DOI: https://doi.org/10.52756/ijerr.2023.v36.006 *Int. J. Exp. Res. Rev.*, Vol. 36: 66-75(2023)

Original Article | *Peer Reviewed* | *Open Access*

International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online)
www.iaph.in
ISSN 2455-4855

Computational Identification and Validation of Non-Synonymous SNPs in Progesterone Receptor Membrane Complex 1 Linked to Lung Cancer

Solaipriya Solairaja, Habeeb Shaik Mohideen and Sivaramakrishnan Venkatabalasubramanian*

Department of Genetic Engineering, SRM Institute of Science and Technology, Kattankulathur, Kanchipuram, Tamil Nadu, Chennai-603203, India

E-mail/Orcid Id:

SS, ss4046@srmist.edu.in; *HSM,* habeeb_skm@yahoo.co.in, https://orcid.org/0000-0003-4217-5063;
SV, sivaramv@srmist.edu.in, https://orcid.org/0000-0002-6498-5561

Article History:

Received: 18th Aug., 2023
Accepted: 8th Dec., 2023
Published: 30th Dec., 2023

Keywords:
Computational, lung cancer, PGRMC1, Progesterone receptor membrane component 1, mutation, single nucleotide polymorphisms

How to cite this Article:
Solaipriya Solairaja, Habeeb Shaik Mohideen and Sivaramakrishnan Venkatabalasubramanian (2023). Computational Identification and Validation of Non-Synonymous SNPs in Progesterone Receptor Membrane Complex 1 Linked to Lung Cancer. *International Journal of Experimental Research and Review*, *36*, 66-75.
DOI:
https://doi.org/10.52756/ijerr.2023.v36.006

Abstract: Numerous gene polymorphisms have been attributed to Lung cancer, but PGRMC1 (Progesterone receptor membrane component 1) is a lesser-known candidate among them. However, emerging research is slowly suggesting the role of polymorphisms in PGRMC1 gene-associated tumorigenesis. Nevertheless, phenotypic changes still need to be studied. The main aim of this study is to identify the most deleterious nsSNPs (non-synonymous single nucleotide polymorphisms) in PGRMC1 that can potentially increase the susceptibility to lung cancer progression. In this work, we scrutinized highly detrimental nsSNPs for PGRMC1 from the available dbSNP database. We further categorized using the FATHMM server to enlist the nsSNPs, which are driver mutants capable of affecting the function of the PGRMC1 protein. We employed clinical evidence from the COSMIC database for further evaluation and confirming the presence of nsSNPs in lung cancer patients. There are 12 nsSNPs reported in lung cancer patients, which are L32M, R47C, D141N, G20W, S57C, R70P, I89V, G118V, A191D, G95V, E157K, and G168V predicted to damage the functions. Conclusively, through a comprehensive comparison of the outcomes obtained through these computational methods, we identified novel I89V, D120E, G95V and G168V nsSNPs that pose substantial risks to the functionality of the PGRMC1 protein. Focusing on the importance of PGRMC1 in lung cancer, analyzing its function was conceded to unveil the interlink between genetic mutation and phenotypic changes. Thus, this study provides insights into the influence of PGRMC1variants in Lung cancer. The evaluated nsSNPs can significantly aid future research on the gene and its association with lung cancer progression in large population distribution frequencies of genotypes among different subgroups.

Introduction

Lung cancer is the most common malignancy worldwide, which accounts for 11.4% of all cancer incidences and 18.0% of all cancer mortalities. It is well-recognized that multiple factors contribute to the development of lung cancer (Saha and Yadav, 2023; Mehta et al., 2023). The etiology of lung cancer is now widely accepted to be influenced by the combined effects of hereditary and various environmental factors. Lung cancer is assessed to have an 18% heritability, suggesting that genetic factors may be significant in lung cancer progression (Wang et al., 2020; Lebrett et al., 2021; Boga and Bisgin, 2022; Reddy and Khanaa, 203).

Progesterone Receptor Membrane Complex 1 (PGRMC1), located on human chromosome Xq22, is a 25 kDa archetypal multi-protein complex with an N terminal transmembrane domain and a large C-terminal cytochrome b5 like heme/ steroid binding domain, belongs to the membrane-associated receptor protein family and is mainly linked with resistance to DNA damage and apoptotic suppression. Unlike the other MAPR family, PGRMC1 is involved in tumour progression, and an increased expression level was

***Corresponding Author:** sivaramv@srmist.edu.in

observed in various cancer types, such as lung, breast, thyroid, and ovarian cancers (McGuire and Espenshade, 2022; Pru, 2022). Due to its high p450 activity and drug-resistance properties, the molecular target against PGRMC1-expressed cancer is still under discussion (Thejer et al., 2020). Mutations on the phosphorylation sites of PGRMC1 significantly impact metabolic changes and varied genomic mutation rates (Thejer et al., 2020).

Furthermore, PGRMC1 serves a vital function in binding, and it is evidentially reported that it nearly binds with 19 Cytochrome P450 from 8 different enzyme families. The pleiotropic function of PGRMC1 is beyond speculation because of its docking property; it is more likely to interact with many other critical proteins, which could lead to regulating cellular functions such as angiogenesis, regulation of cell division, chemoresistance, metabolism, migration and metastasis. Recent studies are focused on establishing the functional attributes of PGRMC1 in many disease conditions (McGuire and Espenshade, 2022; Pru, 2022).

A computational analysis was performed to expand the repertoire of PGRMC1 functional SNPs in lung cancer to identify potential therapeutic targets further down the line. The computational analysis of the most damaging nsSNPs of PGRMC1 in lung cancer has yet to be validated. Thus, we conducted this first study to evaluate the most detrimental and damaging nsSNPs in PGRMC1 and observed their functional stability and oncogenic nature.

Materials and Methods

Dataset collection

The nsSNP data were downloaded from the NCBI dbSNP database, available at https://www.ncbi.nlm.nih.gov/snp/ and includes information such as chromosome loci, protein accession number, nucleic acid position, and amino acid residue changes. The human PGRMC1(ID: O0026) protein information was obtained from UniProtKB. It includes size, FASTA sequence functional domains and regions (Pavithran and Kumavath, 2021).

Computational analysis of nsSNPs of PGRMC1 gene

Using five different in-silico tools permits retrieving SNPs from the database to analyze and predict the functional impact. SIFT (http://sift.jcvi.org/) predicts the deleterious effect of nucleotide substitution on protein function based on the conservancy of the specific position of an amino acid in a sequence (Sim et al., 2012). PROVEAN (http://provean.jcvi.org) is used to specify the missense and insertion/deletion variants, whether they are functionally essential or not (Choi and Chan, 2015). PolyPhen 2.0 (http://genetics.bwh.harvard.edu/pph2/) used the protein structure to identify the changes in the structure and functions into benign, possibly damaging, and probably damaging (Adzhubei et al., 2013). PhD-SNP (http://snps.biofold.org/phd-snp/phd-snp.html) classifies the mutation into either neutral or disease-related polymorphism. SNAP2 (https://www.rostlab.org/services/snap/) includes structural characteristics like secondary structure and solvent accessibility (Hecht et al., 2015).

Prediction of Disease-Associated Mutation of PGRMC1 gene by Mutpred

Mutpred is a tool to determine the disease associated with nsSNP (http://mutpred2.mutdb.org/). It includes the molecular effect of the specific residue substitution. It uses the SVM analysis. It works upon the properties like loss or gain of phosphorylation and alteration in metal binding. FASTA format of the protein sequence and amino acid substitutions were the input. The output provides a probability score for the effect of the variations, whether the changes are disease-causing. P-value ($p < 0.05$ and $p < 0.01$) represents the précised structural and functional features caused by the variants (Pejaver et al., 2020).

Protein stability analysis of disease-associated nsSNP mutations of PGRMC1

Protein structural stability is a fundamental feature that regulates biological molecules, activity, and function. An essential indicator of protein stability is the free energy of protein unfolding. Determining the precision of the mutation's effect on protein stability depends on the impact of the mutation on free energy. Understanding the consequence of mutations on the stability of the protein continues to reflect in function could be analyzed. I-Mutant 2.0: A support vector machine – web server (http://folding.biofold.org/i-mutant/i-mutant2.0.html).
The G value (kcal/mol) can estimate the stability change. If the variant has ΔΔG less than '0', it indicates a decrease in stability, whereas a greater value means the variant elevates the stability (Capriotti et al., 2005).

Biophysical Characteristics Analysis

The impact of PGRMC1 missense substitutions was evaluated using the Grantham Variation (GV) method using the Align-GVGD (A-GVGD) tool (Lim et al., 2022).

Identification of the oncogenic nature of non-synonymous mutation of PGRMC1

Cscape is a web tool (http://fathmm.biocompute.org.uk/) used to predict the oncogenic nature of non-synonymous mutation and the functional effects of a protein caused by the missense

mutation through the HMM model. Based on the FATHMM prediction algorithm, human disease ontology and phenotype ontology were also predicted. The scoring system represents the protein and domain's tolerance level influenced by mutation (Rogers et al., 2017).

Analysing cancer-associated nsSNPs

Mutation 3D, accessible at http://www.mutation3d.org/, is a tool that detects clusters of amino acid variants resulting from somatic cancer mutations. This tool employs 3D clustering methods to analyze the geographical distribution and assess the impact of amino acid substitutions on protein structure. (Feroz and Islam, 2023).

PGRMC1 mutational analysis in Lung Cancer

For PGRMC1 mutation analysis in lung cancer, the data from (https://cancer.sanger.ac.uk/cosmic accessed on August 2023) was explored for identifying the PGRMC1 SNP association with different cancers. This database represented PGRMC1 mutations in Pan-cancer and distribution in clinical samples. The association of highly deleterious PGRMC1 nsSNPs lung cancer was analysed using a mutation profile (Paleri et al., 2020).

Prediction of Structural impact of selected nsSNPs on PGRMC1

Project Hope is an online web server that analyses the structural impact caused by the amino acid substitutions in the protein sequence (https://www3.cmbi.umcn.nl/hope) (Hossain et al., 2020).

Result

SNP Dataset Retrieval

Altogether, 1751 SNPs of the PGRMC1 gene were reported from the dbSNP database, among which 103 were identified as non-synonymous (Supplementary Table 1). We have used all 103 nsSNPs from dbSNP in five different computational approaches to investigate whether all these missenses could cause any influence on the structure or biological function of the protein.

Prediction of deleterious nsSNP of PGRMC1

These five tools predicted the pathogenicity of selected 103 nsSNPs retrieved from the database. As a result of these integrated web tools, outcomes were analyzed according to its scoring matrix (Fig. 1), (Fig. 2).

In SIFT, out of 103 non-synonymous SNPs investigated, 42 (19%) were known to be pathogenic, having a tolerant index (TI) ≥ 0.05. Among these, twenty showed an absolute tolerant score (TI) of 0, which depicted that they were highly damaging. Sixty-one were considered to be tolerated with a TI score of greater than 0.05.

In PROVEAN, if the threshold value is below -2.5 as a final score, then the variants are deleterious. 42 (19%) of nsSNPs scored below the cutoff. The above values are considered neutral.

PolyPhen-2 results indicated that 53 (23%) are considered pathogenic, which means they scored between 0.5- 1. It explains the ranking in a way: less confident prediction scores are in the range between 0.5 – 0.8, and high confident predictions are 0.8 – 1. So out of 103 non-synonymous, 23 are 'possibly damaging', and 30 are 'probably damaging' below the values are found to be 'benign'.

PhD-SNP provides the results based on the probability score of more than 0.5 are marked as disease polymorphism, whereas the remaining are neutral. From the chart, it is counted as 28 are disease-causing.

SNAP2 predicted the impact of amino acid substitution range from – 100 to +100. This tool indicated that 61 variants are significant, while the remaining mutations are neutral.

The bioinformatics approach scrutinized the predicted high-risk nsSNPs that are deleterious. Functional analysis, stability, conservation profile, and physicochemical properties are studied to prioritize the number of possible missense mutation characteristics.

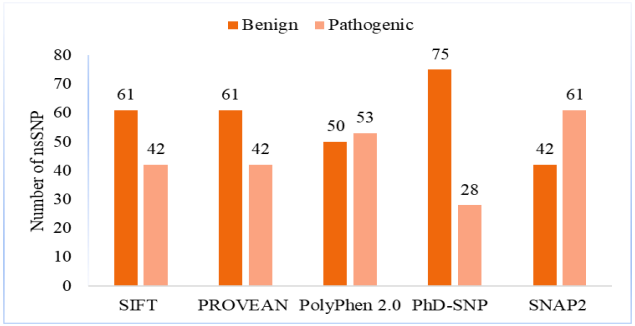

Figure 1. Pathogenicity prediction of nsSNPs by SIFT, PROVEAN, Poly Phen-2, PhD-SNP, and SNAP2. The number of 'benign' and 'pathogenic' nsSNPs identified by each tool.

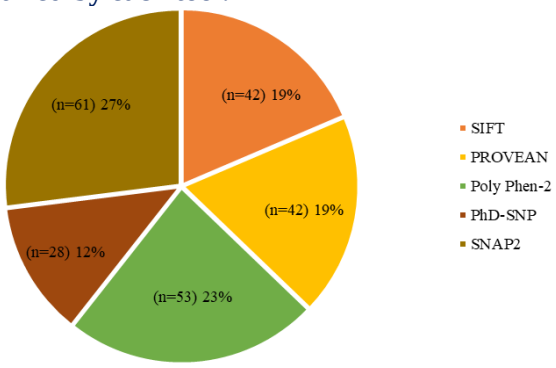

Figure 2. Pie chart representing the percentage of the deleterious effect of nsSNPs predicted. The distribution of deleterious nsSNPs by number (n) and percentage (%) was predicted by 5 *in silico* tools: SIFT, PROVEAN, Poly Phen-2, PhD-SNP, and SNAP2.

DOI: https://doi.org/10.52756/ijerr.2023.v36.005

Diseases associated substitution analysis by Mutpred

The molecular effect of amino acid substitution has been predicted. Based on the default threshold value of 0.5, >0.5 is considered harmful, whereas above 0.75 could be a more confident deleterious prediction. The results for further analysis are given in Table 1.

Table 1. Identification of disease-associated substitution by using Mutpred score.

Amino acid change	Score
K96R	0.517
D131N	0.524
D131H	0.681
P177H	0.636
T74P	0.856
L142P	0.837
D131G	0.709
D120E	0.887
A11P	0.544
G108E	0.905
G48R	0.647
N94D	0.54
F81L	0.655
H166D	0.688
P109S	0.743
F73L	0.799
D140N	0.565
L90F	0.736
R47H	0.531
I45T	0.673
K172T	0.683
D82N	0.581
E110Q	0.541

Stability modification prediction

Modification in protein stability occurs by changes in ΔΔG-free energy. It is projected that the 103 nsSNPs of PGRMC1 will have an effect on the stability of the protein. Out of 103 selected SNPs, 86 showed decreased, and 17 showed increased stability predicted from I-Mutant 2.0. (Supplementary Table 1). Hence, the domain or site-specific mutations might cause protein loss. According to fewer studies on protein stability, there is a possibility of causing damage like a decrease in stability, increasing the misfolding, degradation, and aggregation.

Biophysical property analysis

This measures the biophysical properties and employs the GD value to categorize into C0, C15, C25, C35, C45, C55, and C65 to identify the nsSNPs as neutral, least deleterious or deleterious. Based on Align-GVGD results, the 27 PGRMC1 nsSNPs fall within class C65 (n = 17), class C35 (n = 2), class C25 (n =3), and class C15 (n = 5). C129Y, G174R, G174W, T74I, G174E, P66R, P112L, L135P, R71C, M91T, D131H, P177H, L142P, G108E, P109S, I45T and K172T were predicted to cause functional effect on the protein. The detailed results are shown in Supplementary Table 1.

Analysis of the oncogenic nature and phenotypic impact of nsSNPs

The pathogenicity of a protein substitution was analyzed by the FATHMM tool following the HMM model. For prediction, the FATHMM tool employs two distinct combinations, i.e., coding and non-coding. This is further categorised into germline polymorphisms, cancer-promoting, and disease-specific mutations. The HMM model initially aligns the homology sequences and most conserved proteins with identifying the probability index of mutation promoting amino acid substitution in the protein. Out of all nsSNPs, H165R, L153V, T74I, M91I, M91V, M91L, I89V, P112L, D86N, A76T, E157D, R80H, M91T, K96R, R104H, R88H, T74P, G108E, R79Q, A76V, N94D, F81L, H166D, P109S, F73L, L90F, D82N, E110Q, R80C are identified as a “passenger mutation” whereas D120E is predicted as “cancer driving mutation” (Table 2).

Identification of cancer-causing nsSNPs

The investigation uses Mutation 3D, which identifies the deleterious nsSNPs involved in the development of somatic Cancer. Despite the structural changes, the functional changes in PGRMC1 may result in the development of Cancer. This analysis reported that F73L, T74P, T74I, A76T, A76V, R79Q, R80C, F81L, D82N, D86N, R88H, I89V are clustered mutation. L90F, M91I, M91V, N94D, K96R, R104H, G108E, P109S, E110Q and P112L are covered mutation (Figure 3).

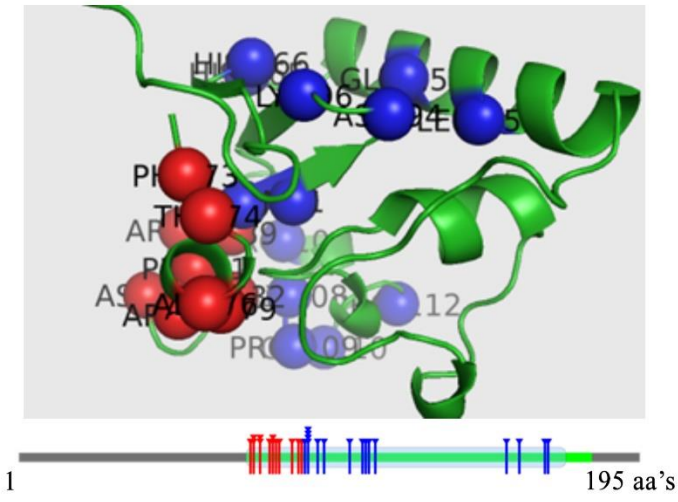

Figure 3. Predicted the association of nsSNPs (red mark) with cancer using Mutation 3D. Red colour represents clustered mutation, while blue represents covered mutation.

DOI: https://doi.org/10.52756/ijerr.2023.v36.005

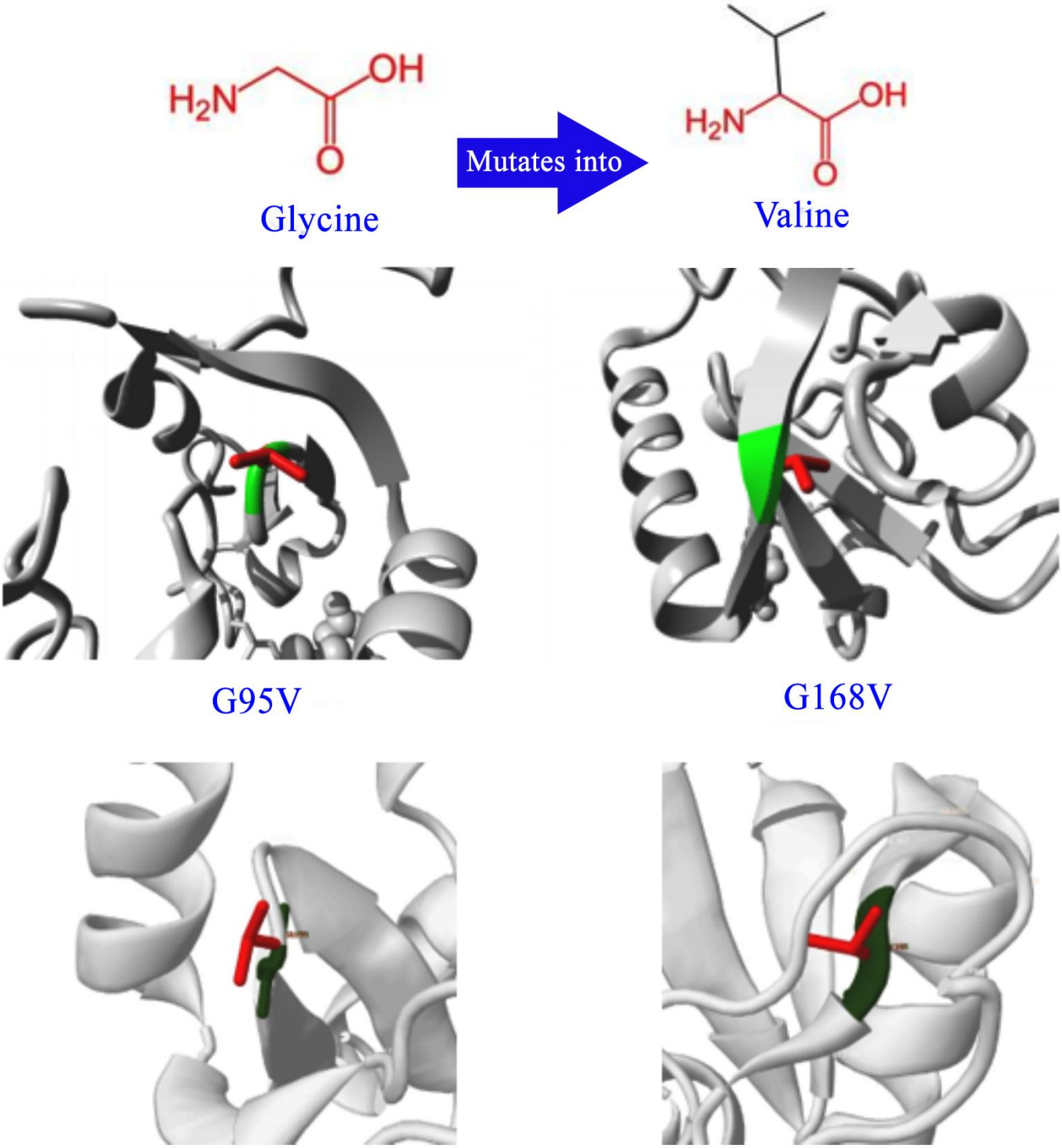

Figure 4. Structural impact of selected nsSNPs using Hope Server. Structural alteration of the wild-type residue G95V and G168V represented by Project Hope. The wild-type residue is presented as green, and the mutant residue is shown in red.

DOI: https://doi.org/10.52756/ijerr.2023.v36.005

Identifying the deleterious PGRMC1 nsSNPs association with Lung Cancer

The analysis of the nsSNPs of PGRMC1 resulted in the report of R47C and R70P in the COSMIC database among the screened 27 nsSNPs. These two mutations have been identified as somatic mutations reported in SCLC (small cell carcinoma) and LUSC. In LUAD, two novel nsSNPs, G95V and G168V, have been confirmed in tumour samples. The details are shown in Table 3, Supplementary Table 2.

Sample Name	AA Mutation	Primary Tissue	Histology Subtype 1	Somatic Status
TCGA-22-4594-01	p.G20W	Lung	Squamous cell carcinoma	Confirmed Somatic
S01542_1	p.L32M	Lung	Small cell carcinoma	Confirmed Somatic
S00501	p.R47C	Lung	Small cell carcinoma	Previously Reported
S00501_1	p.R47C	Lung	Small cell carcinoma	Confirmed Somatic
TCGA-85-A4CN-01	p.S57C	Lung	Squamous cell carcinoma	Confirmed Somatic
AD2315	p.R70P	Lung	Squamous cell carcinoma	Variant of unknown origin
AD2385	p.R70P	Lung	Squamous cell carcinoma	Variant of unknown origin
AD2351	p.R70P	Lung	Squamous cell carcinoma	Variant of unknown origin
TCGA-22-5491-01	p.I89V	Lung	Squamous cell carcinoma	Confirmed Somatic
IGC-04-1143	p.G95V	Lung	Adenocarcinoma	Confirmed Somatic
TCGA-O2-A52S-01	p.G118V	Lung	Squamous cell carcinoma	Confirmed Somatic
585208	p.D141N	Lung	Small cell carcinoma	Confirmed Somatic
LUAD-E00897	p.E157K	Lung	Adenocarcinoma	Previously Reported
TCGA-44-8117-01	p.G168V	Lung	Adenocarcinoma	Variant of unknown origin
TCGA-21-1076-01	p.A191D	Lung	Squamous cell carcinoma	Confirmed Somatic

Structural Impact of nsSNPs on PGRMC1 Protein

The Hope server shows that the substitution of I89V is located on the conserved region of the protein. The mutant residue Valine forms the space in the protein core. Mutant residues of D120E, G95V and G168V are bigger than wild-type residues. The wild-type residue of G95V is flexible to form torsion angles, and mutant residue may lead to unusual conformation and probably disturb the native structure of the protein. In the case of G168V, the mutant residue is bigger than the wild type. However, wildtype residue was in the buried region of the protein. Due to size and location, mutant residue cannot prevent the local structure of the protein. In comparison to all the selected mutants, G95V and G168V are predicted to be pathogenic due to conformational structural changes (Figure 4).

Discussion

Over the past decade, with advanced high-throughput sequencing technology, genomic variation identification in cancer and other rare conditions has emerged rapidly. The identification of biologically significant SNPs plays a crucial role in the formulation of SNP-based genetic profiles. These profiles can serve as essential genetic screening markers, aiding in identifying individuals' susceptibility to various diseases and contributing to the exploration of inheritance patterns. Variations in drug-metabolizing enzymes, drug transporters, and genes responsible for signaling receptors can trigger diverse responses to drugs among individuals. Within the NCBI-maintained dbSNP database, an extensive collection of over a billion SNPs is curated, prominently featuring a subset of 9.6 million SNPs characterized as non-synonymous mutations. The nsSNPs have the potential to influence susceptibility to diseases, alter the structure of proteins, and maintain their significance across different pathological conditions (Bhatnager and Dang, 2018; Zhanget al., 2020; Zhanget al., 2023). The association between PGRMC1 and cancer is supported by a substantial and rapidly increasing form of evidence. Proposed mechanisms driving the upregulation of PGRMC1 in neoplasms include increased hypoxia, augmented responsiveness to growth factors such as EGFR, genetic mutations, downregulated expression of microRNAs that would otherwise modulate PGRMC1 mRNA, and mechanisms commonly associated with elevating the expression of other proto-oncogenes (Cahill and Neubauer, 2021; Pru, 2022; Ruan and Mueck, 2023). PGRMC1 mRNAs and proteins have now been connected to or implicated in the progression of myriad

cancers, such as ovarian, breast, thyroid, lung, liver and head and neck. Upregulated expression of PGRMC1 often correlates with an unfavourable prognosis and increased mortality rates. PGRMC1 proteins have been linked with various attributes frequently contributing to cancer pathology. Nevertheless, our understanding of the molecular mechanism underlying PGRMC1's involvement in the progression of lung cancer remains limited despite its well-known role in the EGFR pathway and cholesterol biosynthetic pathway activation. In the present research, we have followed a pipeline to identify the deleterious nsSNPs of PGRMC1 associated with lung cancer (Ahmed et al., 2010; Solairaja et al., 2022).

This investigation applied various bioinformatics tools to analyze nsSNPs within the PGRMC1 gene. The central aim was to comprehend how these nsSNPs could influence the structure and function of the protein. The dataset encompassing 103 nsSNPs originating from the PGRMC1 gene was derived from the dbSNP database. After this, a meticulous filtration process was enacted to predict the existence of notably detrimental SNPs. This undertaking was facilitated by deploying five distinct tools: SIFT, PROVEAN, PolyPhen 2.0, PhD-SNP and SNAP2. Combining these diverse web servers was strategically employed to heighten the precision and reliability. Ultimately, a specific subset of the study pinpointed 27 nsSNPs categorized as highly damaging, given their potential to cause detrimental effects within the set of 103 PGRMC1 nsSNPs (Avsar, 2022; Bhatnager and Dang, 2018). Additional tools, such as I-Mutant and biophysics analysis, were employed to further refine the selection of potential pathogenic nsSNPs. These tools enabled predictions concerning protein stability, conservation of amino acids across evolution, changes in physical and chemical traits, and alterations in protein structure resulting from the mutations.

To further validate the in-silico findings, an assessment was conducted to determine the impact of the identified 27 deleterious nsSNP protein stability. Upholding protein stability assures the appropriate structure and functionality of the protein (Arshad et al., 2018; Lim et al., 2022; Zhang et al., 2020). This governs its conformational arrangement, a critical factor that dictates its overall functionality. Disruptions in protein stability can result in degradation misfolding or even abnormal protein aggregation. For this purpose, I-Mutant and MUpro were employed to observe the potential influence of these deleterious nsSNPs on the stability of the PGRMC1 protein, aiming to enhance the outcomes' reliability. Remarkably, combining the outcome from these tools concurred that 19 out of the 27 highly deleterious nsSNPs would likely lead to a decrease in the stability of the PGRMC1 protein (Venkata Subbiah et al., 2020).

Cancer-causing nsSNPs are identified using the HMM model tool (Pavithran and Kumavath, 2021; Rogers et al., 2017). The mutation 3D results intuitively represent the difference between functional and non-functional mutation using mutation clusters. Thereby, this server utilizes information from cohort patients to identify the mutation which could induce the same cancer condition since it may be capable of forming a cluster. Such clusters are considered a hotspot in protein, favouring tumour cells by changing their function through structural modification (Meyer et al., 2016). According to the COSMIC database results, twelve nsSNPs in that L32M, R47C, and D141N are identified in SCLC, G20W, S57C, R70P, I89V, G118V, and A191D are in association with LUSC and three nsSNPs G95V, E157K and G168V associated in the risk of LUAD. However, there remains the possibility that other high-risk nsSNPs identified in this work could be linked to lung cancer and other cancer types (Kosaloglu et al., 2016; Paleri et al., 2020). Speculated nsSNPs that corresponded to the PGRMC1gene mutations were analyzed in the COSMIC database to understand the impact of predicted SNPs in lung cancer (Wang et al., 2019). I89V was predicted to be a cluster mutation and was also present in LUSC patients. This mutation is located on the cytochrome b5 domain and present on the interaction surface, which might interrupt the protein function and subsequently decrease the stability of the protein (Mansouri et al., 2008). G95V is located on the surface of the domain with an unknown function, whereas G168V is on the cytochrome b5 domain. Due to structural properties, these mutations are more likely to disturb the native structure and function of the PGRMC1 protein. Nevertheless, G95V's and G168V's mutational impact on a larger population has yet to be studied. Further in vitro studies are required to suggest the role of the unexplored mutations G95V and G168V in lung cancer initiation and progression.

The findings from our study illuminated the high mutational possibility in LUSC compared to LUAD. It could influence patient survival. Prior research has supported that the mutation in PGRMC1 holds significance in driving the onset, progression, proliferation, and overall survival of LUSC and other cancer types.

Conclusion

In conclusion, the exact biological role of the PGRMC1 protein in LUAD has yet to be well

DOI: https://doi.org/10.52756/ijerr.2023.v36.005

understood. However, speculation about the role of PGRMC1 protein in lung cancer has yet to be studied. Though it is secreted in the lung, it is also elevated in a large population of lung tumours and has an impact on promoting cell proliferation, invasion and metastasis. Moreover, PGRMC1 may be involved in the progression of LUAD induced by downstream signalling, regulating the EGFR pathway and other binding partners. Hence, PGRMC1 delineates a considerate target for LUAD, and nsSNPs may directly or indirectly influence LUAD susceptibility.

Interestingly, this is the first comprehensive computational approach to characterizing functional nsSNPs in the PGRMC1 protein. However, more experimental and clinical studies in other lung cancer samples, such as SCLC, LUSC, and other subtypes, should be investigated in the future to validate the results of this study. Moreover, in-silico analysis is needed to interpret the plausible mechanism latent between nsSNPs and susceptibility to LUAD.

Based upon the bioinformatic analysis, this study delineates that out of 103 reported nsSNPs of PGRMC1, the above 12 polymorphisms are identified to have a pathological role in lung cancer patients; hence, this could be possibly play a role in lung cancer progression. Considering the above *in silico* results and analysis, which strongly implicate that I89V, D120E, G168V and G95V could be key candidates in cancer progression. However, in-vitro and in-vivo experiments are required to confirm the impact on the functionality of the PGRMC1 protein. In conclusion, the remaining pathogenic variants could also possibly play a role in the progression of other lung cancer subtypes.

Acknowledgement

We humbly thank the Department of Genetic Engineering, SRMIST, for providing facilities and Infrastructure for this work.

Conflict of Interest

No conflict of interest exists between the authors.

References

Adzhubei, I., Jordan, D. M., & Sunyaev, S. R. (2013). Predicting functional effect of human missense mutations using PolyPhen-2. *Current Protocols in Human Genetics*, *76*(1), 7–20. https://doi.org/10.1002/0471142905.hg0720s76

Ahmed, I. S., Rohe, H. J., Twist, K. E., Mattingly, M. N., & Craven, R. J. (2010). Progesterone receptor membrane component 1 (Pgrmc1): A heme-1 domain protein that promotes tumorigenesis and is inhibited by a small molecule. *Journal of Pharmacology and Experimental Therapeutics*, *333*(2), 564–573. https://doi.org/10.1124/jpet.109.164210

Arshad, M., Bhatti, A., & John, P. (2018). Identification and in silico analysis of functional SNPs of human TAGAP protein: A comprehensive study. *PloS One*, *13*(1), e0188143. https://doi.org/10.1371/journal.pone.0188143

Avsar, O. (2022). Analysis of missense SNPs in the SLC47A1 and SLC47A2 genes affecting the pharmacokinetics of metformin: Computational approach. *Egyptian Journal of Medical Human Genetics*, *23*(1), 92. https://doi.org/10.1186/s43042-022-00306-9

Bhatnager, R., & Dang, A. S. (2018). Comprehensive in-silico prediction of damage associated SNPs in Human Prolidase gene. *Scientific Reports*, *8*(1), 9430. https://doi.org/10.1038/s41598-018-27789-0

Boga, I., & Bisgin, A. (2022). Real-world applications of tumor mutation burden (TMB) analysis using ctDNA and FFPE samples in various cancer types of Turkish population. *International Journal of Experimental Research and Review*, *29*, 89-93. https://doi.org/10.52756/ijerr.2022.v29.010

Cahill, M. A., & Neubauer, H. (2021). PGRMC proteins are coming of age: A special issue on the role of PGRMC1 and PGRMC2 in metabolism and cancer biology. *Cancers*, *13*(3), 512. https://doi.org/10.3390/cancers13030512.

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*(suppl_2), W306–W310. https://doi.org/10.1093/nar/gki375

Choi, Y., & Chan, A. P. (2015). PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*, *31*(16), 2745–2747. https://doi.org/10.1093/bioinformatics/btv195.

Feroz, T., & Islam, M. K. (2023). A computational analysis reveals eight novel high-risk single nucleotide variants of human tumor suppressor LHPP gene. *Egyptian Journal of Medical Human Genetics*, *24*(1), 47. https://doi.org/10.1186/s43042-023-00426-w

Hecht, M., Bromberg, Y., & Rost, B. (2015). Better prediction of functional effects for sequence variants. *BMC Genomics*, *16*(8), 1–12. https://doi.org/10.1186/1471-2164-16-S8-S1

Hossain, M. S., Roy, A. S., & Islam, M. S. (2020). In silico analysis predicting effects of deleterious SNPs of human RASSF5 gene on its structure and functions. *Scientific Reports*, *10*(1), 14542. https://doi.org/10.1038/s41598-020-71457-1

Kosaloglu, Z., Bitzer, J., Halama, N., Huang, Z., Zapatka, M., Schneeweiss, A., … Zörnig, I. (2016). In silico SNP analysis of the breast cancer antigen NY-BR-1. *BMC Cancer*, *16*(1), 1–12. https://doi.org/10.1186/s12885-016-2924-7

Lebrett, M. B., Crosbie, E. J., Smith, M. J., Woodward, E. R., Evans, D. G., & Crosbie, P. A. J. (2021). Targeting lung cancer screening to individuals at greatest risk: The role of genetic factors. *Journal of Medical Genetics*, *58*(4), 217–226. https://doi.org/10.1136/jmedgenet-2020-107399

Lim, E. C., Lim, S. W., Tan, K. J., Sathiya, M., Cheng, W. H., Lai, K.S., & Yap, W.S. (2022). In-silico analysis of deleterious SNPs of FGF4 gene and their impacts on protein structure, function and bladder cancer prognosis. *Life*, *12*(7), 1018. https://doi.org/10.3390/life12071018

Mansouri, M. R., Schuster, J., Badhai, J., Stattin, E.-L., Lösel, R., Wehling, M., & Golovleva, I. (2008). Alterations in the expression, structure and function of progesterone receptor membrane component-1 (PGRMC1) in premature ovarian failure. *Human Molecular Genetics*, *17*(23), 3776–3783. https://doi.org/10.1093/hmg/ddn274

McGuire, M. R., & Espenshade, P. J. (2022). PGRMC1: An enigmatic heme-binding protein. *Pharmacology & Therapeutics*, 108326. https://doi.org/10.1016/j.pharmthera.2022.108326

Mehta, V., Dey, A., Thakkar, N., Prabhakar, K., Jothimani, G., & Banerjee, A. (2023). Anti-cancer Properties of Dietary Supplement CELNORM against Colon and Lung Cancer: An in vitro preliminary study. *International Journal of Experimental Research and Review*, *32*, 1-14. https://doi.org/10.52756/ijerr.2023.v32.001

Meyer, M. J., Lapcevic, R., Romero, A. E., Yoon, M., Das, J., Beltrán, J. F., & Paccanaro, A. (2016). mutation3D: cancer gene prediction through atomic clustering of coding variants in the structural proteome. *Human Mutation*, *37*(5), 447–456. https://doi.org/10.1002/humu.22963

Paleri, A. V., Cherian, I., Suresh, P. S., & Venkatesh, T. (2020). In silico analysis of COSMIC retrieved P body gene mutations in breast cancer. *Gene Reports*, *19*, 100617. https://doi.org/10.1016/j.genrep.2020.100617

Pavithran, H., & Kumavath, R. (2021). In silico analysis of nsSNPs in CYP19A1 gene affecting breast cancer associated aromatase enzyme. *Journal of Genetics*, *100*(2), 23. https://doi.org/10.1007/s12041-021-01274-6

Pejaver, V., Urresti, J., Lugo-Martinez, J., Pagel, K. A., Lin, G. N., Nam, H.J., & Iakoucheva, L. M. (2020). Inferring the molecular and phenotypic impact of amino acid variants with MutPred2. *Nature Communications*, *11*(1), 5918. https://doi.org/10.1038/s41467-020-19669-x

Pru, J. K. (2022). Pleiotropic actions of PGRMC proteins in cancer. *Endocrinology*, *163*(7), bqac078. https://doi.org/10.1210/endocr/bqac078

Reddy, N. S., & Khanaa, V. (2023). Diagnosing and categorizing of pulmonary diseases using Deep learning conventional Neural network. *International Journal of Experimental Research and Review*, *31*(Spl Volume), 12-22. https://doi.org/10.52756/10.52756/ijerr.2023.v31spl.002

Rogers, M. F., Shihab, H. A., Gaunt, T. R., & Campbell, C. (2017). CScape: A tool for predicting oncogenic single-point mutations in the cancer genome. *Scientific Reports*, *7*(1), 11597. https://doi.org/10.1038/s41598-017-11746-4

Ruan, X., & Mueck, A. O. (2023). Clinical importance of PGRMC1 in hormone responsive breast cancer. *Breast Care*, *18*(3), 172–178. https://doi.org/10.1159/000527969

Saha, A., & Yadav, R. (2023). Study on segmentation and prediction of lung cancer based on machine learning approaches. *International Journal of Experimental Research and Review*, *30*, 1-14. https://doi.org/10.52756/ijerr.2023.v30.001

Sim, N.L., Kumar, P., Hu, J., Henikoff, S., Schneider, G., & Ng, P. C. (2012). SIFT web server: Predicting effects of amino acid substitutions on proteins. *Nucleic Acids Research*, *40*(W1), W452–W457. https://doi.org/10.1093/nar/gks539

Solairaja, S., Ramalingam, S., Dunna, N. R., & Venkatabalasubramanian, S. (2022). Progesterone receptor membrane component 1 and its accomplice: Emerging therapeutic targets in lung cancer. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders)*, *22*(6), 601–611. https://doi.org/10.2174/1871530321666211130145542

Thejer, B. M., Adhikary, P. P., Teakel, S. L., Fang, J., Weston, P. A., Gurusinghe, S., & Ludescher, M. (2020). PGRMC1 effects on metabolism, genomic mutation and CpG methylation imply crucial roles in animal biology and disease. *BMC Molecular and Cell Biology*, *21*, 1–19. https://doi.org/10.1186/s12860-020-00268-z

Venkata Subbiah, H., Ramesh Babu, P., & Subbiah, U. (2020). In silico analysis of non-synonymous single nucleotide polymorphisms of human DEFB1 gene. *Egyptian Journal of Medical Human Genetics*, *21*(1), 1–9. https://doi.org/10.1186/s43042-020-00110-3

Wang, Q., Mehmood, A., Wang, H., Xu, Q., Xiong, Y., & Wei, D.Q. (2019). Computational screening and

analysis of lung cancer related non-synonymous single nucleotide polymorphisms on the human kirsten rat sarcoma gene. *Molecules*, *24*(10), 1951. https://doi.org/10.3390/molecules24101951

Wang, Y., Ma, R., Liu, B., Kong, J., Lin, H., Yu, X., & Zhou, B. (2020). SNP rs17079281 decreases lung cancer risk through creating an YY1-binding site to suppress DCBLD1 expression. *Oncogene*, *39*(20), 4092–4102. https://doi.org/10.1038/s41388-020-1278-4.

Zhang, M., Huang, C., Wang, Z., Lv, H., & Li, X. (2020). In silico analysis of non-synonymous single nucleotide polymorphisms (nsSNPs) in the human GJA3 gene associated with congenital cataract. *BMC Molecular and Cell Biology*, *21*(1), 1–13. https://doi.org/10.1186/s12860-020-00252-7

Zhang, R., Akhtar, N., Wani, A. K., Raza, K., & Kaushik, V. (2023). Discovering Deleterious Single Nucleotide Polymorphisms of Human AKT1 Oncogene: An In Silico Study. *Life*, *13*(7), 1532. https://doi.org/10.3390/life13071532

How to cite this Article:

Solaipriya Solairaja, Habeeb Shaik Mohideen and Sivaramakrishnan Venkatabalasubramanian (2023). Computational Identification and Validation of Non-Synonymous SNPs in Progesterone Receptor Membrane Complex 1 Linked to Lung Cancer. *International Journal of Experimental Research and Review*, *36*, 66-75.

DOI : https://doi.org/10.52756/ijerr.2023.v36.006