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Prediction of Methotrexate toxicity in Algerian Rheumatoid Arthritis Patients: Implication of GGH rs11545078 polymorphism

Prédiction de la toxicité au méthotrexate chez des patients algériens atteints de polyarthrite rhumatoïde : implication du polymorphisme GGH rs11545078

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KEY WORDS

GGH, Methotrexate, Rheumatoid arthritis, Algeria, polymorphism

Abstract

Objectives - The aim of this study is to determine the impact of the gamma glutamyl hydrolase (GGH) c.452C>T (dbSNP Id: rs 11545078) polymorphism on the Methotrexate (MTX) adverse drug reactions (ADRs) and on MTX gastro intestinal toxicity (GIT) in rheumatoid arthritis (RA) patients.

Materials and methods - Sixty-one patients with RA are enrolled in this study. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Data were analyzed by x2.

Results - Our findings suggest that the frequencies distributions of alleles/genotypes of this polymorphism were similar in both groups with and without ADRs. However, there is a significant distribution of GGH 452TT (p=0.03) and GGH 452CT+452TT (p=0.02) genotypes between the groups with and without GIT. The same correlation was found for GGH 452T allele (p=0.008).

Conclusion - We have shown for the first time in the West Algerian population that the GGH c.452C>T polymorphism influences the MTX gastro-intestinal toxicity.

MOTS CLÉS

GGH, Méthotrexate, polyarthrite rhumatoïde, Algérie, polymorphisme

Résumé

Objectifs - Le but de cette étude est de déterminer l'impact du polymorphisme gamma-glutamyl hydrolase (GGH) c.452C> T (rs 11545078 dbSNP Id) GIT) chez les patients atteints de polyarthrite rhumatoïde (PR) sur l'apparition des réactions indésirables au

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méthotrexate (MTX) et sur la toxicité gastro-intestinale (TGI).

Matériels et méthodes - Soixante et un patients atteints de PR ont été inclus dans cette étude. Le génotypage a été effectué par la réaction en chaîne par polymérase polymorphisme de longueur de fragment de restriction (PCR-RFLP). Les données ont été analysées par test x2.

Résultats - Nos résultats suggèrent que les distributions de fréquences des allèles et génotypes de ce polymorphisme sont similaires entre les deux groupes avec et sans toxicité. Cependant, il existe une distribution significative des génotypes GGH 452TT (p = 0,03) et GGH 452CT + 452TT (p = 0,02) entre les groupes avec ou sans TGI. La même corrélation à été retrouvez pour l'allèle GGH 452T (p = 0,008)

Conclusion - Nous avons montré pour la première fois dans la population de l'Ouest Algérien que le polymorphisme GGH c.452C> T influence l'apparition de la toxicité intestinale gastro-intestinale du MTX.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease, characterized by progressive damage of synovial-lined joints and variable extra-articular manifestations [1]. One of the most used worldwide among disease-modifying anti-rheumatic drugs (DMARDs) for the treatment of RA is the methotrexate (MTX) [2]. Its effectiveness has been widely demonstrated. However, some patients may develop toxicity to the MTX treatment. Several studies have investigated the individual genetic variations responsible of MTX therapeutic outcomes.

The MTX is an anti-folate drug that inhibits folate dependent enzymes such as dihydrofolate reductase (DHFR), thymidylate synthase (TYMS) and amino-imidazole-carboxamide (ATIC), which is critical for conversion of homocysteine to methionine and purine/pyrimidine synthesis. In this way the MTX reduces the inflammatory and proliferative effects [3].

Intracellular MTX is converted to polyglutamate MTX (MTX-PGs) by foly poly glutamate synthase (FPGS); this active form inhibits the DHFR. The inverse effect of the conversion is obtained by glutamyl hydrolase gamma (GGH), which is essential for the MTX influx.

Intracellular MTX is converted to MTX polyglutamates (MTX-PGs) by foly poly glutamate synthase (FPGS), the opposite effect is obtained by gamma glutamyl hydrolase (GGH) [4]. The GGH c.452C>T (dbSNP Id: rs 11545078) polymorphism in exon 5 causes the change of the amino acid threonine to isoleucine at the position 127 [5], which changes the conformation of the catalytic molecular surface and reduces the affinity binding with MTX-PGs chains [6]. In fact, one study showed that there is a large accumulation of MTX-PGs chains in cells in vivo in the lymphocyte T and B lines which contain this polymorphism in acute lymphoblastic leukemia [7]. Thereby, this polymorphism seems to induce the accumulation of MTX in the cell and may probably contribute to the toxicity of treatment.

The aim of our study was to determine the association of the GGH c.452C>T polymorphism with adverse drug reactions (ADRs) and gastro intestinal toxicity to MTX treatment in RA Algerian patients.

Materials and methods

Patients

This study was developed as a retrospective study including sixty-one RA patients treated with only MTX (as monotherapy, there was no DMARDs therapy combination) for at least six months and was conducted between January 2011 and February 2013 at Rheumatology Department of Oran University Hospital (Algeria). The Institutional Research Ethics Board of molecular and cellular genetics laboratory approved the study. The RA patients were diagnosed according to the revised criteria of the American College of Rheumatology (ACR) in 1987 [8] and reclassified according to ACR/European League against Rheumatism (EULAR) criteria in 2010 [9]. Patients were excluded from this study if they were not treated with MTX for at least six months or had stopped this therapy for different reasons as a recent pregnancy or planning to become pregnant. The MTX was prescribed as a treatment in the first-line at dose of 15 mg/week. The adjustment of MTX therapy also occurred when patients developed ADRs (gastrointestinal, liver, pulmonary, cutaneous mucosa, hematopoietic and renal toxicity) to 10 mg/week. To prevent the toxicity, the folic acid supplementation was prescribed once a week for all patients with the same dose of MTX (mean dose of MTX =11.07 mg/week). The data collected concerned patients medical history and their biological parameters (anti-cyclic citrullinated peptide, body mass index rheumatoid factor, Treatment related, disease activity score 28…. (Table 01).

Methotrexate related ADRs were defined as one or combina-

tion of different events: gastrointestinal (nausea, indigestion or anorexia, diarrhea, mouth ulcers and abdominal pains), liver (hepatic cytolysis, metabolic hepatic steatosis, cirrhosis, hepatic fibrosis and rate of transaminase), pulmonary (presence of cough, difficulty breathing and pneumonia), cutaneous mucosa (hair loss, photosensitivity, erythema of the extremities, rash), hematopoietic (thrombocytopenia and leukopenia) and renal (kidney failure or renal insufficiency) [10].

Genotyping

DNA was isolated from peripheral white blood cells by a standard manual salting-out method [11].

The GGH c.452C>T polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. PCR amplification using Billes illustra Hot Start Mix RTG (Kits PCR Illustra; GE Healthcare) was performed for a final volume of 25 µl containing 0.4 µM of each primer (forward: 5'-GTGCCTATTTGGTTATGACA-3'; reverse: 5'-CTACTTACTAATCCTGCCCA-3') [12] and 100–200 ng of genomic DNA. The PCR conditions consisted of initial denaturation at 95°C during 5 minutes followed by 40 cycles with denaturation for 15 seconds at 94°C, annealing for 15 seconds at 55°C, extension for 45 seconds at 72°C, and a final extension at 72°C during 10 minutes. RFLP was performed at 37°C, overnight, using PshBI (TAKARA Biotechnologie (Dalian) CO., LTD). Individuals with the CC genotype presented 1 fragment with 286 base pairs (bp), whereas individuals with the TT genotype presented 2 fragments with 109 pb and 177 pb.

Statistical analysis

The data were described as means ± standard deviations or numbers (%). For this polymorphism (SNP), the RA patients were classified into 3 groups: wild homozygous, heterozygous and homozygous variants. All statistical analyses were performed with Epi-InfoTM version 7 software. The odds ratios (OR) and 95 % confidence interval (CI) were determined with logistic regression analysis in order to evaluate the association between GGH c.452C>T polymorphism and MTX adverse drug reactions/MTX gastro intestinal toxicity. A p value <0 .05 was considered as statistically significant.

Results

Patients description

The characteristics of 61 RA patients are presented in Table 1. During the MTX therapy, 25 among 61 (40.98%) RA patients developed adverse drug reactions as illustrated in table 2. Gastrointestinal reactions were the most frequent events (20/25 patients, 80%) (Table 3). The adverse drug events were observed during the first three months of treatment (mean duration to observed a ADRs=0.22 year).

Table 01: Demographic and clinical characteristics distribution of RA patients

Characteristics	Patients PR N (%)
General Characteristics	(n=61)
Gender (female/mal)	54/7
Age (years) (mean± SD)	47±14.11
BMI median (IQR) kg/m2	27.01 (10.17-50.91)
Disease related	
Duration of the disease (years) (mean± SD)	10.08 ±7.556
RF n (%positive)	53 (86.18)
Erosion n (%positive)	39 (63.93)
DAS28 (mean± SD)	2.5± 2.3
Individual variable of DAS28	
TJC (out of 28), median (IQR)	2 (0.0-20.0)
SJC (out of 28), median (IQR)	3 (0.0-25.0)
ESR, median (IQR), minutes (1st ho ur)	15.0 (2.0-69.0)
Global health on VAS, median (IQR)	40.0 (0.0-100.0)
HAQ score. median (IQR)	1 (0.0-2.0)
Treatment related	
MTX Dose (mg/week) (mean± SD)	11.07±3.55
Duration of MTX treatment (months) (mean± SD)	29.9 ±31.06
Non- Corticosteroid n (%)	28 (45.09)
Corticosteroid n (%)	33 (54.09)
NSAIDs n (%)	26 (42.62)
Non- NSAIDs n (%)	35 (57.37)

n: number. %: frequency. NPY: number of cigarettes smoked per day × number of years smoking/20. MTX: methotrexate. RA: rheumatoid arthritis. NSAID: non steroidal anti-inflammatory drugs. IQR: interquartile range. Anti-CCP: anti-cyclic citrullinated peptide. BMI: body mass index; DAS28: disease activity score 28. HAQ: health assessment questionnaire. RF: rheumatoid factor. SD: standard deviation. SJC: swollen joints count. TJC: tender joints count. VAS: visual analog scale.

Impact of GGH rs 11545078 on MTX toxicity

Table 2 presents the comparison of GGH c.452C>T polymorphism distribution between ADRs+ and ADRs- groups. Our results showed no significant difference in the frequency distribution of genotypes and alleles.

Impact of GGH rs 11545078 on MTX gastro intestinal toxicity

In table 3, the frequency distribution of GGH c.452C>T polymorphism in patients with and without gastro intestinal toxicity was not similar. A significant difference of frequency distribution regarding the GIT+ or GIT- groups was observed for the genotype GGH 452TT genotype (p=0.03) and the GGH 452CT+452TT (p=0.02). Also, there was a significant difference of the GGH 452T allele distribution between GIT+ and GIT- groups (p=0.008).

Discussion

Since thirty years, methotrexate (MTX) has been the most used worldwide among DMARDs for RA treatment. The adverse drug reactions related to MTX treatment were observed

Table 02: Distribution of genotypes, frequency of alleles of the GGH c.452C>T polymorphism and its correlation with adverse drug reactions (ADRs) of MTX.

	ADRs+ n(%)	*ADRs- n(%)*	*P*	*OR(95% CI)*
GGH c .452C>T	**(n=25)**	**(n=36)**		
Genotypes				
452CC	17(68)	28(77.77)		1[b]
452CT	5(20)	6(16.66)		
452TT	3(12)	2(5.55)	0.37	2.47[0.25-31.08][a]
452CT+452TT	8(32)	8(22.22)	0.39	1.64[0.52-5.20][a]
Alleles				
452C	39(78)	62(86.11)		1[b]
452T	11(22)	10(13.88)	0.24	1.94[0.67-4.50]

n:number. %: frequency. OR: odds ratio. CI: confidence interval. p: significance
b: genotype/ allele /haplotype saved as reference category. RA: rheumatoid arthritis. a: fisher exact test. ADRs: adverse drug reactions. ADRs+: presence of adverse drug reactions. ADRs-: absence of adverse drug reactions

Table 03: Distribution of genotypes, frequency of alleles of the GGH c.452C>T polymorphism and its correlation with gastro intestinal toxicity (GIT) of MTX

	GIT+ n(%)	*GIT- n(%)*	*P*	*OR(95% CI)*
GGH c .452C>T	(n=20)	(n=41)		
Genotypes				
452CC	12(60)	33(80.4)		1[b]
452CT	4(20)	7(17.07)		
452TT	4(20)	1(2.43)	**0.03***	0.09[0.001-1.09][a]
452CT+452TT	8(40)	8(19.51)	**0.02***	0.36[0.11-1.18][a]
Alleles				
452C	28(70)	73(89.02)		1b
452T	12(30)	9(10.97)	**0.008***	0.28[0.1-0.75][a]

n:number. %: frequency. OR: odds ratio. CI: confidence interval. p: significance
b: genotype/ allele /haplotype saved as reference category. GIT: gastro intestinal toxicity. GIT+: presence of gastro intestinal toxicity. GIT-: absence of gastro intestinal toxicity. a: fisher exact test. ADRs: adverse drug reactions. .: p statistically significant.*

with a frequency of 10-60% [13]. Similar results were observed in our cohort, with 40.89% of adverse drug reactions.

In the present investigation, we have explored the impact of GGH c.452C>T polymorphism on the MTX toxicity. Firstly, in the adverse drug reactions study, by the comparison of frequencies between the presence of adverse drug reactions group and absence of adverse drug reactions group; secondly, in the gastro intestinal toxicity study, by the comparison of frequencies between the presence and absence of gastro intestinal toxicity groups.

The current study showed that the genetic polymorphism of GGH c.452C>T does not influence ADRs to MTX treatment in RA patients. A few studies were conducted to study the implication of this polymorphism on MTX toxicity. A recent study on 184 RA Serbian patients, agreed with our findings. There was no association between GGH c.452C>T polymorphisms and MTX toxicity in RA patients [14]. The same result was found on Japanese and Netherlands RA patients [15, 16]. Moreover, a previous study reported that this polymorphism was not a predictive marker of MTX toxicity in Japanese patients with articular-type juvenile idiopathic arthritis [17].

However, our findings demonstrated that the GGH c.452C>T polymorphism influences the MTX gastro intestinal toxicity. Until now, there is no study focused on the impact of this polymorphism on MTX gastro intestinal toxicity. This correlation with these ADRs can be explained by the drug's oral intake.

Multiple factors such as RA disease duration, autoantibody rheumatoid factor (RF) or smoking status can influence the response to different medications in RA patients [18]. These can explain why the genetic variations do not correlate to outcomes.

The most important limitation of our study could be the small

sample size. The small size of a sample mainly affects statistical power: as the sample size increases, power increases.

Conclusion

We have shown, for the first time on an Algerian patients sample, that the GGH c.452C>T polymorphism does not influence the MTX toxicity. However, this genetic polymorphism seems to have an impact on MTX gastro intestinal toxicity in RA patients. These results have to be replicated in a larger sample to confirm their involvement on the MTX toxicity. On the other hand, it would be interesting to explore other polymorphisms involved in the pharmacogenetic of MTX that might be involved in the variability of toxicity response.

Conflict of interest

All authors declare there is no competing interest

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