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The Impact of the ERCC2 Lys751Gln Polymorphism on the Risk of Acute Myeloid Leukemia in an Iraqi Patients

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Abstract

Objectives: AML is the only type of acute leukemia diagnosed in adults and is less common in children. It has the lowest survival rate. Epidemiological risk factors for AML expansion comprise environmental factors, for instance, smoking, and therapy-related factors.

Methods: The study was conducted on 70 acute myeloid leukemia patients–37 females and 33 males and on 30 healthy people–12 females and 18 males–as a control group. DNA was extracted from the study groups' whole blood samples using the gSYNC™ DNA Extraction Kit. The T751G polymorphism of the ERCC2 gene was determined by the PCR-RFLP technique.

Results: In genetic analysis, it was shown that the carriers of allele Lys and genotype Lys/Lys have a lower risk of developing AML, while allele carriers Gln have an increased risk. The results showed the ERCC2 gene, Lys 751 Gln (T/G) heterozygous TG genotypes, and the G allele were significantly higher ($P < 0.05$) in AML patients compared to the control group. In the sequencing of the region we studied, it was found that there is a site of diversity that is located between the CTTCAG and CTGCAG, where a change in nucleotides (T to G) represents the restriction site of the restriction enzyme.

Conclusion: The polymorphic marker 751 Gln> Lys of the ERCC2 gene was associated with the development of AML in Iraqi patients. It was discovered that allele Lys genotype Lys/Lys carriers have a lower risk of developing AML, whereas allele Gln carriers have an increased risk.

Keywords: Leukemia, myeloid, acute, ERCC2, polymorphism, genetic, DNA repair

Introduction

AML is the one type of acute leukemia diagnosed in adults and less common in children and is connected with the lowest survival rate.[1] Epidemiological risk factors for AML expansion comprise environmental factors, for instance, smoking and exposure to benzene, therapy-related factors.[2] AML derives from hematopoietic stem cells with a stepwise acquisition of genetic and epigenetic alterations. These assembled mutations influence normal HSC functions, obstructing differentiation and rising self-renewal capacity.[3] The excision repair cross-complementing group 2 (ERCC2) gene, also known as the xeroderma pigmentosum group D (XPD) gene, is located on chromosome 19q13.3. The ERCC2 gene is consists of 23 exons and stretches about 54,000 base pairs.[4] The ERCC2 gene produces a protein which consists of 760 amino acids with a molecular weight of 86,900 and has been 5'–3' DNA helicase activity that is adenosine triphosphate-dependent. The ERCC2 protein is a part of the core transcription factor IIH, which is particpated in nucleotide excision repair of DNA by opening DNA around the damage.[5]

Materials and Methods

The study group was conducted on 70 acute myeloid leukemia patients 37 females and 33 males at the Department of Hematology, Baghdad Teaching Hospital, Medical City, for the period from March 2022 to July 2022, and 30 healthy people 12 females and 18 males as a control group. The ages of patients and control ranged between 15–82 years.

DNA isolation and Polymerase Chain Reaction (PCR)

Under aseptic conditions, genomic DNA was extracted from nucleated cells. DNA was extracted from the study groups' whole blood samples using the gSYNC™ DNA Extraction Kit from Geneaid. T751G polymorphism of the *ERCC2* gene was determined by PCR-RFLP with the following primers: sense, F: 5'-CCTCTCCCTTTCCTCTGTTC-3' and antisense,

R: 5'-CAGGTGAGGGGGACATCT-3'.[6] The 734 bp product was digested with 5 U of the restriction enzyme *Pst*I.

Amplification was carried out in 25 µl tube of PCR PreMix Reaction Mixture (AccuPower PCR premix, Bioneer) Amplification was performed in a thermal cycler (Cleaver scientific Ltd/UK) programmed for 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 1 min, preceded by an initial denaturation of 5 min at 95°C. Final extension was for 5 min at 72°C. Finally, the gel electrophoresis method was done according to Sambrook and Russell,[7] and 5 µl of each samples was loaded onto 2% agarose gel.

Statistical Analysis

Through the use of the SPSS version 26 software, statistical analyses of all findings were completed. The χ^2-test was used to test Hardy-Weinberg equilibrium in both controls and cases for each polymorphism. Chi-square analysis was used to find the genotype and allele frequency differences between the patients and controls. A measure of the association of the polymorphic sites with AML was also determined using odds ratios (ORs) and 95% confidence intervals (CIs). A *P*-value of 0.05 was considered significant.

Results

An ERCC2 variant with decreased ability to repair DNA breaks has been linked to a single nucleotide genetic polymorphism (SNP) in codon 751 of exon 23 (rs13181), where a change in nucleotides (T to G) leads to an amino acid change (Lys to Gln).

The occurrence of *ERCC2* gene polymorphism was revealed by RFLP-PCR technique. At this position three genotype were found; TT, GG and TG,[6] found that this locus had three genotypes only. The results revealed that GG homozygous genotype relative frequency was found to be 11.5% and 0% in the AML patients and control group respectively that was statically significant. Also the heterozygous genes revealed significant differences where the AML patients 47% contained TG heterozygous genotype, while in the control group this genotype was present in 40%. The TT homozygous genotype was present in 60% of the controls, whereas it was 41.5% in the AML patients as shown in Table 1.

The results showed that the "G" allele is highly prevalent in the AML patients which was 35% as compared to the controls 20%, whereas the relative frequency "T" allele was 65% in the AML patients and 80% in the controls, as shown in Table 2.

Discussion

The present study revealed that the G allele, TG and GG genotype in AML patients were over than the controls, and observed that individuals with the GG genotypes had higher risk for developing AML disease. In contrast, the "T" allele, and TT genotype have a rather preventive role. This may indicate that the "T" allele may be protective. A significant association between polymorphism of *ERCC2* Lys751Gln and AML, overall data analysis revealed that *ERCC2* Lys751Gln may be significantly correlated with elevated leukemia risk. We discovered a strong correlation between the polymorphism Lys751Gln with the risk of developing AML (*P*-value = 0.03, OR = 2.15; 95% CI = 1.05–4.43 for the Gln allele). In the case of Lys751Gln, individuals with AML were more likely to have the combined heterozygous genotypes than controls (OR = 1.34; 95% CI = 0.56–3.19; *P*–value = 0.03). This was also detected when the Gln/Gln genotype was examined (OR = 8.30; 95% CI = 0.46–148.51). In this study, showed that an increase in the Lys/Lys genotype and the Lys allele in the ERCC2 codon 751 polymorphisms play a protective role in AML, and a increase in Gln/Gln genotype in acute leukemia was associated with early relapse Tables 3 and 4.

The relationship between these ERCC2 polymorphisms and leukemia risk has been examined in some case-control studies, but the results of these studies remain confusing rather than conclusive. Although a number of studies have found a link between ERCC2 polymorphisms and the risk of certain types of leukemia, many researchers have discovered that the variant 751Gln allele is associated with an increased risk of AML.[8-16]

Other studies, on the other hand, did not consider the ERCC2 genetic variants to be risk or protective factors for leukemia because they demonstrated that the presence of the ERCC2 751Gln allele had a protective effect in the development of AML.[17-19]

Sequencing of Amplified ERCC22 Gene

In order to check up the genetic variation in a rs 13181; Lys751Gln, T/G in exon 23 *ERCC2* gene. Sequencing was

Table 1. **Comparative analysis of the distribution of genotype frequencies of polymorphic marker *ERCC2* gene; Lys751Gln among patients with AML and in the control group**

Genotypes	Cases	Controls	χ^2	*P*	OR	
	n = 70	*n* = 30			Value	95% CI
Genotype T/T	29(41.5%)	18(60%)			0.47	0.20–1.13
Genotype T/G	33(47%)	12(40%)	4.75	0.03	1.34	0.56–3.19
Genotype G/G	8(11.5%)	0(0%)			8.30	0.46–148.51

Table 2. **Comparative analysis of the distribution of allele frequencies of polymorphic marker *ERCC2* gene; Lys751Gln among patients with AML and in the control group**

Alleles	Cases	Controls	χ^2	*P*	OR	
	n = 70	*n* = 30			Value	95% CI
Allele *T*	91(65%)	48(80%)	4.46	0.03	0.46	0.23–0.96
Allele *G*	49(35%)	12(20%)			2.15	1.05–4.43

Table 3. **Distribution of *ERCC2* gene; Lys751Gln in the study population under Dominant inheritance model**

Genotypes	Cases	Controls	χ^2	*P*	OR	
	n = 70	*n* = 30			Value	95% CI
Genotype *T/T*	29(41.5%)	18(60%)	2.91	0.09	0.47	0.20–1.13
Genotype *T/G+G/G*	41(58.5%)	12(40%)			2.12	0.89–5.07

Table 4. **Distribution of *ERCC2* gene; Lys751Gln in the study population under Recessive inheritance model**

Genotypes	Cases	Controls	χ^2	*P*	OR	
	n = 70	*n* = 30			Value	95% CI
Genotype *T/T+T/G*	62(88.5%)	30(100%)	3.73	0.05	0.12	0.01–2.16
Genotype *G/G*	8(11.5%)	0(0%)			8.30	0.46–148.51

performed to determine the genetic variation in Iraqi patients with AML compared with the apparently healthy control. The complete nucleotide sequence is examined and the results were illustrated in Figures 1-3.

Through the current study of the sequential sequence of the region we studied, it was found that there is a site of diversity which located between the **CTTCAG** and **CTGCAG** where a change in nucleotides (T to G), which represents the restriction site of the restriction enzyme, and this means that the enzyme is cut in the case of the presence of G in the target site of a segment and not cut in the case of the presence of the base T, when compared to the source Reference (Ref.) Or a comparison between patient samples and control samples (Figure 1).

Fig. 1 **Comparison of the alignment of nitrogenous bases in patient samples of a fragment of DNA from the ERCC2 gene, a rectangle indicates the difference site in one of the nitrogenous bases, which is the same as the restriction site of the enzyme.**

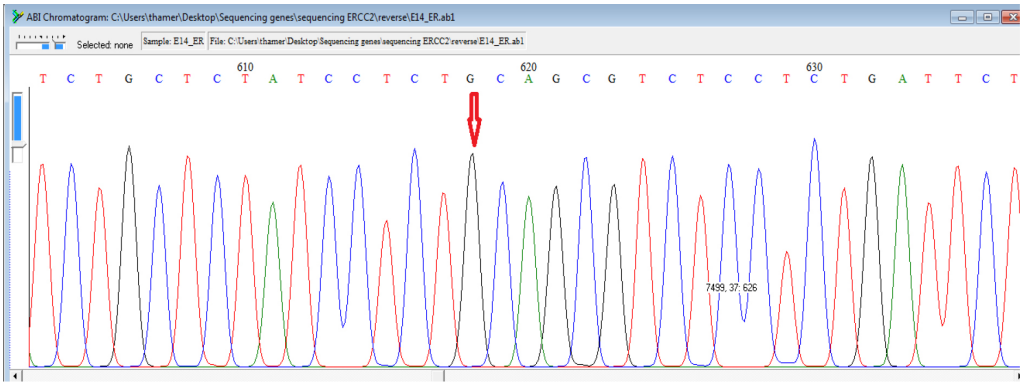

Fig. 2 **The location of the occurrence of genetic diversity in the studied sequence, the arrow indicates the genotype of patient as a result of the presence of one homozygous genetic pattern GG.**

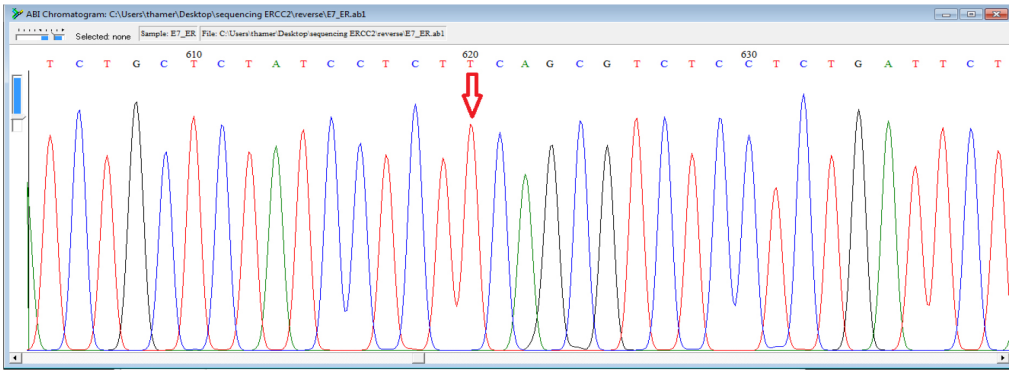

Fig. 3 **The location of the occurrence of genetic diversity in the studied sequence, the arrow indicates the genotype of control as a result of the presence of one homozygous genetic pattern TT.**

For investigated the presence of genetic diversity or mutations in the region we used various and different genetic analysis methods, the results were compared with what was published in the Gene bank website located within the American National Center for Biotechnology Information (NCBI) website, which is http://www.ncbi.nlm.nih.gov/. The BLAST method was searched, which is a tool for searching for matches in sequences on the Gene bank website, after removing excess and non-conforming sequences (Rubbish) from both ends of the sequences and alignment them using the computerized BioEdit program. The target region was obtained and compared with the sequences obtained for the patient and control samples. The ERCC2 region was registered and published on the Gene Bank website located within the National Center for Biotechnology Information (NCBI) website under assigned accession number (LC735410) https://www.ncbi.nlm.nih.gov/nuccore/LC735410.1/.

Conclusion

An association of polymorphic marker 751 Gln> Lys of the ERCC2 gene with the development of AML in Iraqi patients. It was shown that the carriers of allele Lys genotype Lys/Lys have a lower risk of developing AML, while allele carriers Gln have an increased risk.

Conflicts of Interest

"The authors declare no conflicts of interest."

References

1. Yamamoto, J.F. and M.T. Goodman, Patterns of leukemia incidence in the United States by subtype and demographic characteristics, 1997–2002: Cancer Causes Control, 2008. 19(4): 379–90. DOI: 10.1007/s10552-007-9097-2.
2. Zeeb, H. and M. Blettner, Adult leukaemia: what is the role of currently known risk factors. Radiat Environ Biophys,1998. 36: 217–228.
3. Corces-Zimmerman, M. R. and R. Majeti, Pre-leukemic evolution of hematopoietic stem cells: the importance of early mutations in leukemogenesis. Leukemia, 2014. 28(12): 2276–2282.
4. Weber, C.A., et al., ERCC2: cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast RAD3. EMBO J, 1990. 9: 1437–47. DOI: 10.1002/j.1460-2075.1990.tb08260.x.
5. Schaeffer, L., et al., The ERCC2/DNA repair protein is associated with the class II BTF2/TFIIH transcription factor. EMBO J., 1994. 13: 2388–92. doi: 10.1002/j.1460-2075.1994.tb06522.x.
6. Dexi, J., et al., Impact of polymorphisms in DNA repair genes XPD, hOGG1 and XRCC4 on colorectal cancer risk in a Chinese Han Population. Bioscience Reports, 2019. 39: 1–9.
7. Russell, D.W., and J. Sambrook, Molecular cloning: A laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory, 2001.
8. James, M., et al., Genetic variation in XPD predicts treatment outcome and risk of acute myeloid leukemia following chemotherapy Blood, 2004. 104(13): 3872–3877. DOI: 10.1182/blood-2004-06-2161.
9. Ilhan, G., et al., Risk factors and primary prevention of acute leukemia. Asian Pac. J. Cancer Prev. 2006. 7: 515–517.
10. Ali, Ö., et al., Polymorphisms of the DNA repair gene XPD (751) and XRCC1 (399) correlates with risk of hematological malignancies in Turkish population. African Journal of Biotechnology, 2011. 10(44): 8860–8870.
11. Meenaghan, T., et al., Acute leukaemia: making sense of a complex blood cancer. Br. J. Nurs, 2012. 21 (76): 78–83. DOI: 10.12968/bjon.2012.21.2.76.
12. Duo, L., et al., The effect of XPD/ERCC2 Lys751Gln polymorphism on acute leukemia risk: A systematic review and meta-analysis Gene, 2012. 538: 209–216. DOI: 10.1016/j.gene.2014.01.049
13. Liu, D., et al., The effect of XPD/ERCC2 Lys751Gln polymorphism on acute leukemia risk: a systematic review and meta-analysis. Gene, 2014. 538(2): 209–16.
14. Claudia, B., et al., Influence of XPC, XPD, XPF, and XPG gene polymorphisms on the risk and the outcome of acute myeloid leukemia in a Romanian population, Tumour Biol, 2016. 37(7): 9357–66. DOI: 10.1007/s13277-016-4815-6.
15. Dolly, J., et al., Association of XPD (Lys751Gln) and XRCC1 (Arg280His) gene polymorphisms in myelodysplastic syndrome. Ann Hematol, 2016. 95(1): 79–85. DOI: 10.1007/s00277-015-2528-3.
16. Zohreh, S., et al., Genetic variants of nucleotide excision repair pathway and outcomes of induction therapy in acute myeloid leukemia. Per. Med., 2019. 16(6): 479–490. DOI: 10.2217/pme-2018-0077.
17. Mehta, P.A., et al., XPD Lys751Gln polymorphism in the etiology and outcome of childhood acute myeloid leukemia: a Children's Oncology Group report, Blood. 2006. 107(1): 39–45. DOI: 10.1182/blood-2005-06-2305.
18. Sorour, A., et al., The genotype distribution of the XRCC1, XRCC3, and XPD DNA repair genes and their role for the development of acute myeloblastic leukemia. Genet Test Mol Biomarkers, 2013,17: 195–201. DOI: 10.1089/gtmb.2012.0278.
19. Kais, D.et al., Polymorphisms in XPC, XPD and XPG DNA repair genes and leukemia risk in a Tunisian population. Leuk Lymphoma, 2015. 56(6): 1856–62. DOI: 10.3109/10428194.2014.974045.