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In Silico Post Translational Analysis of Functional Single Nucleotide Alterations in Human TERT Gene Associated with Acute Myeloid Leukemia

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ABSTRACT

Acute myeloid leukemia (AML) refers to a diverse assemblage of hematological malignancies that constitute clonal expansion of immature myeloid progenitor cells in the peripheral blood and bone marrow. TERT gene ensures telomeres maintenance, chromosome stability and prevention of malignancy. The TERT gene has several single nucleotide polymorphisms (SNPs) that have been linked to a number of diseases, including AML. **Objective:** To classify the harmful TERT gene mutations, and to analyze them using various computational approaches at structural, functional and translational expression levels **Methods:** National Centre for Biotechnology Information (NCBI) database was used to retrieve nsSNPs of TERT gene (Q53H, V170M, A184T, S255Y, A288V, H412Y, I540M, R631W) reported in AML and they were analyzed using various bioinformatics tools. **Results:** In this *in silico* analysis, it was observed that seven out of eight SNPs had a damaging effect; they could affect the protein stability, protein-protein interactions, hydrophobicity, protein folding, three-dimensional structure, secondary structure and conservation profile. 3D models were generated and validated by various tools and the structural effect of these alterations was observed on protein function that was destabilizing to the RNA folding, protein-protein interactions and other functionally associated proteins. Analysis of post translational modifications showed no significant effect of these mutations. **Conclusions:** These SNPs could be used in future as potential targets in disease diagnosis, biological markers and protein studies.

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy of the bone marrow in which hematopoietic precursors maturation is seized in the early stages of development [1]. A clonal disease characterized by the piling up of somatic acquired genetic mutations in hematopoietic progenitor cells that modify the normal mechanisms of regeneration, proliferation and differentiation [2]. The most prevalent acute leukemia in adults is AML, and as people get older, its extent rises [3]. It remains a fatal disease with a less than 30% 5-year survival rate [4]. AML has variable symptoms, being presented clinically as a combination of cytopenia, which includes weakness, fever, abdominal pain, pallor, shortness of breath, fatigue, easy bruising and bleeding with an elevated infection risk, weight loss, nausea, vomiting and dysphagia [5]. A large and diverse group of genetic and environmental variables have been proposed [6]. The primary cause of AML is thought to be acquired genetic anomalies [7]. In humans at chromosome 5p15.33 TERT gene is located which encodes telomerase reverse transcriptase. The 1132 amino acid polypeptide produced by it is translated into a 130 kD active TERT protein [8]. TERT is an important part and catalytic subunit of the telomerase holoenzyme [9]. The 42 kb long TERT gene contains 15 introns, 16 exons, and a promoter core of 260bp [10]. It has a vital role in the maintenance of telomeres, chromosome stability and preventing malignancy [11]. The Catalytic component's (TERT) expression of telomerase triggers its reactivation

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during carcinogenesis in the majority of human malignancies. This may occur by means of both methylation and mutations at TERT promoter (TERT*p*)[12]. TERT mutations, which occur commonly (2- 19%) in bone marrow failure syndromes, are linked to an elevated risk of MDS/AML [13]. TERT gene amplification is highly related to hematological malignancies, with a greater prevalence in AML patients (53.3%) [14]. Bioinformatics tools are time saving and cost effective [15]. This study focuses on the thorough *in silico* analysis that pinpoint and examine the most pathogenic mutations of the TERT gene.

METHODS

The comprehensive in silico analysis was performed by using different softwares. Human TERT gene's data and TERT protein sequence was retrieved from NCBI (https://www.ncbi.nlm.nih.gov/) and UniProtKB (http://www.uniprot.org/uniprot/) respectively. SNPs found in TERT gene were obtained from dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). To estimate the effect of amino acid alterations on the pathogenicity and the functionality of protein FATHMM (Functional Analysis Through Hidden Markov Models) http://fathmm.biocompute.org.uk [16], PolyPhen-2 (Polymorphism phenotyping v2)(http://genetics.bwh.harvard.edu/ggi/cgi-bin/ggi2.cgi) [17], and SIFT (sorting intolerant from tolerant) algorithm accessed via https://sift.bii.a-star.edu.sg/ [18] were used which showed that whether the mutation is damaging or benign. Mutation Cutoff Scanning Matrix (mCSM) (http://biosig.unimelb.edu.au/mcsm/stability) [19] and MUpro (http://mupro.proteomics.ics.uci.edu/) [20] were used for protein stability analysis which showed the results in the form of ΔΔG values. For the conservation analysis Consurf (http://consurf.tau.ac.il) was used it assigned the scores ranging from 1–4 being variable, 5–6 being average and 7–9 being conserved [21]. Project HOPE (Have (y) Our Protein Explained) available at "https://www3.cmbi.umcn.nl/hope/" was used to evaluate the structural and biochemical effects of single point mutations [22]. SWISS-MODEL (http://swissmodel.expasy.org/) online web service was used for homology modelling of wild and mutant types of TERT protein [23]. 3D structures of proteins were generated and quality evaluation of the generated models was performed by some parameters like GMQE, QMEAN Z-score. SOPMA (Self-Optimized Prediction Method with Alignment) tool (https://npsa- prabi.ibcp.fr/cgibin/npsa_automat.pl?page=/ NPSA/npsa_sopma.html) was used for the secondary structure analysis. It gives the outcome as percentage composition of α- helix, β-sheet, turns, and random coil [24]. The online version of RNAfold Web Server based on the Vienna RNA package available at (http://rna.tbi.univie.ac.at/cgi- bin/RNAWebSuite/RNAfold.cgi) [25] was used to detect single nucleotide alterations' influence on secondary structure of RNA. For the prediction of post translation modifications and mRNA expression cBioPortal (http://www.cbioportal.org/) [26], UALCAN portal (http://ualcan.path.uab.edu/analysis-prot.html) [27], GEPIA (Gene Expression Profiling Interactive Analysis), a web-based tool (http://gepia.cancer-pku.cn/) [28], Gene Set Enrichment Analysis (GSEA) [29] and Cytoscape an open-source software for integration, visualization and analysis of biological networks association analysis were used [30].

RESULTS

Missense SNPs of the TERT include (Q53H, V170M, A184T, S255Y, A288V, H412Y, I540M and R631W) were retrieved from the NCBI. It was predicted by Fathmm that all the SNPs were damaging. SIFT showed that 4 out of 8 SNPs were damaging and only one mutation (A184T) predicted benign by Polyphen-2 (Table 1).

Table 1: Functional analysis of mutations in the TERT gene by using in silico programs

SNP Ids	SNPs	SIFT	Fathmm	Polyphen-2
rs1060503006	Q53H	Not tolerated	Damaging	Damaging
rs387907248	V170M	Not tolerated	Damaging	Damaging
rs773758089	A184T	Tolerated	Damaging	Benign
rs1751207450	S255Y	Tolerated	Damaging	Possibly Damaging
rs774657340	A288V	Tolerated	Damaging	Possibly Damaging
rs34094720	H412Y	Tolerated	Damaging	Damaging
rs797046041	I540M	Not tolerated	Damaging	Damaging
rs1194223999	R631W	Not tolerated	Damaging	Damaging

mCSM and MUpro were employed to examine the impact of these SNPs on protein stability. It was predicted by both the tools that 7 out of 8 mutations were decreasing the protein stability except H412Y (Table 2).

Table 2: Change in protein structural stability of TERT gene by single point mutations estimated through mCSM and Mupro

SNPs	mCSM		Mupro	
	ΔΔG (kcal/mol)	Stability	ΔΔG (kcal/mol)	Stability
Q53H	-0.747	Destabilizing	-1.670	Decrease
V170M	-0.107	Destabilizing	-0.439	Decrease
A184T	-0.512	Destabilizing	-0.465	Decrease
S255Y	-0.743	Destabilizing	-0.497	Decrease
A288V	-0.325	Destabilizing	-0.730	Decrease
H412Y	0.471	Stabilizing	0.0266	Increase
I540M	-0.767	Destabilizing	-0.659	Decrease
R631W	0.188	Stabilizing	-0.393	Decrease

ConSurf predicted the conservation profile of the SNPs. According to the output of the ConSurf web server, it was predicted that 2 out of 8 variants (V170M, R631W) were conserved residues with a conservation score range of 7-9

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shown in Table 3 and Figure 1.

Table 3: Analysis of evolutionary conservation profile of SNPs in TERT gene by ConSurf

SNPs	Conservation score	Conservation scale status
Q53H	5	Average
V170M	7	Conserved
A184T	3	Variable
S255Y	3	Variable
A288V	2	Variable
H412Y	5	Average
I540M	5	Average
R631W	9	Conserved

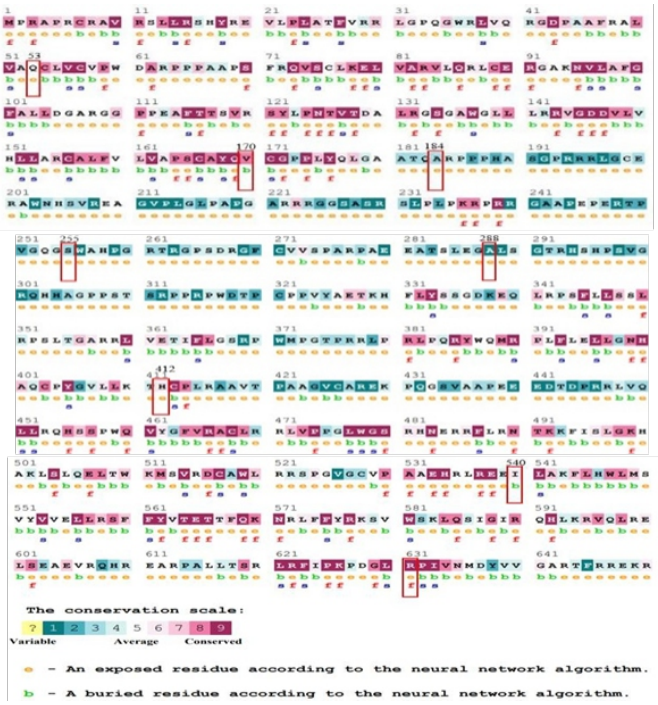


Figure 1: Representation of conservation profile of TERT gene's amino acids in the form of various colors using Consurf.

HOPE was used to examine the structural as well as functional impacts of single amino acid changes on protein. It claimed that all the mutant residues were bigger in size than the wild residue. Only one mutation R631W altered the charge of amino acid from positive to neutral and this difference in charge will disturb the ionic interaction made by the original, wild-type residue. The remaining 7 SNPs were predicted not to affect the charge. As interpreted by HOPE 2 mutant residues (H412Y and R631W) were more hydrophobic than wild-type.

Table 4: Evaluation of amino acid replacement's effect on the structure of TERT protein with reference to wild residue by HOPE

SNPs	Size	Change of Charge	Hydrophobicity
Q53H	W<M	Not affected	Not affected
V170M	W<M	Not affected	Not affected
A184T	W<M	Not affected	Wildtype residue (A) is more hydrophobic
S255Y	W<M	Not affected	Not affected
A288V	W<M	Not affected	Not affected
H412Y	W<M	Not affected	Mutant residue (Y) is more hydrophobic
I540M	W<M	Not affected	Not affected
R631W	W<M	+ve to neutral	Mutant residue (W) is more hydrophobic

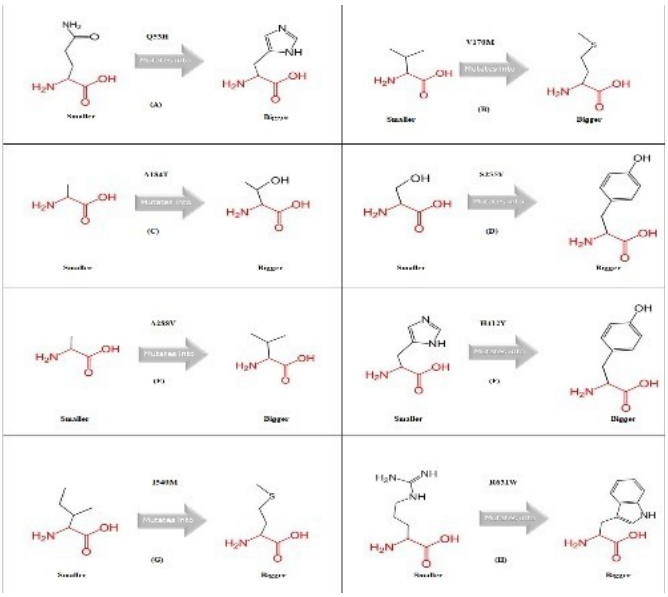
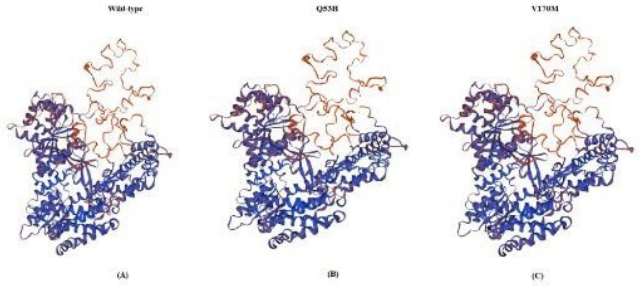


Figure 2: The native amino acid (left) and the mutant amino acid (right) are depicted in schematic form via HOPE

SWISS-MODEL was used to generate the TERT protein homology model. The QMEAN-Z score (-5-0) and GMQE values (0-1) revealed that there was greater compatibility between template and target structure of similar size and the alignment was quite precise (Table 5 and Figure 3).

SNPs	Template Query No.	Sequence Identity (%)	GMQE	QMEAN Z-score
Wildtype	7trd.1. B	100	0.72	-2.62
Q53H	7trd.1. B	99.91	0.72	-2.78
V170M	7trd.1. B	99.91	0.72	-2.65
A184T	7trd.1. B	99.91	0.72	-2.77
S255Y	7trd.1. B	99.91	0.72	-2.43
A288V	7trd.1. B	99.91	0.72	-2.24
H412Y	7trd.1. B	99.91	0.72	-2.67
I540M	7trd.1. B	99.91	0.72	-2.90
R631W	7trd.1. B	99.91	0.72	-2.86



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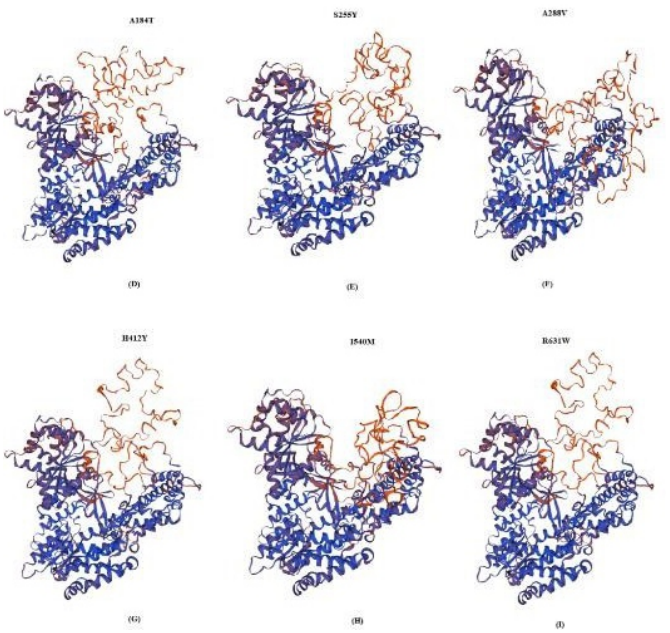

Figure 3: Photographs of Protein structure using Swiss Model. (A) Wildtype (B) Q53H (C) V170M (D) A184T (E) S255Y (F) A288V (G) H412Y (H) I540M (I) R631W

SOPMA was used for the secondary structure analysis and outcome was in the form of percentages of different parameters which are shown in table 6 and figure 4.

Table 6: Prediction of Secondary Structure of TERT protein using SOPMA

SNPs	Alpha Helix (%)	Extended Strand (%)	Beta Turn (%)	Random Coil (%)
Wildtype	44.08	10.51	3.71	41.70
Q53H	43.37	10.78	4.06	41.78
V170M	43.20	11.22	3.80	41.78
A184T	42.76	10.95	4.15	42.14
S255Y	42.40	10.95	3.98	42.67
A288V	42.31	11.31	3.71	42.67
H412Y	42.84	11.04	3.80	42.31
I540M	42.93	11.31	3.89	41.87
R631W	42.58	11.22	3.71	42.49

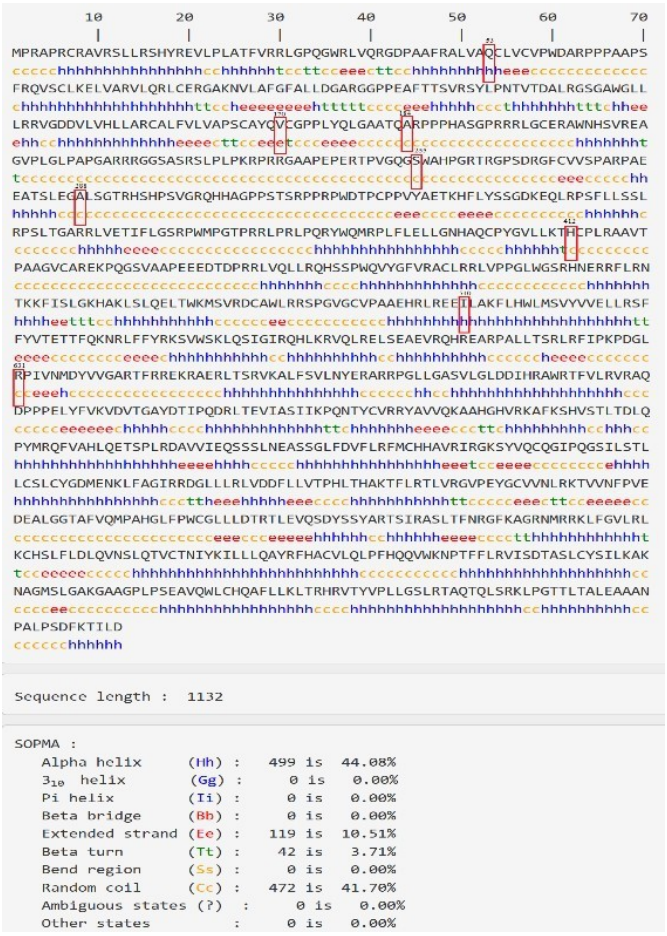

Figure 4: Representing Secondary Structure of TERT protein predicted by SOPMA

The effect of gene mutations on the secondary structure of RNA was examined using the RNAfold tool of the Vienna package. Every mutation resulted in inappropriate RNA folding compared to the wild type, which affects mRNA localization and protein translation (Figure 5).

(A) Wildtype **(B) Q53H** **(C) V170M**

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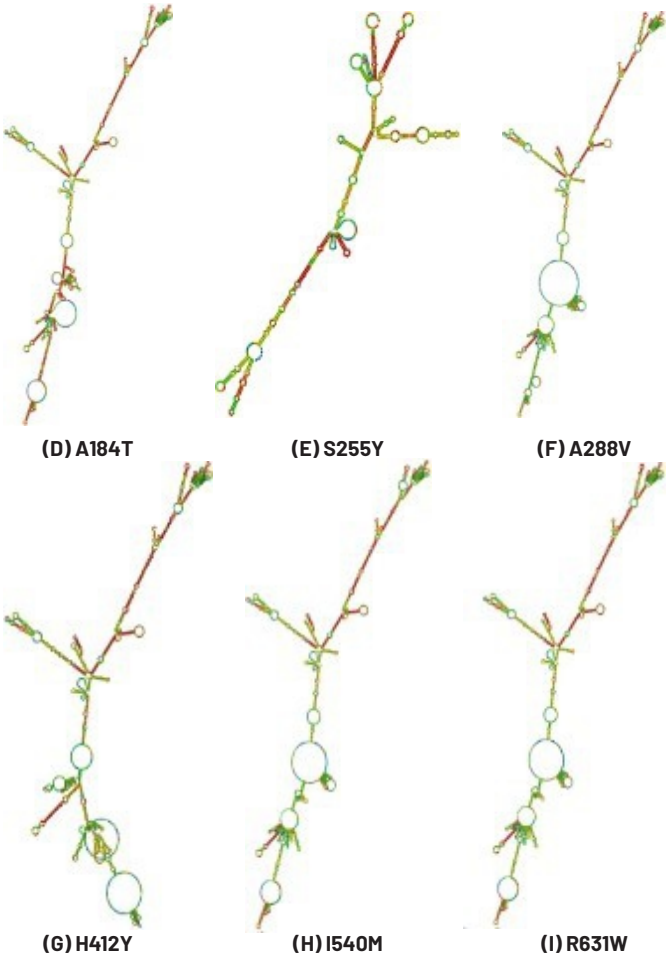

Figure 5: TERT gene's Mutations effect on RNA secondary structure shown by RNA fold server. (A) Wildtype (B) Q53H (C) V170M(D)A184T(E)S255Y(F)A288V(G)H412Y(H)I540M(I)R631W

The secondary structure of RNA with a higher MFE value was one that was more stable(table 7).

Table 7: Estimation of MFE of TERT gene via Vienna Package

SNPs	Minimum Free Energy (kcal/mol)
Wildtype	-797.77
Q53H	-804.47
V170M	-712.70
A184T	-826.07
S255Y	-832.67
A288V	-789.67
H412Y	-788.08
I540M	-797.97
R631W	-795.87

Figure 6: Representation of missense mutations and PTM sites by cBioPortal

However, PTM sites were evaluated in which phosphorylation was the main type of PTM in TERT, with a total of 13 sites, followed by acetylation which had only 1 site. These phosphorylation sites were in the Telomerase-RBD and RVT-1 domain, thus might affect the function of protein (Figure 6). To analyze the expression and clinical significance of TERT gene in AML, UALCAN was used. The level of methylation of TERT gene in the promoter region can lead to the development of AML. TERT promotor was significantly hyper- methylated in both male and female (Figure 7 (A)). Whereas, in individuals with age group 21 years to 100 years were TERT promotor was significantly hypermethylated as shown in Figure 7(B).

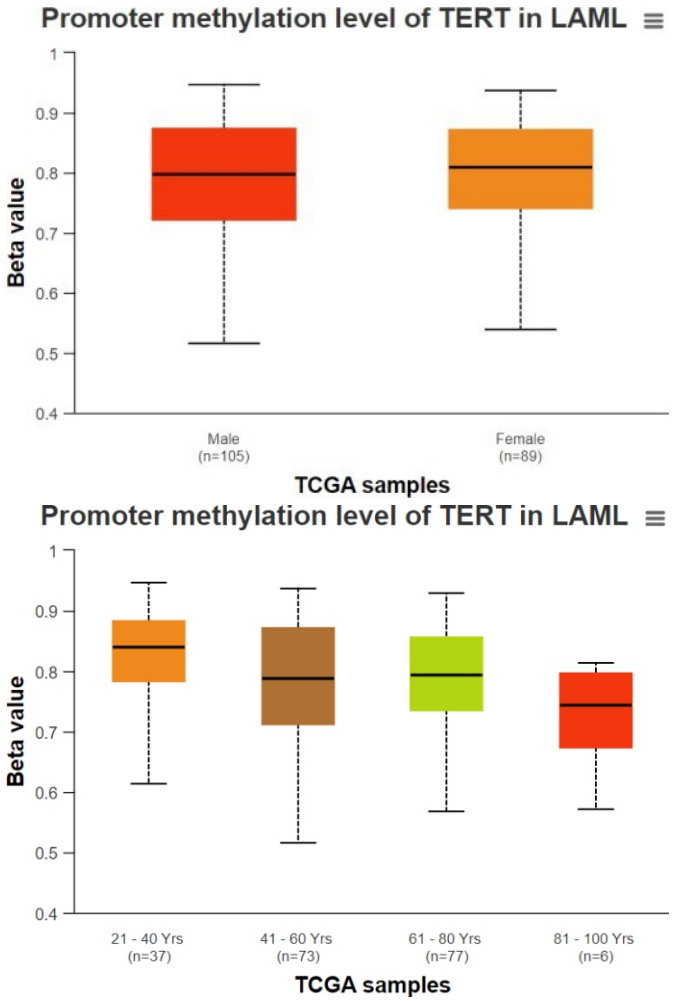

Figure 7: (A) Methylation level of TERT promotor in LAML based on gender. (B) Methylation level of TERT promotor in LAML based on different age group

GEPIA was used to obtain Transcripts per million (TPM) which showed that in tumor tissues T (n=170), the TERT gene's mRNA expression levels were noticeably greater as compared to in normal tissues N (n=70) and were shown as red and green dot plots respectively. To investigate the association between TERT mRNA expression and patient

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prognosis in AML, overall survival rates were obtained using the Cox regression model which determines the relationship between variables and survival rates. TERT mRNA expression levels were visualized using Kaplan–Meier survival curves which showed no significant correlation between TERT expression and percent survival in AML (Figure 8 A and B).

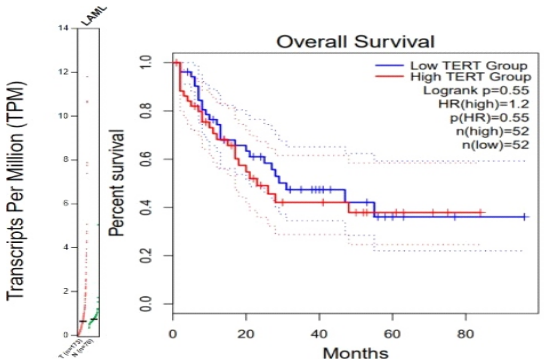
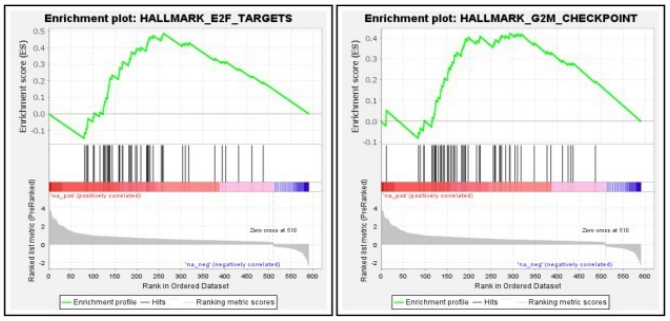
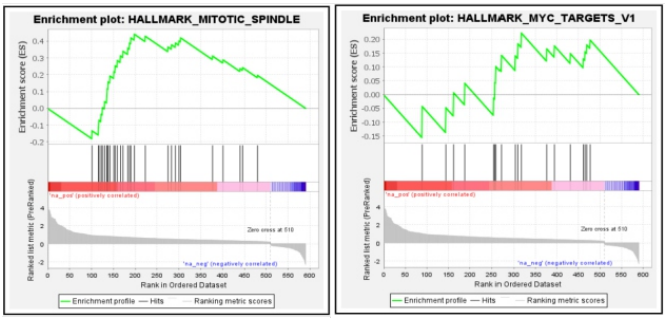


Figure 8: (A) TERT mRNA expression in LAML (B) Overall survival comparison between low and high TERT groups in AML shown by GEPIA

Different biological functional gene sets were analyzed by the GSEA technique to determine the effect of TERT mutations on the protein functionality and different pathways. The GSEA results are shown as enrichment plot in the form of enrichment score. The highest divergence from zero experienced during a random walk is known as the enrichment score (ES) and is plotted against the y-axis in the graph. The biological processes involved in the positive regulation of E2F targets, G2M-Checkpoints, mitotic spindle, and MYC_Target_V1 were considerably enriched in the GSEA analysis of enrichment. This implies that TERT mutations in AML patients may influence transcription, cell growth, apoptosis, and cell adhesion, which may affect the development of the disease and prognosis. All these enrichment plots were positively correlated (Figure 9 A–D).



(A) **(B)**



(C) **(D)**

Figure 9: GSEA representing Enrichment Plots (A) E2F Targets Hallmark (B) G2M checkpoint Hallmarks (C) Hallmark mitotic spindle (D) Hallmark MYC Targets V1

Cytoscape was used to generate a network of association of TERT protein with other related proteins which are involved in AML. A hub of 30 genes was generated which showed the direct link of TERT with 4 other genes and indirect association with many (Figure 10).

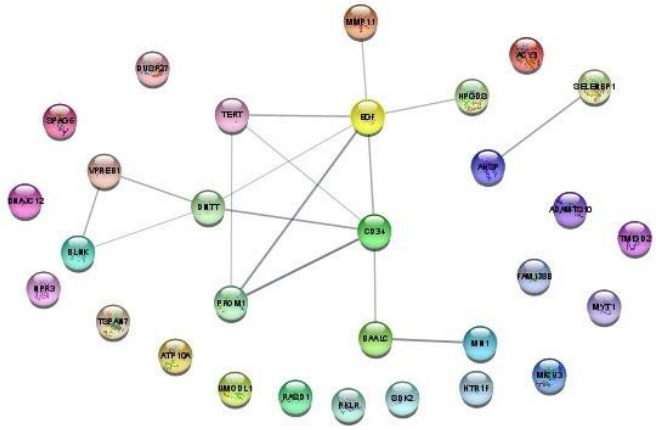


Figure 10: TERT association with other genes in AML via Cytoscape

DISCUSSION

Single nucleotide polymorphisms (SNPs) are single-base alterations that have a role in the pathophysiology of various ailments as well as variation in human biology. Previous research revealed that using experimental methodologies to predict effects of nsSNPs on the structure and functionality might be time-consuming and expensive. Computer simulations (*in silico* analysis) have recently emerged as an excellent method for comprehending disease-related mutations and their consequences in protein structures [31, 32]. TERT gene was selected for the *in-silico* analysis, as previously it has not been carried out. All the SNPs (Q53H, V170M, A184T, S255Y, A288V, H412Y, I540M, R631W) were retrieved from the dbSNP of the NCBI database. The functional analysis was done by SIFT, Fathmm and PolyPhen-2 were employed for the functional analysis which showed all the SNPs were damaging except A184T. The coding region of the TERT

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gene is essential for accurate telomerase activity and telomere length maintenance [33], the functional effect of these coding region mutations leads to overexpression of TERT and abrupt telomerase activity and TL that causes abnormal proliferation and becomes a cause of cancer. Stability analysis showed all mutations were destabilizing to protein structure except one H412Y. Most often, decreased stability is the cause of protein functionality loss brought on by mutations [34]. PROJECT HOPE analyzed that the charge, size, and hydrophobicity values of wild-type residues and mutant residues were different. Two mutations were falling in the conserved region as predicted by conSurf. Previous study by Shaw suggest that evolutionarily conserved regions are the potential sites for disease causing point mutations [35]. SWISS-MODEL quality assessment parameter suggested the accuracy of generated models. SOPMA showed most mutations were in the coiled region and few were in the helical region. Vienna package showed abnormal RNA folding of mutant residues from the wild type due to single base alteration. Covalent modifications of the polypeptides after their synthesis to make them functional are called post translation modifications (PTMs) [36]. Finding disease- associated nsSNPs changing PTM sites can help to assess the various PTM candidates involved in diseases [37]. This study showed the detail analysis of missense substitutions on PTMs. cBioPortal predicted that none of the most deleterious nsSNPs positions correlated with a potential PTM sites. It could be estimate that most of them were the silent mutations. UALCAN showed the promotor methylation level of TERT gene. Hyper-methylation at THOR (TERT hyper- methylated oncological region) was often observed in AML [38]. In malignant tissues expression levels of TERT's mRNA were higher according to GEPIA. Enrichment plot was obtained by GSEA which showed that different hallmarks have an extremely overrepresented TERT gene. Cytoscape showed the association hub of TERT with other proteins involved in AML. Mutations may influence prognosis and contribute to the development of the disease by affecting transcription, apoptosis, cell adhesion as well as cell division in patients of AML.

CONCLUSIONS

This *in silico* analysis of the TERT's functional SNPs offered a substantial understanding of their damaging effects. TERT gene enhanced expression will occur, leading to the development of cancer and an adverse prognosis of AML.

Authors Contribution

Conceptualization: AM
Methodology: AMA
Formal analysis: AT, KJ
Writing-review and editing: AMA, AM

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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