promoted IFNγ secretion. These results suggest that APLs are not Th1 stimulators. It is not clear whether they regulate Th2 cells because no effects on IL4 production were found in the present study. Further studies are necessary to investigate whether APLs effectively inhibit T cell activation in vivo, such as in the HLA-DR4 transgenic animal model.

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Authors' affiliations
L Xia, L Ru, L Zhanguo, Department of Rheumatology and Immunology, People's Hospital, Peking University, 11 Xizhimen South St, Beijing 100044, China

Correspondence to: Dr L Zhanguo, lixia1969@163.com

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Relationship between 5,10-methylenetetrahydrofolate reductase C677T gene polymorphism and methotrexate related toxicity in patients with autoimmune diseases receiving folic acid supplementation

M Speletas, N Papadopoulos, C Daiou, E Katodritou, A Pavlitou-Tsiontsi, V Galanopoulou

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The common polymorphism C677T of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene reduces enzyme activity and it has recently been associated with increased incidence of methotrexate (MTX) related toxicity in patients with cancer and rheumatoid arthritis.[1–5] Considering that folate supplementation may reduce toxicity without affecting MTX efficiency, we conducted a retrospective study to analyse the effect of this polymorphism in patients with autoimmune diseases receiving folate supplementation.

Sixty three patients (F/M: 44/19, mean age 53.6 years, range 20–81) with autoimmune diseases who had been treated with MTX (7.5–15 mg/week, mean duration 35.8 months, range 2–121), were selected from the outpatient clinic between January and June 2004. Five of them had discontinued MTX treatment at the time of selection, because of adverse events or inefficiency. Thirty nine of the patients were receiving a combination of MTX with corticosteroids and/or other disease modifying antirheumatic drugs. All the patients were prescribed supplementary 2.5 mg folic acid the day before and the day after MTX treatment. All participants were informed and consented to take part in the study. Table 1 shows the characteristics of the patients.

Genomic DNA was extracted from peripheral blood, and analysis of the MTHFR C677T polymorphism was performed

Table 1 Patients' characteristics

Characteristics	Adverse effects: Present (n = 15)	Adverse effects: Absent (n = 48)
Age (years)		
Mean (SD)	56.8 (10.8)	52.6 (16.6)
Range	33–72	20–81
Sex (F/M)	11/4 (2.75)	33/15 (2.2)
Disease		
Rheumatoid arthritis	13	33
Psoriatic arthritis	2	10
Ankylosing spondylitis	–	3
Polymyositis	–	2
Disease duration (months)		
Mean (SD)	36.6 (30.1)	35.6 (29.6)
Range	2–121	5–120
Additional drugs (No (%) of patients)		
Corticosteroids (only)	2 (13)	7 (15)
Other DMARDs (± corticosteroids)	8 (53)	22 (46)
MTHFR genotype, No (%)		
CC (wild type)	10 (67)	13 (27)
CT (heterozygous)	3 (20)	28 (58)
TT (homozygous)	2 (13)	7 (15)

DMARDs, disease modifying antirheumatic drugs (included ciclosporin: 9 patients, hydroxychloroquine: 7 patients, and infliximab: 14 patients).

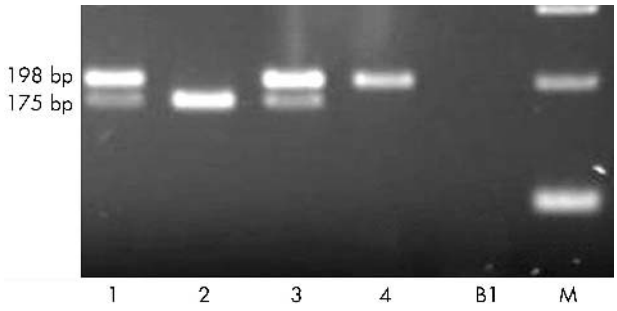

Figure 1 MTHFR C677T gene polymorphism established by PCR-digestion by HinfI. The protocol of Frosst and coworkers was followed,[6] with some modifications. The forward and reverse primers used were 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and 5′-AGGACGGTGCGGTGAGAGTG-3′, respectively. The PCR conditions were 2 minutes at 94°C followed by 32 cycles (94°C for 30 seconds, 62°C for 30 seconds, 72°C for 60 seconds), and 5 minutes at 72°C after the last cycle. A 198 bp fragment was amplified by PCR and subjected to HinfI digestion (New England Biolabs, UK). The 677T allele contains an HinfI site resulting in 175 bp and 23 bp fragments, whereas a C at position 677 (677C) does not. The PCR and digestion products were analysed in 3% TBE agarose gels. Samples were categorised as homozygous for the thermolabile variant (677TT, lane 2), heterozygous for wild type and variant (C677T, lanes 1 and 3), or wild type (677CC, lane 4). Bl, negative control; M, 100 bp ladder molecular weight marker (Invitrogen, UK). The 23 bp fragments were not visible on agarose gels.

by polymerase chain reaction (PCR) amplification followed by restriction digestion analysis (fig 1). The statistical analysis was performed with SPSS statistical software.

The prevalence of the MTHFR C677T genotype in our cohort of patients was 37% for 677CC (23/63 patients), 49% for C677T (31/63 patients), and 14% for 677TT (9/63 patients). Fifteen (24%) patients displayed one or more adverse effects (three nausea, eight neutropenia/pancytopenia, two a rise in transaminases, and three oral mucositis) and six of them discontinued MTX because of toxicity. Moreover, a further four patients discontinued MTX, one because of inefficiency, one because of emergence of secondary amyloidosis, and two because of emergence of neoplasia (total discontinuation 16%). There was no significant difference in the MTX dosage, the demographic and clinical features between the patients with and without adverse effects during MTX treatment.

Interestingly, toxicity was more common in patients with normal genotype than in those with both heterozygotes and homozygotes ($p = 0.005$ analysed by Fisher's exact test). Moreover, in multivariate analysis of variance the MTHFR genotype was the most important independent risk factor predisposing to MTX related toxicity ($p = 0.042$), compared with the other variables analysed—namely, age, sex, duration, and type of treatment (MTX alone or in combination with corticosteroids and/or other disease modifying antirheumatic drugs).

To our knowledge, this is the first study illustrating an inverse relationship between an MTHFR C677T variant and MTX related toxicity, in which the presence of toxicity was more common in patients with the normal 677CC genotype. In previous studies the presence of an MTHFR C677T polymorphism was associated with a higher incidence of MTX related toxicity in patients with rheumatoid arthritis and cancer, and also with an increased risk of discontinuing MTX treatment because of adverse events.[2–5] However, most patients in those studies did not receive folate supplementation, or they received it after the emergence of toxicity. Interestingly, other studies did not support such a correlation.[7 8] A possible explanation of our results, similar to the protective effect of the MTHFR C677T polymorphism in carcinogenesis,[9 10] is that this polymorphism, in the presence of adequate folate supply, results in sustained ability of DNA synthesis and repair through increased synthesis of purines and thymidine, and subsequently in decreased MTX related toxicity.

In conclusion, our data suggest that a folate supply is critical among patients with autoimmune diseases and different MTHFR genotypes. When folic acid is given, subjects with MTHFR 677TT and C677T may be at reduced risk of MTX related toxicity, probably because of the sustained ability of DNA synthesis. This protective effect is absent in subjects with MTHFR 677CC and in subjects with MTHFR 677TT and C677T receiving MTX without folate supply, as has been shown in previous studies. In addition, our results indicate the importance of genotyping to provide useful information for individualised treatment in patients with autoimmune diseases.

Authors' affiliations

M Speletas, C Daiou, E Katodritou, A Pavlitou-Tsiontsi, Haematology and Immunology Department, Papageorgiou General Hospital, Thessaloniki, Greece
N Papadopoulos, V Galanopoulou, Rheumatology Unit, Papageorgiou General Hospital, Thessaloniki, Greece

Correspondence to: Dr M Speletas, Dimarchou K Tsirou 8, 54248 Thessaloniki, Greece; speletas@otenet.gr

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