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The methylenetetrahydrofolate reductase *677T-1298C* haplotype is a risk factor for acute lymphoblastic leukemia in children

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Abstract

The etiology of acute lymphoblastic leukemia (ALL) is complex, linked with both environmental exposures and genetic factors. Functional variants of the methylenetetrahydrofolate reductase (*MTHFR*) gene result in disturbance in folate metabolism and may affect susceptibility to cancer. The study was performed to evaluate whether *MTHFR C677T* and *A1298C* polymorphisms, analyzed separately and together, are associated with the development of ALL in a population under 18 years of age of Caucasian ancestry.

The study included 117 pediatric patients (59% males, mean age at diagnosis 7.4 ± 5.2 years) with ALL, confirmed by conventional immunophenotyping surface-marker analysis and 404 healthy control subjects (48.5% men, mean age 37.7 ± 11.3 years). The *MTHFR C677T* and *A1298C* genotypes were analyzed using allele discrimination tests with Taq-Man fluorescent probes.

The *MTHFR 677TT* genotype was related to a 2-fold increase in risk of ALL ($P = .014$). The *677T-1298C* haplotype was found in ALL patients but not in controls (frequency 0.598%; $P < .0001$). The observed frequency of carriers of this rare haplotype was 12%, including *677CT/1298CC* (1.7%), *677TT/1298AC* (6.0%), and *677CT/1298AC* (4.3%) genotypes.

The *MTHFR 677T* allele alone or in combination with the *MTHFR 1298C* allele significantly increases the risk of development of ALL in Polish population under 18 years of age. Further studies of haplotype composition in subjects with the *677CT/1298AC* genotype are necessary to assess the risk of childhood ALL.

Abbreviations: 5,10-MTHF = 5,10-methyltetrahydrofolate, ALL = acute lymphoblastic leukemia, CI = 95% confidence interval, FAD = flavin adenine dinucleotide, GWAS = genome-wide association study, HWE = Hardy-Weinberg equilibrium, MTHFR = methylenetetrahydrofolate reductase, OR = odds ratio, P = probability, SAM = S-adenosylmethionine.

Keywords: acute lymphoblastic leukemia, ALL risk, MTHFR polymorphism

1. Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous group of neoplasms which develop from rapidly proliferating malignant T or B lymphoid cells. It is the most frequently diagnosed malignancy in children and represents about 30% of all cancers diagnosed in this age group.[1] Peak incidence occurs between 2 and 5 years of age.[2]

The etiology of ALL is complex and not fully understood. It has been linked with both environmental exposures (including ionizing radiation, hydrocarbons, pesticides, and alcohol and cigarette use) and inherited genetic disorders (including Fanconi anemia, Down, and Bloom syndrome). However, these determinants are responsible for <10% of all ALL cases.[3] In children, the most important factors are thought to be interactions between genetic factors and fetal environmental exposures. A genome-wide association study has revealed a few susceptibility loci for childhood ALL, such as 9p21.3 (*CDKN2A*), 7p12.2 (*IKZF1*), 10q21.2 (*ARIDB5*), and 14q11.2 (*CEBPE*).[4,5] However, knowledge of the genetic factors involved in ALL development is still limited, and needs to be expanded.

Methylenetetrahydrofolate reductase (MTHFR, EC 1.5.1.20) is an enzyme which plays a crucial role in folate metabolism. It is responsible for the irreversible conversion of 5,10-methyltetrahydrofolate (5,10-MTHF) into 5-MTHF, which is a cosubstrate for homocysteine to methionine remethylation.[6] The above-mentioned compounds are important for many cellular processes, such as DNA and RNA synthesis, DNA methylation and repair, and cysteine metabolism.[7] Thus, genetic variations in the gene encoding MTHFR may influence enzyme activity and, ultimately, affect susceptibility to cancerogenesis.[8] There are 2 common functional *MTHFR* gene (ENSG00000177000) polymorphisms, *C677T* (rs1801133) and *A1298C* (rs1801131), which result in

Editor: Yuxuan Liu.

EMK and ES contributed equally to this work.

The authors have no conflicts of interest to disclose.

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Medicine (2017) 96:51(e9290)

Received: 8 December 2016 / Received in final form: 19 September 2017 / Accepted: 22 November 2017

http://dx.doi.org/10.1097/MD.0000000000009290

decreased enzyme activity. Literature data on the relationship between these genetic variants and ALL development are conflicting. In addition, there are few studies considering the distribution of *MTHFR 677/1298* genotypes and the *677-1298* haplotype in ALL patients. Therefore, the aim of this study was to evaluate whether *MTHFR C677T* and *A1298C* polymorphisms, analyzed separately and together, are associated with the development of ALL in children.

2. Materials and methods

2.1. Subjects

The study included 117 ALL pediatric patients: 48 females (41%) and 69 males (59%) of Caucasian ancestry, treated in the Department of Pediatric Oncology, Hematology and Bone Marrow Transplantation at the Poznan University of Medical Sciences, Poland. The mean age was 10.4 ± 5.5 years (range: 1–23 years), and mean age at diagnosis was 7.4 ± 5.2 years (range: 0.3–18 years). The ALL diagnosis was made according to the French–American–British criteria, after conventional immunophenotyping surface-marker analysis. Inclusion criteria for the study group were diagnosis at under 18 years of age and absence of other malignancy.

The control group consisted of 404 healthy subjects without ALL: 208 females (51.5%) and 196 males (48.5%) with a mean age of 37.7 ± 11.3 years (age range 19–75 years), selected from an area of northwest Poland (the same part of Poland as for the selection of the ALL patients) in the years 2004 to 2012. Of this group, 95 subjects were selected randomly during the national health program for screening cardiovascular risk factors, and the rest were randomly selected couples whose children were born in hospitals in the Poznan district.

2.2. Ethics statement

The study was performed in accordance with the World Medical Association Declaration of Helsinki and approved by the Bioethics Committee of the Poznan University of Medical Sciences (resolution no. 1430/04, 1140/05, 1502/05, and 595/13).

2.3. Methods

Details of *MTHFR C677T* and *A1298C* genotyping have been described previously.[9] Briefly, DNA was extracted from 5 million cells after lysis of peripheral whole blood, using a DNA extraction kit (DNA Extraction Kit, Stratagene) in accordance with the manufacturer's instructions. Next, the variants studied were identified using commercially available tests: *MTHFR C677T* and *MTHFR A1298C* kit (GeneProof) with fluorophore-labeled probes (Taq-Man hydrolysis probes).

2.4. Statistical analyses

Allele frequencies among patients were compared using the χ^2 test and Fisher exact test (GraphPad Software Inc, San Diego, CA, version 5.03). For risk assessment conferred by a particular variant, the values of the odds ratio (OR) were estimated with 95% confidence intervals (CI). Genotype data were analyzed under dominant, recessive, and additive models to maximize the power of detecting an association. Genotype frequencies were tested for Hardy–Weinberg equilibrium (HWE) using the χ^2 test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl), while linkage disequilibrium between the *MTHFR* variants and haplotype frequencies was tested using Haploview software.[10] The standardized linkage disequilibrium coefficient D' (Lewontin's D') was calculated. The differences were considered significant if the value of probability (P) did not exceed .05.

3. Results

To assess the association between *MTHFR C677T* and *A1298C* polymorphisms and ALL development, the frequencies of genotypes, alleles, and haplotypes from pediatric ALL patients (N = 117) and the control group (N = 404) were compared.

In ALL patients, the observed genotype frequencies for *MTHFR C677T* differed significantly from frequencies calculated for HWE ($P = .006$). Six *MTHFR 677/1298* genotype combinations (*CC/AA*, *CC/AC*, *CC/CC*, *CT/AA*, *CT/AC*, and *TT/AA*) were present in the control group, whereas 8 were present in ALL patients, including additional genotypes of carriers of both variant alleles (*CT/CC* and *TT/AC*; Table 1). The

Table 1

Frequency of *MTHFR 677C>T* and *1298A>C* polymorphisms in the pediatric ALL patients (N = 117) and in the control group (N = 404).

MTHFR polymorphisms	Genotype	ALL patients N = 117	Control group N = 404	Univariate analysis, P
C677T rs1801133	*CC*	54 (46.2)	178 (44.1)	*677TT, P* = .0115*
	CT	41 (35.0)	185 (45.8)	
	TT	22 (18.8)	41 (10.1)	
	P_{HWE}	0.006	0.484	
A1298C rs1801131	*AA*	47 (40.2)	172 (42.6)	NS
	AC	55 (47.0)	194 (48.0)	
	CC	15 (12.8)	38 (9.4)	
	P_{HWE}	0.969	0.112	
C677T/A1298C	*CC/AA*	20 (17.1)	55 (13.6)	*677TT/1298AC, P* < .0001
	CC/AC	21 (17.9)	85 (21.0)	
	CC/CC	13 (11.1)	38 (9.4)	
	CT/AA	12 (10.3)	76 (18.8)	
rs1801133/rs1801131	*CT/AC*	27 (23.1)	109 (27.0)	
	CT/CC	2 (1.7)	0 (0.0)	*677CT/1298CC + 677TT/1298AC, P* < .0001
	TT/AA	15 (12.8)	41 (10.1)	
	TT/AC	7 (6.0)	0 (0.0)	

Variables are expressed as numbers (percentages).

ALL = acute lymphoblastic leukemia, HWE = Hardy–Weinberg equilibrium, MTHFR = methylenetetrahydrofolate reductase, NS = not statistically significant, P = probability value.

* OR = 2.1, 95%CI: 1.2–3.6.

Table 2

***MTHFR C677T* and *A1298C* allele and haplotype frequency in the pediatric ALL patients (N = 117) and in the control group (N = 404).**

MTHFR polymorphisms	Allele or haplotype	ALL patients N = 117	Control group N = 404
C677T rs1801133	*C*	149 (63.7)	541 (67.0)
	T	85 (36.3)	267 (33.0)
A1298C rs1801131	*A*	149 (63.7)	538 (66.6)
	C	85 (36.3)	270 (33.4)
C677T-A1298C	*C-A*	78 (33.3)	271 (33.5)
	C-C	71 (30.3)	270 (33.4)
rs1801133/rs1801131	*T-A*	71 (30.3)	267 (33.0)
	T-C	**14 (5.9)**	**0 (0.0*)**

Variables are expressed as numbers (percentages).
ALL = acute lymphoblastic leukemia, MTHFR = methylenetetrahydrofolate reductase.
* $P < .0001$.

complete linkage disequilibrium between the studied *MTHFR* variants ($D' = 1.0$) was observed only in the control group, in which three *677–1298* haplotypes (*C-A, C-C,* and *T-A*) were found (Table 2). On the other hand, in ALL patients, the value of *D'* for the *MTHFR* variants was <1 (0.59), which suggests that crossing over between these variants occurs in the Polish population. However, the corresponding values of r^2, which is another measure of linkage disequilibrium, were relatively low in both cases ($r^2 = 0.100$), and controls ($r^2 = 0.210$), that corresponds with values observed in the CEU population in the HapMap/1000 genomes sample ($D' = 0.999$, $r^2 = 0.207$). It was estimated that four *677–1298* haplotypes are present in the patients studied, including the rare *T-C* haplotype (frequency 5.7%; Table 2). Altogether in the ALL group, 14 carriers of the *T-C* haplotype were found: 2 patients with the *CT/CC* genotype, 7 patients with the *TT/AC* genotype, and 5 out of 27 patients with the *CT/AC* genotype.

When the variants studied were analyzed separately, it was found that the *677TT* genotype is associated with a 2-fold higher risk of ALL development compared to the *677CC* and *677CT* genotypes (recessive model, $P = .0115$; Table 1). However, the frequency of the *677T* allele did not differ significantly between the groups studied. With regard to the *A1298C* polymorphism, no differences in allele and genotype frequencies between ALL patients and control subjects were found (Table 1). In the analysis of *677/1298* genotype combinations, the presence of the *TT/AC* genotype, as well as one of the rare genotype combinations *CT/CC* or *TT/AC,* was significantly associated with the development of pediatric ALL (*CT/CC+TT/AC,* $P < .0001$), whereas the *CT/CC* genotype alone was associated with ALL at the level of tendency ($P = .050$). Observed frequencies of the 3 major *MTHFR 677–1298* haplotypes (*C-A, C-C, T-A*) were similar in ALL patients and controls (Table 2), while the difference in the distribution of the rare *T-C* haplotype between patients and controls was statistically significant ($P < .0001$).

4. Discussion

The most important result of the present study is the finding that the *MTHFR 677T* allele, alone or in combination with the *MTHFR 1298C* allele, significantly increases the risk of ALL development in a population under 18 years of age. Interestingly, the highest risk was related to the presence of the rare *677T-1298C* haplotype, which carries both variant alleles. This observation is novel in the context of the pediatric ALL development literature.

Despite many studies on the etiology of ALL, the causal mechanism underlying disease development is still unspecified. Recently, there has been growing interest in the significance of folate metabolism in modifying the risk of cancerogenesis. Much attention has been paid to the enzymes involved in the folate pathway, including MTHFR. It is known that genetic variance in *MTHFR* may affect enzyme activity and, in consequence, disturb folate metabolism, DNA synthesis, and methylation. These disturbances may lead to increased cancer susceptibility.[11]

The gene encoding MTHFR is located on the short arm of chromosome 1 (1p36.3), consists of 11 exons, and comprises a region of >20,000 bp.[12] There are 2 common functional *MTHFR* polymorphisms, *C677T* and *A1298C*, which influence enzyme activity. *C>T* transition occurs at nucleotide *677* in exon 4 of the *MTHFR* gene, where the flavin adenine dinucleotide (FAD)-binding site is located. It causes an alanine to valine amino acid substitution (Ala222Val), which results in impaired FAD binding, enzyme thermolability, and decreased activity.[13–15] It has been shown that *677TT* homozygotes and *677CT* heterozygotes have about 70% and 40% reduced MTHFR activity, respectively.[13,16] Moreover, the *677TT* genotype has been found to be associated with a lower level of genomic DNA methylation.[17] In turn, *A>C* transition at nucleotide *1298* occurs in exon 7 within the S-adenosylmethionine-binding domain and results in the substitution of glutamic acid for alanine (Glu429Ala).[18] *1298CC* homozygotes have about 20% reduced MTHFR activity. The exact mechanism underlying the influence of *A1298C* on MTHFR activity and the functional consequences have not been fully elucidated. Some studies show no association with enzyme thermolability or homocysteine concentration.[19,20] However, it was shown that coexistence of the 2 variant alleles, that is, *677T* and *1298C,* results in about 60% to 70% reduction of MTHFR activity, which is comparable to the effect observed in the *677TT* homozygotes.[21]

MTHFR C677T and *A1298C* polymorphisms have been studied extensively in the context of many human diseases and conditions, such as: lung cancer,[22] cervical cancer,[23] rheumatoid arthritis,[24] myocardial infarction,[25] migraine,[26] and recurrent pregnancy loss.[27] There are also studies on the association between the occurrence of *MTHFR* polymorphisms and ALL development in adults and children in different regions and subpopulations. However, data derived from these studies are inconsistent, and very often even conflicting,[6,28–31] which makes the significance of the *MTHFR* gene in the risk of childhood ALL unclear. Moreover, in some studies, only one variant is analyzed, prevalently, the *C677T* polymorphism, or, despite both polymorphisms being genotyped, they are analyzed separately.

In our study, we assessed *MTHFR C677T* and *A1298C* genotypes, allele, and haplotype frequencies. The minor allele frequencies observed in the groups studied were comparable with those for a European subpopulation, which, according to the e! EnsemblGenomes 32 database (released August 4, 2016), were 0.36 for the *677T* allele and 0.31 for the *1298C* allele. In our study, the respective values were 0.36 and 0.33, and did not differ significantly between the ALL group and the control group. In an analysis that takes into account the effects of a single variant, we found that the *677TT* genotype was significantly associated with a 2-fold higher risk of ALL development (a recessive effect of the *677T* allele, $P = .014$). The separate effect of the *A1298C* variant was not statistically significant; however, in combination with the *677T* allele (*677T-1298C* haplotype), it had a significant influence on ALL risk. Moreover, the association of the *677T-1298C*

haplotype with disease development was much stronger ($P < .0001$) than was observed for the *677T* allele alone.

In a meta-analysis Li et al[32] investigated the association between *MTHFR C677T* and ALL risk in children of Caucasian ancestry, and did not find a significant association in any of the five models analyzed (allele contrast, additive, recessive, dominant, and effects of heterozygous genotype).[32] Amigou et al[29] also found no association between both *C677T* and *A1298C* and ALL risk when analyzing a group of 648 children with ALL and 1681 healthy controls. In turn, Lightfoot et al,[6] analyzing these variants in 939 children with ALL and 824 controls in the United Kingdom, did not observe a significant association for *C677T*, but observed that the *1298C* variant is associated with slightly lower risk of ALL (OR = 0.79, $P = .02$). On the other hand, De Jonge et al[33] and Chatzidakis and Goulas[34] observed a protective effect of allele *677T* in regard to ALL in children, with OR = 0.7 and OR = 0.4, respectively. De Jonge et al[33] also analyzed *A1298C* but did not find a significant difference between groups.

One explanation of the inconsistency between the results of various researchers may be the different levels of folates in the populations studied, as well as differences in the selection of patients. It was demonstrated that the association between *MTHFR* polymorphisms and ALL risk in children can be modified by supplementation of folic acid before as well as during pregnancy. Krajinovic et al[35] analyzed *MTHFR* variants in a group of 270 ALL children and 300 healthy controls of French–Canadian origin. The group of ALL patients consisted of those born before as well as after the introduction of mandatory maternal folate supplementation during pregnancy. Only in children born before the introduction of folate supplementation was the protective effect of the *677T* and *1298C* allele found. The authors suggest that the effect of *MTHFR* polymorphisms is less relevant when the folate level is adequate.[35]

As mentioned above, the carriers of the variant alleles of both the *MTHFR* polymorphisms analyzed have decreased enzyme activity up to 30% to 40%. Therefore, it seems reasonable to analyze both variants and genotype combinations. In our study, we observed 8 different *677/1298* genotypes, including 2 which were found only in ALL patients and not in the controls: *677CT/1298CC* and *677TT/1298AC*. Similarly, an additional *677T-1298C* haplotype was only found in the ALL group, and did not occur in healthy subjects. This suggests that the frequency of occurrence of crossing over during the formation of gametes in parents of children suffering from ALL might be higher than observed in the normal population. The inheritance of rare disadvantaging DNA variants resulting from crossing over in leukemia predisposing pathways that could affect overall genomic instability was previously found in the case of Fanconi anemia genes.[36] In contrast, there was no difference in the distribution between the groups studied for other common *MTHFR 677/1298* genotypes.

A study by Goricar et al[31] found a relationship between the *677T-1298A* haplotype and a decreased risk of ALL in pediatric patients (OR = 0.65, $P = .03$). The authors reported only three *677–1298* haplotypes in the groups studied, *C-A*, *C-C*, and *T-A*, and their frequencies are consistent with our results. To the best of our knowledge, most studies on *MTHFR* polymorphisms in childhood ALL do not analyze haplotypes. If haplotypes are analyzed, only the 3 mentioned above are found. However, based on literature data on *MTHFR 677/1298* distribution, we noticed that genotypes *CT/AC, CT/CC*, and *TT/AC,* as well as the *T-C* haplotype were found in different diseases and conditions with frequencies similar to those observed in our ALL patients. Dekou et al[37] observed a *CT/AC* genotype in 19% of a British population sample (23.1% in our ALL patients), *CT/CC* in 1.4% (1.7% in our ALL patients), and *TT/AC* in 1.8% (6.0% in our ALL patients). Isotalo and Donnelly[38] found the *CT/AC* genotype in 32% of patients with venous thrombosis and 53% of healthy volunteers, and *TT/AC* in 9.2% patients and 13% in controls. The higher frequency of the *677T/1298C* haplotype in these populations may be a result of the founder effect. Data collected by Ogino and Wilson[39] revealed that the genotype *TT/CC* has also been found in some studies. *MTHFR 677/1298* genotype frequencies estimated by the authors in the general population are 0.21% for *CT/CC* and 0.22% for *TT/AC*.[39]

This study has some limitations. The first is the ALL sample size. However, because the main results are based on genotypes that were not observed in the controls, the sample was large enough to obtain highly significant results. Moreover, this study includes a relatively large control sample. A second limitation was that we could not distinguish haplotypes in the compound heterozygote *677CT/1298AC*.

Based on the results obtained, it can be summarized that in the Polish pediatric population, carriers of the *MTHFR 677T-1298C* haplotype (i.e. subjects with *677CT/1298CC* and *677TT/1298AC* genotypes) have a 2-fold higher risk of ALL development. As carriers of *677T-1298C* were not present in the control group, and homozygous carriers of the *677T* allele were also observed in >10% of the control group, it might be suggested that both variants should be analyzed in order to estimate the risk of ALL in pediatric patients. As these variants are functional, they may be considered as causal risk factors of ALL. To confirm our finding, additional studies with larger sample sizes are necessary. Further studies of the composition of the haplotype in compound *677CT/1298AC* heterozygotes are crucial to ALL risk assessment. Moreover, there is also a need for further studies on the biological role of both polymorphisms in ALL patients and their parents in order to determine their influence on the pathomechanisms of ALL development.

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