Short Communication: Lack of association between MTHFR gene polymorphisms and response to methotrexate treatment in Pakistani patients with rheumatoid arthritis

Mohammad Perwaiz Iqbal  
Aga Khan University, perwaiz.iqbal@aku.edu

Azra Arif Ali  
Aga Khan University

Naseema Mehboobali  
Aga Khan University, naseema.mehboobali@aku.edu

Khalida Iqbal  
Aga Khan University

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Lack of association between MTHFR gene polymorphisms and response to methotrexate treatment in Pakistani patients with rheumatoid arthritis

Mohammad Perwaiz Iqbal*1, Azra Arif Ali2, Naseema Mehboobali1 and Khalida Iqbal1
1Departments of Biological & Biomedical Sciences and 2Medicine, Aga Khan University, Stadium Road, Karachi, Pakistan

Abstract: Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms have been reported to be associated with response to methotrexate (MTX) in certain populations of patients with rheumatoid arthritis (RA). This study aims at investigating any relationship of two single nucleotide polymorphisms (SNPs) in MTHFR gene, C677T and A1298C with response to therapy with MTX in Pakistani RA patients. Allelic frequencies of the two polymorphisms (C677T and A1298C) were determined in 67 RA patients (9 males and 58 females; mean age 42.87±13.5 years) who had previously participated in a prospective clinical trial. Fifty-one patients had received MTX and were followed up for response up to 6 months. Genotyping of the two MTHFR polymorphisms was carried out using PCR-RFLP, while fasting concentration of plasma homocysteine was determined using a kit method. Twenty-eight patients were found to be “good responders”, while twenty-three were “poor responders”. MTHFR 1298C and MTHFR 677T alleles’ frequencies in “good responders” were not different from frequencies in “poor responders” (0.574 vs. 0.521; p=0.6 and 0.197 vs. 0.196; p=0.75, respectively). Plasma homocysteine levels in female RA patients were significantly higher compared to general population in Karachi (13.1±6.7 µmol/l vs. 11.4±5.3 µmol/l; p<0.001). MTHFR C677T and A1298C polymorphisms are not associated with response to MTX in a population of Pakistani RA patients.

Keywords: Gene polymorphism; Methylenetetrahydrofolate reductase; MTHFR C677T; MTHFR A1298C; Methotrexate; Pharmacogenomics; Rheumatoid arthritis; Response to methotrexate

INTRODUCTION

Methotrexate (MTX) is a commonly used disease modifying antirheumatic drug in treating rheumatoid arthritis (RA) patients. However, the efficacy of this drug in RA varies from one population to another. It has also been reported that nearly one-third of RA patients discontinue this drug because of its adverse reactions (Hider et al., 2007). Studies carried out mostly in the developed countries have shown two single nucleotide polymorphisms (SNPs) in MTHFR gene, C677T and A1298C with response to therapy with MTX in Pakistani RA patients. Allelic frequencies of the two polymorphisms (C677T and A1298C) were determined in 67 RA patients (9 males and 58 females; mean age 42.87±13.5 years) who had previously participated in a prospective clinical trial. Fifty-one patients had received MTX and were followed up for response up to 6 months. Genotyping of the two MTHFR polymorphisms was carried out using PCR-RFLP, while fasting concentration of plasma homocysteine was determined using a kit method. Twenty-eight patients were found to be “good responders”, while twenty-three were “poor responders”. MTHFR 1298C and MTHFR 677T alleles’ frequencies in “good responders” were not different from frequencies in “poor responders” (0.574 vs. 0.521; p=0.6 and 0.197 vs. 0.196; p=0.75, respectively). Plasma homocysteine levels in female RA patients were significantly higher compared to general population in Karachi (13.1±6.7 µmol/l vs. 11.4±5.3 µmol/l; p<0.001). MTHFR C677T and A1298C polymorphisms are not associated with response to MTX in a population of Pakistani RA patients.

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Plasma/serum was analyzed for homocysteine using a kit method (Abbott Laboratories Ltd; Pakistan), while DNA was isolated from the leukocytes using standard procedure as described previously (Ali et al., 2006). Genotyping protocol for the MTHFR C677T and A1298C polymorphisms was based on polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) as described in a previous report (Yakub et al., 2012). The study had been approved by the Ethics Review Committee of the Aga Khan University.

### RESULTS

Mean concentrations of plasma homocysteine in males and females were found to be 16.2±3.0 and 13.1±6.7 µmol/l, respectively. However, mean homocysteine concentration in female RA patients was significantly higher compared to mean homocysteine concentration in general population in Karachi (13.1±6.7 µmol/l vs. 11.4±5.3 µmol/l; p<0.001; Yakub et al., 2010). Twenty eight RA patients were found to be “good responders” to MTX, while 23 were identified as “poor responders”. No significant differences were observed between “good responders” and “poor responders” in terms of gender, age, duration of RA and plasma levels of homocysteine (table 1). Frequencies of MTHFR 1298C and MTHFR 677T alleles in RA patients were not significantly different from their frequencies in general population in Karachi (0.553 vs. 0.55, p=0.977; and 0.196 vs. 0.15; p=0.338, respectively), (Yakub et al., 2012). Genotypes and allele frequencies of the two polymorphisms were not significantly different between “good responders” and “poor responders” (table 2).
DISCUSSION

The advent of pharmacogenomics has opened new vistas in the treatment of diseases by employing efficacious drugs according to the genetic make-up of the patient (Ranganathan and McLeod, 2006). MTX in low-dosages is a drug of choice in the treatment of RA because it exerts its anti-inflammatory effect by inhibiting the enzymes in cellular folate and adenosine pathways (Ranganathan, 2013). However, long-term use of this drug is not without toxic side effects. While quite a few studies have shown that efficacy and toxicity of MTX in RA patients is influenced by two SNPs (C677T and A1298C) in the gene of MTHFR, a key enzyme in folate metabolism, several others did not find any association of these polymorphisms and MTX toxicity in their populations. For example, an association between C677T/A1298C polymorphism(s) and MTX toxicity has been reported in Spanish, Caucasian American, African-American and Korean RA patients (Ranganathan et al., 2008; Plaza-Plaza et al., 2012; Choe et al., 2012). However, no association of these polymorphisms with increased MTX adverse effects was observed in Indian and Briton RA patients (Aggarwal et al., 2006; Owen et al., 2012). Lee et al., 2010 carried out a meta-analysis of 8 studies and found no association of C677T or A1298C polymorphisms with MTX toxicity in Asian RA patients. The results of these studies conform well to our findings that these polymorphisms are not associated with MTX toxicity in Pakistani RA patients. Two studies showed paradoxical results and reported association of these polymorphisms with increased rate of RA remission in patients treated with MTX (Berkun et al., 2004; Kurzawski et al., 2007). All these reports point towards variable response to MTX in RA patients from different populations. Frequency of 1298CC genotype in RA patients of certain populations has been found to be higher than the expected frequency of this genotype in general population (Berkun et al., 2004). Moreover, this genotype was found to be associated with reduced toxicity to MTX (Berkun et al., 2004). However, in Pakistani RA patients, the frequency of 1298CC genotype was not significantly different from healthy controls (Yakub et al., 2012). This could be one of the reasons for lack of association of A1298C polymorphism in Pakistani RA patients.

The mechanism by which MTHFR polymorphisms could be causing MTX toxicity in some RA patients is unclear. However, the role of increased concentrations of plasma homocysteine in RA patients with MTHFR 677CT and MTHFR 677TT genotypes cannot be discounted (van Ede et al., 2002). Increased levels of homocysteine are known to cause endothelial dysfunction, low density lipoprotein oxidation and prothrombotic changes (Hernanz et al., 1999). All these changes can lead to an increase in adverse effects of MTX. Our results should be viewed in the light of certain limitations of the study. Patients enrolled in this study were in a modest number. Moreover, follow-up of these patients was for 6 months only. The possibility that some of the patients classified under “good responders” might develop MTX toxicity on a long term follow-up cannot be ignored. In spite of these limitations, our data did not show any association of MTHFR polymorphisms with efficacy and/or toxicity of MTX.

ACKNOWLEDGEMENTS

We greatly appreciate the help provided by Dr. Ahmed Iqbal in conducting this trial. Technical help provided by Ms. Hadees Farooq Murad and Syed Saad Masroor is also gratefully acknowledged.

REFERENCES


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