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Association between genetic polymorphism of *XRCC7* (G6721T) and risk of acute lymphoblastic leukemia

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Abstract

Background: The DNA non-homologous end joining repair gene *XRCC7* is one of the most important genes in the DNA double-strand break (DSBs) repair. It is supposed that DNA repair gene malfunction is the main risk factor in various malignancies. The *XRCC7* G6721T (rs7003908) polymorphism impact was investigated on the splicing regulation that cause mRNA instability. The goal of the present hospital-based study was to investigate the association between the common genetic polymorphism of *XRCC7* G6721T (rs7003908) and risk of acute lymphoblastic leukemia (ALL). This hospital-based case–control study was performed on 99 ALL patients versus 200 healthy children, as the control group, which were frequent matched by age with cases. The polymorphism of *XRCC7* was determined using an RFLP-PCR technique.

Results: The GT (OR = 1.485, 95% CI 0.765–2.334, $P = 0.243$) and TT (OR = 1.655, 95% CI 0.875–3.128, $P = 0.121$) genotypes had no significant effect on the risk of ALL, in comparison with the GG genotype. However, TT genotype (OR = 1.996, 95% CI 1.033–3.858, $P = 0.04$) after adjusting for the parents' smoking pattern showed a significant impact.

Conclusions: These findings suggest that the TT genotype may increase the ALL susceptibility in children when facing with a tobacco smoke.

Keywords: *XRCC7*, Polymorphism, Acute lymphoblastic leukemia, Susceptibility, Parents smoking statuses

Background

Acute lymphoblastic leukemia (ALL) is the most prevalent type of cancer among children [1]. Its etiology is not yet known; however, several factors are involved in the causality of ALL. As risk factor, childhood ALL is related to some genetic syndromes, ionizing radiation, and genetic susceptibility. Environmental exposure factors can play a part in the accumulation of somatic mutations in children [2].

It is best-known that genes are involved in DNA repair, and crucial in protection against mutations, that preserve the unity of genetic material [3, 4]. Decline in DNA repair capability is related to increased birth defects, cancer, etc. [4]. Although mutations are the most

reason for carcinogenesis, defective DNA repair is also a risk factor for many types of cancer [4]. One of the most harmful types of DNA damage is the double-strand break (DSB), which could lead to DNA missing its physical integrity and data content on both strands [5]. The two important DSBs repair pathways are homologous recombination (HR) and non-homologous end joining (NHEJ) which are provided by exogenous and endogenous carcinogens. It is worth mentioning that for DSBs repair, the NHEJ is that the main pathway in human cells [5].

The human X-ray repair cross-complementing group 7 (*XRCC7*) (GenBank accession no.: NM_001469) plays a fundamental role within the NHEJ pathway [6]. It encodes the catalytic subunit of DNA-activated protein kinase (DNA-PKcs) that is recruited for the DSBs by the KU70/KU80 heterodimer to create an active DNA-PK

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complex. Moreover, it is crucial for the progress of the NHEJ pathway [6, 7]. The *XRCC7* G6721T (rs.7003908) polymorphism is found in intron 8 that might regulate splicing and cause mRNA instability [6]. Although NHEJ is the main pathway for DSBs, only a few studies have investigated the association between *XRCC7* G6721T polymorphism and cancers [7–13].

The aim of the present study was to assess the relationship between *XRCC7* G6721T polymorphism and risk of ALL.

Methods

Patient samples

Ninety-nine patients diagnosed with ALL under 18 years old were recruited at Amir Oncology Hospital, Shiraz, Iran, in 2016–2018. The control group of healthy children without ALL ($n = 200$) matched for age and gender in the same period and selected for this study. The inclusion criteria of the cases included being diagnosed with ALL through bone marrow checking out, immunology, cytology, clinical biochemistry and molecular biology, and having no other hematological disorders and previous cancer. The exclusion criteria of the controls included previous malignant neoplasm and any genetic or familial diseases. A questionnaire was used to obtain demographic data and environmental risk factors such as parents smoking habits and cancer history in the family. Parents of patients or legal guardians provided a written informed consent form for participation in the study. This study was conducted according to the guidelines of the Declaration of Helsinki and its later amendments.

DNA extraction and genotyping analysis

The total genomic DNA of each participant was extracted from the peripheral blood leukocytes using a Genomic DNA Purification Kit (QIAGEN, Germany). Then, they stored at $-20\text{ }^{\circ}\text{C}$ until use. The G6721T polymorphism of *XRCC7* with RFLP-PCR method was performed Taq Green PCR Master Mix (2X) (Thermo-Scientific, Fermentas) with the primers as described previously [11]. PCR conditions were 1 cycle at $94\text{ }^{\circ}\text{C}$ for 5 min; 30 cycles of $94\text{ }^{\circ}\text{C}$ for 30 s, $61.8\text{ }^{\circ}\text{C}$ for 50 s, and $72\text{ }^{\circ}\text{C}$ for 50 s; and a final extension at $72\text{ }^{\circ}\text{C}$ for 10 min. Later, PCR products were digested using 10 units of *Pvu II* (MBI Fermentas Inc.) with incubation at $37\text{ }^{\circ}\text{C}$ for 16 h. Subsequently, fragments of *XRCC7* 6721 G>T were resolved on 2% agarose gels to identify the wild type and polymorphic variant. The wild-type G allele polymorphism had no *Pvu II* cleavage site, and its size was 368 bp, whereas mutant T allele was digested to 274 and 94 bp fragments.

Statistical analysis

The sample groups determined to exhibit Hardy–Weinberg equilibrium for *XRCC7* polymorphism with the chi-square test. The associations between the genotypes of *XRCC7* and risk of ALL were calculated in cases and controls by computing the odds ratios (OR), and 95% confidence intervals (CIs). The multivariate logistic regression was assessed including parents' smoking habits and cancer history in the family. Given the association between the *XRCC7* G6721T polymorphism and the risk of ALL, the genotype data were further stratified by subgroups of cancer history in the family, and parents' smoking habits. Statistical significance was considered P value < 0.05 and two-tailed for all tests. Statistical analysis was performed using the SPSS statistical software package (version 16) for windows (SPSS Inc., Chicago, IL, USA).

Results

The present study involved ninety-nine patients and two-hundred cancer-free controls. Their mean age (SD) of the patients and controls was 5.87 (3.6) and 7.8 (5.2) years. The frequency distributions of the chosen characteristics for the ALL patients and control groups are summarized in Table 1. Family cancer history and parents' smoking habits differed significantly between the cases and controls ($P < 0.05$). The cases had a significantly higher percentage of those characteristics in comparison with the controls. However, the age of diagnosis, blood group and B vs. T immune phenotype distribution did not differ significantly between the cases and controls ($P > 0.05$) (data not shown).

†There are some data missing

Table 2 indicates genotype distributions of the *XRCC7* G6721T (rs7003908) polymorphism among the ALL patients and the control and the associations with the risk of ALL. The genotype frequencies of polymorphism in controls ($\chi^2 = 5.03$, $df = 1$, $P = 0.515$) and patients ($\chi^2 = 2.04$, $df = 1$, $P = 0.165$) were among Hardy-Weinberg equilibrium. *XRCC7* G6721T SNP analysis showed that the wild GG genotype was present in 19/99 patients (19.2%), while the variant genotypes GT and TT were present in 34/99 patients (34.3%) and 46/99 patients (46.5%), respectively. The GT (OR = 1.485, 95% CI 0.765–2.334, $P = 0.243$) and TT (OR = 1.655, 95% CI 0.875–3.128, $P = 0.121$) in comparison with GG had no significant effect on the risk of ALL (Table 2).

The results of the history of familial cancer and positive parents' smoking habits among cases and controls were significant; as a result, we evaluated to study the modulation of ALL risk with regard to *XRCC7* gene polymorphism in the multivariate logistic regression analysis. In addition, insignificant results were obtained for GT and TT genotype compared to those carrying the

Table 1 Frequency distributions of selected variables between the acute lymphoblastic leukemia cases and controls

Variables	Cases (n = 99) n (%)	Controls (n = 200) n (%)	OR (95% CI)	P value*
Gender				
Male	63 (63.6)	104 (52)	1.00	
Female	36 (36.4)	96 (48)	1.1 (0.21–0.75)	0.098
Parents' smoking habit				
No	51 (51.5)	120 (64.2)	1.00	
Yes	48 (48.5)	67 (35.8)	1.77 (1.68–2.94)	0.027
Family cancer history**†				
No	26 (57.8)	149 (78)	1.00	
Yes	19 (42.2)	42 (22)	2.608 (1.31–5.38)	0.006

*P associated with either Student's *t* test or chi-square test and Fisher's exact test

**Family history of acute lymphoblastic leukemia in the first-degree relatives

GG wild-type genotype after adjusting family's history of cancer (data are not shown). The results in Table 3 show the distribution of *XRCC7* G6721T polymorphism between the ALL and control groups among the positive parents' smoking habits. The reference group consisted of individuals with negative parents' smoking habit and GG genotype. The GT (OR = 1.637, 95% CI 0.83–3.229, *P* = 0.155) genotype had no effect on the risk of ALL; however, TT (OR = 1.996, 95% CI 1.033–3.858, *P* = 0.04) genotype had a significant effect on the risk of ALL, in comparison to the GG genotype.

‡ORs were adjusted for parents' smoking status use in a logistic regression model

Discussion

Cancer is a complex disease influenced by multiple genes, lifestyle factors as well as environmental carcinogens. It is known that some hereditary genetic defects result in an increased risk of cancer development. While deficiencies of DNA repair enzymes due to mutations in the genes lead to loss of repair enzymes, the polymorphisms of DNA repair genes may lead to a modulation of cancer susceptibility [4].

The association between ALL cancer risk and *XRCC7* G6721T polymorphism was not assessed to date. Therefore, we investigated this assumption in our hospital-based case-control study in Iranian population. Our

results demonstrated that the *XRCC7* G6721T polymorphism had no effect on increased risk of ALL. A few studies have analyzed the correlation of this polymorphism with the risk of other cancers [7–13]. The assumption was that dysfunction of human *XRCC7* might be included in cancer susceptibility. It seems to be possible, based on the function of the gene and by its product, although the investigated intronic *XRCC7* G6721T (rs.7003908) polymorphism seems to be controversial. Two studies suggested that *XRCC7* G6721T polymorphism would possibly contribute to cancer susceptibility for prostate and renal cell carcinoma [9, 12]. On the contrary, Nasiri et al. [10] study did not show any relationship between *XRCC7* G6721T polymorphism and breast cancer, which was in consistent with our finding.

However, our results showed that cigarette smoking habit of parents to be risk factor for ALL. There was an interaction between TT genotype of *XRCC7* G6721T polymorphism and positive parents' smoking habit with the ALL susceptibility. According to the role of the NHEJ pathway in DNA repair, it is a logical possibility that the *XRCC7* polymorphism may regulate the risk of cancer through environmental risk factors such as smoking habit [11]. Carcinogens would possibly enhance DSBs, leading to the genetic instability by increasing the rate of cancer development. It is well known that the

Table 2 Association between polymorphisms of *XRCC7* and risk of ALL

<i>XRCC7</i> Polymorphism	Controls	Cases	OR (95% CI)	P value
GG	54	19	1	
GT	67	34	1.49 (0.76–2.33)	0.24
TT	79	46	1.66 (0.87–3.13)	0.12

Table 3 The ALL cancer risk by positive parents' smoking habit and the *XRCC7* polymorphism

<i>XRCC7</i> polymorphism	Controls (n = 67)	Cases (n = 48)	OR (95% CI) ‡	P value
GG	27	11	1	
GT	22	30	1.64 (0.83–3.22)	0.15
TT	18	7	1.99 (1.03–3.86)	0.04

XRCC7 gene is responsible for repairing most DSBs [6]. Consequently, the variants of the *XRCC7* gene could be expected to have an effect on DSB repair pathway when people are exposed to carcinogens such as a tobacco smoke.

Conclusions

In summary, this is the first report to investigate the role of *XRCC7* polymorphism in susceptibility to childhood ALL. Interestingly, the TT genotype after being adjusted with positive parents' smoking habit was associated with ALL cancer risk. Therefore, the hospital-based study could improve the representative power by enlarging the sample size of the cases.

Abbreviations

ALL: Acute lymphoblastic leukemia; DSBs: Double-strand break; HR: Homologous recombination; NHEJ: Non-homologous end joining; RFLP: Restriction fragment length polymorphism; *XRCC7*: X-ray repair cross-complementing group 7

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Authors' contributions

F.F., M.R., B.G., and Z.B. conceived and planned the presented experiment. F.F. and Z.B. performed experiments and analyzed the data. Z.B. supervised the research, designed experiments, and wrote the paper. All authors have read and approved the manuscript.

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Ethics approval and consent to participate

Parents of patients or legal guardians provided a written informed consent form for participation in the study. The Institutional Review Board and Human Ethics Committee of Islamic Azad University of Kazerun approved this study (IR.IAU.KAU.REC.1398.182).

Consent for publication

Not applicable

Competing interests

Not applicable

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