Review

Genetic polymorphisms in metabolic pathways of leflunomide in the treatment of rheumatoid arthritis

T. Soukup[1], M. Dosedel[2], J. Nekvindova[3], J. Toms[1], J. Vlcek[2], P. Pavek[4]

[1]Charles University in Prague, Medical Faculty and University Hospital in Hradec Kralove, 2nd Department of Internal Medicine-Gastroenterology;
[2]Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Department of Social and Clinical Pharmacy;
[3]Charles University in Prague, University Hospital in Hradec Kralove, Institute of Clinical Biochemistry and Diagnostics;
[4]Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Department of Pharmacology and Toxicology, Czech Republic.

Tomas Soukup, MD, PhD
Martin Dosedel, PhD
Jana Nekvindova, PhD
Jan Toms, MD
Jiri Vlcek, PhD
Petr Pavek, PhD

Please address correspondence to:
Dr Tomas Soukup,
2nd Department of Internal Medicine - Gastroenterology,
Faculty Hospital Hradec Kralove,
Sokolska 581,
500 05 Hradec Kralove,
Czech Republic.
E-mail: soukutom@seznam.cz

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ABSTRACT

Leflunomide (LEF) is a disease-modifying anti-rheumatic drug used for treating rheumatoid arthritis (RA). More than 50% of patients are withdrawn from LEF treatment within one year, mainly due to AEs. Importantly, it is not possible to predict which patients will respond to LEF therapy nor if adverse outcome occurs.

*Pharmacogenetic studies indicate an impact of single nucleotid polymorphisms (SNPs) on the variability in LEF serum levels with potential relevance to effectiveness and tolera*bility in individual RA patients. *In vitro studies have demonstrated that cytochromes P450 (CYPs), mainly CYP1A2, CYP2C19, and CYP3A4, are involved in LEF metabolite activation. It was shown that CYP1A2*1F allele may be associated with LEF toxicity in patients with RA. In case of dihydroorotate dehydrogenase (DHODH) gene SNP (rs3213422, 19C>A), it was shown that C allele may be associated with LEF toxicity and therapeutic effect. Finally, oestrogen receptor genes SNPs in females may be associated with LEF therapy efficacy.*

In summary, the results of the current studies suggest a possible diagnostic value of genotyping for patients with RA as biomarkers of LEF therapy efficacy or conversely as indicators of serious side effects. In the future, it will be necessary to corroborate these results in studies with larger numbers of patients and longer follow-up. Moreover, it would be appropriate to focus on CYP2C19, ATP5A1 and PKD1L3 genes.

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disorder and the commonest form of chronic inflammatory joint disease. It has an overal prevalence of 0.5–1% in European Caucasian populations, with a female:male ratio of 3:1 (1). Methotrexate (MTX) is still a first-line therapy for monotherapy or combination therapy in RA. However, like other disease modifying anti-rheumatic drugs (DMARDs), one-third of the patients fail to respond to treatment, either because of inefficiency or adverse events (AEs). The need for alternatives in RA treatment is of great interest. Leflunomide (LEF) is one potential drug to replace MTX effectively in treatment of RA if intolerance or lack of effect occurs (2).

Indication for the management of the signs and symptoms of active RA may improve physical function and reduce the progression of structural damage associated with the disease. LEF was licensed for management of RA in 1999 in the European Union. In pivotal trials, LEF achieved similar clinical and radiological responses as compared to MTX and SSZ (sulfasalazine) (3). Therefore, LEF is relatively frequently administered. In RA, the common LEF dose is 20 mg/day, without high start dose (100 mg/day) (2).

In the last decade, studies have reported associations between single nucleotide polymorphisms (SNPs) in genes encoding proteins related to the pharmacokinetics and pharmacodynamics of LEF, its treatment efficiency and AEs (4-9). If it were feasible to select patients at low risk of AEs to LEF or, on the other hand, patients supposed to be resistant to LEF therapy or presumed at high risk of AEs based on genotyping, this would allow us to switch the therapy to another DMARDs.

Mechanism of action

LEF is a prodrug that is rapidly and almost completely metabolised following oral administration to its pharmacologically active metabolite teriflunomide (A77 1726). Its two *in vitro* mechanisms of action vary depending on concentration.

First, its major effect appears to be reversible inhibition of the enzyme dihydroorotate dehydrogenase (DHOODH), which results in inhibition of pyrimidine synthesis (10). DHOODH is a mitochondrial enzyme involved in *de novo* pyrimidine ribonucleotide uridine monophosphate (rUMP) synthesis and has antiproliferative effect. Inhibition of DHODH by A771726 prevents production of rUMP by the *de novo* pathway; such inhibition results to decreased rUMP levels, decreased DNA and RNA synthesis, inhibition of cell proliferation, and G1 cell cycle arrest. LEF inhibits autoimmune T-cell proliferation and production of autoantibodies by B-cells (11, 12).

Second, as to A77 1726 (at higher concentrations) it also inhibits tyrosine kinases, interfering with cell signal transduction (10). In case of RA, activated lymphocytes need to expand their pyrimidine pool 7- to 8-fold, while the purine pool is expanded only 3-fold. To meet the need for more pyrimidines, activated T cells use the *de novo* pathway for pyrimidine synthesis. Therefore, activated T cells, which are dependent on *de novo* pyrimidine synthesis, will be more affected by LEF's inhibition of DHODH than other cell types that use the salvage pathway of pyrimidine synthesis (13, 14).

Therapeutic drug monitoring of A77 1726 may be useful in predicting the efficacy of LEF treatment. Van Roon *et al*. presented that A77 1726 steady state serum concentrations show relation with disease activity score in 28 joints (DAS 28) response (15). Conversely, another study by Chan *et al*. observed no association between A77 1726 serum concentrations and risk for LEF-related AEs (16).

Pharmacokinetics of LEF

Distributions and half-life

The gastrointestinal tract and the liver rapidly and completely convert ingested LEF into A77 1726. Food does not interfere with absorption. Circulating A77 1726 is bound (more than 99%) to plasma proteins, predominantly albumin. Steady state plasma levels are reached in 7 weeks after daily dosing (5 to 25mg) (10).

A77 1726 has a half-life of approximately 2 weeks (mean, 15.5 days), with a low apparent volume of distribution (17). Because of enterohepatic recirculation, LEF has a very long half-life. In healthy subjects, 90% of LEF is excreted by 28 days, but some may be present for a much longer period (17).

Elimination

The active metabolite is eliminated by further metabolism and subsequent renal excretion as well as by direct biliary excretion. In a 28-day study of drug elimination using a single dose of radiolabelled compound, approximately 43% of the total radioactivity was eliminated in urine and 48% was eliminated in the feces (10).

Efficacy of LEF

The efficacy of LEF, initially demonstrated in a phase II randomised, placebo-controlled study in 402 patients, was confirmed in the 3 phase III studies (two placebo controlled and all three active drug controlled), which compared LEF treatment with MTX, SSZ, and/or placebo (17).

Smolen *et al*. presented that both LEF and SSZ were superior to placebo in terms of swollen and tender joint counts, as well as physicians'and patients'overall assessments (18). It is important that both the LEF and SSZ patient groups reported significant effects on slowing radiografic progression of disease compared with placebo (18).

Strnad *et al*. demonstrated that patients receiving LEF therapy displayed an important benefit that was demonstrated by improvement in the clinical signs and symptoms of RA. Benefitial effects were first evident at 1 month and were maintained over a 1-year period, with significant retardation of disease progression confirmed by x-ray analysis and improvement in the performance of physical activities important to patients and in health-related quality of life. The outcomes of this trial suggest that LEF therapy is as effective as MTX therapy and represents an important addition to the treatment armamentarium for patients with active RA (19).

In another study, Emery *et al*. compared LEF therapy (20 mg/day) with MTX therapy (10–15 mg/week). In this trial, MTX was shown to be statistically superior to LEF for the clinical outcomes measured, as well as the rate of radiographic pregression after 2 years (20).

These were the first 6- and 12-month randomised placebo- and active drug-controlled trials to demonstrate retardation of radiographic progression by LEF, as well as two commonly used DMARDs, MTX and SSZ. The phase III clinical trial compared both short-term and long-term (up to two years) clinical efficacy and safety of LEF and MTX in patients with active RA. LEF was shown to be similar to SSZ and superior to MTX for slowing the progression of radiographically assessed joint damage (21, 22).

In conclusion, LEF has shown clinical, functional and structural efficacy. Although MTX doses in respective comparative trials may not have been optimal, LEF has shown efficacies similar to MTX. No recent studies have disproved this conclusion, and LEF has been used effectively in combination with biological agents.

Toxicity or adverse effect of LEF therapy

The experience with the tolerability of LEF in clinical practice analysed in a variety of randomised clinical trials and non-interventional observational studies has been reviewed (23). LEF-related AEs tended to be more frequent in the first year of treatment. LEF is discontinued at a median of 3 months due to AEs compared with a median of 6 months for MTX therapy (24). The most frequent AEs (40–70%) resulting in drug withdrawal during the first year of LEF treatment were increased plasma liver enzyme levels, nausea, diarrhoea, and alopecia. A similar pattern was seen in the patients treated with MTX, but the number of withdrawals due to elevation of plasma liver enzyme levels was twice as high as that seen with LEF. The incidence of drug-related elevations of plasma liver enzyme levels in the first year of the trial was 3-fold higher with MTX than with LEF. During the second year of treatment, arterial hypertension, skin rash, and alopecia were the most common

LEF-related AEs leading to withdrawal, but there was no common cause of withdrawal, nor were there any LEF-related deaths (20).

Moreover, AEs including gastointestinal tract, weight loss (25), hepatotoxicity oral ulcers, arterial hypertension, headache and hair loss, as well as peripheral neuropathy and predisposition to infection have been reported as cause of withdrawal rates of LEF compared with MTX. Most serious AEs of LEF might be liver damage with jaundice and hepatitis, which can be fulminant, severe liver necrosis. The incidence of serious hepatopathy is estimated to be as high as 0.5%, according to the report of the U.S. Food and Drug Administration (FDA) (23). In 2001, the European Medical Association (EMA) reported 296 cases of hepatotoxicity in 104.000 patient-years, with 129 considered as serious, 2 cases of liver cirrhosis, and 15 cases of liver failure. Nine of the patients died. EMA findings suppose that hepatopathy is frequently seen within the first 6 months of therapy and is partially depending on cofactors, because 101 (78%) of the serious cases were concomitantly treated with hepatotoxic drugs; 58% of those with asymptomatic elevations of liver function studies were cotreated with nonsteroidal antiinflammatory drugs and/or MTX. In addition, 33% (27 patients) of the subjets with serious damage had risk factors (history of alcohol abuse, acute heart failure, severe pulmonary or pancreatic disease) (23). Very important is a relatively high incidence of leukopenia, and LEF-related anaemia, and thrombocytopenia. Infections, sometimes as serious as development of active tuberculosis, pneumonia (*e.g.* pneumocystis pneumonia) and severe mycotical or viral infections, possibly leading to sepsis, or death, have been seen (10).

Interstitial lung disease is presented by progressive dyspnoea and typical high resolutiom CT findings. This AE may or may not be reversible upon treatment and may lead to permanent disability or death. If severe AEs are encountered, A77 1726 can be readily removed from the body with oral cholestyramine or activated charcoal to slow or reverse the noted AEs (10).

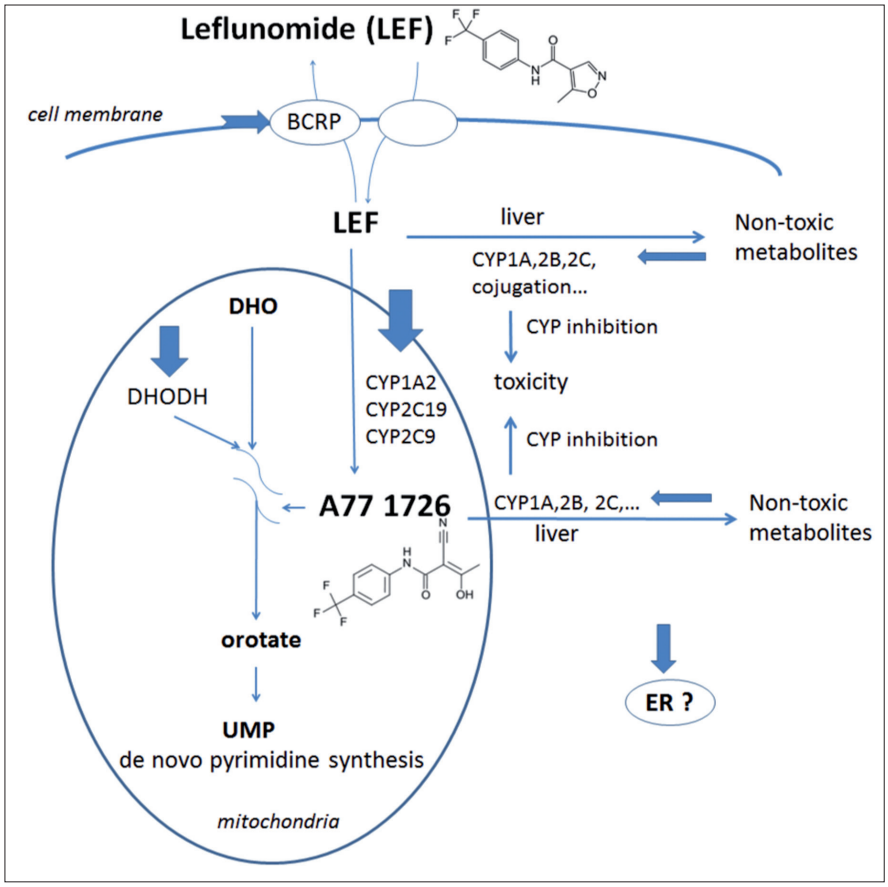

Fig. 1. Simplified mode of metabolism of leflunomidde, single nucleotide polymorphisms modified leflunomide metabolism, adverse events and treatment outcome.
DHO: dihydroorotate; DHODH: dihydroorotate dehydrogenase; CYP: cytochrome; BCRP: ATP-binding cassette (ABC) superfamily of membrane transporters – ABCG2 encoding breast cancer resistence protein (BCRP); A77 1726, active metabolite of leflunomide; ER: oestrogen receptor.

Pharmacogenetics

Cytochromes P450

Active metabolite A77 1726 is formed by cytochrome P450 (CYPs) isoenzymes, such as CYP1A2, CYP2C19 and CYP2C9 (26). Accordingly, genetic polymorphisms of these potential modifiers could play an important role in metabolism, pharmacocinetics and toxicity of LEF.

CYP2C19

CYP2C19 is a clinically important enzyme that metabolises a wide variety of drugs, including the anticonvulsant mephenytoin, anti-ulcer drugs such as omeprazole, certain antidepressants, and the antimalarial drug proguanil (27). Mutation in the CYP2C19 gene causes poor metabolism of these drugs (28). SNPs in CYP2C19 characterise two defective alleles that result in an abolished enzymatic activity (CYP2C19*2, rs4244285 and CYP2C19*3, rs4986893) (29), as well as CYP2C19*17 allele (rs 12248560) that results in increased enzyme activity (30).

CYP1A2

The CYP1A2 gene encodes a P450 enzyme involved in O-deethylation of phenacetin. More than 20 clinically used drugs are partly or predominantly metabolised by CYP1A2 including caffeine, theophylline, imipramine, clozapine, and propranolol. CYP1A2 accounts for nearly 15% of the cytochrome P450 in the human liver (31). CYP1A2 displays higher activity in men than in women, and is inhibited by oral contraceptives. Inducers of CYP1A2 include a number of other Aryl hydrocarbon receptor ligands (32). Cigarette smoking has also been shown to increase CYP1A2 activity (32). According to this fact, CYP1A2 activity may not only be affected due to SNPs in this gene. CYP1A2 -163C>A (rs762551) SNP

characterises the CYP1A2*1F allele that has been presented with a higher enzyme inducibility (33).

CYP2C9

Two common SNPs in the CYP2C9 gene were described characterising the CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) alleles that lead to decreased enzyme activity (34, 35). Although CYP2C9 is not considered to be a major LEF-metabolising enzyme (27), cases of LEF drug interactions and toxicity have been reported in CYP2C9*3 homozygous patients (36, 37). In two case reports, RA patients with CYP2C9*3/*3 genotype that were on ongoing anticoagulant therapy with warfarin developed severe AEs when cotreated with LEF (36).

Clinical influence of CYP1A2 and CYP2C19 SNPs has been first studied by Grabar *et al*. in 2008. The aim of this study was to investigate whether genetic polymorphisms of CYP1A2, CYP2C19, and CYP2C9 are associated with LEF toxicity. The results suggest that the CYP1A2*1F CC genotype may be associated with LEF toxicity in RA patients. In this cohort, occurrence of AEs was transaminase elevation, diarrhoea, rash/pruritus, nausea/vomitus, arterial hypertension, and abdominal pain. Authors observed no significant associations between the CYP2C19 and CYP2C9 genotypes and LEF toxicity (4).

In another study by Grabar *et al*., the authors included 67 RA and psoriatic arthritis patients treated with LEF. The aim of this study was to investigate whether genetic polymorphisms in CYP1A2 and CYP2C19 influence LEF pharmacokinetics, treatment response, and the occurrence of AEs. The steady state plasma concentration of A771726 showed a large inter-individual variability as well as an increase in clearance in carriers of the CYP2C19*2 allele compared with non-carriers. Patients with a more pronounced decrease in C-reactive protein (CRP) reached higher average steady-state plasma concentrations. Nevertheless, there was no association of plasma concentrations of the active drug with the occurrence of AEs (5). The LEF population pharmaco- kinetic studies demonstrated that some of the AEs variability can be explained by variations in patient age, gender, body size, liver function, and smoking status (15).

DHODH

DHODH is a monofunctional protein which, in most eukaryotic organisms, is located on the outer surface of the inner mitochondrial membrane. Mutations in the DHODH gene cause Miller syndrome, an autosomal recessive disorder also known as postaxial acrofacial dysostosis (38). The human DHODH gene sequence is highly conserved and contains only one common missense polymorphism in the coding regions. Pawlik *et al*. reported a study on the SNP (rs 3213422; 19C>A) in 147 RA patients (6). Clinical improvement was evaluated according to the American College of Rheumatology (ACR) 20% and 50% response criteria. The frequency of remission was increased in C allele carriers compared with patients with the A allele. Results suggest that DHODH 19A>C polymorphism may be associated with LEF treatment outcome in RA patients (6).

In another study, Grabar *et al*. reported on risk of toxicity in association with a genetic polymorphism of DHODH. The study included 105 patients with RA. Carriers of the DHODH 19C allele had a 6.8-fold increased risk for development of LEF-induced toxicity. However, no siginicant associatin of DHODH 19AA genotype and CYP2C9*3 allele combination with LEF AEs was observed (5). According to the report by O'Doherty *et al*., DHODH 19A>C SNPe may be associated with a reduced treatment response (p=0.008). The authors analysed six haplotype-tagging SNPs in DHODH in 56 patients with RA treated with LEF. Clinical response was determined by assessing the change in DAS 28 score over the first 3 months of treatment (7).

ABCG2

The ABCG2 gene encodes a membrane transporter belonging to the ATP-binding cassette (ABC) superfamily of membrane transporters, which are involved in the trafficking of biologic molecules across cell membranes. ABCG2, encoding breast cancer resistence protein (BCRP), was initially found to be a xenobiotic transporter that plays a role in the multidrug resistance phenotype (39). The ABCG2 protein is also a high capacity transporter for uric acid excretion in the kidney, liver, and gut (40, 41).

Many commonly used DMARDs (*e.g*. MTX, SSZ, LEF, hydroxychloroquine) are ABCG2 substrates. BCRP is an efflux transporter for both LEF and its active metabolite. The role of ABCG2 polymorphism in LEF pharmacokinetics was studied by Kim *et al*. (8). In a rather small study of 24 healthy volunteers, the influence of the ABCG2 (rs2231142) 421C>A variant on the pharmacokinetics of single dose of 20 mg LEF has been demonstrated and carriers of ABCG2 421C>A SNP exhibited a 30% higher C_{max} than non-carriers (8). In addition, ABCG2 421C>A SNP has potential importance on steady state A77 1726 blood levels via biliary secretion of A77 1726 (42).

Wiese *et al*. (43) showed a trend for ABCG2 421AA genotype to be associated with cessation due to diarrhoea. Although it was not statistically significant, reduced activity of the ABCG2 drug efflux pump appeared to be protective against diarrhoea. This is despite individuals with reduced ABCG2 activity having higher plasma A77 1726 concentrations, which is likely due to less hepatobiliary recycling and fecal elimination (8). Reduced secretion of A77 1726 into the gastrointestinal tract may be protective against diarrhoea through likely reduced local effect of A77 1726 on the bowel wall. Currently there is no known large study studiyng this SNP in RA.

Oestrogen receptor 1 and 2

Numerous studies indicated that women have a poorer LEF response to treatment than men (44, 45). Moreover, it has been indicated that oestrogens may modulate the action of LEF in cell cultures. Montagna *et al*. demonstrated that LEF significantly increased the expression of apoptotic proteins, but that 17β-estradiol significantly decreased the proapoprotic activity of LEF. These authors suggest that the less effective therapeutic effect in women with RA is

due to the action of oestrogens (46, 47). Two oestrogen receptors have been identified and designated as ESR 1 and ESR2. They are members of the superfamily of nuclear receptors. The oestrogen receptor (ESR1) is a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding, and activation of transcription (48). Mosselman *et al*. (1996) identified and characterised a novel human oestrogen receptor, which was called oestrogen receptor-beta (ESR2) (49). ESR-beta is homologous to the previously identified ESR-alpha (ESR1) and has an overlapping but non-identical tissue distribution. Two of the SNPs identified in the ESR1 gene, *i.e*. rs9340799:A>G and rs2234693:T>C, are most widely investigated. In the ESR2, two common SNPs have been investigated: synonymous rs1256049:G>A (Val328Val) in exon 6 and rs4986938: G>A located in 3´-UTR region (50, 51).

Dziedziejko *et al*. presented the first study examining the association between ESR1 and ESR2 gene SNPs and LEF treatment outcome in 115 female RA patients. Results of the study indicate better response to treatment in patients with the ESR1 rs9340799 AA and ESR1 rs2234693 TT genotypes. After 12 months of therapy, the improvement of erythrocyte sedimentation rate, visual analogue scale (VAS), and DAS28 values was greater in patients with ESR1 rs9340799 AA and ESR1 rs2234693 TT genotypes compared with para-meters in patients with rs9340799 AG and GG as well as rs2234693 TC and CC genotypes, respectively. Moreover, concordant associations of erythrocyte sedimentation rate, VAS and DAS28 were observed. In contrast, there were no statistically significant associations of response to treatment with ESR2 SNPs (9).

According to pharmacogenomics knowledge resource PharmGKB, potential association of LEF treatment outcome with genetic variability is also studied in case of ATP5A1 and PKD1L3 genes.

SNPs with no association observed

In case of CYP3A4, no major functionally variant allele has been found in Caucasian at an allele frequency higher than 0.1%, and only limited genotype-phenotype association has been observed (52).

Table I. Potential and clinical influence of SNPs on leflunomide treatment in RA patients.

Gene	Variant	Alternate Names SNPs	LEF toxicity / effect (publication) Number of patients, (n)	Allele
CYP1A2	rs2069526	45831898T>G, 739T>G, -10+103T>G, 31459T>G, 740T>G, 75041341T>G,	**No associations with pharmacokinetics** (Grabar *et al*. 2009) n=67 CYP1A2*1E	T > G
CYP1A2	rs762551	CYP1A2:734C>A, -9-154C>A, 32035C>A, 45832474C>A, 75041917C>A, CYP1A2*1F	**Toxicity risk for A allele (OR=9.7) (*p*=0.002)** (Grabar *et al*. 2008) n=105	C > A
CYP2C19	rs4244285	24154G>A, 24154G>C, 47346080G>A, 47346080G>C, 681G>A, 681G>C, 96541616G>A, 96541616G>C, CYP2C19*2, CYP2C19:681G>A, CYP2C19:G681A, Pro227=	**Pharmacokinetic – Cl/F (OR=1.72) (*p*=0.026)** (Grabar *et al*. 2009) n=67 **No effect** (Grabar *et al*. 2008) n=105	G > C G > A
ESR1	rs2234693	152163335T>C, 156705T>C, 453-397T>C, 56332792T>C, ESR1:PvuII, ESR1:c.454-397T>C	**Effect DAS28 (TT *vs*. CC *p*=0.034)** (Dziedziejko *et al*. 2011) n=115	T > C
ESR1	rs9340799	152163381A>G, 156751A>G, 453-351A>G, 56332838A>G, ESR1:XbaI, ESR1:c.454-351A>G	**Effect DAS28 (K-W test *p*=0.047)** (Dziedziejko *et al*. 2011) n=115	A > G
ESR2	rs1256049	ESR2:1082G>A, ESR2:Val328Val, ESR2:rs1256049, Val328= 45724051C>T, 64724051C>T, 86218G>A, 984G>A,	**No effect DAS28** (Dziedziejko *et al*. 2011) n=115	G > A C > T
ESR2	rs4986938	110453G>A, 1406+1872G>A, ESR2:1730A>G, *39G>A, 45699816C>T, 64699816C>T, ESR2-02	**No effect DAS28** (Dziedziejko *et al*. 2011) n=115	C > T
DHODH	rs3213422	19A>C, 25656881A>C, 5040A>C, 72042682A>C, DHODH: 19C>A, Gln7Lys, Lys7Gln	**Toxicity, risk for AA genotype (OR=6.8) (*p*=0.005)** (Grabar *et al*. 2009) n=105 **Effect ACR 20 (C *vs*. A: OR=1.98) (*p*=0.048)** (Pawlik *et al*. 2009) n=147	A > C
	rs3213423	72042825T>G, NG_016271.1:g.5183T>G, NM_001361.4:c.21+141T>G, NT_010498.15:g.25657024T>G	**Borderline reduced treatment response DAS28 in TT genotype (*p*=0.058)** (O'Doherty *et al*. 2012) n=56	T > G
ABCG2	rs2231142	421C>A	**Possible association with diarrhoea (0 of 14 pts with AC genotype)** (Wiese *et al*. 2010) n=78	C>A

SNPs: single nucleotid polymorphisms; CYP1A2: cytochrome P450 isoenzyme 1A2 gene; CYP2C19: cytochrome P450 isoenzyme 2C19 gene; ESR1: oestrogen receptor 1 gene; ESR2: oestrogen receptor 2 gene; DHODH: enzyme dihydroorotate dehydrogenase gene; ABCG2: membrane transporter ATP-binding cassette gene; CRP: Cl/F, clearance of A77 1726; DAS 28: disease activity score in 28 joints; ACR 20 and 50: American College of Rheumatology 20% and 50% response criteria; KW test: Kruskal-Wallis test; pts: patients.

The results of the recent study suggest that cytokines IL1β, IL6, and TNF genes polymorphisms are not sig-

nificant factors influencing the therapy outcome of RA patients with LEF (7). Results of another study suggest no correlation between androgen receptor gene CAG polymorphism and response to the treatment with LEF in women with RA.

Conclusion

The main four SNP groups have a potential clinical impact on RA patients treated with LEF. First, they are associted with metabolic pathway through CYP450. Reversible inhibition of the enzyme DHODH in LEF treatment represents the second group of SNPs in LEF treatment pharmacogenetics. Consequently, SNPs of oestrogen receptors may modulate the action of LEF and pharmacogenetic studies (ESR1, CYP1A2) confirmed less therapeutic effect of LEF in women with RA. Finally, the activity of ABC transporter in patients should be determined to understand the disposition and pharmacokinetics of the therapy.

However, all the LEF pharmacogenetic studies enrolled small numbers of patients, so that the relevance of their results remains insufficient. Nevertheless, pilot studies demonstrate the potential role of pharmacogenetic impact of LEF metabolism. In summary, the results of the studies suggest a possible diagnostic value for genotyping of patients with RA for prediction of LEF therapy and their ability of tolerance, or conversely to determine patients primarily resistant to the therapy by reason of a predisposition to serioous AEs. A number of SNPs are presented in LEF metabolism and pharmacokinetics, and it is likely that complex and multifactorial interactions, rather than effects of single gene variants, could play a role in determining the risk of AEs in RA patients treated with LEF. Moreover, we expect influence of SNPs on LEF drug interactions. Potential predictive factors for LEF toxicity in RA patients include doses of LEF that exceed 25 mg per day, loading doses of 100 mg on the first three consecutive days, and older population with comorbities. In the future, it will be necessary to analyse the available results in studies with with active metabolite serum levels and larger numbers of patients and longer follow-up.

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