Thymidilate Synthase and Methionine Synthase Polymorphisms in Children with Acute Lymphoblastic Leukemia in Western Iran

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Abstract

Introduction: Polymorphism in genes involved in folate metabolism may cotribute to the susceptibility to acute lymphoblastic leukemia (ALL).
Patients and Methods: To examine the influence of polymorphism in thymidylate synthase (TS 28-bp repeat) and methionine synthase (MS A2756G) genes on the susceptibility to ALL, 73 children with ALL and 128 age and gender matched, unrelated healthy individuals from Kermanshah Province were studied. Detection of TS 28-bp repeat and MS A2756G polymorphisms were performed by PCR and PCR-RFLP, respectively.
Results: The frequency of TS 2R allele in patients and controls were 41.5 and 38%, respectively (OR 1.4, 95%CI 0.76-2.56, P=0.27). The allelic frequency of G allele of MS was higher (25%) in patients compared with healthy subjects (23%) [OR 1.04, 95%CI 0.58-1.87, P=0.8]. Considering MS AA and TS 3R3R genotypes as references indication where that individuals with MS GG+TS 2R2R genotypes have 1.3-fold increase risk of ALL (OR 1.3, 95%CI 0.6-2.7, P=0.5).
Conclusions: For the first time, our study has determined the frequency of polymorphism in two genes involved in the folate metabolism in a homogenous ethnic group of ALL patients. It seems that neither TS 28bp-repeat nor MS A2756G polymorphisms might be risk factors for susceptibility to ALL in western Iran.

Key words: ALL, Gene Polymorphism, MS A2756G, TS 28-bp Repeat, Western Iran

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Introduction

Acute lymphoblatic leukemia (ALL) is the most common malignancy of childhood, accounting for 30% of all cancers among children.(1) In developed countries, approximately 20% of ALL children either relapse or do not respond to treatment which might be related to the presence of polymorphisms having an effect on the activity of drug-metabolizing enzymes and the response to therapy.(1)

Folate is a key element in the one-carbon group metabolism that is required for DNA synthesis and methylation. Its deficiency has been associated with malignancies including the susceptibility to leukemia.(2) Thymidylate synthase (TS) converts dUMP to dTMP which is required for DNA synthesis. The most common polymorphisms in TS are a double (2R) or triple (3R) 28-base pair repeat sequence in the promoter enhancer region of the TS gene which influences protein expression in cancer cells.(2) The presence of a triple versus a double

28-bp repeat is associated with an increased gene expression and the conversion of dUMP to dTMP, decreasing uracil levels and, consequently, the prevention of the erroneous incorporation of uracil into DNA.(3) TS 3R allele is found in 38-54% of caucasian populations.(4)

There are inconsistent results related to the association of TS polymorphism and the risk of ALL in various populations.(3, 5-7)

Methionine synthase (MS) genes encode a vitamin B12-dependent enzyme that catalyzes the remethylation of homocysteine to methionine. A common polymorphism of MS A2756G induces modest homocysteine reduction and DNA hypomethylation.(3,8) There are controversial reports on the role of MS A2756G in ALL.(3, 9)

Kermanshah Province is located in Western Iran and the Kurds are the prominent ethnic group living in this area.(10)

The aim of the present study was to determine the frequency of two folate-related polymorphisms (MS A2756G, and TS28-base pair repeat) and their possible association with a susceptibility to ALL in ALL patients from Western Iran.

Materials and Methods

The patients were comprised of 73 children diagnosed with ALL, who had been referred to the hematologists of the clinics of Kermanshah University of Medical Sciences. The mean age of the patients was 8.23±4.03 years (1 to 16 years old) including 48 males and 25 females. They had been newly diagnosed with ALL, according to French-American-British classification. Cases had been selected from the files of patients who had received a hematological diagnosis from the hematology unit of the clinics of Kermanshah University of Medical Sciences during the period, June 2002 and September 2009. The Control subjects were 128 unrelated healthy individuals with a mean age of 8.58±6.4 years (1 to 20 years) consisting of 70 males and 58 females. The patients and control subjects had been matched by age and gender (P>0.05). Both groups were from the Kermanshah Province of Iran with a Kurdish ethnic background.

Informed written consent was obtained from each individual or their parents before participation. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences and was in accordance with the principles of the Declaration of Helsinki II.

DNA was isolated from the leukocytes of the EDTA treated, whole blood by using the proteinase K treatment followed by a phenol-chloroform extraction and ethanol precipitation.(11)

Genotype Analysis

Thymidylate Synthase: For detection of the tandem repeat sequences in the 5´-terminal of the regulatory region of the TS gene, a polymerase chain reaction, using the forward primer of 5´ GTG GCT CCT GCG TTT CCC CC 3´ and the reverse primer of 5´ CCA AGC TTG GCT CCG AGC CGG CCA CAG GCA TGG CGC GG 3´ was performed, as described by Skibola, et al.(3) The presence of a homozygotes double repeat (2R2R) produced a 220-bp band. Heterozygotes (2R3R) produced two bands of 220-bp and 250-bp. In homozygotes for a triple repeat (3R3R), only a fragment with 250-bp was observed.

Methionine Synthase A2756G: Identification of MS A2756G polymorphism was indicated according to the procedure previously described by Gemmati, et al(8) using a forward primer of 5ˈ TGT TCC AGA CAG TTA GAT GAA AAT C 3´and a reverse primer of 5´ GAT CCA AAG CCT TTT ACA CTC CTC 3´. The 211-bp PCR product (10-15 µl) was digested with 5 units of the restriction enzyme Hae III at 37°C overnight. The A to G substitution in the 211- bp fragment creates a Hae III recognition sequence which digests the 211- bp fragment into 131 and 80-bp fragments.

Statistics

The allelic frequencies were calculated by the chromosome counting method. The distribution of the genotype frequencies in both groups did not deviate from the Hardy-Weinberg expectation. The significance of the difference of observed alleles and genotypes between the groups was tested using the Chi-square analysis. Data on quantitative characteristics are expressed as means ± standard deviations. The Odds ratios (OR) were calculated as estimates of relative risk for disease and 95% confidence intervals which were obtained by SPSS logistic regression. A two-tailed student's t test analysis was used to compare quantitative data. Statistical significance was assumed at the P<0.05 level. The SPSS statistical software package version 11.5 was used for the statistical analysis.

Results

The prevalence and allele frequency of two TS and MS genes are shown in Table- 1. Seventy three patients and 128 healthy individuals were evaluated for MS polymorphism. The wild type genotype (AA) was present in 42 patients (57.5%), a

Table- 1. The frequency of MS and TS genotypes and alleles in ALL patients and controls

Genotype	Patients	Controls	P Value
Methionine synthase, n (%)	73 (100)	128 (100)	
GG	5 (6.8)	6 (4.7)	
AG	26 (35.6)	47 (36.7)	
AA	42 (57.5)	75 (58.5)	>0.05
G	36 (25)	59 (23)	
Thymidylate synthase, n (%)	71 (100)	109 (100)	
3R3R	28 (39.1)	52 (47.7)	
2R3R	27 (38)	30 (27.5)	>0.05
2R2R	16 (22.5)	27 (24.8)	
2R	59 (41.5)	84 (38)	

heterozygous genotype (AG) in 26 patients (35.6%) and a homozygous genotype (GG) in 5 patients (6.8%). In the healthy individual group, there were 75 subjects (58.5%) with an AA genotype, 47 individuals (36.7%) with an AG genotype and 6 individuals (4.7%) with GG genotype. The allelic frequency of G allele was not significantly higher (25%) in patients compared to the healthy subjects (23%) [OR 1.04, 95%CI 0.58-1.87, P=0.8]. TS 28-bp repeat 3R3R was found in 28 out of 71 patients in the study (39.1%), 2R3R in 27 patients (38%) and 2R2R in 16 patients (22.5%), respectively. The frequencies of 3R3R, 2R3R and 2R2R genotypes in 109 healthy individuals were 47.7, 27.5, and 24.8%, respectively. The allelic frequency of 2R allele in patients and controls were 41.5 and 38%, respectively (Table- 1). The 2R allele did not significantly affect the risk of ALL in the study population (OR 1.4, 95%CI 0.76-2.56, P=0.27). Considering MS AA and TS 3R3R genotypes as reference indicated that individuals with MS GG+TS 2R2R genotypes have a 1.3-fold increase in the risk of ALL (OR 1.3, 95%CI 0.6-2.7), although it was not statistically significant (P=0.5).

Discussion

ALL is the most common form of leukemia among children, with a long-term event-free survival rate nearly 80%.(12) The study of Karimi, et al, reported a five-year survival rate of 72.5% in ALL children from Iran as well as an association between white blood cell counts above 50,000/ml and a poor prognostic outcome.(12) In the province of Kermanshah, the study of cancer epidemiology in children indicates a high prevalence of leukemia (49.2%) in which, ALL had the highest frequency (38.2%).(13) There are inconsistent results from numerous studies about the association between genes involved in folate metabolism and susceptibility to ALL. The role of an individual's ethnic background in the various distributions of polymorphisms of genes involved in folate and methionine metabolism has been suggested.(14,15) The prevalence of TS 3R3R has been reported to be 33.1% in Caucasian children with ALL, while in Indonesian samples, its frequency reached 76.1% in ALL children.(15) The frequency of TS genotypes in the Indonesian ALL patients was not significantly different from the control.(15) In the study of Skibola, et al(3), individuals with TS 2R3R demonstrated a 2.8-fold reduction and those with TS 3R3R indicated a 4.0-fold reduction in ALL risk. In contrast, in western European pediatric ALL patients, a protective effect of the TS 2R variant was reported.(7) In the study of Lauten, et al,(5) in Canada, 40 ALL patients compared to 40 controls, the frequency of TS genotypes was not significantly different. Also, in a large number of ALL patients from the United Kingdom Childhood Cancer study, the frequency of TS 28-bp repeat was not significantly different compared to controls.(16) In the present study, the frequency of 2R allele was not significantly higher in All patients compared to the control group and the risk of ALL increased 1.4-fold. An interaction between TS 28-bp repeat polymorphism and folate intake has been reported with a decreased risk of ALL in the 3R3R genotype in combination with high folate intake.(17)

Petra et al,(6) in Slovenia, reported that both TS and MS polymorphisms had no significant effect regarding ALL susceptibility.

A common polymorphism, MS A2756G, might affect enzymatic activity(18) and to induce modest homocysteine reduction(19) and DNA hypermethylation which is significant in acute leukemia.(16) Few studies have investigated its influence with repect to the risk of lymphoid malignancies. Lightfoot, et al,(16) concerning ALL patients participating in the United Kingdom Childhood Cancer study, observed that MS A2756G polymorphism was associated with a significantly greater risk of ALL (1.88-fold). Matsuo, et al,(9) indicated that MS A2756G polymorphism may be considered a risk factor for malignant lymphoma. Also, De Jong, et al,(7) indicated that MS 2756G was a risk allele on ALL in itself, although in combination with serine hydroxymethyltransferase (SHMT1) 1420CT/TT, it caused a 5.6-fold reduction in ALL risk. However, Skibola, et al,(3) reported a protective effect of MS A2756G in combination with SHMT1 C1420T polymorphism on ALL. Further, Gemmati, et al,(8) found a 5.0-fold decreased ALL risk in individuals bearing MS 2756GG. We found no association between MS A2756G polymorphism and a

susceptibility to ALL, although the frequency of G allele tended to be higher in patients compared with controls. However, MS GG, in combination with TS 2R2R, caused a 1.3-fold increase in ALL risk which was not statistically significant. The reasons for different results obtained from various studies are unclear and might be attributed to differences in ethnic backgrounds and the selection of the population studied, differences in sample sizes, and gene-environment interactions, such as diet, chemical exposure, or nutritional intake of folate and related vitamins. Due to limited studies reporting the influence of gene polymorphism involved in folate metabolism on ALL, more investigations is necessary to find a clear relationship of these genes to susceptibility to ALL.(20)

In summary, our study has shown the frequency of two genes involved in folate metabolism with respect to ALL patients within a homogenous ethnic group. It seems that neither TS 28bp-repeat nor MS A2756G polymorphisms are risk factors for a susceptibility to ALL in western Iran. Owing to historical, cultural, religious and linguistic differences, Iranians show wide genetic diversities. Additional analysis with a larger number of individuals is thus needed to clarify the real contribution of these two variants regarding the risk of ALL in different world populations.

Conflict of interest

The authors report no conflict of interest.

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