

Are *Thymidylate synthase* and *Methylene tetrahydrofolate reductase* genes linked with methotrexate response (efficacy, toxicity) in Indian (Asian) rheumatoid arthritis patients?

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Received: 9 January 2008 / Accepted: 16 January 2008
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Abstract Methotrexate (MTX) is among the best-tolerated disease-modifying antirheumatic drugs for the treatment of rheumatoid arthritis (RA); major drawbacks of MTX therapy are the large interpatient variability in clinical response and the unpredictable appearance of a large spectrum of side effects. Several studies have demonstrated gene polymorphism that may regulate intracellular methotrexate metabolic pathway enzymes linked to drug efficacy and safety, but the evidence available is not yet conclusive. We decided to run a pilot study to determine the incidence of *Methylene tetrahydrofolate* (*MTHFR*; C677T, A1298C) and *Thymidylate synthase* (*TS*; 5' UTR repeat, 3' UTR deletion) gene polymorphism in rheumatoid arthritis patients in our community (Indian Asian) and further explore its association with MTX response (efficacy, toxicity). Thirty-four naïve RA patients on supervised MTX therapy and 139 healthy controls were genotyped for A1298C and C677T polymorphism of the *MTHFR* gene and 5' UTR repeat and 3' UTR deletion polymorphism of the *TYMS* gene by polymerase chain reaction-restriction fragment length polymorphism. Association, if any, between gene polymorphism and MTX response in RA

patients was analyzed. The *MTHFR* A1298C 'C' allele incidence among RA patients (46%) was significantly higher ($\chi^2=4.24$, $P<0.05$, OR=1.68). None of the other allele tested showed any association. Although a small sample study, our findings do not suggest a significant association of *MTHFR/TS* allele/genotype with MTX response in our ethnically distinct Indian (Asian) RA patients.

Keywords Gene polymorphism · Indian (Asian) · Methotrexate metabolism · Methotrexate response · Pharmacogenetics · Rheumatoid arthritis

Introduction

Methotrexate (MTX) is the most widely used disease-modifying antirheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA). Although MTX is among the best-tolerated DMARDs, major drawbacks of MTX therapy are the large interpatient variability in clinical response and the unpredictable appearance of a large spectrum of side effects [1].

Several studies have demonstrated polymorphism of the genes regulating enzymes in the intracellular methotrexate metabolic pathway [2]. Many of these gene polymorphisms are being linked to drug efficacy and safety, but the evidence available is not yet conclusive [3]. Pharmacogenetic testing may help to optimize therapy [4].

We decided to run a pilot study to determine the incidence of *Methylene tetrahydrofolate* (*MTHFR*; C677T, A1298C) and *Thymidylate synthase* (*TS*; 5' UTR repeat, 3' UTR deletion) gene polymorphism in rheumatoid arthritis

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patients in our community (Indian Asian) and further explore its association with MTX response (efficacy, toxicity).

Materials and methods

Study population Thirty-four naïve RA patients (27 females) and American College of Rheumatology (ACR) classified [5] completing 6 months of supervised MTX therapy (7.5–17.5 mg weekly) were randomly selected from a community rheumatology referral clinic between March and June 2006. We retrospectively analyzed case record forms and confirmed clinical events. We use ACR 20 improvement index response to evaluate efficacy at 6-month end point.

Methotrexate related adverse effects (AE) were defined as one or combination of the following: nausea/vomiting, abnormal liver functions (greater than twice the upper limit of normal values), aggravated skin nodules, oral ulcers, and cytopenia or any other known MTX-related AE documented in drug literature.

Genotype analysis After informed consent, a peripheral blood sample (4–5 ml) was drawn, and genomic DNA was extracted using Miller's protocol [6]. The A1298C and C677T polymorphism of the *MTHFR* gene and 5' UTR repeat and 3' UTR deletion polymorphism of the *TYMS* gene were analyzed by PCR-RFLP [7] (oligonucleotides—Integrated DNA technologies; restriction enzymes—New England Biolabs). One hundred thirty-nine healthy controls (HC) were included.

Statistical analysis Allelic frequencies and genotype distributions among groups were compared by Chi-square (χ^2) test.

Results

Table 1 shows frequency distribution of *MTHFR* and *TYMS* alleles in different groups.

Allele distribution

We examined *MTHFR* and *TYMS* allele distribution among RA patients and compared them with the allele distribution in HC. Using χ^2 analysis, the *MTHFR* A1298C 'C' allele distribution among RA patients (46%) was significantly different from that of HC population (27%). C allele was associated with RA ($\chi^2=4.24$, $P<0.05$, OR=1.68). While *MTHFR* C677T and none of the *TYMS* allele show any association with RA patients.

Table 1 Frequency distribution of *MTHFR* and *TYMS* alleles in different groups

Polymorphism	RA (n=34)	HC (n=139)	ACR 20		AE	
			Yes (n=17)	No (n=17)	Yes (n=13)	No (n=21)
<i>MTHFR</i> C677T						
C allele	0.82	0.90	0.76	0.88	0.85	0.81
T allele	0.18	0.09	0.24	0.11	0.15	0.19
<i>MTHFR</i> A1298C						
A allele	0.54	0.73	0.58	0.50	0.62	0.38
C allele	0.46 ^a	0.27	0.41	0.50	0.50	0.50
TS 5UTR						
2R allele	0.49	0.36	0.44	0.53	0.58	0.43
3R allele	0.51	0.64	0.56	0.47	0.42	0.57
TS 3UTR						
6 bp allele	0.50	0.42	0.76	0.88	0.42	0.55
–6 bp allele	0.50	0.58	0.23	0.11	0.58	0.45

RA Rheumatoid arthritis, HC healthy controls, AE adverse events

^aChi square (χ^2)=4.24, $P<0.05$, OR=1.68

MTHFR and *TYMS* allele distribution, MTX response (efficacy and toxicity)

Comparison of patients showing ACR 20 response ($n=17$) against those without ACR 20 response ($n=17$) and patients showing AE ($n=13$) against without any AE ($n=21$) was carried out to study any association with *MTHFR* and *TYMS* alleles/genotype. There was no statistically significant association observed for any of the allele/genotype and MTX-related efficacy and toxicity.

Discussion

The *MTHFR* A1298C 'C' allele incidence among RA patients (46%) was significantly higher (Table 1). The C allele was associated with RA ($\chi^2=4.24$, $P<0.05$, OR=1.68). None of the other allele tested showed any association. We also could not find any association between ACR 20 response/AE and tested alleles.

In previous studies, *MTHFR* 677C>T was found to be associated with elevated levels of liver enzymes, hyperhomocysteinemia, and overall MTX toxicity [8–10]. Patients with *MTHFR* 1298AA and *MTHFR* 677CC showed greater clinical improvement with MTX, whereas only the *MTHFR* 1298C allele was associated with toxicity [11]. It has been reported that RA patients homozygous for triple repeat (3R/3R) of *TYMS* 5' UTR gene required significantly higher dosage of MTX to control their disease activity than those with other genotypes (2R/2R and 2R/3R), while patients having –6 bp/–6 bp (homozygous) genotype of *TYMS* 3' UTR showed significantly higher

improvement ($\geq 50\%$) in serum CRP levels after MTX therapy [7]. In contrast, MTHFR C677T and A1298C polymorphisms in some studies have exhibited no association with MTX-related toxicity or efficacy in RA [7, 9].

We could not assess the plasma homocysteine levels. Although a small sample study, our findings do not suggest a significant association of MTHFR/TS allele/genotype with MTX response in our ethnically distinct Indian (Asian) RA patients. We do find some association between MTHFR A1298C, in particular 'C' allele, and RA. But, this had no bearing on AE or clinical efficacy and needs to be validated.

Acknowledgments The authors wish to thank the invaluable assistance from Dr. K. Satyamoorthy, MAHE Manipal, Ms Anuradha V, Ms Manjit S, and lab technicians at CRD Pune. Supported by grants from Council for Scientific and Industrial Research—CSIR-NMITLI (CSIR B 112), Government of India.

Disclosures None

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