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In Silico Assessment of Post Translational Modifications Caused by NRAS Gene SNPs in Acute Myeloid Leukemia

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ABSTRACT

Acute myeloid leukemia (AML) is a blood cancer and a malignant disorder of the bone marrow in which hematopoietic precursors are ceased at an early stage of development, preventing them from differentiating. The NRAS gene plays a vital role in regulating cell division. The mutation in this gene leads to an increased activity of the RAS pathway, increased proliferation and decreased apoptosis rates which causes AML. **Objective:** To identify the deleterious SNPs involved in AML and to further analyze them using bioinformatics tools. **Methods:** The missense nsSNPs (Q61H, Q61L, G13V, G13R, and G12A) of NRAS were retrieved from NCBI databases. **Results:** Using *in silico* analysis, it was found that these pathogenic SNPs could disrupt the protein stability. These mutations were present in the conserved region and had the potential to significantly alter the protein's secondary structure and impair its functionality. The structural effect of mutations was observed by generating 3D models. Post-translational modifications (PTMs) of proteins refers to the chemical modifications that occur after a protein is formed to make it functionally capable. Analyzing PTMs via *in silico* analysis revealed that missense mutations affect protein functionality. The level of methylation was significantly high in AML patients. These SNPs might affect additional proteins which are functionally associated. **Conclusions:** The highlighted SNPs could be suitable targets for future research on proteins, biological markers, and medical diagnosis.

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous group of clonal hematopoietic malignant diseases that are caused due to mutations in Progenitor cells of myeloid lineage which fail to differentiate leading to over-proliferation in the hematopoietic stem cells of bone marrow. It is the most common type of leukemia in adults and its frequency increases with advanced age [1]. Symptoms are widespread and include fever, fatigue, drowsiness, dry mouth, reduced body weight, bone ache, ulcers of mucous membranes, bruising, or bleeding due to thrombocytopenia and anemia [2, 3]. Primary SEER-reported age-adjusted AML incidence was 3.43 per 100,000 annually but has escalated over time, having yearly incidence rates regularly exceeding 4.2 per 100,000 per year as of 2010. According to SEER data from 2016, men are 1.6 times more likely to have an AML diagnosis than women, with a rate of 5.42 and 3.47 per 100,000 person-years for men and women respectively [4]. The risk factors of AML include advanced age, smoking, hazardous chemicals, myelodysplastic syndrome (MDS), chemotherapeutic drugs, high-intensity radiation exposure, genetic disorders, and pedigree of AML patients [5]. Identifying ≥20% myeloid blasts in bone marrow or finding out some specific cytogenetic dysfunctionalities is necessary for the diagnosis of AML [6, 7]. AML is currently treatable in 35–40% of adult patients who are 60 years of age or younger and in 5 to 15% of patients who have reached the age of 60 or more [8]. Chemotherapy, radiation, monoclonal antibodies, or hematopoietic stem cell transplantation is used to treat AML [9]. The NRAS is a powerful oncogene in many human malignancies. It is a small GTPase that regulates cellular growth,

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differentiation, apoptosis, and survival. It encodes a protein that is membrane-bound and serves as a crucial regulatory component in the signal transduction of a variety of hormones, cytokines, and growth factors. On the short arm of chromosome band 1p13.2, NRAS gene is found. It has five isoforms that are present naturally [10]. To find the most harmful missense SNPs, a thorough *in silico* analysis of the NRAS gene must be conducted [11]. The *in silico* techniques are less expensive and more time-saving [12].

METHODS

To perform *in silico* analysis, a total of 14 computational tools were employed.

Table 1: List of tools employed for *In Silico* analysis

In *Silico* Tools	Function
NCBI	For Retrieval of SNPs
SIFT	For Functional Analysis
Mutation Taster	
FATHMM	
I-Mutant Suite	For Prediction of Protein Stability Change
mCSM	
Project HOPE	For Structural Analysis
Consurf	For Conservation Analysis
SWISS-MODEL	For Protein Modeling
SOPMA	For Secondary Structure Analysis
Vienna Package	For RNA Secondary Structure Analysis
cBioportal	For Analysis of Post Translational Modifications
UALCAN	
GEPIA	
GSEA	
Cytoscape	

RESULTS

Pathogenic SNPs (Q61H, Q61L, G13V, G13R and G12A) were retrieved from NCBI. The tools employed for functional analysis predicted these mutations to be non-tolerated, disease-causing and damaging. Whereas 3 SNPs (G13V, G13R and G12A) were found to be tolerated by FATHMM. I-Mutant Suite showed that only 1 SNP (Q61L) did not affect the protein structure while other SNPs decreased the protein stability as shown by negative DDG value. The results of mCSM predicted that 3 mutations (G13V, G13R, and G12A) destabilized the proteins while the other two variants (Q61H, Q61L) did not affect proteins (Table 2).

Table 2: Analysis of the effect of mutations on protein's structural stability of NRAS gene using mCSM, and I-Mutant Suite

SNPs	mCSM		I-Mutant Suite	
	ΔΔG value (kcal/mol)	Stability	DDG value (kcal/mol)	Prediction
Q61H	0.254	Stabilizing	-0.60	Decrease
Q61L	0.139	Stabilizing	0.24	Increase
G13V	-0.374	Destabilizing	-0.42	Decrease
G13R	-0.305	Destabilizing	-0.63	Decrease
G12A	-0.358	Destabilizing	-0.93	Decrease

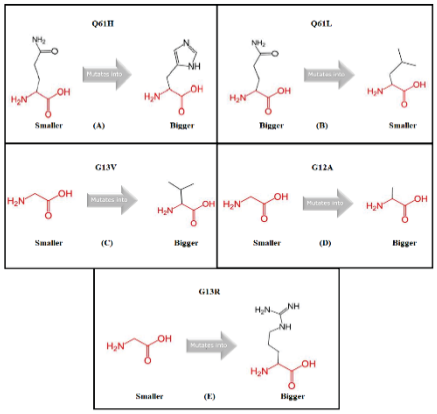

Figure 1: Diagrams representing the wildtype (left) and mutant (right) amino acid residues of the NRAS gene mutations (A) Q61H (B)Q61L(C)G13V(D)G12A(E)G13R

Project HOPE predicted the physiochemical properties of the protein such as hydrophobicity, spatial structure, size and charge. Only one mutant residue (Q61L) was smaller than the wildtype which is shown in figure 1. Whereas, only 1 SNP (G13R) resulted in the change of charge from neutral to positive. The remaining SNPs did not show a change of charge (Table 3).

Table 3: Effect of change of one amino acid on the structural stability of the NRAS protein by Project HOPE

SNPs	Size	Change of charge
Q61H	W<M	Not affected
Q61L	W>M	Not affected
G13V	W<M	Not affected
G13R	W<M	Neutral to +ve
G12A	W<M	Not affected

Figure 2: Using ConSurf to show the amino acid conservation profile of NRAS protein

The Consurf calculated the evolutionary conservation score for each amino acid in the protein sequence. Regions with high conservation scores are likely to be functionally

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important, while regions with low conservation scores are more likely to be structurally or functionally less important (Table 4 and Figure 2).

Table 4: Analysis of evolutionary conservation profile of SNPs by ConSurf

SNPs	Consurf Results		
	Conservation Score	Conservation scale status	Exposed/Buried
Q61H	9	Conserved	Exposed
Q61L	9	Conserved	Exposed
G13V	6	Conserved	Exposed
G13R	6	Conserved	Exposed
G12A	7	Conserved	Exposed

SWISS-MODEL is a protein structure homology-modeling tool that provides us with the 3D structure of proteins which is shown in figure 3. It also provided data in the form of variables to determine the quality of generated model which are shown in table 5. Good agreement between the target and template structures of comparable size is indicated by QMEAN (Qualitative Model Energy Analysis) z-scores near zero. It also provided GMQE (Global Quality Estimate) score which indicates the predictable reliability of alignment of the target template and template structure.

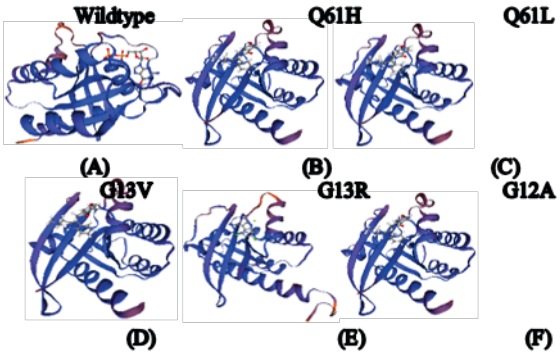

Figure 3: 3D structure of NRAS protein using Swiss Model (A)Wild type(B)Q61H(C)Q61L(D)G13V(E)G13R(F)G12A

Table 5: Representation of the different QMEAN Z-scores, GMQEs, Sequence Identity, and template query No. of wild-type and mutant proteins via SWISS-Model

SNPs	Template Query No.	Sequence Identity (%)	GMQE	QMEAN Z-score
Wild Type	6zio.1. A	99.42	0.82	0.89
Q61H	6ziz.1. A	98.84	0.87	0.49
Q61L	6ziz.1.A	98.84	0.83	0.53
G13V	6ziz.1. A	98.26	0.83	0.17
G13R	6ziz.1. A	98.26	0.84	0.32
G12A	6ziz.1. A	98.26	0.84	0.48

SOPMA predicted the secondary structure properties of the protein including alpha helix, 310 helix, Pi helix, beta bridge, extended strands, beta-turn, bend region, random coil, ambiguous states and other states which are shown in table 6.

Table 6: Prediction of Secondary Structure of NRAS Protein Using SOPMA

SNPs	Alpha Helix (%)	Extended Strand (%)	Beta Turn (%)	Random Coil (%)
Wild type	45.50	18.52	6.88	29.10
Q61H	53.97	17.99	7.41	20.63
Q61L	49.74	15.34	7.94	26.98
G13V	45.50	18.52	6.88	29.10
G13R	52.91	16.93	6.88	23.28
G12A	44.97	18.52	6.88	29.63

Figure 4: Analysis of the Secondary structure of the NRAS gene using SOPMA

Vienna Package determined that all SNPs lead to abnormal RNA folding, which resulted in affected mRNA localization and translation of protein. It calculated the RNA folding energies i.e. Minimum Free Energy (MFE) to predict the stability and secondary structure of wildtype and mutant molecules (Table 7 and Figure 5).

Table 7: Calculation of Minimum Free Energy (MFE) of Wildtype and Mutant Residues using Vienna Package

SNPs	Minimum Free Energy (kcal/mol)
Wildtype	-115.58
Q61H	-117.18
Q61L	-113.88
G13V	-111.18
G13R	-111.38
G12A	-119.68

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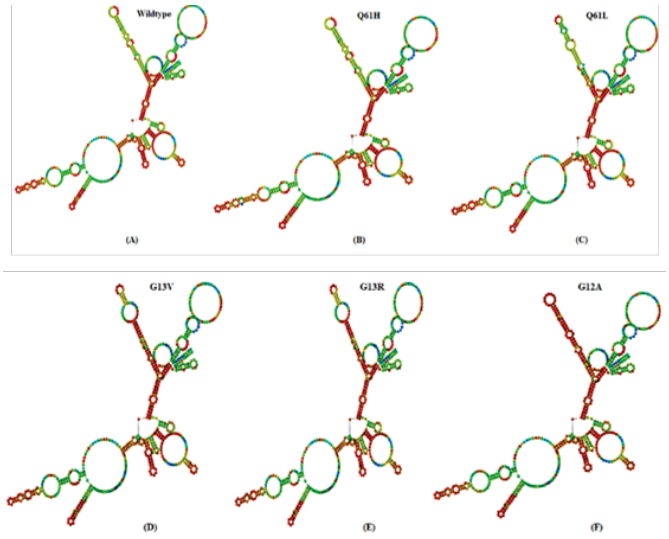

Figure 5: Effect of SNPs of NRAS gene on mRNA folding using the RNA fold program in Vienna Package(A)Wildtype (B)Q61H (C)Q61L (D)G13V(E)G12A(F)G13R

cBioPortal was used to analyze the genetic alterations of NRAS in AML. Figure 6 showed 13% of high and low mRNA expression. The main type of genetically modified sites were 13 missense mutations for NRAS. Furthermore, PTM sites were also evaluated in which Phosphorylation was the main type of PTM with a total of 10 sites, followed by acetylation, ubiquitination, methylation, palmitoylation, and S-nitrosylation. These PTM sites were present on exons 2, 3, 4 and 5 in the RAS domain, thus might cause the altered protein function(Figure 6).

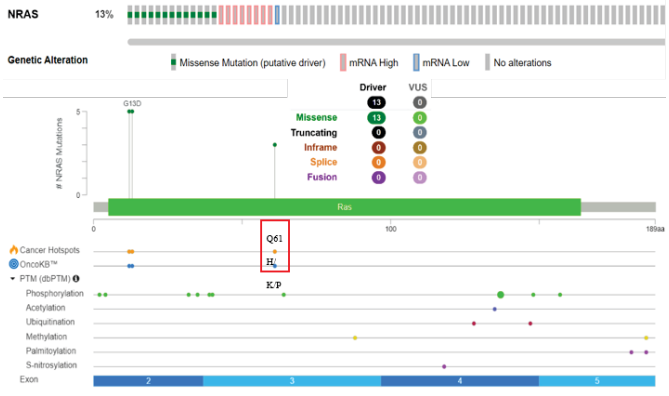

Figure 6: Depiction of missense mutation sites and PTM sites by cBioportal

UALCAN was used to determine the level of methylation in the promoter region of the NRAS gene which can lead to the development of AML. In figure 7(A), NRAS hyper-methylation with no significant variation between males and females is shown. Whereas in figure 7(B) methylation level in different age groups of AML patients is demonstrated. Individuals in the age group 21 years to 80 years were significantly methylated(Figure 7).

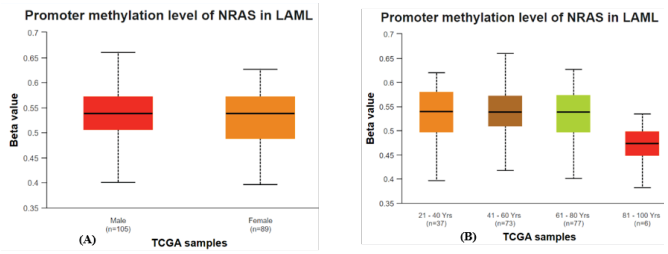

Figure 7: (A) NRAS promoter methylation profile based on patient's gender in LAML (B) NRAS promoter methylation profile based on patient's age in LAML

GEPIA was used to obtain Transcripts per million (TPM) graph in which the mRNA expression levels of the NRAS gene were significantly higher T (n=173) in LAML as compared to in normal tissues N (n=70) and were shown as red and green dot plots respectively which is shown in figure 8(A). Overall survival rates are shown in figure 8(B) to investigate the association between NRAS mRNA expression and patient prognosis in AML. NRAS mRNA expression levels were visualized using Kaplan–Meier survival curves which showed no significant correlation between NRAS expression and percent survival in AML. The graph showed log-rank p-value (0.94), Cox proportional hazard ratio (HR=0.98), p-value of HR (0.94), and number of patients in high (n=53) and low (n=53) NRAS group. Moreover, a 95% confidence interval was marked with dotted lines(Figure 8).

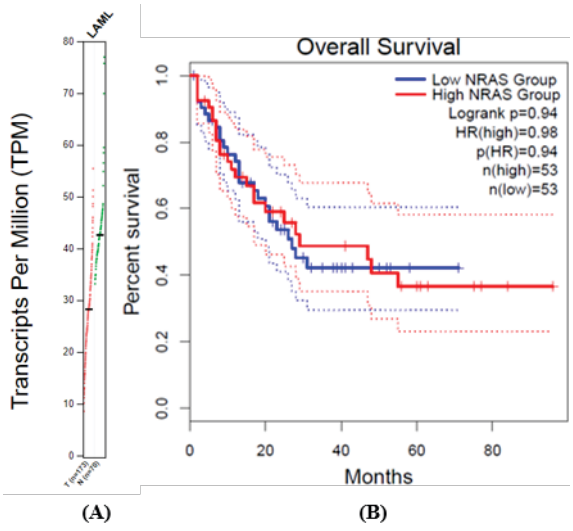

Figure 8: (A) Retrieval of NRAS mRNA expression levels in AML (B) Comparison of overall survival rate between high and low NRAS groups in AML from GEPIA web tool

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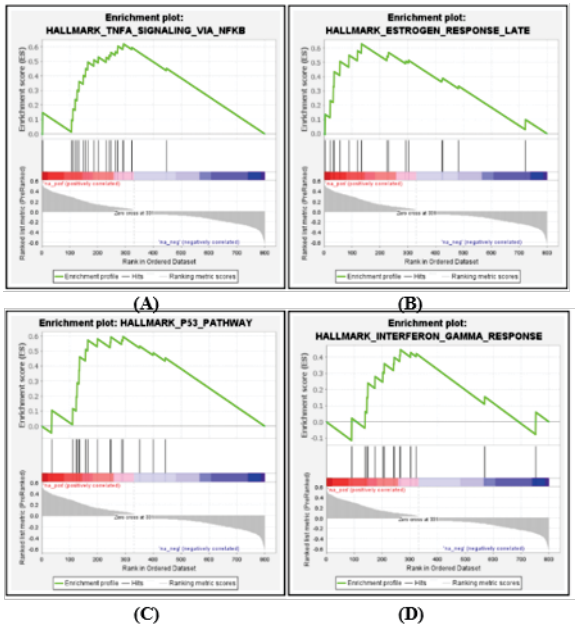

Figure 9: Gene Set Enrichment Analysis (GSEA) representing (A) Hallmark TNFA Signaling via NFKB (B) Hallmark Estrogen Response Late (C) Hallmark p53 Pathway (D) Hallmark Interferon Gamma Response

GSEA was use to analyze the affected mRNA expression levels of the NRAS gene caused by SNPs in various biological functional pathways. The results are shown in the form of an enrichment plot. In Figure 9(A–D), enriched genes associated with DNA-bound NFKB induced transcription of innate immune response genes as well as other genes that protect the cell from TNF-induced cell death. Also, the enriched gene set was upregulated with a late response to estrogen. Enriched genes were also involved in p53 pathways and networks. Furthermore, the most significantly enriched pathways in inducing and modulating an array of immune responses were also observed. All these enrichment plots were positively correlated. Cytoscape was used to generate a network of NRAS protein association with other related proteins which are involved in AML. A SNPs which alter the functionality of NRAS protein will consequently affect the related proteins.

DISCUSSION

Missense non-synonymous SNPs (nsSNPs) might code for various amino acids which can have an impact on the biological functions of the encoded protein as well as the prognosis of a disease. Mutations in the NRAS gene are involved in the pathophysiology of AML patients [13, 14]. Considering this, the NRAS gene was selected for *in silico* analysis as it has not been done previously. All the SNPs (Q61H, Q61L, G13V, G13R, and G12A) were retrieved from the dbSNP-NCBI. Functional analysis of these SNPs showed them to be damaging, disease causing and non-tolerated. NRAS has a domain that binds a GTP molecule and transforms it into GDP to regulate the rate of cell activity. The mutations in the NRAS gene can transform it into an active oncogene with a vital function in cancer such as an increase in the GTPase pathway's activity characterized by altered cellular proliferation, differentiation, survival, and reduced apoptosis [15, 16]. The two mutations (G13R and G13V) with the highest binding affinity to GTP were identified as the most lethal ones [17]. Protein function is determined by protein stability, which controls the protein's conformational shape. Any modification to protein stability may result in protein misfolding, disintegration, or abnormal protein aggregation [18, 19]. Structural analysis of NRAS protein using mCSM and I-mutant suite revealed that SNPs decreases the protein stability. Conformational analysis of NRAS protein was carried out by Project HOPE which predicted the 2D structure of amino acids which was altered by these SNPs. Smaller amino acids may end up losing their external connections whereas larger amino acids may produce bumps. Because the wild-type and mutant residues differ in size, the new residue is not in a position where it may form the same hydrogen bond as the original wild-type residue. Only 2 highly deleterious SNPs (Q61H and Q61L) were found on highly conserved and critical functional domains while others were not highly conserved. SWISS-MODEL was used to generate model of target protein from its amino acid sequence. SOPMA predicted that mutations were dominant in the alpha helix, followed by random coil, extended strand and then beta turn respectively. The effect of these SNP on the secondary structure of protein is highly needed to understand any change in the tertiary structure of NRAS protein [20]. Vienna Package calculated Minimum Free Energy (MFE) to predict the stability and secondary structure of wildtype and mutant molecules [21]. The more negative the MFE value is, the more stable the secondary structure of RNA. Post-translational modifications (PTMs) are covalent modifications that alter the protein structure in a way to perform their vital role in cellular signaling pathways effectively [22]. These PTMs include phosphorylation, ubiquitination, methylation, acetylation and glycosylation [23]. This study performed a detailed examination of how nsSNPs may cause PTMs and how these affected PTM sites are a leading cause of certain malignancies [24]. Therefore, disrupted proteostasis caused by altered PTMs can lead to affected protein functionality [25]. cBioprtal showed that 13 missense mutations were involved in altered PTMs and together they caused affected protein functionality. UALCAN provided the methylation level in the promotor region of the NRAS gene in different age groups of both male and female AML patients. GEPIA showed that mRNA expression levels were elevated in cancerous tissues. Whereas, the Overall

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survival graph showed that mRNA was differentially expressed in low and high NRAS groups. Gene Set Enrichment Analysis (GSEA) is used to identify enriched functional categories of informative biomarkers [26]. It provided data in the form of four enrichment plots which showed that the NRAS gene was significantly overrepresented in different hallmarks [27]. Cytoscape was used to generate the hub gene network of NRAS involved in AML. The mutation in NRAS protein disrupts normal functioning of protein leading to the development of cancer and an adverse prognosis of AML.

CONCLUSIONS

In humans, single nucleotide polymorphisms are the most prevalent type of genetic variation. The *in-silico* analysis of nsSNPs of NRAS predicted them to be deleterious and pathogenic. These SNPs in NRAS may trigger the permanently activated variant (NRAS-GTP), which can cause unstoppable cellular signaling eventually leading to AML.

Authors Contribution

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

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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REFERENCES

[1] Seth R and Singh A. Leukemias in Children. Indian Journal of Pediatric. 2015 Sep; 82(9): 817-24. doi: 10.1007/s12098-015-1695-5.

[2] Zimmermann C, Yuen D, Mischitelle A, Minden MD, Brandwein JM, Schimmer A, *et al*. Symptom burden and supportive care in patients with acute leukemia. Leukemia Research. 2013 Jul; 37(7): 731-6. doi: 10.1016/j.leukres.2013.02.009.

[3] Short NJ, Rytting ME, Cortes JE. Acute myeloid leukaemia. The Lancet. 2018 Aug; 392(10147): 593-606. doi: 10.1016/S0140-6736(18)31041-9.

[4] Howlader NN, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, *et al*. SEER cancer statistics review, 1975–2012. National Cancer Institute. 2014 Nov.

[5] Song X, Peng Y, Wang X, Chen Y, Jin L, Yang T, *et al*. Incidence, survival, and risk factors for adults with acute myeloid leukemia not otherwise specified and acute myeloid leukemia with recurrent genetic abnormalities: analysis of the surveillance, epidemiology, and end results (SEER) database, 2001–2013. Acta Haematologica. 2018 Feb; 139(2): 115-27. doi: 10.1159/000486228.

[6] Cazzola M. Introduction to a review series: the 2016 revision of the WHO classification of tumors of hematopoietic and lymphoid tissues. Blood, The Journal of the American Society of Hematology. 2016 May; 127(20): 2361-4. doi: 10.1182/blood-2016-03-657379.

[7] Tallman MS, Wang ES, Altman JK, Appelbaum FR, Bhatt VR, Bixby D, *et al*. Acute myeloid leukemia, version 3.2019, NCCN clinical practice guidelines in oncology. Journal of the National Comprehensive Cancer Network. 2019 Jun;17(6): 721-49. doi: 10.6004/jnccn.2019.0028.

[8] Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. New England Journal of Medicine. 2015 Sep; 373(12): 1136-52. doi: 10.1056/NEJMra1406184.

[9] Davis AS, Viera AJ, Mead MD. Leukemia: an overview for primary care. American Family Physician. 2014 May; 89(9): 731-8.

[10] Eisfeld AK, Schwind S, Hoag KW, Walker CJ, Liyanarachchi S, Patel R, *et al*. NRAS isoforms differentially affect downstream pathways, cell growth, and cell transformation. Proceedings of the National Academy of Sciences. 2014 Mar; 111(11): 4179-84. doi: 10.1073/pnas.1401727111.

[11] Deller MC, Kong L, Rupp B. Protein stability: a crystallographer's perspective. Acta Crystallographica Section F: Structural Biology Communications. 2016 Feb; 72(2): 72-95. doi: 10.1107/S2053230X15024619.

[12] Pundir H, Joshi T, Joshi T, Sharma P, Mathpal S, Chandra S, *et al*. Using Chou's 5-steps rule to study pharmacophore-based virtual screening of SARS-CoV-2 Mpro inhibitors. Molecular Diversity. 2021 Aug; 25: 1731-44. doi: 10.1007/s11030-020-10148-5.

[13] Asiri A, Alwadaani D, Umair M, Alhamoudi KM, Almuhanna MH, Nasir A, *et al*. Pancytopenia, recurrent infection, poor wound healing, heterotopia of the brain probably associated with a candidate novel de novo CDC42 gene defect: expanding the molecular and phenotypic spectrum. Genes. 2021 Feb; 12(2): 294. doi: 10.3390/genes12020294.

[14] Khosravi SZ, Moonesi M, Moradabadi A, Rajaeinejad M, Heidari MF, Noroozi-Aghideh A. Rapid Detection of N-RAS Gene Common Mutations in Acute Myeloid Leukemia (AML) Using High Resolution Melting (HRM) Method. Asian Pacific Journal of Cancer Prevention: APJCP. 2022 Jan; 23(1): 125. doi: 10.31557/APJCP.20

DOI: https://doi.org/10.54393/fbt.v3i01.33

22.23.1.125.

[15] Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nature Reviews Cancer. 2007 Apr; 7(4): 295-308. doi: 10.1038/nrc2109.

[16] Akram AM, Chaudhary A, Kausar H, Althobaiti F, Abbas AS, Hussain Z, *et al*. Analysis of RAS gene mutations in cytogenetically normal de novo acute myeloid leukemia patients reveals some novel alterations. Saudi Journal of Biological Sciences. 2021 Jul; 28(7): 3735-40. doi: 10.1016/j.sjbs.2021.04.089.

[17] Behairy MY, Soltan MA, Adam MS, Refaat AM, Ezz EM, Albogami S, *et al*. Computational analysis of deleterious SNPs in NRAS to assess their potential correlation with carcinogenesis. Frontiers in Genetics. 2022 Aug; 13: 872845. doi: 10.3389/fgene.2022.872845.

[18] Capriotti E, Fariselli P, Rossi I, Casadio R. A three-state prediction of single point mutations on protein stability changes. BMC Bioinformatics. 2008 Mar; 9(2): 1-9. doi: 10.1186/1471-2105-9-S2-S6.

[19] Witham S, Takano K, Schwartz C, Alexov E. A missense mutation in CLIC2 associated with intellectual disability is predicted by in silico modeling to affect protein stability and dynamics. Proteins: Structure, Function, and Bioinformatics. 2011 Aug; 79(8): 2444-54. doi: 10.1002/prot.23065.

[20] Khan S and Vihinen M. Spectrum of disease-causing mutations in protein secondary structures. BMC Structural Biology. 2007 Dec; 7: 1-8. doi: 10.1186/1472-6807-7-56.

[21] Kishore S, Jaskiewicz L, Burger L, Hausser J, Khorshid M, Zavolan M. A quantitative analysis of CLIP methods for identifying binding sites of RNA-binding proteins. Nature Methods. 2011 Jul; 8(7): 559-64. doi: 10.1038/nmeth.1608.

[22] Aftab A, Khan R, Shah W, Azhar M, Unar A, Jafar Hussain HM, *et al*. Computational analysis of Cyclin D1 gene SNPs and association with breast cancer. Bioscience Reports. 2021 Jan; 41(1): BSR20202269. doi: 10.1042/BSR20202269.

[23] Bhattacharya M, Hota A, Kar A, Chini DS, Malick RC, Patra BC, *et al*. In silico structural and functional modelling of Antifreeze protein (AFP) sequences of Ocean pout (Zoarces americanus, Bloch & Schneider 1801). Journal of Genetic Engineering and Biotechnology. 2018 Dec; 16(2):721-30. doi: 10.1016/j.jgeb.2018.08.004.

[24] Kim Y, Kang C, Min B, Yi GS. Detection and analysis of disease-associated single nucleotide polymorphism influencing post-translational modification. BMC Medical Genomics. 2015 Dec; 8(2): 1-8. doi: 10.1186/1755-8794-8-S2-S7.

[25] Trougakos IP, Sesti F, Tsakiri E, Gorgoulis VG. Non-enzymatic post-translational protein modifications and proteostasis network deregulation in carcinogenesis. Journal of Proteomics. 2013 Oct; 92: 274-98. doi: 10.1016/j.jprot.2013.02.024.

[26] Joly JH, Lowry WE, Graham NA. Differential Gene Set Enrichment Analysis: A statistical approach to quantify the relative enrichment of two gene sets. Bioinformatics. 2020 Nov; 36(21): 5247-54. doi: 10.1093/bioinformatics/btaa658.

[27] Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Systems Biology. 2014 Dec; 8(4): 1-7. doi: 10.1186/1752-0509-8-S4-S11.