JBAAR

Journal of Bioscience and Applied Research

https://jbaar.journals.ekb.eg

SPBH

Single Nucleotide Polymorphism (rs 4012939) *NANOG* gene in Iraqi patients with Chronic Myeloid Leukemia

Hassnien Samer Al-Hashemi[1], Noor Tariq Naeem [2], Zeyad A. Shabeeb[1]

[1] National Center of Hematology/ Mustansiriyah University, Iraq

[2]Forensic DNA Center for Research and Training, Al-Nahrain University, Jadria, Baghdad, Iraq.

noortarik8@gmail.comhassnhashmi@uomustansiriyah.edu.iq dr.zeyad.ahmed@uomustansiriyah.edu.iq

Corresponding author: Dr.Hassnien Samer Al-Hashemi

National Center of Hematology/ Mustansiriyah University, Iraq

Email; hassnhashmi@uomustansiriyah.edu.iq

DOI: 10.21608/jbaar.2025.453513

ABSTRACT

Background : NANOG is a transcription factor that is crucial for maintaining pluripotency in embryonic stem cells. It is involved in self-renewal and differentiation processes. Beyond its role in embryonic development, NANOG has been implicated in various cancers. The study aimed to detect the polymorphism rs9607340 in the NANAO gene and the progression of the disease. **Method:** The study consists of two groups: fifty CML patients (male 26, female 24) and fifty apparently healthy volunteers (male 25, female 25) from the National Center of Hematology/ Mustansiriyah University. **Result:** The result suggests that the SNP rs4012939 heterozygous genotype TC shows significant differences, $P \leq 0.05$, it was 48% in chronic CML patients 60% in controls (and OR = 2.8). The heterozygous CT genotype for rs4012937 showed a highly significant difference with $P \leq 0.01$ and OR= 7.7.

Conclusion: Polymorphism in the Nanog gene is associated with progression of the disease.

Keywords: Chronic Myeloid Leukemia, polymorphism(rs4012939), NANOG gene.

Introduction

Nanog transcription factors are essential for sustaining the pluripotent state and promoting self-renewal in embryonic stem (ES) cells (1). Nanog, identified as a master regulator governing pluripotency and developmental processes, represented the initial pluripotency gene wherein variability and temporal fluctuations were documented (2). The majority of human tumors contain a subset of cancer cells known as cancer stem cells (CSCs), exhibiting biological traits akin to normal stem cells, including self-renewal and differentiation (3). CSCs are believed to be endowed with immortality, persisting in tumors and contributing to relapse and metastasis (4). Given that CSCs may potentially arise from oncogenic reprogramming, the identification of molecules associated with stem cell characteristics, such as transcription factors, cell surface proteins, stemness genes, and microRNAs, is a pivotal inquiry. This exploration can lead to the recognition of novel therapeutic targets for cancer. Accumulating evidence suggests that NANOG is a pivotal factor capable of imparting certain CSC properties to cancer cells, including self-renewal, tumorigenicity, metastasis, and drug resistance (5). Nanog is a good

Received: May 15, 2025. Accepted: Aug 5, 2025. Published: August 25, 2025

prognostic and predictive factor in various human cancers (6).

Material and Method

This study consists of two groups. The first group includes 50 patients with chronic myeloid leukemia CML (male 26, female 24), and the second group includes 50 apparently healthy volunteers (male 25, female 25). All samples were diagnosed in the National Center of Hematology, Mustansiriyah University.

Blood sample collection

Three mL of blood was obtained from each group for complete blood count (CBC) and polymorphism rs4012939.

Genomic DNA isolation

EasyPure® Genomic DNA Kit (TransGen, Biotech. EE101-01) used for Deoxyribonucleic acid extraction.

Conventional PCR

PCR reactions were carried out in a final volume of 25 µL according to the manufacturer's instructions. The protocol employs 2xEasyTaq® PCR SuperMix.

Agarose gel electrophoresis

Amplified PCR fragments were separated on an agarose gel and then seen under UV light after ethidium bromide staining, PCR product, and ladder, as shown in Figure 1.

Statistical analysis

The result used haplotype combination between patients and controls was compared using the chi-square test, OR, and 95% CI.

Result

DNA sequencing and genotyping

The amplified PCR fragments were subjected to Sanger sequencing using an ABI3730XL automated DNA sequencer (Macrogen Corporation, Korea).

One hundred blood samples were used to amplify the targeted region of the Nanog gene of CML patients and controls. The primer used for sequencing Nanog rs 4012939 Sequence (5′→3′ direction) forward-TGGCCAGGAATAAAAGTTAGC Reverse –GTGTCCAGACTGAAATTGAGT.

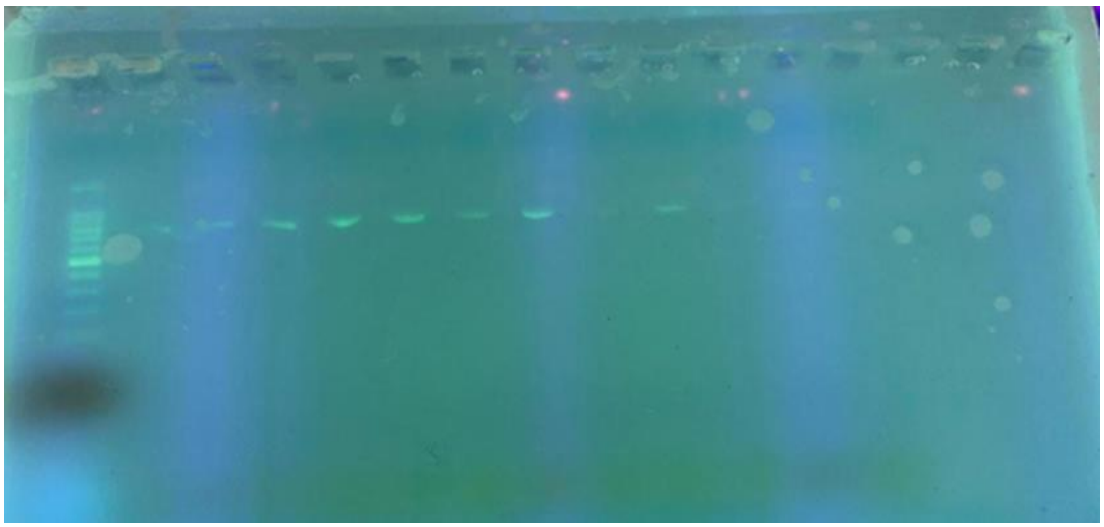

Figure (1): PCR product and ladder, ladder size 100-1500, voltage 90V, time 100 min, band size 388bp.

Figure 2: Representative sequence alignment of Nanog gene amplification results with NCBI Blast. The arrow is for mutant rs4012939 (T/G) from the CML sample.

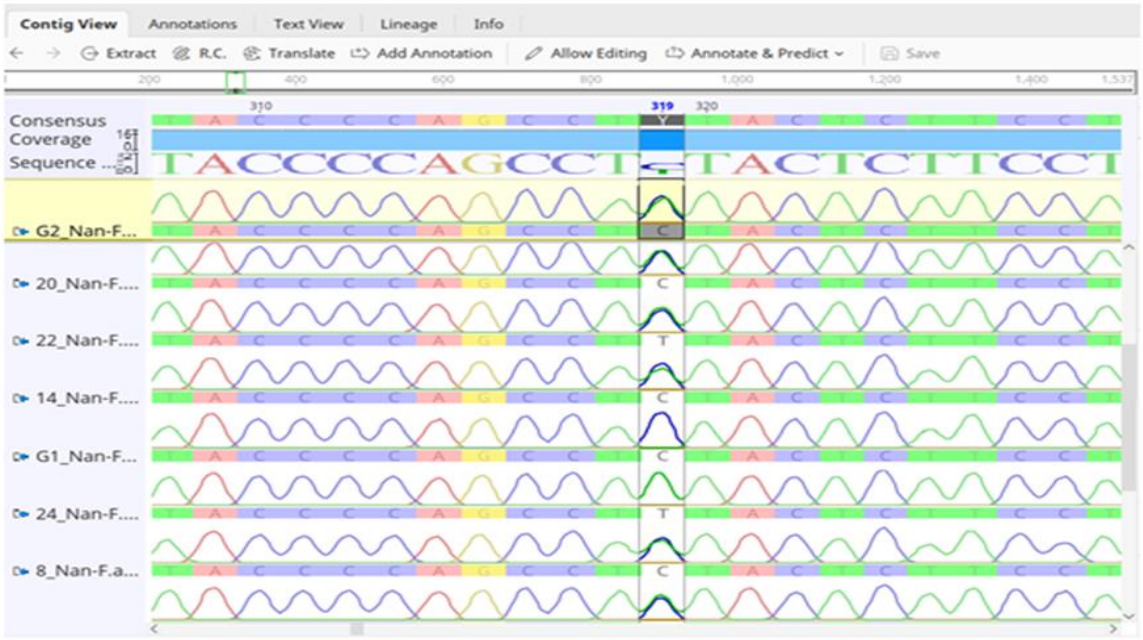

Figure (3): The genus sequence alignment findings for the Homo sapiens NANOG fragments confirmed the compatibility of sample sequences with a reference sequence from the Gene Bank.

Allele frequency and genotype distribution of the NANOG gene rs4012939 T>G

Distribution of genotypes and allele frequency of the single-nucleotide polymorphism rs4012939 of the NANOG Gene in Iraqi patients with CML compared to the control group. This SNP was located in intron 3 on chromosome 12.

According to genotype frequencies Homozygous wild TT genotype was Ref, and the highest percentage was in apparently healthy controls 30% and 0% in CML patients. The frequency of the heterozygous TC genotype was 84% in CML patients and 60% in apparently healthy controls, showing significant differences with $P \leq 0.05$. Homozygous mutant CC genotype frequency was

16% in CML patients and 10% in controls, and showed no significant differences. The frequency of the T allele was lower in CML patients compared to apparently healthy controls, whereas the frequency of the C allele was higher in CML patients compared to apparently healthy controls.

Another SNP was found in the fourth exon rs4012937. According to genotype frequencies Homozygous wild TT genotype was Ref and the highest percentage was in apparently healthy control 50% and 20% in CML patent, the frequency of the heterozygous CT genotype was 50% in CML patent and 16% in apparently healthy control shows high significant differences with P ≤ 0.01 and OR= 7.7 Homozygous mutant TT genotype frequency was 30% in CML patients and 34% in Control and shows no significant differences. The frequency of the C allele was lower in CML patients compared to apparently healthy controls, whereas the frequency of the T allele was higher in CML patients compared to apparently healthy controls. The result suggests that the presence of the T allele is associated with decreased risk of the disease, and the C allele could have a susceptibility association with increased risk of having the disease.

Table (1): Genotype and allele frequency among patient groups compared with the healthy group of NANOG gene rs4012939 T>C.

rs4012939	Frequencies (%)			P value	Odd ratio (95% CI)
	Patients (n=50)	Control (n= 50)			
TT	0 (0%)	15 (30%)	-	---	1.00 (Reference)
TC	42 (84%)	30 (60%)		0.01*	2.8 (2.736 to 23.06)
CC	8 (16%)	5(10%)		0.1	1.2 (0.9025 to 5.0659)
T	40 (40)	42 (42)	-	---	1.00 (Reference)
C	60 (60)	58 (58)		0.06	1.4 (0.973 to 2.852)

Table (2): Genotype and allele frequency among patient groups compared with the healthy group of NANOG gene rs4012937 C> T.

rs4012937	Frequencies (%)			P value	Odd ratio (95% CI)
	Patients (n=50)	*Control (n= 50)*			
CC	10 (20%)	25 (50%)	-	---	1.00 (Reference)
CT	25 (50%)	8 (16%)		0.002*	7.7 (2.65 to 23.06)
TT	15 (30%)	17(34%)		0.1	2.2 (0.8015 to 4.0548)
C	40 (40%)	42 (42%)	-	---	1.00 (Reference)
T	60 (60%)	58 (58%)		0.06	1.4 (0.973 to 2.852)

Discussion

Polymorphisms in the NANOG gene rs4012939 were evaluated for susceptibility in Chronic Myeloid Leukemia. The NANOG gene, which encodes a homeobox transcription factor, plays an important role in the maintenance of pluripotency of ESC (7). SNP rs4012939 was located in intron 3; the heterozygous TC genotype was significantly increased in CML patients compared to healthy controls, so the C allele could have a susceptibility association with an increased risk of having the disease. The heterozygous CT genotype showed a highly significant difference in CML patients with $P \leq 0.01$ and OR= 7.7. Polymorphisms in the NANOG gene affect breast cancer risk (8). Polymorphisms in the NANOG gene can lead to variations in gene expression and function. These genetic variations may influence susceptibility to CML and the disease's clinical outcomes (9,10).

In the context of chronic myeloid leukemia (CML) and other m,10alignancies, research has suggested that specific polymorphisms in the NANOG gene, including rs4012939, could be associated with differences in disease risk, progression. However, findings can be mixed, and further studies are needed to clarify the exact role of this SNP in CML and its potential as a biomarker for prognosis or therapeutic response.

Acknowledgments

Only to the authors for their support of the article.

Funding: None

Conflict of Interest

The authors declare that there is no conflict of interest for the authors of the manuscript.

REFERENCES

1- Loh YH, Wu Q, Chew JL, Vega VB, Zhang W, Chen X, Bourque G, George J, Leong B, Liu J, Wong KY, Sung KW, Lee CW, Zhao XD, Chiu KP, Lipovich L, Kuznetsov VA, Robson P, Stanton LW, Wei CL, Ruan Y, Lim B, Ng HH. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. Nat Genet. 2006 Apr;38(4):431-40. doi: 10.1038/ng1760. Epub 2006 Mar 5. PMID: 16518401.

2- Lucia Marucci, "Nanog Dynamics in Mouse Embryonic Stem Cells: Results from Systems Biology Approaches", *Stem Cells International*, vol. 2017, Article ID 7160419, 14 pages, 2017. https://doi.org/10.1155/2017/7160419

3- Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer. 2008 Oct;8(10):755-68. doi: 10.1038/nrc2499. Epub 2008 Sep 11. PMID: 18784658.

4- Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, Silasi DA, Steffensen KD, Waldstrom M, Visintin I, Mor G. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle. 2009 Jan 1;8(1):158-66. doi: 10.4161/cc.8.1.7533. PMID: 19158483; PMCID: PMC3041590.

5- Gong S, Li Q, Jeter CR, Fan Q, Tang DG, Liu B. Regulation of NANOG in cancer cells. Mol Carcinog. 2015 Sep;54(9):679-87. doi: 10.1002/mc.22340. Epub 2015 May 27. PMID: 26013997; PMCID: PMC4536084.

6- Grubelnik, G., Boštjančič, E., Pavlič, A., Kos, M., & Zidar, N. (2020). NANOG expression in human development and cancerogenesis. Experimental Biology and Medicine, 245(5), 456-464.

7- Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ (2008) Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451:141–146.

8- Liao, Y. C., Wang, L. H., Hung, M. C., Cheng, T. C., Lin, Y. C., Chang, J., ... & Ho, Y. S. (2024). Investigation of the α9-nicotinic receptor single nucleotide polymorphisms induced oncogenic properties and molecular

mechanisms in breast cancer. Human Molecular Genetics, ddae132.

9- Bruzzoni-Giovanelli, H., González, J. R., Sigaux, F., Villoutreix, B. O., Cayuela, J. M., Guilhot, J., & Rousselot, P. (2015). Genetic polymorphisms associated with increased risk of developing chronic myelogenous leukemia. Oncotarget, 6(34), 36269.

10- Al-Hashemi HS, Rahman SAHA, Shabeeb ZA. Expression of immune checkpoint molecules in Iraqi acute myeloid leukemia patients. Iraqi J Hematol. 2021;10(1):1-16. https://doi.org/10.4103/ijh.ijh_46_20.