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Analysis of Single Nucleotide Polymorphisms of Methylenetetrahydrofolate Reductase in Japanese Psoriasis Patients

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Abstract Methotrexate (MTX) is an effective drug for the treatment of psoriasis as well as rheumatoid arthritis. We investigated the correlation between single nucleotide polymorphisms (SNP) in methylenetetrahydrofolate reductase (*MTHFR*) and susceptibility to psoriasis in 175 psoriasis patients and 150 healthy controls and between *MTHFR* SNP and responsiveness to psoriasis treatment in 7 psoriasis patients. The frequencies of the 677C allele in the patient and healthy control groups were 0.61 and 0.57, respectively, while those of the 677T allele were 0.39 and 0.43, respectively. Both allele frequencies in both groups were not significantly different. Moreover, both groups showed the same frequency of 1298A allele (0.83 vs. 0.83) and 1298C allele (0.17 vs. 0.17). The frequency of *MTHFR* SNP and susceptibility to psoriasis were not correlated. Additionally, *MTHFR* SNP frequency and the psoriasis lesion were not correlated. Investigation of the MTX treatment efficacy in 7 patients revealed that the drug was effective in psoriasis patients with positive presumed 677C – 1298A SNP haplotypes (N = 3), and they developed no side effects. Although *MTHFR* SNP involved in folate metabolism is not related to susceptibility to psoriasis and its severity, SNP could become a predictive index to use MTX safely and effectively in psoriasis treatment.

Key words: folic acid, methotrexate, methylenetetrahydrofolate reductase, polymorphism, disease susceptibility to psoriasis

Introduction

Recent advances in the analysis of genomic information have enabled the prediction of not only the onset of some diseases such as rheumatoid arthritis but also the susceptibility to the treatment drugs and the incidence of adverse reactions to them.¹⁻⁵⁾ Regarding methotrexate (MTX),⁶⁾⁷⁾ which is one of the most important drugs used in the area of rheumatoid arthritis, single nucleotide polymorphisms (SNP) in enzymes involved in folic acid metabolism have been utilized to predict patient responses to treatment with this drug.⁷⁻¹⁰⁾

Methylenetetrahydrofolate reductase (*MTHFR*) is an enzyme that catalyzing the reduction of methylenetetrahydrofolate into methyltet-

rahydrofolate. 5,10-Methylenetetrahydrofolate (5,10-CH₂-THF) serves as the substrate for methylenetetrahydrofolate reductase (EC 1.5.1.20) to form 5-methyltetrahydrofolate. The *MTHFR* is known to have several polymorphisms, including C677T and A1298C.¹¹⁾ With the C677T polymorphism, the change of cytosine (C) to thymine (T) induces the substitution of alanine with valine, which makes the enzyme unstable against heat. In addition, the presence of the T allele causes a decrease methyltetrahydrofolate which is reduced by *MTHFR*. With the A1298C polymorphism, the change of adenine (A) to cytosine (C) induces the substitution of glutamine with alanine, which also lowers the enzymatic activity.²⁾¹²⁻¹⁴⁾

In order to examine whether the C677T and

A1298C polymorphisms are related to psoriasis susceptibility and safely and effectively use of MTX for the disease, we recently conducted a SNP analysis of the gene (one of the rate-limiting enzymes for folic acid) in relation to psoriasis vulgaris and MTX (one of the drugs used for treatment of this disease).¹⁵⁻¹⁸⁾

Subjects and methods

This study involved 175 patients with psoriasis vulgaris and 150 healthy individuals. Patient age ranged from 6 to 90 years, with a median of 52 years. One hundred and forty-seven patients (84%) were male and twenty-eight (16%) were female. Age of the healthy individuals ranged from 20 to 77 years, with a median of 28 years. Seventy-seven patients (51%) were male and seventy-three (49%) were female. Age and sex ratio of both groups is not coordinated. The study was approved in advance by the Ethics Review Committee of Yamaguchi University School of Medicine. After each patient was informed of the study in writing and his/her consent to the study was obtained in writing, blood was sampled from each patient for the analysis of the *MTHFR* polymorphisms C677T and A1298C.

From each blood sample, DNA was extracted with a QIAamp DNA Blood Maxi Kit (QIAGEN, Hilden, Germany). The extracted DNA was amplified by polymerase chain reaction (PCR). The primers used were 677F (5'-TGAAGGA GAAGGTGTCTGCGGGA-3') and 677R (5'-AG ACGGTGCGGTGAGAGTG-3'). The amplified DNA was treated with the restriction enzyme *HinfI*, which was followed by polyacrylamide gel electrophoresis for the analysis of the *MTHFR* C677T polymorphisms CC, CT, or TT. Another set of primers used were 1298F (5'-GGGAGGAGCTGACCAGTGCAG-3') and 1298R (5'-GGGGTCAGGCCAGGGGCAG-3'). The DNA was amplified and treated with the restriction enzyme *Fnu4HI*, which was followed by polyacrylamide gel electrophoresis for the analysis of the *MTHFR* A1298C polymorphisms AA, AC, or CC. The distribution of the C677T and A1298C polymorphisms was analyzed in both the patient and the healthy volunteer groups. To confirm a genotype, we performed genetic typing by the other methods (Big Dye Terminator 3.1 Cycle Sequencing

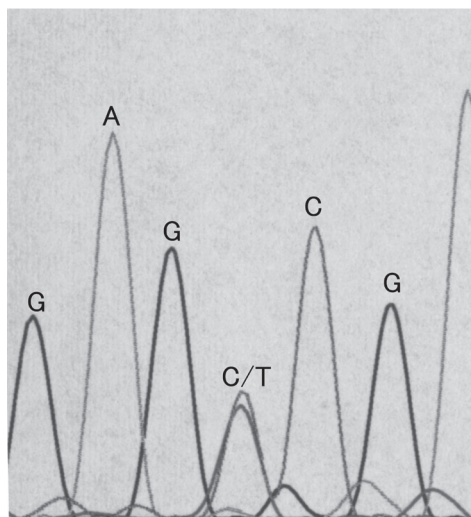


Fig. 1 *MTHFR* 677CT genotype identified by the Big Dye Terminator 3.1 Cycle Sequencing Kit.

Kit) (Fig. 1). We examined genotype by two different technique. Both technique showed the same result. The data collected were used to analyze the association of the 2 *MTHFR* polymorphisms with the presence/absence and severity of psoriasis vulgaris. In addition, the association of the *MTHFR* polymorphisms with the responses and adverse reactions to MTX (one of the therapies for psoriasis vulgaris) was analyzed in 7 patients with psoriasis who had been previously treated with this drug in accordance with the Weinstein method.¹⁹⁾ Fisher's exact test was employed for statistical analysis of data between the psoriasis and the healthy volunteer groups.

Results

Frequencies of *MTHFR* C677T polymorphisms and alleles

For the C677T genotypes, the frequency in the psoriasis group ($n = 175$) was 34% (60/175) for C/C, 53% (92/175) for C/T and 13% (23/175) for T/T, and the frequency in the healthy control group ($n = 150$) was 31% (47/150) for C/C, 51% (77/150) for C/T and 17% (26/150) for T/T (Table 1). There were no significant differences between the 2 groups in terms of the frequency of any type (C/C, C/T, or T/T). The proportions of genotypes were generally in agreement with Hardy-Weinberg's equilibrium in the Table 1 ($\chi^2 = 0.34$; $p > 0.8$).

Table 1 Genotype and allele frequencies of *MTHFR* C677T in Psoriasis

SNP	Psoriasis (n=175)	Normal control* (n=150)
Genotype		
C/C	34.3% (=60/175)	31.3% (=47/150)
C/T	52.6% (=92/175)	51.3% (=77/150)
T/T	13.1% (=23/175)	17.3% (=26/150)
Allele	(2n=350)	(2n=300)
C	60.6% (=212/350)	57.0% (=171/300)
T	39.4% (=138/350)	43.0% (=129/300)

* All allele frequencies conformed to Hardy-Weinberg equilibrium ($\chi^2 = 0.34$; $P > 0.8$).

Table 2 Genotype and allele frequencies of *MTHFR* A1298C in Psoriasis

SNP	Psoriasis (n=175)	Normal control* (n=150)
Genotype		
A/A	68.0% (=119/175)	68.0% (=102/150)
A/C	30.9% (=54/175)	30.7% (=46/150)
C/C	1.1% (=2/175)	1.3% (=2/150)
Allele	(2n=350)	(2n=300)
A	83.4% (=292/350)	83.3% (=250/300)
C	16.6% (=58/350)	16.7% (=50/300)

* All allele frequencies conformed to Hardy-Weinberg equilibrium ($\chi^2 = 1.42$; $P > 0.4$).

For the alleles, the frequency in the psoriasis group was 0.61 for the C allele and 0.39 for the T allele, where as the frequency in the healthy control group was 0.57 for the C allele and 0.43 for the T allele (Table 1). There were no significant inter-group differences in the frequencies of the C or T alleles.

Frequencies of *MTHFR* A1298C polymorphisms and alleles

For the analysis of the distribution of the *MTHFR* A1298C genotypes, the frequency in the psoriasis group was 68% (119/175) for A/A, 31% (54/175) for A/C and 1% (2/175) for C/C and the frequency in the healthy volunteer group was 68% (102/150) for A/A, 31% (46/150) for A/C and 1% (2/150) for C/C (Table 2). There were no significant differences between the 2 groups in the distribution of the *MTHFR* A1298C polymorphisms. The proportions of genotypes were generally in agreement with Hardy-Weinberg's equilibrium in the Table 2 ($\chi^2 = 1.42$; $p > 0.4$).

For the alleles, the frequency in the psoriasis group was 0.83 for the A allele and 0.17 for

the T allele and the frequency in the healthy control group was 0.83 for the A allele and 0.17 for the C allele (Table 2). There were no significant inter-group differences in the frequency of the A or C alleles.

Distribution of eruption and its association with *MTHFR* SNPs

An analysis of the distribution of the eruption extent of psoriasis and the *MTHFR* SNPs was conducted in 96 patients with psoriasis in whom the course of eruption could be followed in detail. Psoriatics with erythroderma were excluded. The data were compared among the severe psoriasis group (25 patients or 26%), who had extensive erythema covering 50% or more of the entire body surface, the mild to moderate psoriasis group (71 patients or 74%), who had erythema covering less than 50% of the entire body surface, and the healthy control group (n = 150). There were no significant differences in the distribution of the *MTHFR* polymorphisms (Fig. 2). These results suggest that the extent of the eruption does not correlate with the distribution of the *MTHFR* poly-

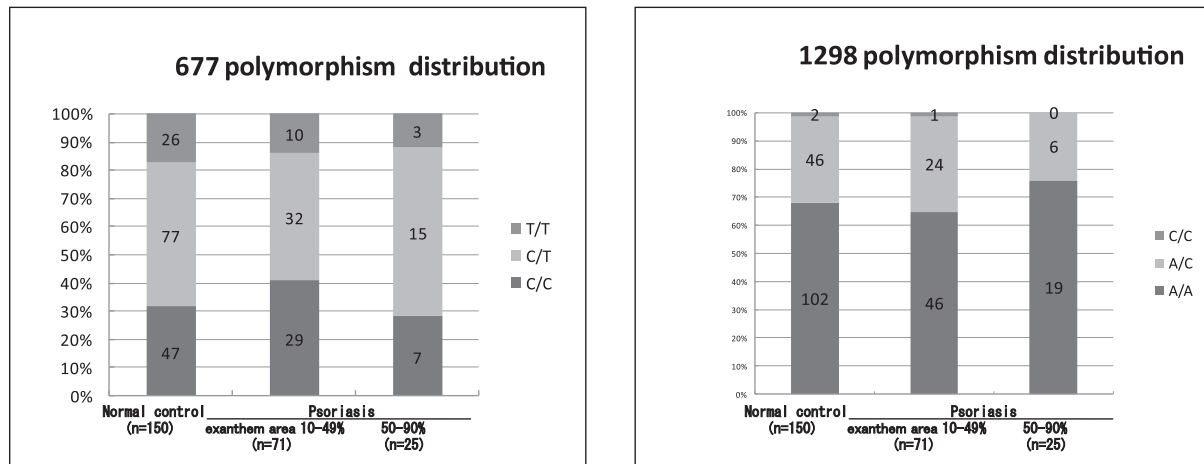


Fig. 2 *MTHFR* genotype frequency according to the erythema area in the psoriasis patients.

Table 3 Summary of the psoriasis patients used MTX in the past clinical records

Cases	Diagnosis	Genotype		Side effect	Curative effect
		677	1298		
1	Psoriasis vulgaris	C/C	A/A	–	+
2	Psoriatic arthritis	C/C	A/A	–	+
3	Psoriasis vulgaris	C/T	A/C	liver damage※	+
4	Psoriatic arthritis	C/C	A/C	–	+
5	Psoriatic erythroderma	C/C	A/A	–	+
6	Psoriatic arthritis	T/T	A/A	liver damage※	–
7	Psoriasis vulgaris	C/T	A/C	–	–

※Case3 AST 53 IU/l ALT 61 IU/l
Case6 AST 60 IU/l ALT 83 IU/l

morphisms.

Association between clinical efficacy of MTX and *MTHFR* SNPs

Next, we investigated whether or not the *MTHFR* polymorphisms affect the incidence of adverse reactions to MTX²⁰⁾ or the efficacy of this drug²¹⁾ in patients with psoriasis, similar to that seen in patients with rheumatoid arthritis.¹⁴⁾³⁹⁾ Responses to MTX treatment were analyzed in 7 patients who had received MTX treatment in accordance with the Weinstein method¹⁹⁾ (3 oral doses of 2.5 mg/dose at 12 h intervals) among the 175 patients with psoriasis managed at our facility (Table 3). For the A1298C polymorphism, the CC type was not seen in any case.

Differences in response to treatment were analyzed in relation to the *MTHFR* polymorphisms. Cases showing a 50% or more reduc-

tion in the eruption-covered area were rated as responders. Because the number of patients studied was not large enough in the present study, it was not possible to detect a significant difference in the response to treatment with MTX depending on the *MTHFR* polymorphism in patients with psoriasis, although such a difference has previously been reported for patients with rheumatoid arthritis.¹⁴⁾³⁹⁾ The drug was effective in psoriasis patients with positive presumed 677C-1298A SNP haplotypes (Case 1, 2, and 5), and they developed no side effects. Although we did not perform a family analysis for Case 6 to determine the haplotype, we considered that the *MTHFR*677 polymorphism was TT homozygote and the 1298 polymorphism was AA homozygote. Therefore, we presumed that the haplotype for Case 6 was 677T-1298A, and the patient showed adverse reactions (liver damage) to MTX.

Discussion

The present study was designed to analyze the association of *MTHFR* polymorphisms with the onset and severity of psoriasis,²²⁾ the treatment responses to MTX, and the incidence of adverse reactions to MTX.²³⁾ No evident associations were noted, and it was not possible to conclude if the *MTHFR* polymorphisms serve as genetic factors that can determine susceptibility to psoriasis. *MTHFR* has been reported to be associated with the onset of various diseases such as colorectal cancer,²⁾²⁴⁻²⁷⁾ and it was recently reported that gastric cancer in females correlated with the 677TT polymorphism.²⁸⁾ In addition, a correlation with schizophrenia has been pointed out by some investigators.²⁹⁾

A shortage of folic acid makes DNA more susceptible to damage, and thereby probably stimulates the onset of various diseases including malignant neoplasms.¹²⁾ Folic acid is degraded with enzymes involved in folic acid metabolism. Of these enzymes, *MTHFR* and MTR (methionine synthetase) are known to have gene polymorphisms. The *MTHFR* can be divided into the C677T and A1298C types, and SNP mutants of both types are known to affect the enzymatic activity of *MTHFR*.¹³⁾²⁴⁾

MTX is an anti-tumor and anti-rheumatic drug classified as an anti-folate drug.¹⁵⁾¹⁶⁾³⁰⁻³²⁾ This drug inhibits the activity of enzymes involved in the conversion of folic acid into active folic acid and thus inhibits the nucleic acid synthesis of folic acid, which results in the suppression of cell proliferation. Gubner et al.³³⁾ reported that MTX used for the treatment of arthritis simultaneously alleviated the symptoms of psoriasis. Ree et al.³⁴⁾ reported that the alleviation of psoriasis occurred after treatment with another anti-folate drug, aminopterin. Following these reports, anti-folate drugs began to be used extensively for the treatment of psoriasis.

In patients with rheumatoid arthritis, associations with *MTHFR* have been studied.³⁵⁾³⁶⁾ The following findings have been reported about the association of *MTHFR* polymorphisms with responses to MTX treatment and adverse reactions to MTX.¹⁴⁾³⁷⁻⁴⁰⁾ First, MTX was effective without no side effects in psoriasis patients with positive presumed 677C-1298A SNP haplotypes (Case 1, 2, and 5). Second,

in the present analysis of the association between MTX therapy for psoriasis and *MTHFR* SNP polymorphisms, Case 6 possessed the presumed 677T-1298A haplotype in which adverse reactions of liver damage to MTX were seen. The incidence of adverse reactions such as hepatopathy was significantly increased in patients possessing the T allele of the C677T polymorphism (CT, TT).¹⁴⁾

The result in Cases 6 is consistent with the above-mentioned findings from the previous studies on the association between *MTHFR* polymorphisms and the prediction of adverse reactions to MTX in patients with rheumatoid arthritis. In the *MTHFR* 1298AA cases, the MTX dose level was high and the CRP was also high 1 year after the start of treatment; this suggests that the alleviation of inflammation in these cases was less.¹⁰⁾

The incidence of adverse reactions such as hepatopathy was significantly increased in the patients having the 677T-1298A haplotype, as shown in the Case 6. This may be viewed as one of the haplotypes that determine the response and adverse reactions to MTX, regardless of the type of disease for which this drug is used. In the patients analyzed in the present study, the MTX dose level was fixed at 7.5 mg/week in accordance with the method reported by Weinstein et al. who explored the methods of MTX treatment that could reinforce efficacy while still suppressing adverse reactions.¹⁹⁾ If the MTX dose level had been increased for the severe cases, there might have been a difference in the MTX treatment efficacy due to *MTHFR* polymorphisms in the present study; this finding would have been similar to that reported for patients with rheumatoid arthritis.¹⁴⁾ Regarding the C677T polymorphism, the present study revealed that the frequency of CC was 34% for patients with psoriasis vulgaris, suggesting the possibility that MTX therapy can be performed relatively safely in this group of patients with psoriasis.

In conclusion, the results from the present study do not conclusively prove that *MTHFR* polymorphisms serve as a factor determining the susceptibility to psoriasis. However, the results suggest that the treatment response to drug therapy in patients with psoriasis may be determined by *MTHFR* polymorphisms, with *MTHFR* 677CC serving as a predictor of high

drug sensitivity, and the *MTHFR* 677(CT, TT) and *MTHFR* 677T-1298A haplotypes serving as possible predictors of adverse effects for MTX.

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