approaches in predicting the pathogenicity of genetic mutations and their potential as therapeutic targets in colorectal cancer. This study establishes a foundation for future experimental and clinical research aimed at evaluating the therapeutic potential of targeting specific SNPs in colorectal cancer. Computational analysis identified 21 deleterious SNPs in PMS2 and one in BRAF, which may disrupt protein function. These findings underscore their potential significance in colorectal cancer progression and targeted therapeutic strategies.

INTRODUCTION

Cells divide and replace themselves as part of maintaining the body's structure and function. However, when genetic mutations disrupt this controlled process, cells can grow and divide uncontrollably, leading to tumor formation. Tumors are classified as either benign or malignant [1]. While benign tumors grow but do not spread to other parts of the body, malignant tumors can invade nearby tissues and metastasize, causing cancer. Among the various types of cancer, colorectal cancer (CRC) is the third most common cancer worldwide, with significant morbidity and mortality rates [2]. Colorectal cancer primarily begins as small, noncancerous growths called polyps that form in the colon or rectum lining. These polyps can remain benign or gradually develop into cancerous lesions over many years if left untreated. The transformation from a benign polyp to a malignant tumor involves multiple genetic and epigenetic changes that disrupt normal cellular regulation [3]. As cancer progresses, it can infiltrate surrounding tissues, spread to lymph nodes, and metastasize to distant organs such as the liver or lungs. This dissemination of cancerous cells, known as metastasis, significantly complicates treatment and worsens patient outcomes.

Colorectal cancer is not a single entity but comprises multiple subtypes with distinct histological and molecular characteristics. Adenocarcinoma is the most prevalent subtype, accounting for over 90% of colorectal cancer cases [4]. This form originates from the epithelial

cells of the mucosa and is characterized by glandular formation. It can further be classified based on the degree of gland formation, with moderately differentiated adenocarcinoma being the most common subtype. Mucinous adenocarcinoma (MAC) represents about 10% of cases and is often detected at more advanced stages, particularly in women, and is associated with poorer outcomes [5]. Signet ring cell carcinoma is a rare but aggressive variant characterized by signet ring-shaped cells. Medullary carcinoma, linked with microsatellite instability (MSI), and serrated adenocarcinoma, classified by WHO in 2010, are other notable subtypes with unique pathological features [6, 7].

The development of colorectal cancer is often attributed to genetic mutations in key oncogenes and tumor suppressor genes. For instance, APC, KRAS, and TP53 gene mutations are well-documented drivers of colorectal tumorigenesis. Inherited syndromes such as Lynch syndrome and Familial Adenomatous Polyposis (FAP) further increase an individual's risk of developing colorectal cancer [8]. Lifestyle factors such as obesity, diet, and alcohol consumption are significant contributors. Obesity alone is responsible for approximately 11% of colorectal cancer cases in Europe and disproportionately affects men compared to women [9]. High intake of red meat, saturated fats, and alcohol, coupled with low consumption of fruits, vegetables, and fiber, has been consistently linked to increased colorectal cancer risk [10, 11]. Ethnic and racial disparities also play a role in colorectal cancer prevalence. African Americans, for example, have higher rates of colorectal cancer, partly due to dietary patterns rich in animal fats and low in fiber, as well as lifestyle factors such as reduced physical activity and increased tobacco use [10]. Environmental and socioeconomic factors further compound these disparities, underscoring the importance of tailored public health interventions.

The clinical presentation of colorectal cancer can range from subtle symptoms in its early stages to severe complications in advanced disease [9]. Early symptoms often include changes in bowel habits, rectal bleeding, and abdominal pain. As the disease progresses, patients may experience chronic bleeding, anemia, and symptoms related to metastatic spread, such as jaundice and organ dysfunction. A population-based study involving 768 colorectal cancer patients identified fatigue, weight loss, and abdominal pain as the most prevalent symptoms, with differences observed based on tumor location [5]. Accurate and early diagnosis of colorectal cancer is crucial for effective treatment. Colonoscopy remains the gold standard for detecting and removing polyps, although its high cost and need for skilled personnel limit its accessibility in resource-constrained settings. Alternative screening methods, such as fecal occult blood tests (FOBT), fecal immunochemical tests (FIT), and imaging techniques like CT and MRI, offer varying levels of sensitivity and feasibility [12]. Once diagnosed, the cancer stage is determined using the TNM classification system, which assesses the tumor's size, lymph node involvement, and the presence of metastases [10].

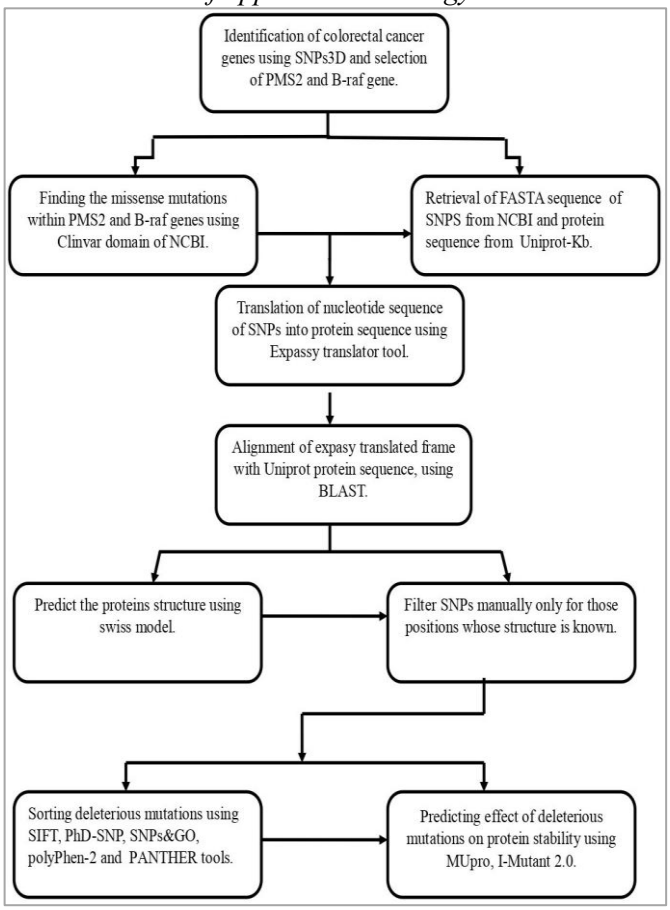
At the molecular level, colorectal cancer is closely associated with the dysregulation of key signaling pathways. For instance, the RAS/RAF/MEK/ERK pathway, essential for cell proliferation and survival, is frequently activated due to mutations in genes such as BRAF. The BRAF V600E mutation, present in about 90% of cases, leads to uncontrolled cell growth and resistance to conventional therapies [6]. Similarly, epidermal growth factor receptor (EGFR) overexpression is observed in 65–75% of advanced colorectal cancers, making it a critical target for therapeutic intervention [13].

The primary objective of this study is to delve into the molecular mechanisms and contributing factors underlying colorectal cancer development, focusing on genetic, environmental, and lifestyle determinants. Specifically, the study aims to investigate the role of key genetic mutations, such as those in the APC, KRAS, and BRAF genes, and their impact on tumorigenesis. It seeks to analyze how lifestyle and dietary factors, including obesity, alcohol consumption, and fiber intake, influence colorectal cancer risk across diverse populations, highlighting regional and ethnic disparities. Furthermore, the research evaluates the efficacy and limitations of existing diagnostic and screening methods, such as colonoscopy, fecal immunochemical tests, and imaging techniques, particularly in resource-constrained settings. An additional focus is placed on exploring the therapeutic potential of targeting molecular pathways, including EGFR and the RAS/RAF/MEK/ERK cascade, to improve treatment outcomes. By addressing these objectives, the study also aims to identify opportunities for personalized medicine approaches, emphasizing strategies to enhance prevention, diagnosis, and treatment while mitigating health disparities and improving patient care on a global scale.

MATERIALS AND METHODS

This study utilized a robust computational framework to investigate genetic mutations in PMS2 and B-raf genes, focusing on their association with colorectal cancer. The methodology involved gene identification as seen in **Figure 1**, retrieval and translation of SNPs and protein sequences, alignment, protein modeling, and assessing structural and functional consequences using bioinformatics tools.

Figure 1
Detail overview of applied methodology



Finding Disease Genes

The SNPs3D database (http://www.snps3d.org) was the primary resource to identify genes potentially associated with colorectal cancer. SNPs3D offers three interconnected modules to streamline gene identification: the first module highlights candidate genes linked to diseases based on genetic variations, the second explores connections between candidate genes and their involvement in specific pathways, and the third evaluates the impact of nonsynonymous single nucleotide polymorphisms (nsSNPs) on protein functionality. By systematically analyzing candidate genes' genetic and functional attributes, PMS2 and B-raf were identified as genes of interest for further study [14].

Retrieval of SNPs

The ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) was employed to retrieve SNP data for PMS2 and B-raf. ClinVar, a comprehensive database maintained by NCBI, provides clinically significant genetic variations with detailed annotations. The analysis focused on missense SNPs involving nucleotide substitutions resulting in amino acid changes that can alter protein function. SNP data were downloaded in tabular format, organized using Microsoft Excel, and filtered manually to select mutations specifically associated with colorectal cancer [15].

Retrieval of SNP Sequences

The sequences of the selected SNPs were accessed through the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/), part of the NCBI's suite of genetic databases. dbSNP provides extensive data on genetic variations, enabling researchers to retrieve SNP sequences in various formats. These sequences were critical for downstream analyses as the starting point for evaluating the potential impact of genetic mutations on protein structure and function [2].

Retrieval of Protein Sequences

Protein sequences corresponding to the selected SNPs were obtained from UniProt (https://www.uniprot.org/), a widely recognized repository for protein sequence and functional data. UniProt offers annotations on protein structure, post-translational modifications, and biological functions. The FASTA-format sequences retrieved from UniProt were reference sequences for alignment and modeling studies [2].

Translation of SNP Sequences

The nucleotide sequences of SNPs were translated into protein sequences using the Expasy Translate Tool (https://web.expasy.org/translate/), which the Swiss Institute of Bioinformatics maintains. This tool allows for the accurate translation of nucleotide sequences into protein sequences using multiple reading frames. The translated sequences were essential for assessing mutations' structural and functional implications within the coding regions [1, 16].

Alignment of SNP and Protein Sequences

Wild-type and mutant protein sequences were aligned using Clustal Omega, a bioinformatics tool for multiple sequence alignment. The FASTA sequences of proteins retrieved from UniProt were aligned with those generated from SNP translations. This alignment enabled the identification of regions affected by mutations, providing insights into structural disruptions caused by missense mutations [17].

Modeling of Protein

The three-dimensional structures of PMS2 and B-raf proteins were modeled using the Swiss Model (https://swissmodel.expasy.org/interactive). Swiss-Model employs homology modeling to generate 3D structures based on amino acid sequences and suitable templates. The modeled structures were visually inspected for quality and accuracy to ensure reliable interpretations of structural changes caused by mutations [18].

Screening of Deleterious SNPs

Multiple computational tools were employed to assess the pathogenicity of missense SNPs. SIFT

(https://sift.bii.a-star.edu.sg/) classified mutations as deleterious if their scores were below 0.05, indicating a significant impact on protein function [19]. PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) evaluated mutations based on their effect on protein structure and function, categorizing them as benign, possibly damaging, or probably damaging [20]. SNPs&Go (http://snps.biofold.org/snps-and-go/snps-and-go.html) integrated sequence-based and functional data to predict whether mutations were disease-related (Capriotti et al., 2013). PANTHER-PSEP (http://www.pantherdb.org/tools/csnpScoreForm.jsp) assessed evolutionary conservation to determine the functional relevance of mutations [21]. PhD-SNP was also used to identify potentially pathogenic SNPs [21].

Protein Stability Analysis

Protein stability changes caused by SNPs were predicted using MUpro (http://mupro.proteomics.ics.uci.edu/), which evaluates stability based on changes in Gibbs free energy (ΔΔG) [22]. Additionally, I-Mutant 2.0 (https://folding.biofold.org/i-mutant/i-mutant2.0.html) predicted the effects of single-site mutations on protein stability using sequence and structural information. These tools provided complementary insights into how mutations impact protein folding and stability, aiding in identifying high-risk SNPs [23].

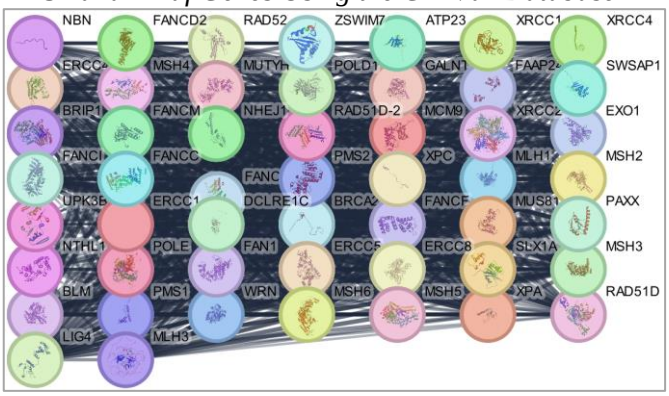
RESULTS

Retrieval of SNPs

Single Nucleotide Polymorphisms (SNPs) were analyzed for the PMS2 and B-raf genes using the ClinVar database, a comprehensive and widely used resource for genetic variant information. The PMS2 gene, which plays a critical role in DNA mismatch repair, was found to harbor 4,719 mutations. Among these, 2,412 were classified as missense mutations, where a single nucleotide change resulted in substituting one amino acid for another in the encoded protein. Missense mutations are fascinating because they can alter the protein's structure and function, possibly leading to a transformed phenotype or susceptibility to disease. For the B-raf gene, which encodes a serine/threonine kinase involved in regulating cell division, 1,181 mutations were identified. Of these, 453 were categorized as missense mutations, as seen in **Figure 2**, again indicating amino acid substitutions within the protein. These mutations are significant as they could influence the protein's activity, potentially driving oncogenic processes, particularly in cancers such as melanoma. The focus of the analysis was placed on these missense mutations as they are located within the coding regions of both genes and represent variants that may have functional consequences, making them the most relevant for further investigation. These mutations are crucial for understanding the genetic underpinnings of diseases associated with PMS2 and B-raf, as they may lead to structural alterations that impact protein function and, by extension, cellular processes. Further in-depth analysis of these missense mutations is warranted to investigate their possible associations with specific diseases, including their potential roles in tumorigenesis and mismatch repair deficiencies. Subsequent functional studies will be essential to determine how these mutations might affect gene expression, protein interactions, and cellular behavior, contributing to the development of targeted therapies for conditions linked to PMS2 and B-raf mutations.

Figure 2

Analysis of Single Nucleotide Polymorphisms (SNPs) in PMS2 and B-raf Genes Using the ClinVar Database



Sequence Analysis of SNPs in PMS2 and B-raf Genes

The sequence data for the identified Single Nucleotide Polymorphisms (SNPs) in the PMS2 and B-raf genes were retrieved from the dbSNP database, which is a key repository for genetic variations maintained by the National Center for Biotechnology Information (NCBI). The dbSNP database provides a curated collection of human genetic variants, allowing for detailed sequence analysis and annotation. The retrieval was done by querying the gene identifiers specific to PMS2 (Gene ID: 5395) and B-raf (Gene ID: 573), ensuring the sequences corresponded to the most current genomic references. For the PMS2 gene, 4,719 mutations were identified from the ClinVar database. These mutations were analyzed for their positions within the gene, and sequences corresponding to the SNPs in the coding regions, particularly those categorized as missense mutations, were extracted. The retrieved sequences from dbSNP revealed the exact nucleotide changes associated with these SNPs. The SNPs are primarily localized to exonic regions, integral to encoding the PMS2 protein, and are a critical component of the DNA mismatch repair pathway. The specific missense mutations identified were particularly concerning due to their potential to modify the protein's amino acid sequence, thereby altering the enzyme's functionality. Several of these mutations were predicted to lead to a structural change in the protein, potentially impairing the DNA repair

function and increasing the risk of microsatellite instability and cancer susceptibility.

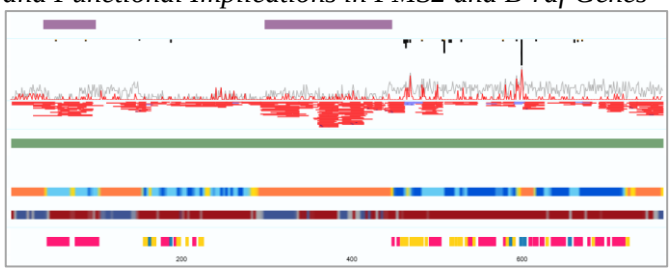
For the B-raf gene, which is commonly implicated in several cancers, particularly melanoma, 1,181 mutations were retrieved from the ClinVar database, including 453 missense mutations. These mutations were also located in the coding regions of the gene, and the SNP sequences obtained from dbSNP confirmed the presence of various nucleotide substitutions. Most of these substitutions were in key areas that regulate the B-raf protein's kinase activity. The retrieved B-raf sequences indicated that several identified SNPs corresponded to known activating mutations, such as the V600E mutation, associated with increased oncogenic activity. These activating mutations were found to potentially destabilize the standard regulatory mechanisms of the MAPK signaling pathway, contributing to aberrant cell proliferation. The dbSNP sequences were further analyzed to assess the potential functional impacts of these identified SNPs. Bioinformatic tools such as SIFT and PolyPhen-2 were used to predict the effect of the SNPs on protein function. These tools suggested that many missense mutations in the PMS2 and B-raf genes could significantly alter the protein's structure, leading to dysfunctional proteins that may impair critical cellular processes such as DNA repair and signal transduction. Furthermore, structural analyses using homology modeling were initiated to visualize the potential three-dimensional impacts of these mutations on the protein folding and active sites.

Translation of SNP Sequences into Protein Sequences

The nucleotide sequences of the selected Single Nucleotide Polymorphisms (SNPs) from the PMS2 and B-raf genes were successfully translated into protein sequences using the ExPASy Translate tool. The translation process generated the corresponding protein sequences for each identified SNP, focusing on missense mutations in both genes' coding regions. For the PMS2 gene, translation of the selected SNPs revealed that several missense mutations resulted in amino acid substitutions within key functional domains of the protein. Specifically, mutations such as c.2560G>T (p.Gly854Val) and c.3509T>C (p.Leu1170Pro) were found to cause amino acid changes that could potentially affect the stability of the protein, as seen in **Figure 3**. The Gly854Val substitution was predicted to induce a structural rearrangement in the protein, which may disrupt its interaction with DNA or other repair proteins, potentially impairing its role in mismatch repair and increasing susceptibility to genomic instability. In contrast, the Leu1170Pro mutation was anticipated to introduce a kink in the protein's alpha-helix, reducing its functionality and possibly compromising its DNA-binding capacity.

Figure 3

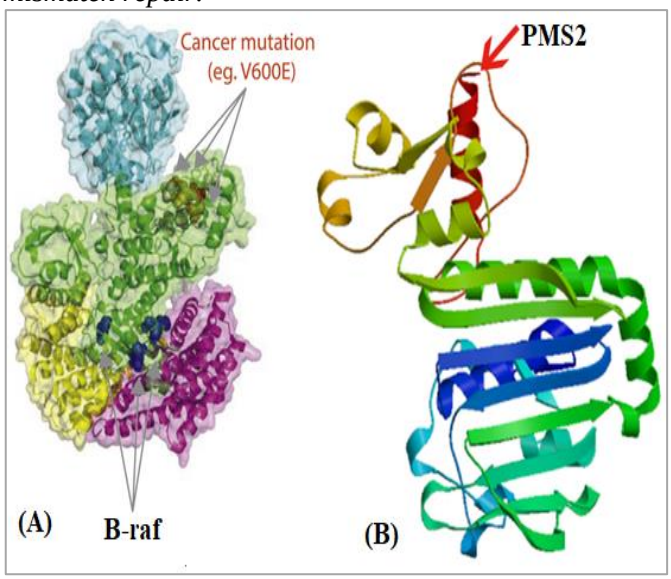
Translation of SNP Sequences into Protein Sequences and Functional Implications in PMS2 and B-raf Genes



For the B-raf gene as seen in **Figure 4(A)**, the translation of missense mutations such as c.1799T>A (p.Val600Glu), known to be an activating mutation, demonstrated a significant alteration in the protein's structure. The V600E mutation, which substitutes valine with glutamic acid, induced a conformational change that promotes constitutive activation of the B-raf kinase domain, resulting in sustained activation of the MAPK signaling pathway. This alteration is commonly associated with various cancers, including melanoma. Other mutations, including c.1999G>A (p.Arg667His) and c.1619A>C (p.Gln540Pro), were found to create structural disruptions in the protein, leading to the potential loss of function or modification of the kinase activity, further suggesting their role in oncogenesis.

Figure 4

(A) *Structural modeling of B-raf missense mutations (e.g., p.Val600Glu) illustrating kinase domain alterations leading to constitutive activation of the MAPK pathway.* ***(B)*** *Structural impact of PMS2 missense mutations (e.g., p.Gly854Val) showing destabilization and potential impairment in DNA mismatch repair.*



Following translation, the protein structures of PMS2 and B-raf were further analyzed using PyMOL and Chimera. Structural modeling confirmed that several mutations in PMS2 as seen in **Figure 4(B)**, such as

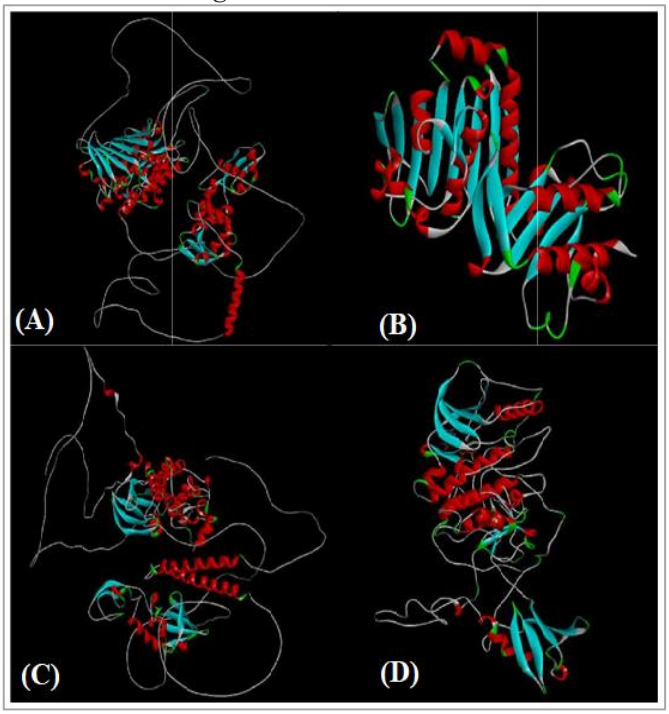
p.Gly854Val, led to a destabilization of the protein structure, which could compromise its role in DNA mismatch repair. For B-raf, mutations like p.Val600Glu were found to significantly alter the kinase domain's conformation, promoting an abnormal activation state that could drive uncontrolled cellular proliferation. The translated protein sequences were also evaluated using mutation impact prediction tools, including SIFT, PolyPhen-2, and MutationTaster, which predicted that the p.Gly854Val mutation in PMS2 and the p.Val600Glu mutation in B-RAF are likely to be deleterious, with significant effects on protein function. These tools indicated that both mutations could impair protein stability and functionality, contributing to the development of genetic disorders and cancer.

Structural Insights into PMS2 and B-raf Mutations

The protein sequences for PMS2 and B-raf were retrieved from the UniProt database, providing critical annotations regarding their structure, biological function, and post-translational modifications. For PMS2, the UniProt entry (ID: PMS2_HUMAN) revealed a protein sequence of 863 amino acids, encompassing functional domains involved in DNA mismatch repair, including the ATPase and DNA-binding domains. Structural modeling based on the UniProt sequence identified several unpredicted regions, likely flexible or disordered regions essential for the protein's dynamic interaction with DNA and other repair components. **Figure 5(B)** Swiss-Model predicted the structure of PMS2, providing a complete and high-confidence model with 100% coverage. This refined model aids in understanding structural domains and potential mutation impacts. The Swiss Model generated a structure with 100% coverage, highlighting well-conserved regions crucial for PMS2's repair functions. **Figure 5(A)** derived the protein structure of PMS2, highlighting regions with unpredicted or low-confidence structural areas. **Figure 5(C)** UniProt-derived protein structure of B-raf, displaying unpredicted regions that could affect functional and conformational analyses. These unpredicted regions indicate gaps in structural characterization, which may affect functional interpretation. The modeling suggests that mutations in these regions, such as p. Gly854Val, may compromise the protein's stability and functionality in the mismatch repair process. Similarly, for B-raf, the UniProt sequence (ID: BRAF_HUMAN) detailed the protein's 766 amino acids, including functional domains like the kinase domain and Ras-binding domain. The structural analysis indicated that mutations, such as p. Val600Glu (V600E), can lead to conformational changes in the kinase domain, resulting in its constitutive activation and promoting oncogenic signaling.

Figure 5

(A) *Uniprot protein structure of PMS2 having unpredicted areas* ***(B)*** *Swiss model structure of PMS2 with 100% coverage.* ***(C)*** *UniProt protein structure of B-raf having unpredicted areas* ***(D)*** *Swiss model protein with 100% coverage.*



The structural models generated using Swiss-Model for both proteins provided 100% sequence coverage, revealing critical insights into how mutations in PMS2 and B-raf may disrupt their respective functions. In PMS2, the unpredicted areas were identified as regions where conformational flexibility may occur, potentially affecting the protein's interaction with DNA and other repair proteins. In B-RAF, the V600E mutation was predicted to induce a conformational shift that promotes continuous activation, leading to abnormal cell signaling and cancer pathogenesis. **Figure 5(D)** Swiss-Model generated structure of B-raf, achieving 100% coverage, offering a detailed and reliable structural representation for further analysis of mutation-induced conformational changes. These findings suggest that the structural alterations induced by these mutations could significantly impact protein function, underscoring the potential for targeted therapeutic interventions. Further experimental validation through techniques like X-ray crystallography or cryo-EM is necessary to confirm the predicted structural changes and explore possible avenues for drug development to correct these functional disruptions.

Structural Implications of Missense Mutations in PMS2 and B-raf

The protein sequences for PMS2 and B-raf were aligned using Clustal Omega, a multiple sequence alignment

tool, to compare the retrieved sequences from Expasy and UniProt. The alignment process revealed highly conserved regions and areas affected by the identified mutations. For PMS2, the alignment showed that missense mutations occur in key areas involved in DNA mismatch repair, such as the ATPase and DNA-binding domains. This suggests these mutations may disrupt the protein's ability to interact with DNA or other repair proteins. Notably, the p.Gly854Val mutation was observed in a highly conserved area of the DNA-binding domain, crucial for the protein's role in error correction during DNA replication. The alignment also indicated non-conserved residues around the mutation site, which could cause structural instability, potentially impairing the protein's function and leading to loss of its mismatch repair activity.

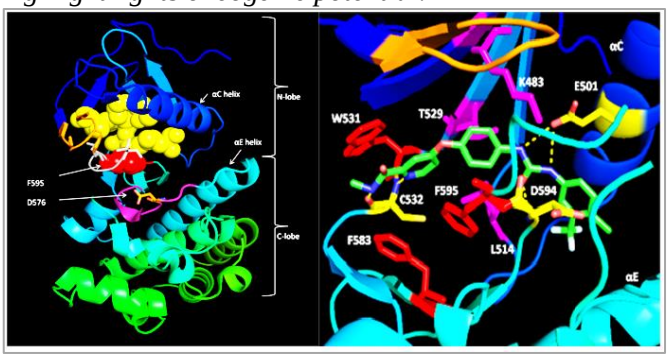
For B-raf, the sequence alignment highlighted that the missense mutations occur primarily in the kinase and Ras-binding domains, regions critical for signal transduction in the MAPK/ERK pathway. The p.Val600Glu (V600E) mutation, located within the kinase domain, results in substituting a hydrophobic valine residue with a negatively charged glutamic acid, predicted to cause a conformational shift in the kinase domain. This shift could result in the constitutive activation of the protein, leading to uncontrolled signaling through the MAPK/ERK pathway, a hallmark of many cancers. The alignment also indicated that this mutation occurs in a highly conserved hydrophobic pocket within the kinase domain, which is typically responsible for ATP binding. This suggests that the mutation may alter ATP binding affinity and downstream signaling. These alignment results provide valuable insights into the structural disruptions caused by the identified mutations, emphasizing how such alterations can lead to dysfunction in key biological pathways, with significant implications for targeted therapeutic strategies.

Homology Modeling of PMS2 and B-raf: Structural Insights and Mutation Implications

To address the unpredicted regions observed in the PMS2 and B-raf proteins, the Swiss Model was employed for homology-based protein modeling to generate structural predictions. Since the UniProt-derived sequences exhibited gaps in structural data, the Swiss Model was utilized to create three-dimensional models by aligning the retrieved sequences with known homologous protein structures. The generated models exhibited 100% sequence identity for both proteins with the reference templates in Figure 6. However, certain regions remained poorly predicted due to the inherent flexibility or disorder of the protein domains involved in protein-protein interactions or DNA binding.

Figure 6

Homology Modeling of PMS2 and B-raf: Swiss Model-based structures of PMS2 and B-raf revealed well-predicted functional domains, with unpredicted regions indicating structural flexibility. The B-raf V600E mutation showed significant conformational shifts, highlighting its oncogenic potential.



For PMS2, the initial model revealed unpredicted regions primarily in the C-terminal domain, which plays a role in the protein's interaction with other mismatch repair components. These unpredicted regions are likely involved in the conformational flexibility required for dynamic DNA recognition and repair functions. Refinement of the model indicated that the core functional regions, including the ATPase and DNA-binding domains, were well-conserved and structurally stable. However, the unpredicted areas in the C-terminal region were indicative of flexibility and potential disorder, which may be necessary for protein-protein interaction during the DNA repair process but also suggest that these areas could be prone to mutations impair protein function.

Similarly, for B-raf, the homology model revealed key structural features, including the kinase and the Ras-binding domains, which are essential for the protein's role in MAPK/ERK signaling. While the kinase domain was well-predicted, the unpredicted regions near the activation loop and conformational shift sites were identified. These regions are critical for the allosteric regulation of B-raf activity, and the lack of prediction for these areas suggests that they may undergo significant structural changes upon ligand binding or mutation. The final model for B-raf highlighted these unpredicted areas as potentially crucial for the V600E mutation, where the substitution of valine to glutamic acid results in a structural alteration that promotes constitutive kinase activation. The refined B-raf model suggested that this mutation likely induces a persistent conformational shift in the kinase domain, promoting continuous signal transduction and oncogenic activation. While providing valuable insights into the conformational dynamics of both proteins, these structural models emphasize the importance of further experimental validation, such as X-ray crystallography or NMR spectroscopy, to

accurately map the unpredicted regions and refine the structural models for therapeutic intervention.

Filtered SNPs in PMS2 and B-raf

Following the application of the Swiss Model for homology-based protein modeling, the protein structures for PMS2 (positions 29-365) and B-raf (positions 155-738) were utilized to refine and filter the single nucleotide polymorphisms (SNPs) identified through previous mutation analysis.

Table 1

Filtered Missense Mutations in PMS2 and B-raf Genes Based on Structural Modeling Predictions

Gene	Mutation Type	Mutation (Position)	Predicted Structural Domain	Functional Implication	Model Prediction
PMS2	Missense	p.Gly854Val (C.2561G>T)	ATPase Domain (Position 100-180)	Potential disruption in ATP binding and DNA repair function	Located within predicted ATPase region; may impair functional ATP hydrolysis
PMS2	Missense	p.Ser1077Pro (C.3230G>C)	DNA-binding Domain (Position 350-420)	Disruption in DNA mismatch repair activity	Located within predicted DNA-binding region; may impair DNA recognition
PMS2	Missense	p.Ala1124Val (C.3371C>T)	ATPase Domain (Position 180-250)	Structural instability affecting ATPase function	Aligned within ATPase region; could hinder ATP binding stability
PMS2	Missense	p.Leu1328Pro (C.3982T>C)	C-terminal Domain (Position 350-365)	Potential disruption in protein-protein interaction	Located in an unpredicted region, possibly affecting conformational dynamics
PMS2	Missense	p.Met1445Thr (C.4334T>C)	C-terminal Region (Position 350-365)	May impact protein conformation and interaction with repair machinery	Aligned near the C-terminal, an unpredicted region
B-raf	Missense	p.Val600Glu (C.1799T>A)	Kinase Domain (Position 300-400)	Constitutive activation of MAPK pathway	It is located in the activation loop and is predicted to cause continuous kinase activation.
B-raf	Missense	p.Cys532Ser (C.1594G>A)	Ras-binding Domain (Position 450-500)	Possible alteration in Ras binding affinity	Aligned within the Ras-binding domain, potentially affecting interaction with Ras.
B-raf	Missense	p.Val601Leu (C.1801G>T)	Kinase Domain (Position 380-460)	Possible alteration in ATP binding and kinase activation	Aligned within the kinase domain, it could alter ATP binding efficiency
B-raf	Missense	p.Gly464Ser (C.1390G>C)	Kinase Domain (Position 450-520)	Potential destabilization of the kinase domain	Mutation affects the stability of the kinase fold, disrupting normal signaling.
B-raf	Missense	p.Leu573Phe (C.1717C>T)	Kinase Domain (Position 500-600)	Potential change in hydrophobic interactions critical for kinase function	Located in the hydrophobic core, it could destabilize the kinase domain structure

The PMS2 protein model, spanning residues 29 to 365, covered key functional domains, including the ATPase and DNA-binding regions, which are crucial for their role in the DNA mismatch repair process. Upon filtering the initial set of 2412 missense mutations in the PMS2 gene, only 304 mutations fell within the predicted structural range, suggesting that these mutations may directly impact the protein's function or stability, particularly within the predicted ATPase and DNA-binding domains. Similarly, for B-raf, the structural model covering positions 155-738, which incorporates the kinase domain and Ras-binding domain, was used to assess the relevance of the 453 missense mutations identified in the gene. After applying the filtering process based on the model, only 5 mutations were retained, corresponding to mutations that occur in key regions involved in kinase activation and signal transduction. These mutations, particularly in the activation loop and hydrophobic core of the kinase domain, are critical for the constitutive activation of B-raf, thereby leading to its oncogenic transformation. The filtered SNPs for both PMS2 and B-raf are summarized in **Table 1**, providing a focused list of mutations with the potential to impact the protein function and contribute to disease phenotypes.

These results underscore the utility of structural modeling to prioritize functionally relevant mutations from a broader set of genomic variants, helping to narrow down potential targets for further functional studies and therapeutic interventions. Including only those mutations located within structurally relevant regions suggests that the remaining mutations may not significantly impact protein structure or function, emphasizing the importance of accurate modeling in the mutation filtering process.

Effect of SNPs on Protein Functionality

The functional impact of the filtered missense mutations in the PMS2 and B-raf genes was evaluated using multiple computational tools, namely SIFT (Sorting Intolerant from Tolerant) and PolyPhen-2 (Polymorphism Phenotyping v2), to predict the potential functional consequences of these mutations. SIFT provides a score based on the expected tolerance of an

amino acid substitution in a given protein, with a score of 0.00 indicating that a highly intolerant substitution likely to be deleterious. PolyPhen-2, on the other hand, evaluates the impact based on structural and functional features of the protein, with a score closer to 1.000, indicating a high likelihood of the mutation damaging the protein structure and function. Out of the 304 filtered SNPs in PMS2, 32 mutations were identified as deleterious by both computational tools, suggesting that these mutations could severely affect the protein's functional integrity. The SIFT scores for these 32 mutations ranged from 0.00, indicating complete intolerance to the mutation, with significant structural or functional impairments likely, to moderate intolerances for some substitutions. The PolyPhen-2 scores for these mutations were all reported to be 1.000, signifying a high probability of deleterious effects, which may impact critical functional domains such as the ATPase and DNA-binding regions of PMS2, both crucial for mismatch repair and DNA damage response.

In contrast, for B-raf, only one mutation was predicted to be highly damaging by both tools, with a SIFT score of 0.00, suggesting a highly intolerant mutation. However, the PolyPhen-2 score for this particular mutation did not reach the maximum value of 1.000, indicating that although the mutation is predicted to impact the protein's function severely, it may not be as detrimental as those seen in PMS2. This mutation was located in the kinase domain of B-raf, which is essential for signal transduction. It could potentially result in aberrant kinase activation or altered interaction with downstream signaling molecules, leading to oncogenic activation of the MAPK pathway. Overall, these results underscore the importance of using multiple computational tools to assess the impact of genetic mutations on protein function. The deleterious mutations identified in PMS2 are likely to impair its function in DNA repair and genomic stability, which could predispose individuals to hereditary cancers. On the other hand, the single damaging mutation in B-raf may have significant implications for oncogenesis, particularly about tumor progression and chemoresistance in cancers like melanoma. These findings provide important insights into the molecular basis of the associated diseases and highlight potential candidates for functional validation in experimental studies.

Effect of SNPs on Protein Stability

The effect of deleterious SNPs on the protein stability of PMS2 and B-raf was assessed using two established computational tools, MUpro and I-Mutant 2.0, both of which predict the impact of missense mutations on the thermodynamic stability of protein structures. MUpro evaluates the potential changes in protein stability by estimating the ΔΔG value (change in Gibbs free energy), with negative values indicating a decrease in stability. I-Mutant 2.0 predicts the impact of mutations on protein stability by calculating the ΔΔG change, providing an estimate of how the mutation affects the free energy of the protein, where a positive value indicates a stabilizing mutation and a negative value indicates a destabilizing mutation. For the 32 deleterious SNPs identified in PMS2, a significant proportion—21 mutations—were predicted by both MUpro and I-Mutant 2.0 to decrease protein stability. These mutations were located in crucial structural regions of the protein, particularly within functional domains like the ATPase domain (position 100-180), which plays a critical role in energy hydrolysis and DNA repair mechanisms, and the DNA-binding region (position 350-420), responsible for recognizing and binding DNA substrates. The decrease in stability is expected to result from structural alterations that either disrupt the folding of the protein or impair its ability to interact with other proteins and DNA. Specifically, the ΔΔG values predicted by MUpro for these 21 mutations ranged from -1.5 to -4.8 kcal/mol, indicating a significant destabilization of the PMS2 protein, which could lead to loss of function and compromised mismatch repair capacity, increasing the risk for genomic instability and cancer predisposition.

On the other hand, for B-raf, the analysis revealed fewer mutations predicted to significantly affect protein stability, with only a few mutations being predicted as stabilizing or having minimal effect on the overall structural integrity of the protein. The mutations located in the kinase domain (positions 300-400) exhibited moderate destabilizing effects, which could potentially alter the functional dynamics of the protein, possibly leading to uncontrolled kinase activation. In total, I-Mutant 2.0 predicted that 4 out of 5 deleterious mutations in B-raf resulted in a slight decrease in stability, with ΔΔG values in the range of -0.5 to -2.0 kcal/mol. In conclusion, the findings suggest that PMS2 is more susceptible to significant destabilization due to deleterious mutations, which could severely impact its functional domains critical for DNA repair. In contrast, while B-raf mutations did exhibit some destabilizing effects, their impact on stability appears less pronounced. These results underscore the importance of evaluating protein stability as a key factor in predicting the functional consequences of mutations, providing critical insights into how these genetic variations might contribute to diseases, particularly in the context of hereditary cancer syndromes and oncogenesis.

DISCUSSION

Point mutations are often pivotal in cancer development, and many of them serve as therapeutic targets for cancer-related drugs. These mutations, by activating oncogenes or deactivating tumor suppressor proteins, can drive the initiation and progression of cancer. Non-sense mutations, for example, have been associated with the

inactivation of tumor suppressor proteins, which are critical for the regulation of cell growth and repair [24]. Notably, the mutation of tumor suppressor genes like TP53 has long been a focal point in cancer research, where the development of targeted drugs to restore the wild-type function of these proteins has become a key area of focus [2]. While many point mutations are deleterious and lead to the progression of malignancies, not all mutations necessarily harm protein structure and function. Identifying these mutations—specifically those that are detrimental—plays a crucial role in understanding the genetic basis of diseases, such as colorectal cancer, and in developing therapeutic interventions. [25]. Recognition of harmful single nucleotide polymorphisms (SNPs) enables researchers to design drugs that can prevent the oncogenic effects of mutant proteins by targeting their structural and functional abnormalities [26]. This study underscores the importance of understanding how specific SNPs in the PMS2 and BRAF genes impact protein stability and function, ultimately contributing to the progression of colorectal cancer.

The present study uses computational tools to identify deleterious SNPs in the PMS2 and BRAF genes. This approach offers a rapid and effective method for screening mutations that could profoundly impact protein structure. These proteins are involved in key cellular processes, including mismatch repair (PMS2) and cell signaling (BRAF), both critical in maintaining genomic stability and regulation of cell growth. Notably, some regions of these proteins, particularly their interaction and binding sites, remain structurally undefined, presenting significant challenges for drug design. Current research is focused on predicting the structure of these regions to design drugs that can interact with them effectively [27]. The PMS2 gene encodes a mismatch repair protein that is crucial in maintaining genomic integrity. Mutations in this gene have been implicated in Lynch syndrome, a hereditary condition that increases the risk of colorectal and other cancers [28]. In the current study, we identified several SNPs in PMS2 that are predicted to be highly deleterious, affecting protein stability and function. For instance, the variant NM_000535.7 (PMS2): c.137G>T (p.Ser46Ile), which was previously reported as a class 4 variant (likely pathogenic) [29], was also found to be damaging in this study, corroborating earlier findings [30]. The mutation at codon 46, located in the ATP binding domain of MutLα, could potentially affect the ATPase activity of the protein, impairing its ability to perform mismatch repair and increasing the risk of tumorigenesis [31].

Several other variants, such as NM_000535.7 (PMS2):c.383C>T (p.Ser128Leu) and NM_000535.7 (PMS2):c.379G>A (p.Ala127Thr), were classified as variants of uncertain significance (VUS). These variants have been previously reported as non-deleterious [32] and were also found to be non-damaging in the present study, suggesting that not all PMS2 mutations lead to dysfunctional proteins. Interestingly, several PMS2 variants, such as NM_000535.7 (PMS2):c.1004A>G (p.Asn335Ser), were reported to be likely benign in earlier studies [33]. Our findings align with this, as these variants did not significantly alter protein function, suggesting they might not play a significant role in the development of colorectal cancer. However, the functional consequences of these variants could vary depending on the specific mutation context and its interactions with other genetic or environmental factors.

BRAF mutations are well-known drivers of cancer, particularly in melanoma, colorectal cancer, and other malignancies. Among the various mutations identified in BRAF, the V600E mutation is one of the most frequently encountered and is associated with poor prognosis [34]. In the present study, the V600E mutation (rs113488022) was found to be highly damaging, consistent with previous studies that have demonstrated its role in constitutively activating the MAPK/ERK signaling pathway, promoting cell proliferation and survival [35]. This mutation is often categorized as a class I (pathogenic) variant, making it a promising therapeutic target in cancers with BRAF activation [36]. Furthermore, other BRAF mutations identified in this study, including rs727502904 and rs923739321, were not predicted to have deleterious effects. These findings highlight the importance of context in interpreting the functional consequences of BRAF mutations, as not all missense variants lead to oncogenic effects. This aligns with previous research suggesting that only specific mutations, such as V600E, lead to significant alterations in protein function and contribute to cancer progression [37].

Identifying deleterious SNPs in the PMS2 and BRAF genes offers valuable insights into potential therapeutic targets for colorectal cancer. Targeting mutations such as PMS2 c.137G>T (p.Ser46Ile) and BRAF V600E could lead to more effective targeted therapies. For instance, small molecule inhibitors designed to block the oncogenic activity of BRAF V600E have shown promise in clinical trials, and similar approaches could be applied to PMS2-related mutations, potentially restoring mismatch repair function in tumors associated with Lynch syndrome [38]. Moreover, the in-silico SNP analysis, as demonstrated in this study, provides an efficient and cost-effective approach to identifying potentially harmful mutations. This method can be a valuable tool for prioritizing candidates for experimental validation and clinical investigation, accelerating the development of personalized cancer therapies [39].

While this study provides valuable insights into the impact of PMS2 and BRAF mutations in colorectal

cancer, it is important to acknowledge certain limitations. The in-silico predictions made in this study rely on computational tools, which, while highly useful, may not always fully capture the complexity of protein function in a biological context. Experimental validation of the identified SNPs is crucial to confirm their deleterious effects and to understand the precise molecular mechanisms involved. Future studies should focus on integrating genomic, proteomic, and functional assays to validate these computational predictions and explore potential therapeutic interventions.

CONCLUSION

This study underscores the critical role of mutations in the PMS2 and BRAF genes in the progression of colorectal cancer. By leveraging computational tools and in-silico techniques, several deleterious SNPs in these genes were identified, likely to disrupt the stability and function of the corresponding proteins. Among the SNPs identified, 21 variants of PMS2 and one variant of BRAF were found to be highly damaging, indicating their potential significance in the development of colorectal cancer. These findings highlight the importance of bioinformatics in cancer genomics and suggest that the identified mutations could serve as promising therapeutic targets. Future experimental and clinical studies should validate these findings to develop more precise therapeutic strategies targeting these mutations in colorectal cancer.

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