Relationship between Genetic Polymorphisms and mRNA Expression of Dihydrofolate Reductase Enzyme in a Healthy Japanese Population

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Aim: We aimed to investigate the relationship between genetic polymorphisms and mRNA expression of dihydrofolate reductase (DHFR) in a healthy Japanese population.

Method: Two gene polymorphisms of DHFR (19-bp deletion allele and 3′UTR 829C>T) and mRNA expression of DHFR were evaluated in 100 unrelated healthy Japanese adults (47 men and 53 women). The genotype for DHFR 19-bp deletion was determined using the complete deletion/insertion method and that for DHFR 3′UTR 829C>T using the PCR-restriction fragment length polymorphism method. The mRNA expression of DHFR was determined by real-time PCR using RNA extracted from peripheral blood mononuclear cells.

Results: Allelic frequencies of DHFR 19-bp deletion in healthy Japanese adults were: wild allele 38% and deletion allele 62%. Allelic frequencies of DHFR 3′UTR 829C>T were: C allele 83% and T allele 17%. Median (25th-75th percentile) mRNA expression levels of DHFR intron 1 in wild/wild, wild/deletion, and deletion/deletion individuals were 0.53 (0.33-0.61), 0.28 (0.20-0.44), and 0.33 (0.24-0.52), respectively, with a significant difference between wild/wild and wild/deletion (P=0.010). Median mRNA expression of DHFR 3′UTR 829C>T in C/C and C/T genotypes were 0.29 (0.20-0.45) and 0.41 (0.25-0.62), respectively, with a significant difference between C/C and C/T (P=0.024).

Conclusion: Our healthy Japanese adults showed statistically significant differences in distribution of allelic frequencies for DHFR 19-bp deletion and 3′UTR 829C>T, and in mRNA expression of DHFR according to genotype. Therefore, the genetic polymorphisms and/or differences in mRNA expression of DHFR might contribute to the variation in efficacy and toxicity of methotrexate in patients with rheumatoid arthritis and other diseases.


Key words: dihydrofolate reductase, healthy Japanese population, genetic polymorphism, mRNA expression, methotrexate

Background

Methotrexate (MTX), which is widely used for the treatment of rheumatoid arthritis (RA) and acute lymphoblastic leukemia, inhibits dihydrofolate (DHF) reductase (DHFR) [EC1.5.1.3] and folate-dependent enzymes. The pharmacological effects of MTX have been shown to vary widely among individuals and show ethnic differences. The reasons for these differences are thought to involve several mechanisms in the cellular pathway of MTX, including single nucleotide polymorphisms (SNPs) in transport-
ers, glutamation, the folate pathway, and adenosine pathway\(^1\). We previously studied two gene polymorphisms at the site of action of MTX; TYMS 5’UTR (2R/3R) and 3’UTR 6-bp deletion as well as MTHFR 677C>T and 1298A>C, as factors related to interindividual, ethnic, and gender differences\(^2\).

Recently, DHFR, another site of action of MTX, has been reported to have a 19-bp deletion polymorphism located within DHFR intron 1 and a polymorphism for DHFR 3’UTR 829C>T\(^3\)\(^\text{−}^6\). A major mechanism of MTX action involves competitive inhibition of DHFR. This leads to impaired regeneration of tetrahydrofolate from DHF, resulting in a deficit of folate coenzymes and impairment of purine and pyrimidine synthesis\(^7\). DHFR may play an important role in the development of MTX resistance. In both experimental and clinical settings, altered levels of DHFR and/or decreased DHFR-MTX complex formation are found in relapsed patients and in cells manifesting the MTX-resistant phenotype\(^8\)\(^\text{−}^10\). Changes in DHFR expression level and consequently alteration in sensitivity to MTX may also be due to genetic polymorphisms, particularly those located in the regulatory elements. Therefore, the activity and/or quantity of enzyme may be affected by these genetic polymorphisms, although the relationship between DHFR genetic polymorphisms and the activity or quantity of DHFR is unclear. Moreover, there are few reports on DHFR 19-bp deletion polymorphism and the association between 3’UTR 829C>T genotype and DHFR gene expression in Japanese population\(^6\)\(^\text{−}^11\). We therefore undertook this study to clarify the relationship between genetic polymorphisms and mRNA expression of DHFR in a healthy Japanese population.

**Methods**

**Volunteers**

A total of 100 unrelated healthy adult Japanese volunteers (47 men and 53 women) older than 20 years of age were recruited from Honjo Clinic (Tokyo) and enrolled in this study. Their mean (± standard deviation) age was 30 ± 8 years (men 27 ± 7 years; women 32 ± 9 years). The study protocol was approved by the Ethics Committee of the Clinical Pharmacology Center, Honjo Clinic, and written informed consent was given by all volunteers prior to enrollment.

**Preparation of peripheral blood mononuclear cells and RNA extraction**

Ten milliliters of heparinized blood was collected from each volunteer, mixed well, and diluted in an equal volume of phosphate-buffered saline (PBS). Peripheral blood mononuclear cells (PBMCs) were then separated using Lymphoprep (Nycomed Pharma AS, Oslo, Norway) density centrifugation, washed twice in PBS, and then made up to a density of 2 × 10^6 cells/mL using PBS. The cell pellets were homogenized in Buffer RLT (Qiagen, Valencia, CA, USA). Total RNA was extracted from PBMCs using the Qiagen RNeasy Mini Kit (Qiagen). RNA extraction procedures were performed following the manufacturer’s instructions.

**DNA extraction**

For genetic analysis, a peripheral blood sample was collected from each volunteer and stored at −20°C until DNA extraction. DNA was extracted using the agglutination partition method (Sepa Gene, Sanko Junyaku, Ltd., Tokyo, Japan).

**Genotyping of alleles of the DHFR 19-bp deletion polymorphism**

Genotyping of the 19-bp deletion polymorphism in intron 1 of DHFR was performed according to the method previously described by Johnson et al\(^3\). For the nondeleted allele, the 3’ portion of primer #1 (forward) (5’-CCACGGTCGGGTACCTGGG-3’) rests within the 19-bp deletion region, while primer #3 (reverse) (5’-AAAAGGGGAATCCAGTCGG-3’) binds sufficiently far in the 3’ direction to give a product of 113 bp. For the deleted allele, primer #2 (forward) (5’-ACGGTCGGGGTGGCCGACTC-3’) bridges the gap caused by the 19-bp deletion, and primer #3 (reverse) is the same as that for the nondeletion allele resulting in a product of 113 bp. For the deleted allele, primer #2 (forward) (5’-ACGGTCGGGGTGGCCGACTC-3’) bridges the gap caused by the 19-bp deletion, and primer #3 (reverse) is the same as that for the nondeletion allele resulting in a product of 92 bp. The 50-μL PCR reaction mixture consisted of 20 ng of template (genomic DNA), 0.25 μL of 100 μM each of the three primers, 4 μL of 2.5 mM dNTP, 0.25 μL of Ex Taq, and 5 μL of Ex Taq Buffer (in MgCl2). Amplification was performed as follows: initial denaturation for 4 min at 94°C; 35 cycles at 94°C/55s, 68.8°C/55s, and 72°C/55s; and final extension for 10 min at 72°C. The PCR product was separated on an 8% acrylamide 1×TBE gel. Gels were stained with ethidium bromide for visualization of the separated DNA bands. Samples were genotyped in duplicate.
Genotyping of alleles of the DHFR 3’UTR 829C>T polymorphism

Genotyping of the DHFR 3’UTR 829C>T polymorphism was performed according to the method described by Goto et al. Total RNA was extracted from PBMCs using Lymphoprep. The first-strand cDNA was generated using 2 μg of total RNA, random hexadeoxynucleotide primer (Takara Bio Inc., Shiga, Japan), and reverse transcriptase (Takara Bio Inc.). The oligonucleotide primers for the DHFR gene were: forward primer, 5’-ATGAAGGTGTTTTCTAGT-3’ (sense); and reverse primer, 5’-AAGCTTTGGTTATTTCCA-3’ (antisense). The 50-μL PCR reaction mixture consisted of 0.5 μg (500 ng) of template (cDNA), 0.2 μL of 100pM of each primer, 4 μL of 2.5 mM dNTP, 0.25 μL of Ex Taq, and 5 μL of Ex Taq Buffer (in MgCl₂). Amplification was performed as follows: initial denaturation for 5 min at 94℃; 40 cycles at 95℃ /30s, 51℃ /30s, and 72℃ /60s; and final extension for 5 min at 72℃. The PCR product (10 μL) was digested with 1 μL of TspR (Takara Bio Inc.) at 65℃ for 6h. Finally, the digested PCR product was separated on 3% NuSieve GTG agarose gel and visualized with ethidium bromide staining. Samples were genotyped in duplicate.

Complimentary DNA synthesis

Complimentary DNA was synthesized using an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA USA). The eluted RNA was denatured for 3 min at 70℃ and then cooled on ice. The 20-μL reaction mixture consisted of 0.5 μg of total RNA, 4 μL of 5×iScript Reaction Mix, and 1 μL of iScript Reverse Transcriptase. Complimentary DNA was amplified using the following procedure: denaturation for 5 min at 25℃; extension for 30 min at 42℃; extension for 5 min at 85℃. The amplified DNA was stored at −20℃ until use.

Real-time PCR

TaqMan probes were synthesized by the Applied Biosystems (Life Technologies Japan, Tokyo, Japan). Real-time PCR was performed using the TaqMan probe method, in which the oligonucleotide probes are modified with a fluorescent substance (FAM) at the 5’-end and a quencher substance at the 3’-end (TAMRA). Real-time PCR amplification in DHFR was performed in a 20-μL reaction mixture consisted of 2 μL of cDNA, 0.4 μL each of forward primer (5’-AAACTGCATCGTGCTGTGTC-3’), reverse primer (5’-AAACCATATTACCGAGATTCTGT-3’), and probe [5’ (FAM)-CGTTCTTGCCGATGCCCATGTTCT-3’ (TAMRA)], and 10 μL of Master Mix. Amplification was performed as follows: AmpErase uracil N-glycosylase treatment for 2 min at 50℃; initial denaturation for 10 min at 95℃; and 40 cycles at 95℃ for 15s and 60℃ for 1 min.

Real-time PCR amplification in GAPDH was performed in a 20-μL reaction mixture consisted of 2.0 μL of cDNA, 0.4 μL each of forward primer (5’-GAAGGTGAAGTGGAGTGC-3’), reverse primer (5’-GAAGATGGTGATGGGATTTC-3’) and probe [5’ (FAM)CAAGCTTCCGGTTCAGCC(TAMRA)-3’], and 10 μL of Master Mix. The PCR was performed under the same conditions as for DHFR.

The reaction product was quantified by the standard curve method. A standard curve with predetermined concentrations and the respective PCR amplification products serially diluted from 1×10⁻² to 1×10⁻⁷ was constructed for each transcript analysed. The relative expression levels of DHFR mRNA were normalized to the GAPDH values.

Statistical analysis

Evaluation of the distribution of allelic frequencies among genotypes and/or gender differences was performed using the chi-square test and Fisher’s exact test. The Kruskal-Wallis test was used to determine differences between the expression levels of DHFR mRNA and a specific genotype. A P value less than 0.05 was considered significant in all statistical analyses. For comparisons among three groups, the statistically significant value of 0.05 was corrected using Bonferroni’s inequality. All statistical analyses were performed using SPSS for Windows software ver.14.0 J (SPSS Inc., Chicago, IL, USA).

Results

Allelic frequencies of DHFR intron 1 (wild/19-bp deletion) and DHFR 3’UTR 829C>T in healthy Japanese adults

The distribution of DHFR intron 1 (wild/19-bp deletion) and DHFR 3’UTR 829C>T polymorphisms in 100 healthy Japanese adults are summarized in Table. Allelic frequencies of DHFR intron 1 19-bp deletion were: wild allele 38% and deletion allele 62%. Allelic frequencies of DHFR 3’UTR 829C>T
were: C allele 83% and T allele 17%. The proportions of genotypes at each site of DHFR intron 1 were generally in agreement with Hardy-Weinberg equilibrium. Therefore, these data confirmed that the proportions of genotypes at each site of DHFR intron 1 followed the Mendelian principle. However, the proportions of genotypes at each site of DHFR 3’UTR 829C>T were not in agreement with Hardy-Weinberg equilibrium, and therefore they did not follow the Mendelian principle.

Gender-specific distribution of genotypes in healthy Japanese adults

The distribution of allelic frequencies of DHFR intron 1 and DHFR 3’UTR 829C>T by gender are also summarized in Table. For the DHFR intron 1 genotypes, no significant difference in the distribution of allelic frequencies between men and women was found. On the other hand, the allelic frequencies of the DHFR 3’UTR 829C>T allele were 88/12% in men and 77/23% in women, and this difference was significant ($P=0.018$, Fisher’s exact test). In addition, the proportions of genotypes at each site in women did not conform to Hardy-Weinberg equilibrium. Therefore, these data confirmed that the frequencies of genotypes at each site did not follow the Mendelian principle in women.

Relationship between DHFR intron1 (wild/19-bp deletion) genotypes and DHFR mRNA expression

The DHFR mRNA expression level measured by real-time quantitative PCR analyses for each DHFR intron 1 (wild/19-bp deletion) genotype in 100 healthy Japanese adults is shown in Figure 1. The relative DHFR and GAPDH expression (DHFR/GAPDH normalized) was compared among genotypes. Median value is indicated as horizontal bar, and 25th–75th percentile as vertical bar.

![Fig. 1 Relationship between DHFR intron 1 (wild/19-bp deletion) genotypes and DHFR mRNA expression](image)

The distribution of DHFR enzyme gene polymorphisms in healthy Japanese adults

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele frequency (%)</th>
<th>HWE**</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHFR intron1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15 (15)</td>
<td>39 (39)</td>
</tr>
<tr>
<td>Men</td>
<td>9 (19)</td>
<td>22 (47)</td>
</tr>
<tr>
<td>Women</td>
<td>6 (12)</td>
<td>24 (45)</td>
</tr>
<tr>
<td>3’UTR 829C&gt;T</td>
<td>C/C</td>
<td>C/T</td>
</tr>
<tr>
<td>Total</td>
<td>65 (65)</td>
<td>35 (35)</td>
</tr>
<tr>
<td>Men</td>
<td>36 (77)</td>
<td>11 (23)</td>
</tr>
<tr>
<td>Women</td>
<td>29 (55)</td>
<td>24 (45)</td>
</tr>
</tbody>
</table>

Values are given as N (%); total N=100; 47 men and 53 women.

* $P<0.05$, significantly different DHFR 3’UTR allele frequency in men and women.

** HWE = $P$ value in $\chi^2$ test for agreement with Hardy-Weinberg equilibrium.

HWE: Hardy-Weinberg equilibrium; DHFR: dihydrofolate reductase. Genotypes: wild; wild-type allele, del; 19-bp deletion allele.

Gender-specific distribution of genotypes in healthy Japanese adults

The distribution of allelic frequencies of DHFR intron 1 and DHFR 3’UTR 829C>T by gender are also summarized in Table. For the DHFR intron 1 genotypes, no significant difference in the distribution of allelic frequencies between men and women was found. On the other hand, the allelic frequencies of the DHFR 3’UTR 829C>T allele were 88/12% in men and 77/23% in women, and this difference was significant ($P=0.018$, Fisher’s exact test). In addition, the proportions of genotypes at each site in women did not conform to Hardy-Weinberg equilibrium. Therefore, these data confirmed that the frequencies of genotypes at each site did not follow the Mendelian principle in women.

Relationship between DHFR intron1 (wild/19-bp deletion) genotypes and DHFR mRNA expression

The DHFR mRNA expression level measured by real-time quantitative PCR analyses for each DHFR intron 1 (wild/19-bp deletion) genotype in 100 healthy Japanese adults is shown in Figure 1. The relative expression [median(25th–75th percentile)] in healthy Japanese samples with wild/wild (N=15), wild/deletion (N=46), and deletion/deletion (N=39) was 0.53 (0.33–0.61), 0.28 (0.20–0.44), and 0.33 (0.24–0.52), respectively. The distribution (median) of mRNA expression of the DHFR transcript was significantly different between the wild/wild and wild/deletion genotypes ($P=0.010$).

Comparing the combination of wild/deletion and deletion/deletion genotypes with the wild/wild genotype, the (median) mRNA expression level of the DHFR transcript was significantly higher in the wild/wild (0.53 [0.33–0.61], N=15) genotype than in that of the wild/deletion + deletion/deletion (0.32 [0.21–0.52]).
The DHFR mRNA expression level measured by real-time quantitative PCR analyses for each DHFR 3'UTR 829C>T genotypes is shown in Figure 2. The relative expression (median) in healthy Japanese samples with C/C (N=65) and C/T (N=35) was 0.29 (0.20-0.45) and 0.41 (0.25-0.62), respectively. The distribution (median) of mRNA expression of the DHFR transcript was significantly different between the C/C and C/T genotypes (P=0.024).

### Relationship between DHFR 3'UTR 829C>T genotypes and DHFR mRNA expression

The DHFR mRNA expression levels for combinations of DHFR intron 1 (wild/19-bp deletion) and 3'UTR 829C>T genotypes are shown in Figure 3. The relative expression (median) in healthy Japanese samples with wild/wild and C/C (N=11), wild/deletion and C/C (N=33), deletion/deletion and C/C (N=21), wild/wild and C/T (N=4), wild/deletion and C/T (N=13), and deletion/deletion and C/T (N=18) was 0.53 (0.29-0.61), 0.28 (0.20-0.38), 0.31 (0.19-0.36), 0.49 (0.41-0.76), 0.32 (0.23-0.51), and 0.40 (0.33-0.66), respectively. There were no statistically significant differences among all combination groups. However, for the combination of wild/deletion, deletion/deletion in C/C genotypes (N=54), the distribution (median) of mRNA expression of the DHFR transcript in samples with wild/wild and C/C (N=11) was high, and there was a statistically significant difference between wild/wild, wild/deletion, and deletion/deletion in C/C genotypes [0.49 (0.41-0.76), N=54] (P=0.028). For the combination of wild/deletion and deletion/deletion in C/T genotypes, there was no statistically significant difference between the two groups; the expression level was 0.49 (0.41-0.76) in wild/wild and C/T (N=4) and 0.39 (0.25-0.62) in wild/deletion, deletion/deletion and C/T (N=31) genotypes.

### Gender-specific DHFR mRNA expression in DHFR 3'UTR 829C>T genotypes

The DHFR mRNA expression levels according to gender are shown in Figure 4. The relative expression (median) in samples with C/C and C/T in men and women was 0.32 (0.20-0.44) (N=36), 0.28 (0.21-0.50) (N=29), 0.36 (0.23-0.47) (N=11), and 0.47 (0.27-0.83) (N=24), respectively. There were no significant differences between men and women for the three genotypes.
Gender-specific DHFR mRNA expression in DHFR intron 1 (wild/19-bp deletion) genotypes

The DHFR mRNA expression levels according to gender are shown in Figure 5. The relative expression (median) in samples with wild/wild, wild/deletion, and deletion/deletion in men and women were 0.56 (0.35–0.64) (N = 9), 0.40 (0.28–0.77) (N = 6), 0.28 (0.19–0.38) (N = 22), 0.28 (0.20–0.55) (N = 24), 0.32 (0.20–0.39) (N = 16), and 0.39 (0.23–0.73) (N = 23), respectively. There were no statistically significant differences between men and women for all three genotypes.

Comparison of DHFR mRNA expression for combinations of DHFR intron 1 (wild/19-bp deletion) and 3’UTR 829C>T genotypes in men and women

The DHFR mRNA expression levels for the combinations of DHFR intron 1 (wild/19-bp deletion) and 3’UTR 829C>T genotypes according to gender are shown in Figure 6. The relative expression (median) in samples with wild/wild and C/C, wild/deletion and C/T, wild/deletion and C/C, deletion/deletion and C/T, and deletion/deletion and C/C were 0.28 (N = 2), 0.41 (0.20–0.93) (N = 8), 0.28 (0.20–0.47) (N = 16), 0.45 (0.28–0.81) (N = 12), and 0.33 (0.19–0.73) (N = 11), respectively. There were no significant differences between men and women for all the genotype combinations.

Discussion

In this study, we investigated the frequencies of two gene polymorphisms: DHFR intron 1 (wild/19-bp deletion) and 3’UTR 829C>T in healthy Japanese adults, which affect the therapeutic response to MTX. We also analyzed the relationship between the genotypes and/or gender with DHFR mRNA expression. A previous report has described the DHFR intron (wild/deletion) gene in this population. In the present study, we found that the frequencies of DHFR intron wild/wild, wild/deletion, and deletion/deletion genotypes were 15%, 46%, and 39%, respectively, in healthy Japanese adults. Allelic frequencies of the wild and deletion alleles were 38% and 62%, respectively. No difference in allelic frequency was found between men and women. The genotype frequency of DHFR intron (wild/deletion) in this
The intron 1 sequence is known to be involved in transcriptional regulation by binding to a transcription factor in the mouse dhfr gene, which is 76.5% homologous to the human DHFR gene\textsuperscript{12}. Moreover, the 19-bp deletion mutation of DHFR intron 1 is believed to decrease the expression of mRNA by deleting the binding site of the transcription factor and thereby decreasing the activity of DHFR\textsuperscript{3}.

In this study, DHFR mRNA expression (median) in DHFR intron 1 (wild/19-bp deletion) genotypes was high [0.53 (0.33-0.61) in wild/wild versus 0.28 (0.20-0.44) in wild/deletion], and there was a statistically significant difference between wild/wild and wild/deletion ($P=0.010$). Our results thus showed that there was an association between DHFR intron 1 (wild/19-bp deletion) genotypes and DHFR mRNA expression. Therefore, we compared wild/wild homozygotes with a combination of wild/deletion and deletion/deletion and found that the DHFR mRNA expression of wild/wild homozygotes was higher compared with a combination of wild/deletion + deletion/deletion ($P=0.019$). These results suggest that DHFR mRNA expression decreases in the presence of the 19-bp deletion mutation in DHFR intron 1.

Goto et al.\textsuperscript{6} investigated DHFR 3’UTR 829C>T gene polymorphisms in 37 patients with childhood leukemias/lymphomas and 83 healthy Japanese children. Our study had no T/T genotype, while Goto et al. found frequencies of 5.4% and 6.0% in patients with childhood leukemias/lymphomas and healthy Japanese children, respectively. However, the frequency of C allele in our study was 83%, while it was 89% (childhood leukemias/lymphomas patients) and 84% (healthy children) in the study by Goto et al. Although there was no statistically significant difference in these allele frequencies, our results tend to confirm the results of Goto et al.\textsuperscript{6} When gender difference was analyzed, the frequency of C allele was 77% in women and 88% in men ($P=0.018$).

In the analyses of the DHFR 3’UTR 829C>T gene polymorphism and its effect on DHFR mRNA expression, we were not able to evaluate DHFR mRNA expression in the T/T genotype because none of our samples had this genotype. DHFR mRNA expression (median) in DHFR 3’UTR 829C>T was 0.29 (0.20-0.45) in C/C genotype and 0.41 (0.25-0.62) in C/T genotype samples ($P=0.024$). Since DHFR mRNA expression in the C allele in DHFR 3’UTR 829C>T is less frequent than that in the T allele and the activity of DHFR is lower in the C allele\textsuperscript{6}, it is believed that the therapeutic response to
MTX is higher in individuals with the C allele than those with the T allele. Several groups have reported that men respond well to MTX treatment\(^{13−15}\). In this study, the C allele frequency was higher in male samples, and this may be one reason for the finding that overall men are better responders to MTX than women\(^{13−15}\). Further study in large populations and/or patients with RA is necessary to evaluate the relationship between the 3’UTR 829C>T gene polymorphism and DHFR mRNA expression.

This study had several limitations. The proportions of genotypes at each site for DHFR 3’UTR 829C>T were not in agreement with Hardy-Weinberg equilibrium, especially in women. Therefore, the proportions of genotypes at each site for DHFR 3’UTR 829C>T do not follow the Mendelian principle. A possible reason may have been our small sample size (100 healthy volunteers). Therefore, we need to increase the study population in future investigations. Moreover, since there was no T/T genotype for, we were not able to evaluate DHFR mRNA expression in this genotype. It is therefore necessary to evaluate the combinations of these two gene polymorphisms and the relevance of DHFR mRNA in the future. The relationship among DHFR mRNA expression, gene polymorphisms, and DHFR enzyme activity also should be evaluated. In addition, we were not able to evaluate the effects of DHFR intron 1 (wild/19-bp deletion) and DHFR 3’UTR 829C>T gene polymorphisms on DHFR mRNA expression in patients with RA and other diseases in this study.

Conclusion

Our healthy Japanese adults showed statistically significant differences in the distribution of allelic frequencies of DHFR intron 1 and 3’UTR 829C>T. In addition, the median mRNA expression of DHFR differed according to genotype. Therefore, these genetic polymorphisms and/or mRNA expression of DHFR might contribute to the variations in efficacy and toxicity of MTX in patients with RA and other diseases.

Acknowledgement

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Conflict of Interest

The authors have no conflict of interest to declare.

References