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Methylenetetrahydrofolate reductase (MTHFR) C677T and thymidylate synthase promoter (TSER) polymorphisms in Indonesian children with and without leukemia

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Abstract

Genetic variations in the polymorphic tandem repeat sequence of the enhancer region of the *thymidylate synthase* promoter (*TSER*), as well as in *methylenetetrahydrofolate reductase* (*MTHFR*) *C677T* polymorphism, influence methotrexate sensitivity. We studied these polymorphisms in children with acute lymphoblastic leukaemia (ALL) and in subjects without malignancy in Indonesia and Holland.

The frequencies of *TT* and *CT* genotypes were two-fold higher in Dutch children. The *TSER 3R/3R* repeat was three-fold more frequent in the Indonesian children, while the *2R/2R* repeat was only 1% compared to 21% in the Dutch children. No differences of these polymorphisms were found between ALL cells and normal blood cells, indicating an ethnic rather than leukemic origin. These results may have implications for treatment of Indonesian children with ALL.

Keywords: Acute lymphoblastoid leukemia; Paediatric patients; Methotrexate; MTHFR C677T; TSER 2R/3R; Indonesia

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy that affects children, representing nearly one-third of all paediatric cancers. Annual incidence of ALL is about 30 cases per million people, with a peak incidence in patients aged 2–5 years [1]. The optimisation of the use of antileukemic agents that were developed from the 1950s through the 1980s, has resulted in a substantial reduction in death rates from ALL, particularly in children [2]. An important drug in the treatment of ALL is methotrexate (MTX), which inhibits several enzymes involved in folate homeostasis. MTX is an inhibitor of dihydrofolate reductase and decreases intracellular levels of reduced folates,

Abbreviations: ALL, acute lymphoblastic leukemia; CH_2-THF, 5,10-methylenetetrahydrofolate; DDH, double distilled water; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine monophosphate; dTTP, deoxythymidine triphosphate; dUMP, deoxyuridine monophosphate; FdUMP, fluoro-dUMP; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; MTRR, methionine synthase reductase; MTX, methotrexate; PCR, polymerase chain reaction; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SHMT, serine-hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase; TSER, tandem repeat sequence of the enhancer region of the *TYMS* promoter

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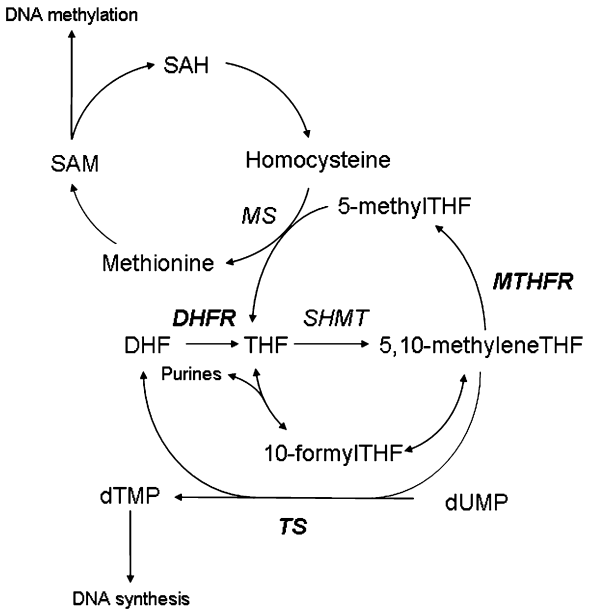

Fig. 1. Overview of the human folate metabolic pathway. *S*-adenosylmethionine (SAM); *S*-adenosylhomocysteine (SAH); dihydrofolate (DHF), dihydrofolate reductase (DHFR); tetrahydrofolate (THF); serine hydroxymethyltransferase (SHMT); 5,10-methylenetetrahydrofolate (5,10-methyleneTHF); 5,10-methylenetetrahydrofolate reductase (MTHFR); 5-methyltetrahydrofolate (5-methylTHF); 10-formyltetrahydrofolate (10-formylTHF); methionine synthase (MS); thymidylate synthase (TS); deoxythymidine monophosphate (dTMP); deoxyuridine monophosphate (dUMP); fluoro-dUMP (FdUMP).

such as 5,10-methylenetetrahydrofolate (CH_2-THF), which is required for DNA synthesis and for maintaining the balance of the deoxynucleotide pool [3]. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-CH_2-THF to 5-CH_3-THF, the predominant circulatory form of folate and carbon donor for the remethylation of homocysteine to methionine (Fig. 1). *MTHFR* is located on the short (p) arm of chromosome 1, at position 36.3 (1p36.3). Up to 12–15% of Caucasian individuals are homozygous for a C → T polymorphism located at nucleotide 677 (referred to as the TT genotype). The resulting substitution of alanine to valine increases thermolability and reduces the activity of MTHFR [4]. Accumulation of 5,10-CH_2-THF resulting from the *MTHFR C677T* polymorphism may have an effect on the response to MTX, and recent studies have suggested that the TT genotype may be associated with an increased toxicity of MTX in leukemia patients [5,6]. The risk of cardiovascular disease, neural tube defects and several cancers, including ALL, may also be increased, although the evidence to support these relationships is controversial [4,7,8].

Thymidylate synthase (TS) is a key-enzyme in *de novo* DNA synthesis, which catalyses the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), and is another critical target for MTX [3]. Inhibition of this enzyme results in deoxythymidine triphosphate (dTTP) depletion, chromosome breaks and cell death [9]. The *TYMS* gene is located on chromosome 18p11.32 and has a unique tandem repeat sequence in the enhancer region (*TSER*) that has been shown to be polymorphic [10], containing either two (*2R*) or three (*3R*) 28-bp repeats. The presence of the *3R* versus *2R* was shown to influence gene expression *in vitro* and *in vivo* [10], and in childhood ALL, homozygosity for the *3R*, was reported to be associated with poorer outcome compared to the presence of at least one *2R* allele [10].

The prevalence of the *MTHFR C677T* and *TSER* polymorphisms may be different among various populations and may affect the efficacy of MTX, a chemotherapeutic agent that is extensively used for the treatment of paediatric ALL. Therefore, identification of these polymorphisms may be an important pharmacogenetic determinant to predict response or toxicity to chemotherapy in children affected by leukaemia. Ethnic variations in the frequency of both the *C677T MTHFR* and the *TSER* polymorphic tandem repeat sequences have been described earlier [11,12]. However, to our knowledge, identification of the *MTHFR C677T* and *TSER* polymorphisms in Indonesian ALL patients has not been performed before. ALL treatment protocols in Indonesia are analogous to the Dutch protocols (DCLSG ALL-6 and ALL-9), which are based on tolerability and efficacy in this Caucasian population. Since ethnic differences may exist between Dutch and Indonesian children, which may affect drug sensitivity, we determined the ethnic variations in *TSER* and *MTHFR* polymorphisms in Dutch and Indonesian children with ALL, in Dutch adult controls, and in Indonesian children without malignancies.

2. Materials and methods

2.1. Patient and control samples

The patients of Indonesian origin included in this study were 71 children diagnosed with ALL treated at Dr. Sarjito Hospital, Yogyakarta, Indonesia and Dr. Soetomo Hospital, Surabaya, Indonesia with the Wijaya Kusuma ALL 2000 protocol. Control samples were from 44 patients without any malignancy selected within the range of 3–8 years of age, while the mean age of the children affected by ALL was 6.1 years (Table 1). For DNA isolation we used isolated mononuclear cells frozen on DMSO and stored in liquid nitrogen ($n = 24$) and cells from bone marrow slides ($n = 91$). Furthermore, we used as a comparison the results obtained in 157 samples of ALL children of Caucasian origin [13].

2.2. Isolation of mononuclear cells from fresh bone marrow or blood

The sample was diluted 1:1 with wash medium (Phosphate Buffered Saline, pH 7.4 containing 1% Fetal Calf Serum) and put on a ficoll-gradient with ratio 2:1. The interphase was collected and the cells were washed two times with wash medium. The mononuclear cell pellet was dissolved