GENETIC POLYMORPHISM IN DIHYDROFOLATE REDUCTASE IMPACTS METHOTREXATE POLYGLUTAMATION IN ADULT RHEUMATOID ARTHRITIS

Thierry Dervieux[1], Marie Grosjean[2], Chuang Jiang[3,4], Kelley Brady[1], Kjeld Schmiegelow[2], Joel Kremer[5] and Jun Yang[4]

[1]Exagen Diagnostics, Vista, CA, USA; [2]University Hospital Rigshospitalet, Copenhagen, Denmark;
[3]Shanghai Jiaotong University School of Medicine, Shanghai Children's Medical Center,
[4]Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis TN USA;
[5]The Center of Rheumatology, Albany, NY, USA

P31025 (10/18)

ABSTRACT

PURPOSE

Methotrexate (MTX) is an anti-folate activated to MTX polyglutamates (MTXPGs). MTX metabolism includes multiple enzyme-mediated reactions and genetic polymorphisms in these genes are linked to MTX disposition and effects. The objective of this study was to examine the influences of single nucleotide polymorphism in the dihydrofolate reductase gene (DHFR: rs1382539 G/A) on MTX and clinical efficacy in rheumatoid arthritis (RA).

METHODS

Two U.S. based cohorts of consented adult RA patients were analyzed, the first cohort of 187 patients (median age 65 years under median 15 mg/weekly MTX) was enrolled at a single visit for MTXPG levels (>3 months), and a second cohort consisting of 38 patients (mean age 55 years) was enrolled in a dose escalation study (starting 7.5 mg/week) for 6 months (206 study visits). RBC MTXPG levels (MTXPG1 [MTX] up to 5 glutamic residues [MTXPG5]) were measured using liquid chromatography. MTXPG3 (the preponderant MTXPG species) was expressed as nmol/L packed RBC; percent long-chain MTXPG3-5 (over total MTXPG1-5) and dose normalized MTXPG3 levels (polyglutamation rate, nmol/L per mg) were also estimated. Real time-PCR was used to genotype the rs1382539 G/A variant in DHFR. Differences in MTXPG accumulation by genotypes were analyzed using Mann-Whitney test. Linear mixed effect with random intercept and fixed slope were used to analyze the impact of the variant on longitudinal changes in MTXPG levels and response (per DAS-28).

RESULTS

In the first cohort, carriers of the rs1382539 A/A genotype (n=15 patients [8%], mean age 64±12 years under 15 mg/week, respectively) presented with 15 nmol/L lower MTXPG3 levels (median 26 nmol/L [IQR: 18-55] vs 41 nmol/L [IQR: 28-60]; p=0.04) for those with G/G or GA genotypes (total 173 patients mean age 64±12 years under 15 mg/week). Similarly, 16% lower long-chain MTXPG3-5 (median 36% [IQR 18-48%] vs median 52% [IQR 40-68%]; p<0.01) and 0.8 nmol/L per mg lower MTX polyglutamation rate (median 2.1 nmol/mg MTX [IQR 1.2-3.1] vs 2.9 nmol/mg MTX [IQR 2.0-4.3]; p=0.02) were measured in carriers of the AA genotype vs GG or GA genotypes, respectively (Figure, panel A). In the second cohort, linear mixed effect models revealed that carriers of the rs1382539 A/A genotype (n=4 patients [10%]) also presented with lower RBC MTXPG3 levels (estimate=-11±5 nmol/L; p=0.03), lower percent long-chain MTXPG3-5 (estimate=-15±1%; p=0.02) and lower polyglutamate rate (estimate=-0.9±0.4 nmol/mg; p=0.01) than carriers of the GG or GA genotype. While the rs1382539 variant was not significantly associated with clinical efficacy (p>0.05), lower RBC MTXPG3 level and percent long-chain MTXPG3-5 levels associated with higher DAS28 in that cohort (p<0.05).

CONCLUSION

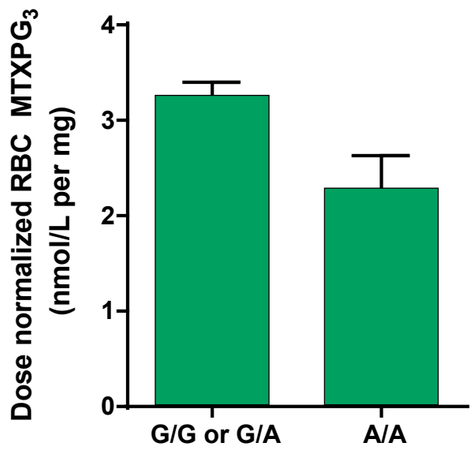
Our data indicate that the rs1382539 G/A variant in DHFR impacts MTX polyglutamation in adult RA, and may indirectly contribute to clinical efficacy in some patients

OBJECTIVE AND METHODS

- To examine the influences of variant in the dihydrofolate reductase gene (DHFR: rs1382539 G/A) on MTX accumulation
- Two US based cohorts of consented adult RA patients were analyzed
- Cohort1: N= 187 patients (median age 65 years under median 15 mg/weekly MTX) evaluated at a single visit for MTXPG levels (>3 months).
- Cohort 2: N= 38 patients (mean age 55 years) enrolled in a dose escalation study (starting 7.5 mg/week) for 6 months (206 study visits).
- RBC MTXPG levels (MTXPG1 [MTX] up to 5 glutamic residues [MTXPG5]) were measured using liquid chromatography.
- MTXPG3 was expressed as nmol/L packed RBC; percent long-chain MTXPG3-5 (over total MTXPG1-5) and dose normalized MTXPG3 levels (polyglutamation rate, nmol/L per mg) were also estimated.
- Real time-PCR was used to genotype the rs1382539 G/A variant in DHFR.
- Statistics: Mann-Whitney test. Linear mixed effect with random intercept and fixed slope.

RESULTS

- A/A genotype (n=15) vs G/G or G/A genotypes (n=172)
- 15 nmol/L lower MTXPG3 levels (median 26 nmol/L [IQR: 18-55] vs 41 nmol/L [IQR: 28-60]) (p<0.05)
- 16% lower long-chain MTXPG3-5 (median 36% [IQR 18-48%] vs median 52% [IQR 40-68%]) (p<0.05)
- 0.8 nmol/L per mg lower MTX polyglutamation rate (median 2.1 nmol/mg MTX [IQR 1.2-3.1] vs 2.9 nmol/mg MTX [IQR 2.0-4.3] (p<0.05)

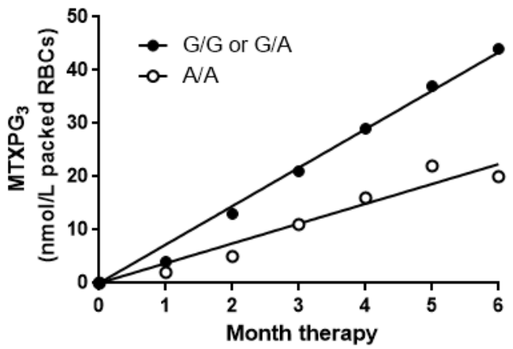


IMPACT OF DHFR VARIANT ON MTXPGs

Cohort 2:

- LInear mixed effect models revealed that carriers of the rs1382539 A/A genotype (n=4 patients [10%]) demonstrated the following relative to carriers of the GG or GA genotype

- Lower RBC MTXPG3 levels (estimate=-11±5 nmol/L; p=0.03)
- Lower percent long-chain MTXPG3-5 (estimate=-15±1%; p=0.02)
- Lower polyglutamate rate (estimate=-0.9±0.4 nmol/mg; p=0.01)
- While the rs1382539 variant was not significantly associated with clinical efficacy (p>0.05), lower RBC MTXPG3 level and percent long-chain MTXPG3-5 levels associated with higher DAS28 in that cohort (p<0.05).



CONCLUSION

rs1382539 G/A variant in DHFR impacts MTX polyglutamation in adult RA, and may indirectly contribute to clinical efficacy in some patients.

Contact: tdervieux@exagen.com