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Original Article

DROSHA rs642321 Polymorphism Influence Susceptibility to Childhood Acute Lymphoblastic Leukemia: A Preliminary Report

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Abstract

Introduction: It has been well known that the microRNA biogenesis is involved in the pathogenesis of various diseases. We investigated the possible association between *DROSHA* rs642321 variant and risk of acute lymphocytic leukemia (ALL). **Materials and Methods:** We genotyped 75 children diagnosed with ALL and 115 age- and sex-matched children with no history of cancer of any type (as the control group) by the tetra amplification refractory mutation system-polymerase chain reaction. **Results:** We found that *DROSHA* rs642321 C > T variant significantly decreased the risk of ALL in codominant (TT vs. CC: odds ratio [OR] = 0.33, 95% confidence interval [CI] = 0.14–0.80, $P = 0.020$) and dominant (TT + CT vs. CC: OR = 0.51, 95% CI = 0.27–0.94, $P = 0.037$) inheritance model tested. The rs642321 T allele was associated with protective against ALL (OR = 0.58, 95% CI = 0.38–0.88, $P = 0.011$) in comparison with C allele. **Conclusion:** The study findings revealed that *DROSHA* rs642321 variant decreased the risk of pediatrics ALL in an Iranian population. Larger sample sizes with different ethnicities are needed to validate our findings.

Keywords: *Childhood acute lymphoblastic leukemia, DROSHA, polymorphism*

Introduction

Acute lymphocytic leukemia (ALL) is the most common malignancy in childhood, and it is the main cause of morbidity and mortality in childhood. Although the pathogenesis of the disease is not fully understood, it is known that genetic polymorphisms play a critical role in ALL development.[1,2]

MicroRNAs (miRNAs) are a class of single-stranded, small noncoding RNA molecules, which are approximately 22 nucleotides in length.[3] They are involved in regulation of gene expression through binding to the 3'untranslated region (3'UTR) of target mRNAs, resulting in mRNA degradation or translational inhibition. It is now clear that miRNAs contribute to most diverse biological processes such as differentiation, cell proliferation, and apoptosis.[4,5] Besides, miRNAs can function as either oncogenes or tumor suppressors and play an important role in the pathogenesis of cancer.[6,7]

Biosynthesis of miRNAs involves various miRNA machinery genes and occurs in multiple steps. RNA polymerase II produces primary miRNA (~500–3000 nucleotides), which is processed by a multiprotein complex that includes DROSHA to form a precursor miRNA hairpin (~60–100 nucleotides, pre-miRNA). Then, pre-miRNA transported to the cytoplasm by Ran GTPase which subsequently processed by DICER1 to generate a single strand mature miRNA.[8-11]

The roles of miRNAs and miRNA biosynthesis pathway genes have been reported in cancers.[12-17] Several studies have shown the association between genetic polymorphisms in the miRNAs biogenesis genes and risk of various cancers.[15,18-22]

In this study, for the first time, we evaluated the impact of *DROSHA* rs642321 polymorphism on risk of childhood ALL in a sample of Iranian population.

Materials and Methods

Patients

In this case–control study, we recruit 75 children diagnosed with ALL and 115 age- and sex-matched healthy children in a southeast Iranian population. The study design and the enrolment procedure have been described previously.[23,24] Ethical approvals for recruitment were obtained

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from Local Ethics Committee of Zahedan University of Medical Sciences, and informed consent was obtained from parents of patients and healthy individuals in accordance with our institutional guidelines. Peripheral blood sample was collected in ethylenediamine tetraacetic acid-containing tubes from patients and healthy controls and DNA were extracted using salting out method as described previously.[25]

Genotyping

We used tetra amplification refractory mutation system – polymerase chain reaction (PCR), which is a simple and rapid method for detection of single nucleotide polymorphisms (SNPs),[26-29] for genotyping *DROSHA* rs642321 polymorphism.

We used four primers, two outer primers (forward outer: 5′-AAAGCTATAAACCCTCCTCCTTCCTGC-3′, reverse outer: 5′-CCCAGATTGTGACCCTAGACTTTCAATT-3′) and two allele-specific internal primers (forward inner [T allele]: 5′-CCCCTTCTCCCATTAGCCAATTACTT-3′, reverse inner [C allele]: 5′-GTGTTTTGTAGAAGGAATCCGTTTACCG-3′).

In each 0.20 ml PCR reaction tube, 1 µl of genomic DNA (~100 ng/ml), 1 µl of each primer (10 µM), 10 µl of 2 × Prime Taq Premix (Genet Bio, Korea) and 5 µl ddH_2O were added.

Amplification was done with an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C with a final step at 72°C for 10 min. PCR products were verified on a 2.5% agarose gel contained 0.5 µg/ml ethidium bromide, and photographs were taken [Figure 1]. Product sizes were 245 bp for T allele, 294 bp for C allele, and 486 bp for the two outer primers (control band). To verify genotyping quality, all polymorphisms in random samples were re-genotyped.

Table 1: Genotypic and allelic frequency of *DROSHA* rs642321 polymorphism in childhood acute lymphocytic leukemia and control subjects

Genotypes	ALL, *n* (%)	Control, *n* (%)	OR (95% CI)	*P*
Codominant				
CC	30 (40.0)	29 (25.2)	1.00	-
CT	35 (46.7)	57 (49.6)	0.59 (0.31-1.15)	0.133
TT	10 (13.3)	29 (25.2)	0.33 (0.14-0.80)	0.020
Dominant				
CC	30 (40.0)	29 (25.2)	1.00	-
CT + TT	45 (60.0)	86 (74.8)	0.51 (0.27-0.94)	0.037
Recessive				
CC + CT	65 (86.7)	86 (74.8)	1.0	-
TT	10 (13.3)	29 (25.2)	0.46 (0.21-1.00)	0.065
Allele				
C	95 (63.3)	115 (50.0)	1.00	-
T	55 (36.7)	115 (50.0)	0.58 (0.38-0.88)	0.011

ALL – Acute lymphocytic leukemia; OR – Odds ratio; CI – Confidence interval

Statistical analysis

Statistical analyses were performed using the SPSS software for Windows, version 18.0 (SPSS Inc., Chicago IL, USA). Student's *t*-test and ANOVA was used to test for differences in continuous variables. The categorical data were analyzed using χ^2 test. The values of $P < 0.05$ were considered to be statistically significant. The Hardy–Weinberg equilibrium was determined using the χ^2 test in both cases and controls.

Results

In this study, we genotyped *DROSHA* rs642321 polymorphism in 75 ALL children and 115 healthy children. The findings demonstrated that *DROSHA* rs642321 C > T variant significantly decreased the risk of ALL in codominant (TT vs. CC: odds ratio [OR] = 0.33, 95% CI = 0.14–0.80, P =0.020) and dominant (TT + CT vs. CC: OR = 0.51, 95% CI = 0.27–0.94, P =0.037) inheritance model tested. The rs642321 T allele was associated with protective against ALL (OR = 0.58, 95% CI = 0.38–0.88, P = 0.011) in comparison with C allele [Table 1]. The *DROSHA* rs642321 genotypes in cases and controls were in HWE (χ^2 = 0.002, P = 0.966, and χ^2 = 0.008, P = 0.925, respectively).

We analyzed possible association between *DROSHA* rs642321 variant and patients' demographic and clinical data. As shown in Table 2, no association was found between ages of patients, white blood cell count, hemoglobin concentration, platelet count, sex, organomegaly, lymphadenopathy as well as cerebrospinal fluid involvement and rs642321 genotypes.

Discussion

In recent years, it has been revealed that miRNAs are

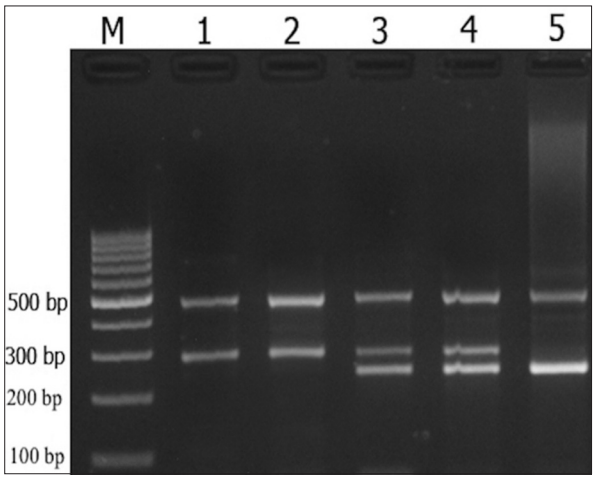

Figure 1: Agarose gel electrophoresis pattern of tetra amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) for detection of DROSHA rs642321 polymorphism. Product sizes were 245 bp for T allele, 294 bp for C allele, and 486 bp for the two outer primers. M: DNA marker; Lanes 1,2: rs642321 CC; Lanes 3,4: rs642321 CT; Lane 5: rs642321 TT

Table 2: Association of *DROSHA* rs642321gene polymorphism with demographic and clinical features of childhood acute lymphocytic leukemia patients

Factors	rs642321			*P*
	CC	CT	TT	
Sex				
Male	16	25	9	0.074
Female	14	10	1	
Age at diagnosis (year)	6.3±3.8	6.5±3.7	6.4±3.6	0.981
WBC ($\times10^3$/μL)	18.5±33.6	39.2±61.8	39.7±59.6	0.246
Hemoglobin (mg/dl)	7.4±2.7	8.0±2.1	7.8±2.1	0.596
Platelet ($\times10^3$/μL)	53.5±32.7	60.9±62.2	60.2±40.4	0.835
Organomegaly				
Positive	22	28	8	0.796
Negative	8	7	2	
Lymphadenopathy				
Positive	16	17	5	0.928
Negative	14	18	5	
CSF involvement				
Positive	1	1	1	0.579
Negative	29	34	9	

WBC – White blood cell; CSF – Cerebrospinal fluid

important components of complex gene regulatory networks. miRNA are a class of small noncoding RNA molecules involved in a variety of cellular functions.[5] SNPs in miRNA sequences, miRNA target genes as well as miRNA biogenesis may affect the miRNAs regulation.[17,18,30,31]

In this study, we aimed to find out the impact of *DROSHA* rs642321 polymorphism on childhood ALL. The study findings showed an association between *DROSHA* rs642321 and ALL. The *DROSHA* rs642321 TT as well as TT + CT genotype decreased the risk of ALL in comparison with CC genotype. The rs642321 T allele was significantly associated with protective against ALL. Gutierrez-Caminors *et al*.[31] have found that *DROSHA* rs10035440 variant increased the risk of pediatric ALL.

DROSHA is a member of RNase III superfamily and playing a pivotal role in the pathway of miRNAs biogenesis.[32,33] Sung *et al*.[20] evaluated the impact of rs644236, rs874332, rs7737174, rs10461898, rs11748548, and rs16901096 polymorphisms of *DROSHA* on breast cancer and found no significant association between these variants and breast cancer risk. They reported that *DROSHA* rs644236 TT genotype as well as rs7737174 AA genotype was associated with breast cancer risk in postmenopausal women.[20] Weng *et al*.[21] have found an association between *DROSHA* rs10719 variant and risk of malignant peripheral nerve sheath tumor.

Lin *et al*.[34] stated the haplotypes of DROSHA and DICER, but not individual variant, is associated with altered survival and recurrence of renal cell carcinoma. Yuan *et al*.[18] have found no significant association between *DROSHA* rs642321 polymorphism and bladder cancer risk, while rs10719T > C variant increased the risk of the disease in a Chinese population.

The aberrant expression of DROSHA at mRNA or protein level has been reported in numerous types of cancer, including skin cancer,[35] cervical cancer,[36] ovarian cancer,[15] breast cancer,[37] nonsmall-cell lung cancer,[38] and neuroblastoma.[39]

Conclusion

The study finding showed that rs642321 variant located in *DROSHA* 3'UTR decreased the risk of childhood ALL. The association and functional study are necessary to certify our result.

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Conflicts of interest

There are no conflicts of interest.

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