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In-silico Analysis of Single Nucleotide Polymorphisms (SNPs) in Human Pten Gene

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Abstract

Phosphatase Tensin Homolog deleted on Chromosome 10 is a tumour suppressor gene frequently inactivated in human cancers. Single Nucleotide Polymorphisms (SNPs) are the substitution of one DNA nucleotide base with another and a commonly occurring genomic alteration. This study involved analyzing various missense SNPs of the PTEN gene using five different bioinformatics tools -SIFT, POLYPHEN, CADD, META LR, and MUTATION ASSESSOR to identify the tolerated from intolerant ones. String analysis of PTEN protein-protein interactions was also done using the STRING database. A total of 8298 missense SNPs were retrieved and analyzed using the five bioinformatics tools. Out of 8298 missense SNPs analyzed, SIFT categorized 5281 SNPs as deleterious, while POLYPHEN identified 4490 as damaging. CADD showed that 909 were disease-causing SNPs. META LR identified 2995 as damaging, and the MUTATION ASSESSOR identified 897 high-risk missense SNPs. This study shows that the various in silico tools are a good preliminary approach to identifying the harmful missense SNPs.

Keywords: *Bioinformatics, PTEN, Single Nucleotide Polymorphisms.*

Introduction

The term “in silico” is a term similar to the biological terms “in vivo” and in vitro which mean ‘in the living body and ‘in the test tube respectively. It refers to data or knowledge acquired with the help of computer simulations and model analysis done in a virtual environment [1].

PTEN, phosphatase and tensin homolog, deleted on chromosome 10, is an important tumour suppressor gene located on chromosome 10q23.3 and is mutated in a wide variety of cancers. PTEN encodes a protein phosphatase comprised of 403 amino acids and has dual specificity on phosphoryl/threonyl and phosphotyrosine residues. PTEN causes dephosphorylation of PIP3 (phosphatidyl inositol 3,4,5-triphosphate to phosphatidylinositol 4,5-bisphosphate (PIP2) and hence suppresses Protein kinase B/Akt signalling, thus becoming the main down-regulator of the PI3K pathway. When PTEN is

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inactivated, the PI3K/Akt-mediated signalling cascade pathway is unchecked and causes uncontrolled cell growth and proliferation. The main effect of PTEN is its lipid-dephosphorylating property [2, 3].

Location of PTEN Protein

PTEN was thought to be localized to primarily the cellular cytoplasm but recent studies have confirmed its presence in subcellular compartments like mitochondria, nucleus and even in extracellular tissue spaces. In the cytoplasm, apart from its role in the inhibition of the PI3K/AKT pathway, PTEN also has a role in promoting apoptosis. Nuclear PTEN plays a vital role in maintaining chromosome integrity, DNA repair mechanisms, cell cycle regulation and genomic stability [4].

Regulation of PTEN Protein Function

Regulation of PTEN protein function occurs mainly by protein-protein interactions and post-translational modifications. However, it is important to note that new ways of regulation may also exist owing to the presence of PTEN in various cellular as well as extracellular compartments [5].

Metabolic Role of PTEN

Apart from its role in cell growth and signalling as a negative regulator, recent studies have shown that PTEN also plays a key role in metabolic pathways. Loss of PTEN function leads to tumorigenesis in humans. However recent studies indicate that loss of PTEN influences glucose metabolism and finally improves insulin sensitivity. In addition to this, the PI3K/Akt signalling pathway promotes lipid synthesis by activating SREBP-1(Sterol Regulatory Element Binding Protein -1), which increases the expression of key enzymes involved in de novo lipogenesis. In the context of **PTEN loss**, this pathway becomes overactive, leading to excessive lipid accumulation [6].

Other Important Functions

Evidence suggests that PTEN expression is regulated by transcription factors responsible for epithelial-mesenchymal transition (EMT). Also, PTEN has regulatory functions in various signalling pathways involved in EMT, metastasis and modulation of the tumour microenvironment. (Fedorova et al) Interestingly, PTEN also regulates the development and maintenance of Cancer Stem Cells (CSC) by affecting various signalling pathways like NOTCH, WNT, NF-kB, PI3K/AKT and MAP kinase pathway [7].

Germline Mutations of PTEN

Tumor syndromes such as Cowden syndrome, and PHTS (PTEN hamartoma tumor syndrome) show germline mutations in the PTEN gene. PHTS also includes individuals with Bannayan Riley Ruvalcaba (BRRS) Proteus and Proteus-like syndromes with germline mutation of the PTEN gene. Studies have shown that individuals with PHTS have a high risk of malignancies in the breast, thyroid, kidney, and colon along with an increased risk for melanoma [8].

The Roles of PTEN in Various Biological Processes are Still to be Explored

PTEN Gene in Various Cancers

PTEN gene alterations are frequently found in Endometrial cancers, glioblastoma, skin and prostatic carcinomas. Nearly forty-five per cent of endometrial carcinomas show PTEN gene alterations and most of them are missense substitutions. Endometrial carcinomas are classified as type 1 and type 2. While Type 1 endometrial carcinomas are associated with alterations involving PTEN, KRAS, and PI3KCA genes to name a few, Type II endometrial carcinomas usually show involvement of the P53 gene. In other gynecological cancers too, PTEN gene alterations like missense, and nonsense substitutions are seen, but the percentage is much lesser [9].

Glioblastoma (GBM) shows PTEN gene alterations commonly and most of them are missense mutations involving the phosphatase domain and truncating mutations which are mostly seen in the C2 domain. The study by Choi et al suggested that a PTEN mutation is more disastrous when compared to a PTEN deletion in a malignancy [10]. In Breast cancer, PTEN gene SNPs have been implicated in Breast cancer susceptibility, chemotherapeutic response as well as prognosis [11].

Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms are the most common genomic alteration that occurs in the human genome. In SNP, there is a substitution of one base of a DNA nucleotide. SNPs can occur at different locations of the gene, such as exon, intron, UTR, promoter regions, etc. SNPs situated in the coding regions i.e., exons, are called Non-Synonymous and Synonymous coding SNPs. Missense SNP is a type of Nonsynonymous SNP (nsSNP), and it causes an amino acid substitution leading to a potential alteration in the protein product, its structure and even function [12, 13].

This study aims to use computational bioinformatics tools to analyze various missense SNPs of the PTEN gene classify them into tolerated SNPs and identify the potentially disease-causing ones.

Objectives

To use bioinformatics tools such as SIFT, POLYPHEN, CADD, META LR and MUTATION ASSESSOR to analyze SNPs of the PTEN gene and to classify them into disease-causing ones and neutral ones. To identify the protein-protein interactions of PTEN protein using the String database.

Materials and Methods

Ethics and Consent

The study is a secondary analysis of human genomic data and does not meet the criteria of human experiments.

Inclusion Criteria

The missense SNPs in the PTEN gene for which the SIFT, POLYPHEN, CADD, META LR, and MUTATION ASSESSOR scores were available were included in this study.

Exclusion Criteria

Missense SNPs for which scores were not available were not included in the study. The number of such SNPs was, however, mentioned in the results section.

Methodology

Retrieval of SNP Data

The following steps were followed to retrieve data regarding missense SNPs from the Ensembl database (in the public domain).

1. After entering into the ensemble database, the human PTEN gene was selected, followed by selecting the menu 'VARIANT TABLE'.
2. In the variant table, filters were applied and only data about 5 *in-silico* tools i.e., SIFT CLASS', 'POLYPHEN CLASS', 'CADD CLASS', 'META LR CLASS', 'MUTATION ASSESSOR CLASS' were selected along with the rs IDs
3. Also, in the 'CONSEQUENCES' menu, filters were applied for all except Missense SNPs.

The entire data was exported to a CSV file and then into Microsoft Excel for data analysis. The data was analyzed using IBM SPSS software version 20 and expressed as descriptive statistics.

Statistical Analysis

The following in-silico tools were used:

SIFT (Sorting Intolerant from Tolerant)

Sorting Intolerant from Tolerant is a software tool used in bioinformatics to assess the functional significance of missense mutations in proteins. SIFT predicts whether a particular amino acid substitution will be tolerated or not by the protein. Tolerated Mutation: If the SNP

has a high probability of being tolerated (score >0.05), it suggests that the SNP is unlikely to cause a significant impact on protein function. Intolerated Mutation: If the SNP has a low probability of being tolerated (SIFT score <0.05), it suggests that the SNP may disrupt protein function and could be pathogenic.

POLYPHEN

Polyphen is yet another tool used to predict the impact of missense SNPs as probably damaging, possibly damaging or benign. The score ranges from 0 to 1.

CADD (Combined Annotation Dependent Depletion)

The CADD score ranges from 0 to 99 where higher scores indicate a greater chance that a variant is deleterious. CADD v1.7 integrates various features to enhance the accuracy of these predictions across different molecular functions.

META LR

Meta LR is a logistic regression-based scoring system that ranges from 0 to 1 where the higher the values, the more the chance that the variant is disease-causing. This prediction score has 10 other scores incorporated into it.

MUTATION ASSESSOR

Like the other tools above, the mutation assessor also predicts the impact of amino acid substitution on the structure as well as the function of human protein. This score ranges from 0 to 1 with higher values indicating the deleterious nature of the variant. STRING analysis of PTEN protein was done using the data from the STRING database which revealed the protein-protein interactions of PTEN with other proteins.

Results

In the present study, a total of 8298 missense SNPs were retrieved and 5 silico tools were used to analyze the SNPs. The results obtained are as follows.

SIFT

Table 1. Evaluation of Missense SNPs Using SIFT

SIFT_CLASS	Frequency	Per cent
Not available	2	0.0
Deleterious	5281	63.6
Tolerated	3015	36.4
Total	8298	100.0

Table 1 shows the functional impact of missense SNPs using the sequence homology tool SIFT (Sorting Intolerant from Tolerant). Out of the 8298 missense SNPs studied, 5281 were deleterious, and 3015 were tolerated. Values were not available for 2 SNPs. This indicates that SIFT categorizes most missense variants as deleterious, with a smaller proportion as tolerated, and very few cases where predictions were unavailable.

Polyphen

Table 2. Evaluation of Missense SNPs Using POLYPHEN

POLYPHEN_CLASS	Frequency	Per cent
Not available	2	0.0
Benign	3735	45.0

possibly damaging	1244	15.0
probably damaging	3246	39.1
Unknown	71	0.9
Total	8298	100.0

Table 2 summarizes the classification of missense variants analyzed by the PolyPhen (Polymorphism Phenotyping) tool, which predicts the functional impact of amino acid substitutions based on structural and evolutionary information. Out of the 8298 missense SNPs analysed using POLYPHEN, 3735 were benign, and 4490 were damaging. A smaller portion of variants falls into the 'possibly damaging' category, suggesting they might impact protein function, but the evidence is not very strong. Values were unavailable for 73 SNPs.

CADD

Table 3. Evaluation of Missense SNPs Using CADD

CADD_CLASS	Frequency	Per cent
Not available	16	0.0
likely benign	7373	88.9
likely deleterious	909	11.0
Total	8298	100.0

Table 3 summarizes the classification of missense variants analyzed by the CADD (Combined Annotation Dependent Depletion) tool, which provides a scaled score to predict the deleteriousness of genetic variants. CADD analysis of missense SNPs of the PTEN gene shows that the majority of SNPs (7373 SNPs) were likely benign, indicating they are unlikely to have harmful effects whereas 909 were likely deleterious with disease-causing potential.

META LR

Table 4. Evaluation of Missense SNPs Using META LR

META_LR_CLASS	Frequency	Per cent
Not available	37	0.4
Damaging	2995	36.1
Tolerated	5266	63.5
Total	8298	100.0

Table 4 provides a summary of the classification of missense variants analyzed by the MetaLR (Meta Logistic Regression) tool, which combines predictions from multiple algorithms to assess the functional impact of genetic variants. Meta LR analysis of the SNPs shows that 2995 were identified as damaging and 5266 as tolerated, indicating that they are not likely to be harmful.

MUTATION ASSESSOR

Table 5. Analysis of Missense SNPs Using Mutation Assessor

Mutation_assessor_class	Frequency	Per cent
Not available	39	.5
High	897	10.8
Low	2774	33.4
Medium	2906	35.0
Neutral	1682	20.3
Total	8298	100.0

Table 5 summarizes the classification of missense variants analyzed by the Mutation Assessor tool. This tool predicts the functional impact of variants based on evolutionary conservation and structural properties of proteins. The analysis of the 8298 missense SNPs using the Mutation assessor tool was done. This tool categorizes the SNPs into low, medium and high risk.897 SNPs were of high-risk type and 2906 of medium risk.

String Analysis of PTEN Protein

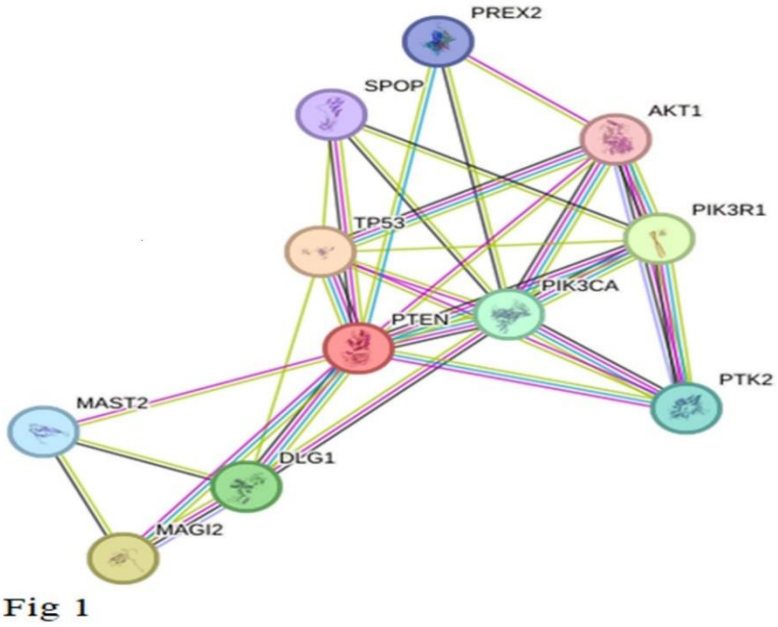

Figure 1. Network of Protein-Protein Interactions of PTEN Gene Generated by the String Database

This analysis using string (Fig 1) was done to understand the protein-protein interactions of the PTEN protein using the STRING database. It reveals the PTEN protein at the centre of the network. Surrounding the PTEN protein are the remaining proteins that interact with PTEN. The colored circles are called nodes, which are the proteins in this network and the colored lines are called edges, which indicate that there is evidence of interaction between the proteins(nodes). The green lines indicate co-expression, the purple line indicates experimentally determined interaction, the pink or red line indicates the actual biochemical signaling pathway interaction, and the blue lines indicate curated interactions from database sources.

The number of nodes was 11, the edges were 30 (expected edges,22), the average node degree was 5.45 and average local clustering coefficient value was 0.821 and the PPI enrichment p-value was found to be 0.0551.

Discussion

Missense Single Nucleotide Polymorphism is a type of ns SNP and is characterized by an amino acid change that has the potential to form a mutated protein with an alteration in its structure or function. This can cause many diseases. This deleterious change in the protein is by alteration of various properties of proteins such as decreased protein solubility, destabilizing of its tertiary structure, etc. The clinical consequence of each missense SNP can be done by conducting wet-lab experiments but can be tedious and time-consuming. Using a silicon approach to analyse these SNPs would help in identifying the deleterious ones [14].

This study shows the computational analysis of various SNPs on the deleteriousness of the PTEN gene. As per SIFT, the score for deleterious SNP is less than 0.05. A score of more than 0.05 indicates that the SNP is tolerated. Polyphen divides the prediction scores into three categories and they are Benign, possibly damaging and probably damaging. The scores of polyphenol range from 0 to 1. If the score is between 0.9 and 1, then that missense SNP comes under the category of 'probably damaging'. A score of 0.4 to 0.8 comes under possibly damaging and others come under the 'Benign' category [15]. In the present study, out of the 8298 polymorphisms analysed, 5281 were found to be deleterious.

CADD (Combined Annotation Dependent Depletion) is also another in-silico tool for estimating the deleterious nature of SNPs as well as insertions and deletions in the genome. It is built with nearly 60 genomic features and uses a machine-learning algorithm model. A higher score points towards the deleteriousness [16]. In the present study, as per CADD scores, 909 SNPs were likely deleterious.

Meta LR integrates nine independent deleteriousness scores using a logistic regression model to predict the deleterious nature of missense SNPs [17]. Analysis of the PTEN missense SNPs in the present study by Meta LR revealed 2995 damaging variants and 5266 tolerated ones.

MUTATION ASSESSOR was designed by the Memorial Sloan Kettering Cancer Centre in 2011, and this tool helps distinguish between high, medium and low-risk variants. It uses information from the sequence homology of protein families and subfamilies between the species and also within the species. [1]. The present study showed that there were 897 high-risk, 2906 medium-risk and 2774 low-risk SNPs. In the study by Khan et al., an analysis of 35 significant ns SNPs of the PTEN gene was conducted, and among them, five ns SNPs were found to be deleterious. The study used computational predictive tools and molecular dynamics simulations in their methodology [18].

Impact of SNPs in the PTEN Gene on Specific Cancers

The results in the study by Naidu CK et al, three deleterious nSNPs rs121909218 (G129E), rs121909229 (R130Q), and rs57374291 (D107N) were analyzed using computational tools and the results indicated their role in change of stability of PTEN protein. These SNPs were associated with breast cancer phenotype [19].

In a meta-analytical study done by Song D et al, two SNPs in the PTEN gene namely rs701848 (CC) and rs2735343 (GG) polymorphisms were analyzed and they were reported to be associated with an increased risk of cancer [20]. The study by Han et al suggested that PTEN rs3830675 SNP was linked to colorectal carcinomas in those patients who had habits of smoking and alcohol consumption [21].

The study by Andreassen KE et al showed that the SNP in PTEN gene i.e., rs11202586 showed association with Testicular Germ Cell tumors [22].

Interpretation of String Analysis of PTEN Protein

In the string analysis, 30 edges were seen as compared to the expected number of 22 and this suggests a stronger connection than expected. The average node degree indicates that each node (protein) in the network interacts with an average of 5.45 nodes and this points towards a relatively well-interconnected network. An average Local Clustering Coefficient value of 0.821 implies strongly that it is a well-interconnected network with many formed triangles. This p-value (0.0551) suggests the probability that the observed connections occurred by chance. Still, this near-significant enrichment suggests the dataset's structure is not entirely random and warrants further exploration for underlying patterns or functional relationships.

Conclusion

In-silico analysis is a useful computational technique to assess the deleterious nature of SNPs. In this study, the potential disease-causing polymorphisms of PTEN genes were identified that can drive further studies.

Outcomes of the Study

This study gives an insight into the potential pathogenic SNPs of the PTEN gene, and this can help in driving further experimental research. The advantage of this analysis is that polymorphisms that are unlikely to cause diseases can be identified and avoided while considering future research.

The Rationale of the Study

The rationale behind the study is to predict the effect of various polymorphisms on protein function. This computational analytic method helps to assess the potential impact of missense SNPs on PTEN protein structure and function as these SNPs cause amino acid substitutions. PTEN is a key tumour suppressor gene that plays an important role in cell growth and survival. Utilizing these computational methods together also helps in identifying disease-causing variations and prioritizing the SNPs for functional validation studies.

Limitations of the Study

Though computational analysis generates predictions about gene functions and pathways, it requires further validation by experimental research. Moreover, these in silico tools don't account for specific phenotypic or clinical outcomes. Also, Epigenetic modifications and post-translational modifications are not taken into consideration. Further research can be done to overcome limitations by using clinical databases and population databases to cross-check predictions for known pathogenic variants.

Conflict of Interest

Nil.

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References

[1]. Garcia, F. A., de O., de Andrade, E. S., & Palmero, E. I., 2022, Insights on variant analysis in silico tools for pathogenicity prediction. *Frontiers in Genetics*, *13*, 1010327. https://doi.org/10.3389/fgene.2022.1010327

[2]. Weng, L. P., Smith, W. M., Dahia, P. L., Ziebold, U., Gil, E., Lees, J. A., & Eng, C., 1999, PTEN suppresses breast cancer cell growth by phosphatase activity-dependent G1 arrest followed by cell death. *Cancer Research,* 59(22), 5808–5814.

[3]. Fusco, N., Sajjadi, E., Venetis, K., Gaudioso, G., Lopez, G., Corti, C., Rocco, E. G., Criscitiello, C., Malapelle, U., & Invernizzi, M., 2020, PTEN alterations and their role in cancer management: Are we making headway on precision medicine? *Genes*, *11*(7), 719. https://doi.org/10.3390/genes11070719

[4]. Liu, T., Wang, Y., Wang, Y., & Chan, A. M., 2019, Multifaceted regulation of PTEN subcellular distributions and biological functions. *Cancers*, *11*(9), 1247. https://doi.org/10.3390/cancers11091247

[5]. Hopkins, B. D., Hodakoski, C., Barrows, D., Mense, S. M., & Parsons, R. E., 2014, PTEN function: the long and the short of it. *Trends in Biochemical Sciences*, *39*(4), 183–190. https://doi.org/10.1016/j.tibs.2014.02.006

[6]. Chen, C.-Y., Chen, J., He, L., & Stiles, B. L., 2018, PTEN: Tumor suppressor and metabolic regulator. *Frontiers in Endocrinology*, *9*, 338. https://doi.org/10.3389/fendo.2018.00338

[7]. Luongo, F., Colonna, F., Calapà, F., Vitale, S., Fiori, M. E., & De Maria, R., 2019, PTEN tumor-suppressor: The dam of stemness in cancer. *Cancers*, *11*(8), 1076. https://doi.org/10.3390/cancers11081076

[8]. Ngeow, J., & Eng, C., 2020, PTEN in hereditary and sporadic cancer. *Cold Spring Harbor Perspectives in Medicine*, *10*(4), a036087. https://doi.org/10.1101/cshperspect.a036087

[9]. Nero, C., Ciccarone, F., Pietragalla, A., & Scambia, G., 2019, PTEN and gynecological cancers. *Cancers*, *11*(10), 1458. https://doi.org/10.3390/cancers11101458

[10]. Choi, S. W., Lee, Y., Shin, K., Koo, H., Kim, D., Sa, J. K., Cho, H. J., Shin, H.-M., Lee, S. J., Kim, H., Chung, S., Shin, J., Lee, C., & Nam, D.-H., 2021, Mutation-specific non-canonical pathway of PTEN as a distinct therapeutic target for glioblastoma. *Cell Death & Disease*, *12*(4), 374. https://doi.org/10.1038/s41419-021-03657-0

[11]. Wang, J., Zhang, S., Zhang, J., Zhang, Z., Ma, Q., Fu, W., Chen, X., Zhao, D., Zhao, M., Di, C., & Xie, X., 2023, A novel PTEN mutant caused by polymorphism in cis-regulatory elements is involved in chemosensitivity in breast cancer. *American journal of cancer research*, *13*(1), 86–104.

[12]. Deng, N., Zhou, H., Fan, H., & Yuan, Y., 2017, Single nucleotide polymorphisms and cancer susceptibility. *Oncotarget*, *8*(66), 110635–110649. https://doi.org/10.18632/oncotarget.22372

[13]. Ng, P. C., & Henikoff, S., 2003, SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Research*, *31*(13), 3812–3814. https://doi.org/10.1093/nar/gkg509

[14]. Behairy, M. Y., Soltan, M. A., Adam, M. S., Refaat, A. M., Ezz, E. M., Albogami, S., Fayad, E., Althobaiti, F., Gouda, A. M., Sileem, A. E., Elfaky, M. A., Darwish, K. M., & Alaa Eldeen, M., 2022, Computational analysis of deleterious SNPs in NRAS to assess their potential correlation with carcinogenesis. *Frontiers in Genetics*, *13*, 872845. https://doi.org/10.3389/fgene.2022.872845

[15]. Ajith, A., & Subbiah, U., 2023, In silico screening of non-synonymous SNPs in human TUFT1 gene. *Journal, Genetic Engineering & Biotechnology*, *21*(1), 95. https://doi.org/10.1186/s43141-023-00551-4

[16]. Rentzsch, P., Witten, D., Cooper, G. M., Shendure, J., & Kircher, M., 2019, CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Research*, *47*(D1), D886–D894. https://doi.org/10.1093/nar/gky1016

[17]. Liu, X., Wu, C., Li, C., & Boerwinkle, E., 2016, DbNSFP v3.0: A one-stop database of functional predictions and annotations for human nonsynonymous and splice-site SNVs. *Human Mutation*, *37*(3), 235–241. https://doi.org/10.1002/humu.22932

[18]. Khan, I., Ansari, I. A., Singh, P., & Dass J, F. P., 2017, Prediction of functionally significant single nucleotide polymorphisms in PTEN tumor suppressor gene: An in-silico approach. *Biotechnology and Applied Biochemistry*, *64*(5), 657–666. https://doi.org/10.1002/bab.1483

[19]. Naidu, C. K., & Suneetha, Y., 2016, Prediction and analysis of breast cancer related deleterious non-synonymous single nucleotide polymorphisms in the PTEN gene. *Asian Pacific Journal of Cancer Prevention: APJCP*, *17*(4), 2199–2203. https://doi.org/10.7314/apjcp.2016.17.4.2199

[20]. Song, D.-D., Zhang, Q., Li, J.-H., Hao, R.-M., Ma, Y., Wang, P.-Y., & Xie, S.-Y., 2017, Single nucleotide polymorphisms rs701848 and rs2735343 in PTEN increases cancer risks in an Asian

population. *Oncotarget*, *8*(56), 96290–96300. https://doi.org/10.18632/oncotarget.22019

[21]. Han, M., Wu, G., Sun, P., Nie, J., Zhang, J., & Li, Y., 2015, Association of genetic polymorphisms in PTEN and additional interaction with alcohol consumption and smoking on colorectal cancer in Chinese population. *International Journal of Clinical and Experimental Medicine*, *8*(11), 21629–21634

[22]. Andreassen, K. E., Kristiansen, W., Karlsson, R., Aschim, E. L., Dahl, O., Fosså, S. D., Adami, H.-O., Wiklund, F., Haugen, T. B., & Grotmol, T., 2013, Genetic variation in AKT1, PTEN and the 8q24 locus, and the risk of testicular germ cell tumor. *Human Reproduction (Oxford, England)*, *28*(7), 1995–2002. https://doi.org/10.1093/humrep/det127