Genetic polymorphisms of enzyme proteins and transporters related to methotrexate response and pharmacokinetics in a Japanese population

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Abstract

Background: Methotrexate (MTX) is currently the anchor drug widely used worldwide in the treatment of rheumatoid arthritis (RA). However, the therapeutic response to MTX has been shown to vary widely among individuals, genders and ethnic groups. The reason for this has been not clarified but it is considered to be partially due to several mechanisms in the cellular pathway of MTX including single-nucleotide polymorphisms (SNPs). The purpose of this study was to investigate the allelic frequencies in different ethnic and/or population groups in the 10 polymorphisms of enzyme proteins and transporters related to the MTX response and pharmacokinetics including MTHFR, TYMS, RFC1, FPGS, GGH, ABCB1, ABCC2 and ABCG2 in unrelated healthy Japanese adults and patients with RA.

Methods: Ten polymorphisms, methylenetetrahydrofolate reductase (MTHFR) 1298, thymidylate synthase (TYMS) 3'-UTR, reduced folate carrier 1 (RFC1) 80 and −43, folypolyglutamyl synthase (FPGS) 1994, γ-glutamyl hydrolase (GGH) −401, the ABC transporters (ABCB1 3435, ABCC2 IVS23 + 56, ABCG2 914) of enzyme proteins and transporters related to MTX response and pharmacokinetics in 299 unrelated healthy Japanese adults and 159 Japanese patients with RA were investigated to clarify their contributions to individual variations in response and safety to MTX and establish personalized MTX therapy. SNPs were evaluated using real-time polymerase chain reaction (PCR).

Results: Comparison of allelic frequencies in our study with other ethnic/population groups of healthy adults and RA patients showed significant differences in 10 polymorphisms among healthy adults and 7 among RA patients. Allelic frequencies of MTHFR 1298 C, FPGS 1994A and ABCB1 3435 T were lower in Japanese than in Caucasian populations and those of ABCC2 IVS23 + 56 C and ABCG2 914A were higher in Japanese than in Caucasian/European populations in both healthy adults and RA patients. Allelic frequencies of MTHFR 1298 C, GGH−401 T, ABCB1 3435 T, and ABCC2 914A were higher in healthy Japanese adults than in an African population, and those of RFC1 80A, RFC1−43C and ABCC2 IVS23 + 56 C in healthy Japanese adults were lower than in Africans. However, no significant differences were seen in the distribution of allelic frequencies between healthy Japanese adults and RA patients.

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Background

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disease and its pathogenesis remains unclear. Although no curative therapy for RA has been established, the therapeutic goal is to delay symptom progression using disease-modifying anti-rheumatic drugs, gold preparations and biologics and achieve pain relief using nonsteroidal anti-inflammatory drugs (DMARDs). Among the therapeutic options, methotrexate (MTX) is currently the anchor drug widely used worldwide in the treatment of RA because it is inexpensive, effective and safe. In the 2014 Japanese therapeutic guidelines for RA treatment published by the Japan College of Rheumatology (JCR) [1], MTX was also cited as a first-line drug. However, the therapeutic response to MTX has been shown to vary widely among individuals, genders and ethnic groups [2, 3]. The reason for this has been not clarified but it is considered to be partially due to several mechanisms in the cellular pathway of MTX including single-nucleotide polymorphisms (SNPs) in transporters, glutamation, the folate pathway and adenosine pathway [2] such as reduced folate carrier 1 (RFC1), folypolyglutamyl synthase (FPGS), γ-glutamyl hydrolase (GGH), the ABC transporters ABCB1, ABCC2 and ABCG2, methenyltetrahydrofolate reductase (MTHFR), and thymidylate synthase (TYMS). We previously reported that the distribution of MTHFR C677T between black and Japanese populations, of TYMS 3'-UTR alleles between Caucasian or black and Japanese populations, and of TYMS 3'-UTR alleles between Caucasian and Japanese populations showed significant differences, as well as gender differences in TYMS 3'-UTR allelic frequency in Japanese [3].

Up to the present, a clear genetic difference has not been confirmed between healthy individuals and RA patients which would indicate susceptibility to RA, as such genetic variations do for many other diseases. There are few data on the possible genetic differences in RA, and therefore, it is important to clarify them to obtain useful information from the viewpoint of human genetics and/or population genetics, leading to the establishment of personalized medicine for those susceptible to the development of RA.

The purpose of this study was to investigate the allelic frequencies in different ethnic and/or population groups in healthy adults and RA patients in the 10 polymorphisms of enzyme proteins and transporters related to MTX response and pharmacokinetics including MTHFR, TYMS, RFC1, FPGS, GGH, ABCB1, ABCC2 and ABCG2 in unrelated healthy Japanese adults and patients with RA.

Methods

Volunteers

A total of 299 unrelated healthy Japanese adult volunteers (148 men and 151 women) were recruited from Sumida Hospital (Tokyo) and 159 unrelated Japanese adult patients with RA (40 men and 119 women) admitted to PS Clinic (Fukuoka, Kyushu) were enrolled in this study. All were older than 20 years of age. MTX was administered to 99 (62.3%) of 159 RA patients, while 43 (27%) were receiving DMARDs, and 55 (34.6%) biologics as combination drugs. The daily dosage of MTX was 4 mg (3 patients), 6 mg (11 patients), 8 mg (75 patients), 10 mg (7 patients), and 12 mg (3 patients).

DNA extraction

For genetic analysis, a 5-mL peripheral blood sample was obtained from each study participant using the standard venipuncture technique. The samples were placed in tubes containing ethylenediaminetetraacetic disodium salt (EDTA 2Na) and stored at −20 °C until DNA extraction. DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer’s instructions. Total genomic DNA was quantified and its purity and integrity were analyzed using the NanoDrop 1000 spectrophotometer v3.7 (Thermo Scientific, Wilmington, DE, USA).

Allele genotyping

Genotyping of alleles of MTHFR 1298A > C (rs1801131), TYMS 3'-UTR (−6/+6) (rs16430), RFC1 80G > A (rs1051266), RFC1−43 T > C (rs1131596), FPGS 1994G > A (rs10106), GGH 452C > T (rs11545078), GGH 401C > T (rs3758149), ABCB1 3435C > T (rs1045642), ABCC2 IVS23 + 56 T > C (rs4148396), and ABCG2 914C > A (rs2231142) was performed using the TaqMan SNP Genotyping Assay from Applied Biosystems (Foster City, CA, USA) with fluorogenic binding probes. PCR amplification with the real-time PCR method was performed in 25 μL of reaction mixture including genomic DNA 20 ng (60 ng for FPGS 1994G > A), 0.63 μL of 40 × TaqMan SNP
Genotyping Assay Mix (0.32 μL for RFC1 80G > A), and TaqMan Universal PCR Master Mix 12.5 μL. The PCR reaction conditions were: initial denaturation for 10 min at 95 °C; 40 cycles at 92 °C/15 s; and annealing and extension for 1 min at 60 °C (or 55 °C for FPGS 1994G > A). For genotyping of alleles of ABCB1 3435C > T and ABCG2 914C > A, the PCR reaction conditions were: initial denaturation for 10 min at 95 °C; 50 cycles at 92 °C/15 s; and annealing and extension for 1 min at 60 °C. The PCR system used was the StepOnePlus real-time PCR system (Applied Biosystems).

Assessment of ethnic or population and gender differences

We identified eligible comparative studies by searching MEDLINE (1966–May 2016), Embase (1974–May 2016), the Cochrane Library (Issue 1 of 12, January 2016), Japana Centra Revuo Medicina (Ichushi) (1981–2016), the Cochrane Library (Issue 1 of 12, January 2016), and the database of the HapMap Project (accessed in May 2016) [4] on the assumption that it contained healthy adult data. The search terms were MTHFR, methylene tetrahydrofolate reductase, TS, TYMS, thymidylate synthase, dihydrofolate reductase, RFC1, reduced folate carrier 1, Solute carrier family 19 (folate transporter) member 1, SLC19A1, folylpolyglutamate synthase, FPGS, gamma-glutamyl hydrolase, GGH, ATP-binding Cassette Sub-family B Member 1, ABCB1, ATP-binding cassette sub-family C member 2, ABCG2, ATP-binding cassette transporter G2 and ABCG2, in combination with genotype and polymorphism. All languages were included. Additionally, a manual search of reference listings from all of the articles retrieved from the electronic databases was performed. Selection criteria were case–control, cross-sectional or prospective cohort studies, which assessed distributions of the MTHFR 1298A > C, RFC1 80 G > A, RFC–43 T > C, FPGS 1994 G > A, GGH–401 C > T, ABCC2 IVS23 + 56 T > C in healthy adults and RFC1 80 G > A, and ABCG2 914 C > A alleles in healthy adults between our Japanese population and Caucasians (Table 2). The number of individuals and published genotype frequency data for each ethnic/population group and comparisons with our Japanese allelic frequency results are shown in Tables 1 and 2.

Results

Allelic frequencies in healthy adult populations and RA patients

Among the 298 studies retrieved from the electronic databases and those retrieved from their references by manual search, a total of 14,000 healthy adults from 16 studies [4–19] and 4284 RA patients from 25 studies [5, 6, 9, 14, 20–40], involving HapMap Projects [4] that described Japanese, Asian, European and African groups, were included in the comparative analysis (Tables 1 and 2). Comparisons of allelic frequencies of MTHFR 1298 A > C allele in Caucasian [23–31] in RA patients were also not in agreement with Hardy-Weinberg equilibrium. The distributions of the MTHFR 1298 A > C allele in Caucasian [23–31] in RA patients were also not in agreement with Hardy-Weinberg equilibrium.

Statistically significant differences were found in the distributions of the MTHFR 1298A > C, RFC1 80 G > A, RFC–43 T > C, FPGS 1994 G > A, GGH–401 C > T, ABCCB1 3435 C > T, ABCG2 IVS23 + 56 T > C alleles in healthy adults between our Japanese population and Caucasians and Africans. Statistically significant differences were found in the distributions of the RFC1 80 G > A and ABCG2 IVS23 + 56 T > C alleles in healthy adults between our Japanese population and Asians (Table 1).

Statistically significant differences were found in the distributions of the MTHFR 1298 A > C, TYMS 3’-UTR −6/+6, RFC1 80 G > A, RFC1–43 T > C, FPGS 1994 G > A, GGH 452 C > T, ABCB1 3435 C > T, ABCG2 IVS23 + 56 T > C in healthy adults and RA patients in which ethnic group was clearly identified. In case–control studies, only the allelic frequencies of the healthy adult control group were extracted. If we could not identify the ethnic group in the literature, population groups were assumed to be from the country of residence of the lead author.

Statistical analysis

Comparisons of ethnic and gender differences in the distribution of allelic frequencies among genotypes, and/or gender differences and tests for Hardy-Weinberg equilibrium were carried out using the chi-square test. A p-value of <0.05 was considered to represent a significant difference in all statistical analyses. All statistical analyses were performed using JMP 12.0.1 software (SAS Institute Inc., Cary, NC, USA).
Table 1 Comparison of distribution of MTX-related enzyme gene and transporter polymorphisms among ethnic or population group in healthy adults

| Ethnic/population group | n   | Genotype frequency (n) | HWE | Allele frequency (%) | p-value | Reference No. |
|-------------------------|-----|------------------------|-----|----------------------|---------|---------------|
|                         |     |                        |     |                      |         |               |
| MTHFR 1298              |     |                        |     |                      |         |               |
| Japanese (Our study)    | 299 | 206 (68.9)             | 84  | (28.1)               | 9 (3.0) | 0.902 83      |
| Japanese                | 477 | 316 (66.3)             | 146 | (30.6)               | 15 (3.1)| 0.707 82      |
| Asian                   | 176 | 114 (64.8)             | 54  | (30.7)               | 8 (4.5) | 0.623 80      |
| Caucasian               | 1315| 636 (48.3)             | 548 | (41.7)               | 131 (10.0)| 0.418 69   |
| African                 | 346 | 270 (78.0)             | 74  | (21.4)               | 2 (0.6) | 0.198 89      |
|                         |     |                        |     |                      |         |               |
| TYMS 3’-UTR             |     |                        |     |                      |         |               |
| Japanese (Our study)    | 299 | 127 (42.5)             | 137 | (45.8)               | 35 (11.7)| 0.833 65   |
| Japanese                | 239 | 106 (44.4)             | 114 | (47.7)               | 19 (7.9) | 0.123 68      |
| Asian                   | 176 | 114 (64.8)             | 54  | (30.7)               | 8 (4.5) | 0.623 80      |
| Caucasian               | 1315| 636 (48.3)             | 548 | (41.7)               | 131 (10.0)| 0.418 69   |
| African                 | 346 | 270 (78.0)             | 74  | (21.4)               | 2 (0.6) | 0.198 89      |
|                         |     |                        |     |                      |         |               |
| RFC1 80                 |     |                        |     |                      |         |               |
| Japanese (Our study)    | 299 | 52 (17.4)              | 155 | (51.8)               | 92 (30.8)| 0.336 43     |
| Japanese                | 172 | 34 (19.8)              | 84  | (48.8)               | 54 (31.4)| 0.897 44     |
| Asian                   | 164 | 46 (27.4)              | 78  | (47.6)               | 42 (25.6)| 0.533 51     |
| Caucasian               | 226 | 64 (28.3)              | 128 | (56.6)               | 34 (15.1)| 0.021 57     |
| African                 | 220 | 20 (9.1)               | 82  | (37.3)               | 118 (53.7)| 0.299 28   |
|                         |     |                        |     |                      |         |               |
| RFC1-43                 |     |                        |     |                      |         |               |
| Japanese (Our study)    | 299 | 52 (17.4)              | 155 | (51.8)               | 92 (30.8)| 0.336 43     |
| Japanese                | 172 | 34 (19.8)              | 84  | (48.8)               | 54 (31.4)| 0.897 44     |
| Asian                   | 164 | 46 (27.4)              | 78  | (47.6)               | 42 (25.6)| 0.533 51     |
| Caucasian               | 226 | 64 (28.3)              | 128 | (56.6)               | 34 (15.1)| 0.021 57     |
| African                 | 220 | 20 (9.1)               | 82  | (37.3)               | 118 (53.7)| 0.299 28   |
|                         |     |                        |     |                      |         |               |
| FPGS 1994               |     |                        |     |                      |         |               |
| Japanese (Our study)    | 299 | 140 (46.8)             | 128 | (42.8)               | 31 (10.4)| 0.828 68     |
| Japanese                | 170 | 74 (43.5)              | 74  | (43.5)               | 22 (13.0)| 0.606 65     |
| Asian                   | 630 | 315 (50.0)             | 251 | (39.8)               | 64 (10.2)| 0.185 70     |
| Caucasian               | 226 | 104 (46.0)             | 92  | (40.7)               | 30 (13.3)| 0.943 36     |
| African                 | 220 | 100 (45.5)             | 118 | (52.2)               | 74 (32.8)| 0.241 41     |
|                         |     |                        |     |                      |         |               |
| GGH 452                 |     |                        |     |                      |         |               |
| Japanese (Our study)    | 299 | 249 (83.3)             | 47  | (15.7)               | 3 (1.0) | 0.641 91     |
| Japanese                | 170 | 74 (43.5)              | 74  | (43.5)               | 22 (13.0)| 0.606 65     |
| Asian                   | 564 | 453 (80.3)             | 104 | (19.7)               | 7 (1.2) | 0.710 90     |
| Caucasian               | 209 | 155 (74.2)             | 49  | (23.4)               | 5 (2.4) | 0.633 86     |
| African                 | 332 | 284 (85.5)             | 44  | (13.3)               | 4 (1.2) | 0.135 92     |
|                         |     |                        |     |                      |         |               |
| GGH–401                 |     |                        |     |                      |         |               |
| Japanese (Our study)    | 299 | 175 (58.5)             | 110 | (36.8)               | 14 (4.7) | 0.531 77     |
| Japanese                | 86  | 34 (39.5)              | 42  | (48.8)               | 10 (11.7)| 0.583 64     |
| Asian                   | 564 | 453 (80.3)             | 104 | (19.7)               | 7 (1.2) | 0.710 90     |
| Caucasian               | 120 | 54 (45.0)              | 58  | (48.3)               | 8 (6.7) | 0.145 69     |
| African                 | 120 | 90 (75.0)              | 26  | (21.7)               | 4 (3.3) | 0.232 86     |
|                         |     |                        |     |                      |         |               |
| ABCB1 3435              |     |                        |     |                      |         |               |
| Japanese (Our study)    | 299 | 94 (31.4)              | 156 | (52.2)               | 49 (16.4)| 0.242 58     |
| Japanese                | 716 | 239 (33.4)             | 357 | (49.9)               | 120 (16.7)| 0.494 58   |
| Asian                   | 84  | 32 (38.1)              | 34  | (40.5)               | 18 (21.4)| 0.125 58     |
The distributions of MTHFR 1298 A > C, and TYMS 3'-UTR (−6/+6), RFC1 80G > A, RFC1−43 T > C, FPGS 1994G > A, GGH 452C > T, GGH−401C > T, ABCB1 3435C > T, ABC2 IVS 23 + 56 T > C, and ABCG2 914C > A polymorphisms in 159 Japanese RA patients are summarized in Table 3. The proportions of genotypes at each site for their transporter carrier proteins and enzymes were generally in agreement with Hardy-Weinberg equilibrium, following Mendelian principles. Only three genotype combination patterns occurred for RFC1 80G > A and RFC1−43 T > C, consisting of the 80G/G,−43 T/T genotype, 80G/A,−43 C/C genotype, and 80A/A,−43C/C genotype, respectively and a strong linkage disequilibrium was observed in healthy Japanese adults.

Gender differences in the allelic frequencies were also summarized in Table 3. Allelic frequencies of RFC1 80G > A and RFC1−43 T > C were 61% in men and 52% in women for the A and C alleles and those of ABC2 IVS23 + 56 T > C were 80% in men and 69% in women for the C allele. These differences in distribution were statistically significant (p = 0.0226 for RFC1 80G > A and RFC1−43 T > C, p = 0.0009 for ABC2 IVS23 + 56 T > C, chi-square test). No gender differences in the distribution of other genotypes were observed (Table 3), although a tendency for a difference by gender was noted in the distribution of FPGS 1994 G > A (p = 0.0775, chi-square test) and GGH 452 C > T (p = 0.097, chi-square test).

### Allelic frequencies and gender differences in frequencies in Japanese RA patients

The distributions of MTHFR 1298A > C, and TYMS 3'-UTR (−6/+6), RFC1 80G > A, RFC1−43 T > C, FPGS 1994G > A, GGH 452C > T, GGH−401C > T, ABCB1 3435C > T, ABC2 IVS 23 + 56 T > C, and ABCG2 914C > A polymorphisms in 159 Japanese RA patients are summarized in Table 3. The proportions of genotypes at each site for their transporter carrier proteins and enzymes were generally in agreement with Hardy-Weinberg equilibrium, following Mendelian principles. Only three genotype combination patterns occurred for RFC1 80G > A and RFC1−43 T > C, consisting of the 80G/G,−43 T/T genotype, 80G/A,−43 C/C genotype, and 80A/A,−43C/C genotype, respectively and a strong linkage disequilibrium was observed in Japanese RA patients. Allelic frequencies of TYMS 3'-UTR−6 > +6 were 38% in men and 32% in women for the +6 allele, and a significant gender difference in TYMS 3'-UTR−6 > +6 (p = 0.0064, chi-square test) was seen, but the proportions of genotypes in men were not in agreement with Hardy-Weinberg equilibrium (p = 0.003, chi-square test). Gender differences in the distribution of other genotypes were not observed (Table 3), although a tendency toward gender differences was seen in the distribution of MTHFR 1298 A > C (p = 0.0736, chi-square test).

### Comparison of allelic frequencies between healthy Japanese adults and RA patients

The distributions of MTHFR 1298A > C, TYMS 3'-UTR (−6/+6), RFC1 80G > A, RFC1−43 T > C, FPGS 1994G > A, GGH 452C > T, GGH−401C > T, ABCB1 3435C > T, ABC2 IVS 23 + 56 T > C, and ABCG2 914C > A polymorphisms in 299 healthy Japanese healthy adults and 159 Japanese RA patients are summarized in Table 3. There were no statistically significant differences in allelic frequencies between the healthy and RA groups.

### Discussion

We comprehensively investigated the allelic frequencies in the gene polymorphisms of enzymes and transporter proteins including MTHFR, TYMS, RFC1, FPGS, GGH, ABCB1, ABC2 and ABCG2, which affect
Table 2: Comparison of distribution of MTX-related enzyme gene and transporter polymorphisms among ethnic or population group in the patients with rheumatoid arthritis

| Ethnic/Population group | n    | Genotype frequency (n) | HWE | Allele frequency (%) | p-value | Reference No. |
|-------------------------|------|------------------------|-----|----------------------|---------|---------------|
|                         |      | A/A                   | A/C | C/C                  |         |               |
| MTHFR                  |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 109 (68.6)             | 42 (26.4) | 8 (5.0)         | 0.149   | [5, 6, 20, 21]|
| Japanese                | 357  | 232 (65.0)             | 107 (30.0) | 18 (5.0)       | 0.224   | [20, 22]      |
| Chinese                 | 93   | 63 (67.7)              | 29 (31.2) | 1 (1.1)        | 0.237   | [23]          |
| Caucasian               | 1828 | 809 (44.3)             | 785 (42.9) | 234 (12.8)     | 0.045   | [24]          |
| African-American        | 138  | 102 (74.0)             | 35 (25.0) | 1 (1.0)        | 0.278   | [25]          |
| TYMS 3'-UTR             |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 72 (45.3)              | 67 (42.1) | 10 (6.6)       | 0.477   | [26]          |
| Japanese                | 409  | 135 (19.8)             | 348 (51.1) | 198 (29.1)     | 0.421   | [27]          |
| Caucasian               | 106  | 10 (10.2)              | 37 (37.8) | 51 (52.0)      | 0.053   | [28]          |
| RFC1 80                 |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 34 (21.4)              | 74 (46.5) | 51 (32.1)      | 0.461   | [29]          |
| Japanese                | 681  | 135 (19.8)             | 348 (51.1) | 198 (29.1)     | 0.421   | [30]          |
| RFC1-43                 |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 34 (21.4)              | 74 (46.5) | 51 (32.1)      | 0.461   | [31]          |
| Caucasian               | 196  | 10 (10.2)              | 37 (37.8) | 51 (52.0)      | 0.053   | [32]          |
| RFC1-43                 |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 34 (21.4)              | 74 (46.5) | 51 (32.1)      | 0.461   | [33]          |
| Caucasian               | 196  | 10 (10.2)              | 37 (37.8) | 51 (52.0)      | 0.053   | [34]          |
| FPGS 1994               |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 134 (84.3)             | 22 (13.8) | 3 (1.9)        | 0.081   | [35]          |
| Japanese                | 142  | 129 (90.8)             | 13 (9.2)  | 0 (0.0)       | 0.568   | [36]          |
| Caucasian               | 571  | 479 (83.9)             | 91 (15.9) | 1 (0.2)        | 0.119   | [37]          |
| GGH 452                 |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 88 (55.3)              | 62 (39.0) | 9 (5.7)        | 0.654   | [38]          |
| Japanese                | 257  | 169 (65.8)             | 78 (30.3) | 10 (3.9)       | 0.790   | [39]          |
| ABCB1 3435              |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 54 (34.0)              | 82 (51.6) | 23 (14.4)      | 0.363   | [40]          |
| Japanese                | 174  | 61 (35.0)              | 80 (46.0) | 33 (19.0)      | 0.460   | [41]          |
| Caucasian               | 769  | 178 (23.1)             | 381 (49.5) | 210 (27.3)    | 0.838   | [42]          |
| ABCB2 IVS23 + 56        |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 6 (3.8)                | 55 (34.6) | 98 (61.6)      | 0.614   | [43]          |
| Caucasian               | 309  | 122 (39.5)             | 149 (48.2) | 38 (12.3)     | 0.467   | [44]          |
| ABCG2 914               |      |                       |     |                      |         |               |
| Japanese (Our study)    | 159  | 77 (48.4)              | 67 (42.1) | 15 (9.5)       | 0.939   | [45]          |
| Japanese                | 55   | 30 (54.5)              | 20 (36.4) | 5 (9.1)        | 0.537   | [46]          |
| Caucasian               | 190  | 149 (78.4)             | 40 (21.1) | 1 (0.5)        | 0.330   | [47]          |

Values are given as n (%). HWE: p-value for chi-square test for agreement with Hardy-Weinberg equilibrium. p-value: comparison our study (Japanese) with each ethnic/population group.

**MTHFR**: methylenetetrahydrofolate reductase, **TYMS**: thymidylate synthase, **RFC1**: reduced folate carrier 1, **FPGS**: folypolyglutamayl synthase, **GGH**: γ-glutamyl hydrolase, **ABCB1**: ATP binding cassette subfamily B member 1, **ABCC2**: ATP binding cassette subfamily C member 2, **ABCG2**: ATP binding cassette subfamily G member 2
| Genotype | Allele frequency (%) | p-value* | HWE | Genotype | Allele frequency (%) | p-value* | HWE |
|----------|-----------------------|-----------|-----|----------|-----------------------|-----------|-----|
| MTHFR 1298 |                       |           |     |          |                       |           |     |
| A/A      | 206 (68.9)            | 0.902     |     | A/A      | 109 (68.6)            | 0.149     |     |
| A/C      | 84 (28.1)             |           |     | A/C      | 42 (26.4)             |           |     |
| C/C      | 9 (3.0)               |           |     | C/C      | 8 (5.0)               |           |     |
| Total    | 299                   |           |     | Total    | 159                   |           |     |
| Men      | 107 (72.3)            |           |     | Men      | 60 (40.0)             |           |     |
| Women    | 99 (65.6)             |           |     | Women    | 49 (32.5)             |           |     |
| TYMS 3'-UTR |                   |           |     |          |                       |           |     |
| −6/−6    | 127 (42.5)            | 0.833     |     | −6/−6    | 72 (45.3)             | 0.478     |     |
| −6/+6    | 137 (45.8)            |           |     | −6/+6    | 67 (42.1)             |           |     |
| +6/+6    | 35 (11.7)             |           |     | +6/+6    | 20 (12.6)             |           |     |
| Total    | 309                   |           |     | Total    | 169                   |           |     |
| Men      | 64 (43.2)             |           |     | Men      | 34 (22.5)             |           |     |
| Women    | 63 (41.7)             |           |     | Women    | 30 (19.0)             |           |     |
| RFC1 80 |                       |           |     |          |                       |           |     |
| G/G      | 52 (17.4)             | 0.336     |     | G/G      | 34 (21.4)             | 0.461     |     |
| G/A      | 155 (51.8)            |           |     | G/A      | 74 (46.5)             |           |     |
| A/A      | 92 (30.8)             |           |     | A/A      | 51 (32.1)             |           |     |
| Total    | 299                   |           |     | Total    | 169                   |           |     |
| Men      | 17 (11.5)             |           |     | Men      | 9 (22.5)              |           |     |
| Women    | 35 (23.2)             |           |     | Women    | 25 (21.0)             |           |     |
| RFC1−43 |                       |           |     |          |                       |           |     |
| T/T      | 52 (17.4)             | 0.336     |     | T/T      | 34 (21.4)             | 0.461     |     |
| T/C      | 155 (51.8)            |           |     | T/C      | 74 (46.5)             |           |     |
| C/C      | 92 (30.8)             |           |     | C/C      | 51 (32.1)             |           |     |
| Total    | 299                   |           |     | Total    | 169                   |           |     |
| Men      | 17 (11.5)             |           |     | Men      | 9 (22.5)              |           |     |
| Women    | 35 (23.2)             |           |     | Women    | 25 (21.0)             |           |     |
| FPGS 1994 |                    |           |     |          |                       |           |     |
| G/G      | 140 (46.8)            | 0.828     |     | G/G      | 70 (44.0)             | 0.632     |     |
| G/A      | 128 (42.8)            |           |     | G/A      | 73 (45.9)             |           |     |
| A/A      | 31 (10.4)             |           |     | A/A      | 16 (10.1)             |           |     |
| Total    | 299                   |           |     | Total    | 169                   |           |     |
| Men      | 76 (51.3)             |           |     | Men      | 37 (23.5)             |           |     |
| Women    | 64 (42.4)             |           |     | Women    | 32 (19.9)             |           |     |
| GGH 452 |                       |           |     |          |                       |           |     |
| C/C      | 249 (83.3)            | 0.641     |     | C/C      | 134 (84.3)            | 0.081     |     |
| C/T      | 47 (15.7)             |           |     | C/T      | 22 (13.8)             |           |     |
| T/T      | 3 (1.0)               |           |     | T/T      | 3 (1.9)               |           |     |
| Total    | 299                   |           |     | Total    | 169                   |           |     |
| Men      | 124 (83.8)            |           |     | Men      | 88 (55.3)             |           |     |
| Women    | 125 (82.8)            |           |     | Women    | 56 (34.7)             |           |     |
| GGH−401 |                       |           |     |          |                       |           |     |
| C/C      | 175 (58.5)            | 0.531     |     | C/C      | 88 (55.3)             | 0.654     |     |
| C/T      | 110 (36.8)            |           |     | C/T      | 62 (39.0)             |           |     |
| T/T      | 14 (4.7)              |           |     | T/T      | 9 (5.7)               |           |     |
| Total    | 299                   |           |     | Total    | 169                   |           |     |
| Men      | 91 (61.5)             |           |     | Men      | 49 (30.4)             |           |     |
| Women    | 84 (55.6)             |           |     | Women    | 49 (32.5)             |           |     |
| ABCB1 3435 |                   |           |     |          |                       |           |     |
| C/C      | 94 (31.4)             | 0.242     |     | C/C      | 54 (34.0)             | 0.363     |     |
| C/T      | 156 (52.2)            |           |     | C/T      | 82 (51.6)             |           |     |
| T/T      | 49 (16.4)             |           |     | T/T      | 23 (14.4)             |           |     |
| Total    | 299                   |           |     | Total    | 169                   |           |     |
| Men      | 45 (30.4)             |           |     | Men      | 45 (30.4)             |           |     |
| Women    | 49 (32.5)             |           |     | Women    | 49 (32.5)             |           |     |
Table 3  Distribution of MTX-related enzyme gene and transporter polymorphisms in healthy Japanese adults and patients with rheumatoid arthritis (Continued)

|             | ABCC2 | IVS23 + S6 |  |  |  |  |  |  |  |  |  |
|-------------|-------|-----------|---|---|---|---|---|---|---|---|---|
|             | T/T   | T/C       | C/C | T | C   | T/T | T/C | C/C | T  | C  | T/T |
| Total       | 25 (8.4) | 103 (34.4) | 171 (57.2) | 26 | 74 | 0.099 | 6 (3.8) | 55 (34.6) | 98 (61.6) | 21 | 79 | 0.614 |
| Men         | 4 (2.7)  | 51 (34.5)  | 93 (62.8)  | 20 | 80 | 0.333 | 1 (2.5)  | 17 (42.5) | 22 (55.0) | 24 | 76 | 0.273 |
| Women       | 21 (13.9) | 52 (34.4)  | 78 (51.7)  | 31 | 69 | 0.0009 | 5 (4.2)  | 38 (31.9) | 76 (63.9) | 20 | 80 | 0.4574 |

|             |  |  |  |  |  |  |  |  |  |  |  |
|-------------|-------|-------|---|---|---|---|---|---|---|---|---|
|             | ABCG2 | 914   |  |  |  |  |  |  |  |  |  |
|             | C/C   | C/A   | A/A | C  | A   | C/C | C/A | A/A | C  | A  | C/C |
| Total       | 166 (55.5) | 111 (37.1) | 22 (7.4) | 74 | 26 | 0.565 | 77 (48.4) | 67 (42.2) | 15 (9.4) | 69 | 31 | 0.939 |
| Men         | 78 (52.7) | 57 (38.5) | 13 (8.8) | 72 | 28 | 0.579 | 49 (75) | 67 (40.0) | 5 (12.5) | 68 | 32 | 0.576 |
| Women       | 88 (58.3) | 54 (35.7) | 9 (6.0) | 76 | 24 | 0.5003 | 58 (48.7) | 51 (42.9) | 10 (8.4) | 70 | 30 | 0.7525 |

Values are given as n (%).

HWE: p-value for chi-square test for agreement with Hardy-Weinberg equilibrium. MTHFR: methylenetetrahydrofolate reductase, DHFR: dihydrofolate reductase, TYMS: thymidylate synthase, RFC1: reduced folate carrier 1, FPGS: folypolyglutamyl synthase, GGH: γ-glutamyl hydrolase, ABCB1: ATP binding cassette subfamily B member 1, ABCC2: ATP binding cassette subfamily C member 2, ABCG2: ATP binding cassette subfamily G member 2.

* p-value: comparison of men and women

** p-value: comparison of healthy adults and RA patients
MTX pharmacokinetics and the therapeutic response to it, between a healthy Japanese population and Japanese RA patients to obtain the fundamental data for the personalized MTX therapy for RA.

Characteristics of MTX-related enzyme gene and transporter polymorphisms are summarized in Table 4. In the comparison of allelic frequencies in ethnic and/or population groups in healthy adults and RA patients, the frequency of the MTHFR 1298 C allele in our Japanese study was significantly lower than in Caucasian [4, 7, 8], but higher than in Africans [4]. The MTHFR gene is associated with the generation of 5-methyl THF, the MTHFR 1298A > C polymorphism decreases MTHFR activity, and is thereby associated with MTX efficacy [41, 42] and toxicity [26, 43]. In terms of the effect of each SNP on MTX efficacy and toxicity, it is considered to be the greatest in Caucasians [4, 7, 8], followed by Japanese and Africans [4]. However, currently there are few data comparing Japanese with Africans in relation to the efficacy and toxicity of MTX treatment. As such one example, Hughes et al. [26] reported that there was an association between scores of MTX toxicity and the rs4846051 C allele, that is, a higher mean toxicity score in Japanese than in African-Americans, among African-Americans than among Caucasians, and haplotypes containing this allele in African-Americans, but not in Caucasians.

The frequency of RFC1 80 A and RFC1−43C alleles in the present Japanese study was found to be higher than in Caucasian healthy adults [4, 7, 8, 11] and RA patients [24, 34–36]. The present study found it to be lower in Japanese than in African healthy adults [4]. RA patients with the RFC1 80 A/A genotype had increased MTX-PG concentrations in RBCs [9, 41], and the mean MTX-PG concentration in RBCs in RA patients with that genotype was reported to be 3.4-fold greater than in other genotypes [44]. Those patients showed a good clinical response to MTX treatment [35]. On the other hand, the −43 T > C change decreased the expression of RFC1 protein in patients with RA [34]. The directions in MTX-PG influx between RFC1 80 G > A and −43 T > C are completely opposite. To clarify the contributions of these two genotypes to MTX-PG concentrations in RBCs, it will be necessary to measure the concentrations in RFC1 80G > A and RFC1−43 T > C RA patients.

The frequency of the FPGS 1994 A allele in our Japanese study was significantly lower than reported in Caucasian [4] and Africans [4]. FPGS 1994 A > G polymorphism reported to have no effect on MTX efficacy or toxicity [45], although there was an association between FPGS mRNA expression in peripheral blood mononuclear cells and a poor response to MTX in RA patients with that genotype [46]. Therefore, it is unknown whether the FPGS 1994 A > G polymorphism is associated with the inter-individual difference in MTX efficacy or toxicity.

For the frequency of the GGH 452 T allele, our Japanese study found it to be significantly lower than reported in Caucasian healthy individuals [4] and was the almost same as in Caucasian RA patients [27, 37, 38]. The GGH—401 T allelic frequency in our study was also lower than in HapMap-JPT [4] and in Caucasians [4], but it was higher than in Africans [4]. The GGH gene is

| Gene | SNP ID | SNP allele | Effects on gene product/ enzyme | Clinical significance |
|------|--------|------------|-------------------------------|----------------------|
| MTHFR | rs1801131 | 1298 A > C | Decreases MTHFR activity | Associated with MTX efficacy, not associated with MTX toxicity, or associated with MTX toxicity |
| TYMS | rs16430 | 3'-UTR - 6/6 | Decreases TYMS mRNA expression | Associated with MTX toxicity |
| RFC1 | rs1051266 | 80 G > A | Affects transcriptional activity of RFC1 gene and MTX entry into cells | Increases MTX-PG concentrations and good clinical response to MTX treatment |
| | rs1131596 | −43 T > C | Decreases the expression of RFC1 protein | Unknown |
| FPGS | rs10106 | 1994 G > A | Associated with FPGS mRNA expression | Not associated with MTX efficacy/toxicity, or poor response to MTX treatment |
| GGH | rs11545078 | 452 C > T | Associated with lower GGH activity, greater accumulation of long-chain MTX-PGs | Not associated with MTX efficacy/toxicity |
| | rs3758149 | −401 C > T | Associated with greater GGH promoter activity, decrease polyglutamimation | Affects MTX toxicity |
| ABCB1 | rs1045642 | 3435 C > T | Decreases stability/expression of mRNA, reduces the activity of efflux transporters | Associated with MTX efficacy |
| ABCB2 | rs4148396 | IVS23 + 56 T > C | Affects ABCB2 enzyme activity and MTX efflux from cell | Associated with MTX toxicity (adverse GI effects) |
| ABCG2 | rs2231142 | 914 C > A | Increases MTX-PG1/MTX-PG2 concentrations | Associated with MTX toxicity |

MTX: methotrexate, MTHFR: methylenetetrahydrofolate reductase, TYMS: thymidilate synthase, RFC1: reduced folate carrier 1, FPGS: folypolyglutamayl synthase, GGH: γ-glutamyl hydrolase, ABCB1: ATP-binding cassette sub-family B member 1, ABCB2: ATP-binding cassette subfamily C member 2, ABCG2: ATP-binding cassette sub-family G member 2, GI: gastrointestinal
involved in reversing polyglutamation by removing glutamate moieties, and a specific functional SNP (452C > T) in the human GGH gene is associated with lower catalytic activity and greater accumulation of long-chain MTX-PGs in leukemia cells of patients treated with high-dose MTX [47]. The −401C > T mutation resulting in greater GGH promoter activity increases the hydrolytic activity of MTX-PGs and decreases polyglutamation in the−401TT genotype compared with the−401CC or CT genotype [44]. Thus, MTX might be more effective in Caucasian than in Japanese RA patients at the same dose, when we speculate on the intracellular MTX-PG concentration.

The frequency of the ABCB1 3435 T allele in our study was lower than reported in Caucasians [4] but higher than in African healthy adults [4]. Conversely, the ABCC2 IVS23 + 56 C allelic frequency was higher in our study than reported in Caucasians [4] but lower than in Africans [4]. We also found a higher frequency of the ABCG2 914 A allele in our study than reported in Caucasians [4] and Africans [4]. The ABCB1 3435C > T mutation resulting in decreased stability and expression of mRNA reduces the activity of efflux transporters, may affect P-glycoprotein function and MTX efflux from cells [48], and is associated with MTX efficacy. It was reported that the length of time before it became necessary to reduce the MTX dose or discontinue administration due to the occurrence of toxicity was 2 months in patients with the T/T genotype, 23 months in those with the C/C genotype, and 29 months in those with the C/C genotype and the time period was significantly correlated with genotype [40]. Therefore, it is considered that the ABCC2 IVS23 + 56 C > T mutation may affect ABCC2 enzyme activity involved in MTX efflux from cells [40]. The ABCG2 914C > A mutation was reported to increase MTX-PG1 and MTX-PG2 concentrations in RA patients [27]. It is considered that MTX efficacy is the highest in Caucasians, followed by Japanese and Africans, from the viewpoint of ABCB1 3435C > T; conversely, it is the highest in Caucasians, followed by Japanese and Africans, from the viewpoint of ABCC2 IVS23 + 56 T > C and the highest in Japanese, followed by Caucasians and then Africans, from the viewpoint of ABCG2 914 C > A. However, we need to investigate the net contribution in MTX-PG efflux among three ABC transporters polymorphism.

There were no previous reports of RFC1−43 T > C, FPGS 1994 G > A, GGH−401 C > T, and ABCC2 IVS23 + 56 T > C in a healthy Japanese population except for HapMap-JPT and RFC1−43 T > C, FPGS 1994 G > A, and ABCC2 IVS23 + 56 T > C in Japanese patients with RA for the evaluation of allelic frequency. Therefore, this is the first comprehensive report on these genetic polymorphisms in healthy Japanese adults and Japanese RA patients.

When comparing healthy Japanese adults with Japanese RA patients, there were no significant differences in allelic frequencies of the MTHFR 1298A > C, TYMS 3′-UTR (−6/+6), RFC1 80G > A, RFC1−43 T > C, FPGS 1994G > A, GGH 452C > T, GGH−401C > T, ABCB1 3435C > T, ABCC2 IVS 23 + 56 T > C, and ABCG2 914C > A polymorphisms in 299 healthy Japanese adults and 159 Japanese RA patients. Therefore, we confirmed that the allelic frequencies of those SNPs in healthy Japanese adults were almost the same as in Japanese patients with RA in this study.

The allelic frequencies of SNPs in Japanese RA patients in this study were in agreement with Hardy-Weinberg equilibrium and were considered representative of the Japanese population as a whole. Chatzikyriakidou et al. [34] reported a strong linkage disequilibrium between RFC1 80 G > A and RFC1−43 T > C genotypes for only three patterns in the combination of the RFC1 80 G > A with RFC1−43 T > C genotype, that is, 80G/G and−43 T/ T, 80G/A and−43 T/C, and 80A/A and−43 C/C in Greek patients with RA. In agreement with the results of Chatzikyriakidou et al. [34], we also confirmed that the three patterns in the combination of the RFC1 80 G > A with RFC1−43 T > C genotype had a strong linkage disequilibrium between RFC1 80 G > A and RFC1−43 T > C genotypes in healthy Japanese adults and RA patients.

Gender differences in allelic frequencies of RFC1 80G > A, RFC1−43 T > C, and ABCC2 IVS23 + 56 T > C in healthy Japanese adults and TYMS 3′-UTR−6 + +6 in Japanese RA patients were observed. Meanwhile, Kameda et al. [48] reported that the tender joint count, swollen joint count and decrease in serum C-reactive protein (CRP) levels as indices of MTX treatment response in Japanese patients with RA were better in men than in women and gender differences in the MTX therapeutic response were suggested in Japanese RA patients. Currently, however, we cannot clearly explain the reason for the gender differences in these allelic frequencies between healthy Japanese adults and Japanese RA patients, but Hardy-Weinberg disequilibrium in the distribution of those SNPs in either men or women may be one factor responsible for the differences.

This study had several limitations: 1) There were only a small number of reports in the literature allowing comparisons of ethnic and/or population groups, because most did not clearly describe the ethnicity/race and/or population groups studied. These were assumed based on the country of residence of the lead author, and therefore might have misclassified ethnicity/race and/or population groups. 2) We collected allelic frequency data from the HapMap Project for comparisons with our Japanese study results in cases when sufficient data could not be retrieved from the literature search. Although the HapMap data contained the same rs numbers,
they included different ss numbers. 3) Several papers used for comparison with our results did not report the rs numbers for ethnic/race and/or population groups. Therefore, our comparisons may not be completely accurate in using the same rs numbers of SNPs. 4) All SNPs studied here could not be compared among ethnic/race and/or population groups due to a lack of data. This study may also include mixed ethnicities/races in a population group that we treated as a single national population, because that was assumed based on the lead author’s country of residence. 5) The distributions of several SNPs in the ethnic/population group in healthy adults and RA patients were also not in agreement with Hardy-Weinberg equilibrium. 6) We did not evaluate allelic frequencies using combinations of SNPs such as diplotypes or haplotypes in our Japanese population. 7) There are few studies of MTX pharmacokinetics among ethnic/population groups. We also did not investigate MTX-PGs pharmacokinetics among ethnic/population groups by comparing the genotypes of MTX-related SNPs to clarify interethnic variations in MTX pharmacokinetics and therapeutic response. This is the greatest limitation in this study.

Conclusion
This study identified allelic frequencies in ethnic and/or population groups compared with healthy Japanese and RA patients. The differences in frequencies may be variables in the interethnic variations in MTX response. Further study is needed to confirm the association of these genetic/phenotypic factors and clinical outcomes and whether these SNPs may be useful in determining personalized MTX therapy for RA.

Abbreviations
3′-UTR: 3′-untranslated region; ABCB1: ATP binding cassette subfamily B member 1; ABCB2: ATP-binding cassette subfamily C member 2; FCPS: Focopolyglutamyl synthase; GCPR: γ-glutamyl hydrolase; IVS: Intervening sequence; MTHFR: Methylenetetrahydrofolate reductase; RFC1: Reduced folate carrier 1; TYMS: Thymidylate synthase

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Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. Please contact the authors for data requests.

Authors’ contribution
MH, KM, JH, OT and MS carried out the genotype determination. TT, TT, MS, TC, JH and SI carried out the clinical study. MH, JH, KM and MS performed the statistical analyses. MH, KM, TT, SI, MS, JM and MW participated in study design and coordination and drafted the manuscript. MH, MS and MM completed the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The study protocol was approved by the Ethics Committee of the Clinical Pharmacology Center, Sumida Hospital (B-28) and PS Clinic (C-72), and written informed consent was given by all volunteers and patients prior to enrollment.

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References
1. Japan College of Rheumatology. Guideline for the management of rheumatoid arthritis. Osaka: Medical Review Co., Ltd. 2014.
2. Ranganathan P. An update on methotrexate pharmacogenetics in rheumatoid arthritis. Pharmacogenomics. 2006;9:439–51.
3. Inoue S, Hashiguchi M, Chiyoda T, Sunami Y, Tanaka T, Mochizuki M. Pharmacogenetic study of methylenetetrahydrofolate reductase and thymidylate synthase in Japanese and assessment of ethnic and gender differences. Pharmacogenomics. 2007;8:411–7.
4. National Center for Biotechnology Information SNP database (https://www.ncbi.nlm.nih.gov/snp). Accessed 1 May 2016.
5. Fukino K, Kawashima T, Suzuki M, Ueno K. Methylenetetrahydrofolate reductase and reduced folate carrier-1 genotypes and methotrexate serum concentrations in patients with rheumatoid arthritis. J Toxicol Sci. 2007;32(4):449–52.
6. Kumagai K, Hiyama K, Oyama T, Maeda H, Kohno N. Polymorphisms in the thymidylate synthase and methylenetetrahydrofolate reductase genes and sensitivity to the low-dose methotrexate therapy in patients with rheumatoid arthritis. Int J Mol Med. 2003;11(5)593–600.
7. Herrlinger KR, Cummings JR, Barnardo MC, Schwab M, Ahmad T, Jewell DP. The pharmacogenetics of methotrexate in inflammatory bowel disease. Pharmacogenet Genomics. 2005;15(10):705–11.
8. Kotopoulous J, Zhang WW, Zhang S, et al. Polymorphisms in folate metabolizing enzymes and transport proteins and the risk of breast cancer. Breast Cancer Res Treat. 2008;112(3):585–93.
9. Takatori R, Takahashi KA, Tokunaga D, et al. ABCB1 C3435T polymorphism influences methotrexate sensitivity in rheumatoid arthritis patients. Clin Exp Rheumatol. 2006;24(5):546–54.
10. Hiraoka M. Folate intake, serum folate, serum total homocysteine levels and methylenetetrahydrofolate reductase C677T polymorphism in young Japanese women. J Nutr Sci Vitaminol. 2004;50(4):238–45.
11. Summers CM, Mitchell LE, Stanislawksa-Sachadyn A, et al. Genetic and lifestyle variables associated with homocysteine concentrations and the distribution of folate derivatives in healthy premenopausal women. Birth Defects Res A Clin Mol Teratol. 2010;88(8):679–88.
12. Oppeneer SJ, Ross JA, Koh WP, Yuan JM, Robien K. Genetic variation in folypolyglutamyl synthase and gamma-glutamyl hydrolase and plasma homocysteine levels in the Singapore Chinese Health Study. Mol Genet Metab. 2012;105(1):73–8.
13. Hayashi H, Fujimaki C, Inoue K, Suzuki T, Itoh K. Genetic polymorphism of C452T (T1278) in human gamma-glutamyl hydrolase in a Japanese population. Biol Pharm Bull. 2007;30(4):839–41.
14. Hamada A, Kato T, Saito H, Mori S. Pharmacogenetic study to develop the personalized medicine of methotrexate monotherapy in patients with rheumatoid arthritis. Recent Adv Clin Pharmacol. 2010;31:154–62.

15. Komoto C, Nakamura T, Sakeeda T, et al. MDR1 haplotype frequencies in Japanese and Caucasian, and in Japanese patients with colorectal cancer and esophageal cancer. Drug Metab Pharmacokinet. 2006;21(2):126–52.

16. Honda M, Ogura Y, Toyoda W, et al. Multiple regression analysis of pharmacogenetic variation of caridilil disposition in 54 healthy Japanese volunteers. Bio Pharm Bull. 2006;29(4):772–28.

17. Sakaeda T, Nakamura T, Horinouchi M, et al. MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. Pharm Res. 2001;18(10):1400–4.

18. Tanabe M, Ieiri I, Nagata N, et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. J Pharmacol Exp Ther. 2001;297(3):1137–43.

19. Yamagishi K, Tanigawa T, Kitamura A, Köttingen A, Folsom A, Ishi H. CIRCS Investigators. The n232114 variant of the ABCG2 gene is associated with uric acid levels and gout among Japanese people. Rheumatology. 2010;49(8):1461–5.

20. Kato T, Hamada A, Mori S, Saito H. Genetic polymorphisms in metabolic and cellular transport pathway of methotrexate impact clinical outcome of methotrexate monotherapy in Japanese patients with rheumatoid arthritis. Drug Metab Pharmacokinet. 2011;27(2):192–9.

21. Hayashi H, Fujimaki C, Daimon T, Tsuboi S, Matsuyama T, Itoh K. Genetic polymorphisms in folate pathway enzymes as a possible marker for predicting the outcome of methotrexate therapy in Japanese patients with rheumatoid arthritis. J Clin Pharmacol Ther. 2009;34(3):355–61.

22. Xiao H, Xu J, Zhou X, et al. Associations between the genetic polymorphisms of MTHFR and outcomes of methotrexate treatment in rheumatoid arthritis. Clin Exp Rheumatol. 2010;28(5):728–33.

23. Palomo-Morales R, González-Juante C, Vazquez-Rodriguez TR, et al. A1298C polymorphism in the MTHFR gene predisposes to cardiovascular risk in rheumatoid arthritis. Arthritis Res Ther. 2010;12(2):R71.

24. James HM, Gillis D, Hisarla P, et al. Common polymorphisms in the folate pathway predict efficacy of combination regimens containing methotrexate and sulfasalazine in early rheumatoid arthritis. J Rheumatol. 2008;35(4):562–71.

25. Kurzawski M, Pawlik A, Safranow K, Herczynska M, Drodzak M. 677C > T and 1298A > C MTHFR polymorphisms affect methotrexate treatment outcome in rheumatoid arthritis. Pharmacogenomics. 2007;8(11):1551–9.

26. Hughes LH, Beasley TM, Patel H, et al. Racial or ethnic differences in allele frequencies of single-nucleotide polymorphisms in the methylene tetrahydrofolate reductase gene and their influence on response to methotrexate in rheumatoid arthritis. Ann Rheum Dis. 2006;65(9):1213–8.

27. Stamp LC, Chapman PT, O’Donnell JL, et al. Polymorphisms within the folate pathway predict folate concentrations but are not associated with disease activity in rheumatoid arthritis patients on methotrexate. Pharmacogenomics. 2010;11(6):567–76.

28. Pawlik A, Kurzawski M, Gawronska-Szklarz B, et al. The effect of 677C > T and 1298A > C MTHFR polymorphisms on sulfasalazine treatment outcome in rheumatoid arthritis. Braz J Med Biol Res. 2009;42(7):660–4.

29. Lee YC, Cui J, Costenbader KH, Shadick NA, Weinblatt ME, Karlson EW. Investigation of candidate polymorphisms and disease activity in rheumatoid arthritis patients on methotrexate. Rheumatology. 2009;48(6):613–20.

30. Pawlik A, Kurzawski M, Górnik W, Drobniak-Babkowska-Zamojcin E, Drodzak M. 677C > T and 1298A > C MTHFR polymorphisms affect arachin treatment outcome in rheumatoid arthritis. Pharmacol Rep. 2007;59(6):721–6.

31. Berkun Y, Levartovsky D, Rubinson A, et al. Methotrexate related adverse events in patients with rheumatoid arthritis are associated with the A1298C polymorphism of the MTHFR gene. Ann Rheum Dis. 2004;63(10):1227–31.

32. Hayashi H, Itoh J, Tsuboi S, et al. A single nucleotide polymorphism of reduced folate carrier 1 predicts methotrexate efficacy in Japanese patients with rheumatoid arthritis. Drug Metab Pharmacokinet. 2013;28(2):164–8.

33. Ando Y, Shimada H, Matsunoto N, et al. Role of methotrexate polyglutamation and reduced folate carrier 1 (RFC1) gene polymorphisms in clinical assessment indexes. Drug Metab Pharmacokinet. 2013;28(5):442–5.

34. Chatziykiadou A, Georgiou I, Voulgaris P, Papadopoulos CG, Tzavaras D, Drosos AA. Transepithelial regulatory polymorphism—43 T > C in the 5′-flanking region of SLCO1A1 gene could affect rheumatoid arthritis patient response to methotrexate therapy. Rheumatol Int. 2007;27(11):1057–61.

35. Drodzak M, Rudas T, Pawlik A, Górnik W, Kurzawski M, Herczynska M. Reduced folate carrier-1 B0G > A polymorphism affects methotrexate treatment outcome in rheumatoid arthritis. Pharmacogenomics J. 2007;7(6):404–7.