Outcomes of methotrexate therapy for psoriasis and relationship to genetic polymorphisms

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Summary

Background The use of methotrexate is limited by interindividual variability in response. Previous studies in patients with either rheumatoid arthritis or psoriasis suggest that genetic variation across the methotrexate metabolic pathway might enable prediction of both efficacy and toxicity of the drug.

Objectives To assess if single nucleotide polymorphisms (SNPs) across four genes that are relevant to methotrexate metabolism [folypolyglutamate synthase (FPGS), gamma-glutamyl hydrolase (GGH), methylenetetrahydrofolate reductase (MTHFR) and 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC)] are related to treatment outcomes in patients with psoriasis.

Methods DNA was collected from 374 patients with psoriasis who had been treated with methotrexate. Data were available on individual outcomes to therapy, namely efficacy and toxicity. Haplotype-tagging SNPs (r² > 0.8) for the four genes with a minor allele frequency of > 5% were selected from the HAPMAP phase II data. Genotyping was undertaken using the MassARRAY spectrometric method (Sequenom®).

Results There were no significant associations detected between clinical outcomes in patients with psoriasis treated with methotrexate and SNPs in the four genes investigated.

Conclusions Genetic variation in four key genes relevant to the intracellular metabolism of methotrexate does not appear to predict response to methotrexate therapy in patients with psoriasis.

Methotrexate is a first-line systemic therapy for psoriasis; however, its use is limited by unpredictable efficacy and significant hepatotoxicity and gastrointestinal symptoms. Studies in patients with psoriasis and rheumatoid arthritis treated with methotrexate suggest that functional single nucleotide polymorphisms (SNPs) in genes relevant to methotrexate metabolism may influence both efficacy and toxicity of the drug.1–12

Such studies have focused on isolated functional polymorphisms in only a few genes relevant to methotrexate metabolism and results have been variable, especially in the most investigated gene methylenetetrahydrofolate reductase (MTHFR); (Table 1).

We investigated 47 haplotype-tagging and three functional SNPs in four genes coding for enzymes involved in methotrexate intracellular metabolism: folypolyglutamate synthase (FPGS); gamma-glutamyl hydrolase (GGH); MTHFR; and 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC); (Fig. 1) in a large cohort of patients with psoriasis treated with methotrexate.

Methods

This study adheres to the declaration of Helsinki Guidelines and was approved by the relevant local research ethics committees. Subjects provided written, informed consent.

Adult patients who had received methotrexate for treatment of psoriasis were recruited retrospectively from The Dermatology Centre, Salford Royal Hospital, Manchester, and St John’s Institute of Dermatology, London.

Evaluation of efficacy

Patients were stratified as: (i) ‘responders’—those with clearly documented clinical improvement, using the Psoriasis Area and
Severity Index (PASI), i.e. > 75% reduction in PASI from the start of methotrexate therapy; and (ii) ‘nonresponders’—those patients who showed no clear improvement, i.e. < 50% improvement in PASI while on therapy. In cases where the PASI score was not recorded, an explicit statement of response to therapy recorded in the clinical records was acceptable.

Evaluation of toxicity

The adverse effects of methotrexate were assessed by clinical records:

Hepatotoxicity. Alanine transaminase (ALT) three times the upper limit of normal on two consecutive outpatient visits and/or serum level of aminoterminal of type III procollagen (P3NP) elevated above 4 µmol L⁻¹ on three consecutive occasions or 8 µmol L⁻¹ on one occasion and/or liver biopsy changes consistent with methotrexate hepatotoxicity within 3 years of starting therapy.

Gastrointestinal. Severe nausea, vomiting or diarrhoea, which required cessation of methotrexate or the addition of an anti-emetic.

Bioinformatics

SNPS were located and downloaded from the public HAPMAP Phase II, October 2005 database (http://www.hapmap.org/). Haplotype-tagging SNPS (n = 47; r² threshold of 0.8) were identified and filtered through the Haploview software omitting all markers having a minor allele frequency < 5%. Additional SNPs previously studied in MTHFR (rs1801133/677C>T and rs1801131/298A>C) and ATIC (rs2372536/347C>G) were also genotyped.
Genotype analysis

DNA was extracted from white blood cells using a standard phenol–chloroform extraction procedure. Fifty SNPs were genotyped using Sequenom® MassARRAY™ (Sequenom Inc., San Diego, CA, U.S.A.) technology.

Statistical analyses

The statistical software package STATA v. 8.2 (StataCorp LP, College Station, TX, U.S.A.) was used to compare genotype frequencies (χ² test for trend) in order to evaluate the association of each SNP with each defined treatment outcome. Logistic regression analysis was performed using the software PLINK15 to adjust for age of onset of psoriasis, sex, race and, in 294 of the 374 patients, the presence or absence of folic acid supplementation.

FPGS and GGH

No significant (P < 0.05) genotypic associations were detected between SNPs in FPGS or GGH and either the efficacy or toxicity of methotrexate. After adjusting for age, sex and ethnicity the results remained nonsignificant for all SNPs other than rs10987746 in FPGS (adjusted P = 0.01). No significant associations were detected upon adjustment for folic acid supplementation.

MTHFR

No significant genotypic associations were found between SNPs in MTHFR and either efficacy or toxicity of methotrexate before or after adjustment for age, sex, ethnicity and folic acid. Genotypic data for the previously investigated SNPs rs1801133 and rs1801131 are shown in Table 2. Combined analysis was performed for SNP rs1801133 T allele and SNP rs1801131 C allele with no significant (P > 0.1) association found.

ATIC

No significant genotypic associations were detected between SNPs in ATIC and the efficacy of methotrexate. However, two of the 15 SNPs were significantly (P < 0.05) associated with onset of toxicity (Table 2). The most significant of these is the coding SNP rs2372536, with a χ² test for trend of P = 0.01. These two SNPs have a very high degree of correlation with an r² of 1 and remain associated with onset of toxicity (P = 0.01) after adjustment for the variables age, sex and gastrointestinal toxicity occurred in 65 and 79 patients, respectively.

Data were obtained for 50 SNPs in the cohort with a genotype success rate of > 90%.

Table 2 Genotypic associations and odds ratios (OR) for the carriage of allele 2 [with 95% confidence interval (CI)] for previously investigated single nucleotide polymorphisms in the genes methylenetetrahydrofolate reductase (MTHFR) and 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC) and their association with the efficacy and toxicity of methotrexate in our psoriasis cohort.

| Gene/ Rs number | Genotype frequencies (%) | Trend test, P-value | Carriage of allele 2 (OR, 95% CI) |
|-----------------|--------------------------|---------------------|----------------------------------|
| MTHFR rs1801133 | 115 (48) 100 (42) 23 (10) | 0.05 0.8 (0.4–1.4) |                                  |
| MTHFR rs1801131 | 118 (51) 86 (37) 28 (12) | 0.04 1.1 (0.7–2.0) |                                  |
| ATIC rs2372536  | 77 (52) 57 (38) 15 (10)  | 0.01 0.6 (0.3–0.9) |                                  |
| ATIC rs4672768* | 76 (51) 57 (38) 16 (11)  | 0.02 0.6 (0.4–0.9) |                                  |

*No known function but has a borderline association with the onset of toxicity. 1, Major allele; 2, minor allele. Bold font indicates association with toxicity.
ethnicty. A further SNP, rs4672768 was associated (p = 0.01) with the onset of toxicity after adjustment for the above three variables. No associations remained after adjusting for folic acid supplementation. Following correction for the multiple statistical tests (Bonferroni correction factor, n = 50) these associations became nonsignificant (p > 0.05). These SNPs were not associated with either hepatic or gastrointestinal toxicity (data not shown).

Discussion
In this study, we found no significant (p < 0.05) associations between SNPs in FPGS, GGH and MTHFR and either methotrexate efficacy or toxicity in patients with psoriasis. Borderline associations (p > 0.01 to ≤ 0.05) were detected between methotrexate toxicity and two SNPs in ATIC. Multivariate analysis accounting for age at onset of psoriasis, sex and ethnicity showed four borderline associations between three SNPs in ATIC and one in FPGS and methotrexate toxicity. When the Bonferroni correction was performed or when analysing the subgroups of hepatotoxicity and gastrointestinal toxicity, these associations became nonsignificant (p > 0.05). Adjustment for the supplementation with folic acid revealed no associations between any SNPs and outcomes to methotrexate treatment. We were unable to replicate any of the previously reported associations in patients with rheumatoid arthritis and two functional SNPs in MTHFR and one functional SNP in ATIC. No associations were detected for carriage of combinations of previously described functionally independent risk alleles in the genes MTHFR (rs1801133 T allele and rs1801131 C allele) and ATIC (rs2372536 G allele).

Clearly there are important limitations to the current study. Firstly, it was performed in a retrospective cohort of patients therefore not allowing a systematic, prospective and objective collection of phenotypic data. Furthermore, a diverse range of endpoints was used in studies reported in the literature which makes direct comparison difficult, particularly as the majority were performed in patients with rheumatoid arthritis. It is possible that we may have missed genetic variation across each of the genes studied which could influence response to methotrexate treatment in psoriasis patients. However, we estimate that we had 80% gene coverage of all SNPs reported on the phase II HAPMAP data.

We conclude that genetic variation across the genes FPGS, GGH, MTHFR and ATIC is not predictive of either efficacy or toxicity of methotrexate in patients with psoriasis.

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