The Quarterly Scientific Journal of Applied Biology

Vol.37, No.4, Sering.82, Winter 2024, p. 16-19

Journal homepage: https://jab.alzahra.ac.ir

doi.org/10.22051/jab.2024.45923.1605

Association Between ERCC5 Gene Polymorphism (SNP: rs1047768 T>C) and the Risk of Breast Cancer in Northwest of Iran

Neda Shah Abbasi, Sara Ghaffarian*

Department of Cellular and Molecular Biology, Faculty of Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran

***Corresponding author: s.ghaffarian@azruniv.ac.ir**

Introduction

According to the GLOBOCAN reports, in 2020, breast cancer was the most common cancer in the world (Sung et al., 2021). DNA damages are very abundant and their repair is essential in preventing the stabilization of mutation and cancer (Ghoncheh et al., 2016).

Mutations in DNA repair genes can lead to the loss of DNA repair ability. There are at least 5 main DNA repair pathways and the Nucleotide Excision Repair (NER) is the most diverse pathway that has a high correlation with the risk of cancer (Chatterjee & Walker, 2017). During NER process, at first DNA damage is detected, then the DNA strand is cut by endonucleases at both 12-24 nucleotides upstream and downstream of the damage, and then the removed region is re-synthesized (Friedberg & Zaher, 2021). The ERCC5 (Excision Repair Cross Complementing Group 5) gene, also known as XPG gene, encodes a special endonuclease with 1168 amino acids, which is a key component of the NER pathway (Friedberg & Zaher, 2021).

Previous studies have shown a wide association between more than 1000 single nucleotide polymorphisms with a wide range of human tumors (Collins et al., 1997). According to our review, and the lack of previous reports, in this study, the association between rs1047768 SNP of ERCC5 gene polymorphism and the risk of breast cancer in the north west of Iran was investigated.

Material and Methods

Population The study population comprised 100 breast cancer patients and 100 healthy controls with no history of cancer. The DNA extraction performed by Suguna et al., (2014) protocol using proteinase K.

Genotyping rs1047768 polymorphism of ERCC5 gene was genotyped by Tetra ARMS-PCR. Primers were designed based on Malik et al. (2018) and were synthesized by Metabion (Germany). The primers sequence was:

5´CACTTAAAGGAGTCCGGGATCGCAAT3´ for forward inner primer, 5´GAAGATGAGGATTTTCTATTGAGTTCACG3´ for reverse inner primer, 5´GATGAAGAGAAAAATCCCGGAGTTTTTT3´ for forward outer primer, and 5´GTCTGTTTCTTCAATAGTGGAGCATCCC3´ for reverse outer primer. PCR amplifications were performed in a total volume of 12 µL, consisting of 1.5 µl DNA, 7 µl master mix (AMPLIQON, Denmark), 0.3 µl of each inner primer, 0.5 µl of each outer primer and 1.9 µl of H_2O, using the following profile: a 4 min denaturation at 94°C and 35 cycles of 40 sec denaturation at 94°C, 30 sec annealing at 59°C, 30 sec extension at 72°C, followed by a final extension at 72°C for 5 min. The amplification products were separated on 3 % agarose gel and detected by DNA safe stain.

Data and in silico analysis based on the size of products, the genotype of patients and controls were determined. Genotype and allele frequency were statistically analyzed via javastat online statistics package (www.statpages.info/ctab2x2.html) and SPSS V.26. Also, the spatial structure of RNA for rs63749820 polymorphism of MLH1 gene was analyzed using RNAsnp software.

The study protocol follows the ethical guidelines of the 1975 Declaration of Helsinki and approved by the Ethics Committee of Azarbaijan Shahid Madani University (https://ethics.research.ac.ir/IR.AZARUNIV.REC.1402.004).

Result and Discussion

In the case group, the frequency of CC, CT, and TT genotypes were 26.43 %, 44.83 %, and 28.73 %, respectively and they were 24.44 %, 15.55 %, and 60 % for the control group. Statistical analysis showed a significant association between TT genotype (p-value= 0.000) (OR= 0.269, CI 95 %= 0.144-0.503) and CT genotype (p-value= 0.000) (OR= 4.411, CI 95 %= 2.169-8.967) with breast cancer risk. Also, C and T allele frequency in the case group was 48.85 % and 51.14 % and those in the control group was 32.22 % and 67.77 % respectively. There was a significant association between T (p-value= 0.001) and C (p-value= 0.001) allele frequency and breast cancer risk in Northwestern Iran. There was no significant association between this SNP frequency and the patient's pathological traits.

In this study, the outer primers amplified a 220 bp fragment, and specific primers, designed for T and C alleles, amplified 148 bp and 126 bp fragments respectively. So, persons with TT, CC and TC genotypes in addition to the 220 bp control band, had 148, 126 and both 148 and 126 bp bands on agarose gel. The study showed that 25 (28.73%) patients and 54 (60 %) healthy people had TT genotypes. 39 (44.83 %) patients and 14 (15.55 %) healthy people had heterozygote TC genotypes, 23 (26.43 %) patients and 22 (24.44 %) healthy people had homozygote CC genotype. A significant correlation between the frequency of TT and TC genotypes for single-nucleotide polymorphism rs1047768 between patients and control group was observed ($P < .05$).

In the control group, the frequency of the T allele was 67.77 % and the frequency of the C allele was 32.22 %. In the patient group, the frequency of the T allele was 51.14 % and the frequency of the C allele was 48.85 %. There was a significant correlation between the frequency of T and C allele for single-nucleotide polymorphism rs1047768 between the patient and control group ($P < .05$).

Patients were also evaluated for pathological diagnosis including age, body side, tumor size, tumor stage, tumor type and lymph node involvement. No significant correlation between genotype and pathological information of patients was found. This analysis was performed by the RNA structure program and showed that free energy was changed when C > T alteration happened as ΔG was changed but it was not significant ($p= 0.27$).

In this study, TT and TC genotypes frequency showed a significant correlation between the patient and control group. Malik et al (2018) studied the correlation between rs1047768 polymorphism and breast cancer risk in a population from Pakistan and reported a significant correlation. But Na et al (2015) reported a non-significant correlation between this SNP polymorphism and breast cancer risk in a population from Han. This study provides a good view of the difference between populations from different countries in genotypic frequencies and cancer risk depending on the gene. Also, each of these frequencies may provide different results by conducting more extensive studies in wider areas.

Conclusions

According to the results of this study and the significant correlation between TT and TC genotypes of the rs1047768 T>C of the ERCC5 gene frequency in control and patient, this SNP SNP may contribute to the susceptibility of breast cancer in northwestern Iran, and its study may be useful in screenings for the breast cancer risk in this population.

Considering the diversity in rs1047768 SNP correlation with breast cancer risk in populations from different countries, as well as the diverse results regarding different cancers, authors are

recommending its study in a larger statistical population of sick and healthy people and from different races independently and also defining dedicated risk factors for each population.

Keywords: Case-control study, Nucleotide excision repair, Pathological traits, Single nucleotide polymorphism, XPG gene.

Acknowledgement

The current research has been financially supported by the Azarbaijan Shahid Madani Universitty.

Declaration of conflict of interest

The authors declare that they have no conflicts of interest.