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Review Article

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Variations in Genetic Factors Impacting the Effectiveness and Side Effects of Methotrexate with Rheumatoid Arthritis

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ABSTRACT

This review provides a comprehensive overview of genetic variations influencing the effectiveness and side effects of methotrexate (MTX) treatment in rheumatoid arthritis (RA) patients. It synthesizes findings on associations between genetic polymorphisms and MTX therapy outcomes, aiming to identify potential genetic markers for enhancing treatment and personalized strategies in RA. The review highlights genetic variations associated with MTX therapy effects. Variants in genes involved in MTX transport, including reduced folate carrier 1 (RFC1) and ABC transporters, correlate with treatment response. Specific RFC1 and ABCB1 variants are linked to improved MTX efficacy. Polymorphisms in genes regulating MTX metabolism, such as thymidylate synthase (TYMS) and methylenetetrahydrofolate reductase (MTHFR), predict effectiveness and toxicity risks of MTX. Additionally, genes influencing MTX's mechanistic pathways, like the adenosine signaling cascade, impact clinical outcomes. While the evidence is preliminary, this review suggests the potential of genetic testing to guide personalized MTX therapy in RA, leading to improved effectiveness and reduced adverse events. However, further research with diverse cohorts is necessary to validate these findings and establish the utility of pharmacogenomic-based treatment approaches for RA patients receiving MTX. RA is a chronic autoimmune disease treated with MTX as the cornerstone therapy, but patient responses vary. This comprehensive review examines genetic variations influencing MTX's efficacy and toxicity in RA patients. Through a rigorous literature review using databases like PubMed and Web of Science, this study synthesizes findings from pharmacogenetic research on genetic polymorphisms and MTX outcomes.

Key words: *Methotrexate, Rheumatoid arthritis, Polymorphism, Pharmacogenetics*

INTRODUCTION

Rheumatoid arthritis (RA) is a prevalent chronic inflammatory autoimmune disease that affects approximately 1% of the global population, with a higher incidence in women and older individuals [1-3]. RA primarily impacts the synovial joints, leading to progressive joint destruction, pain, and disability [4, 5]. Nevertheless, systemic signs may result from the inflammation spreading to other organs such as the skin, eyes, lungs, and blood vessels [5, 6]. Although the specific cause of RA is still unknown, a complex interaction of hormonal, environmental, and genetic variables is thought to be the cause [6, 7]. Pathologically, RA involves the thickening of the synovial lining, the proliferation of synovial cells, and the infiltration of inflammatory cells, contributing to joint erosion and damage [8, 9].

In the therapeutic landscape of RA, methotrexate (MTX) has established its role as a linchpin, with a significant percentage of patients experiencing marked improvements in symptoms, disease activity, and functional ability

[10, 11]. The treatment paradigm for RA has dramatically evolved, with disease-modifying antirheumatic drugs (DMARDs) and biological agents, including MTX, forming the cornerstone of disease management [10, 12]. However, patient responses to MTX treatment exhibit considerable variability, with up to one-third of patients failing to respond due to ineffectiveness [13-17]. Moreover, many patients experience at least one adverse drug reaction, including gastrointestinal issues, liver toxicity, skin reactions, neurological problems, and hematological toxicity [16, 18].

This variation in response and adverse effects may be attributed to genetic polymorphisms, particularly genes encoding proteins involved in MTX transport and metabolism [19-21]. Numerous pharmacogenetic studies have embarked on a quest to identify correlations between therapeutic outcomes of MTX and genetic polymorphisms in genes designated for MTX carrier-mediated transport systems [19-21]. These studies primarily focus on genes involved in MTX influx, efflux, and metabolism pathways [19, 20]. Recent findings have linked 120 SNPs in 34 genes with MTX response, although contradictory data and discrepancies in study populations and methodologies have clouded clear interpretations [20, 22].

Aim

This review aims to shed light on the genetic variations of various proteins and their subsequent impact on MTX's efficacy and toxicity profiles in RA patients. Given the critical nature of early diagnosis and appropriate treatment in preventing irreversible joint damage and improving patients' overall quality of life, a thorough understanding of genetic factors influencing MTX response and toxicity is critical. This knowledge is also integral to paving the way for personalized treatment strategies in RA.

MATERIALS AND METHODS

This comprehensive review aimed to provide an in-depth understanding of the genetic factors influencing MTX treatment's efficacy and side effects in RA patients. To achieve this, a systematic and methodical approach was undertaken, synthesizing insights from prior pharmacogenetic studies and focusing on the associations between genetic polymorphisms and clinical outcomes of MTX treatment in August 2023.

Literature review

Our study's initiation involved a rigorous literature review. Employing databases like PubMed, Web of Science, Embase, Scopus, and Cochrane, we conducted exhaustive searches using a combination of keywords such as "Methotrexate," "Genetic Polymorphisms," "Rheumatoid Arthritis," "Adverse Reactions," "Efficacy," "Pharmacogenetics," "Pharmacogenomics," "SNP," and relevant gene names, and we searched with all synonyms. This extensive approach enabled us to gather a repository of both historical and contemporary studies, offering a panoramic view of the topic.

Inclusion & exclusion criteria

A stringent set of predefined criteria was established to screen the amassed literature. We sought studies that addressed genetic variations influencing MTX's efficacy and side effects in RA patients. Inclusion was limited to studies that had undergone genotyping analysis for polymorphisms in MTX pathway genes and had statistically analyzed associations between genotypes and clinical outcomes. On the contrary, studies were excluded if they weren't focused on humans, exhibited unclear methodologies, failed to offer relevant genetic insights, or did not evaluate MTX's clinical efficacy and/or adverse effects.

Data extraction

Data extraction was a meticulous process. Relevant information from the included studies, such as gene names, associated polymorphisms, MTX metabolism, response impacts, study characteristics, treatment protocols, genotyping methods, clinical measures, and primary findings, was methodically extracted. The aim was to comprehensively represent the genetic landscape under investigation through tables, figures, and summary points, enabling a coherent understanding of the topic.

Comparative analysis

With the extracted data in hand, a comparative analysis was conducted. This allowed us to discern patterns, associations, and contradictions in the findings across various studies. Such an analysis was pivotal in understanding the multifaceted interplay of genetics and the MTX response.

RESULTS AND DISCUSSION

The genetics of rheumatoid arthritis

Significant genes associated with RA susceptibility

RA has a complicated genetic backdrop characterized by numerous genes and their interplay. Much research has delved into critical genes linked to RA risk: the HLA class II genes, IL23R, PTPN22, STAT3, MBL, and DPB1 [23-28]. The HLA class II genes, vital for moderating immune responses, are intrinsically tied to RA susceptibility. Comprehensive genome studies have spotlighted common variants in these genes that incrementally influence RA development. Specific alleles of the HLA-DRB1 gene, in particular, play decisive roles in RA vulnerability [2, 29].

The IL23R gene is also noteworthy, as its variations are associated with a heightened chance of seropositive RA onset. These variations, researchers suggest, may tamper with microRNA binding spots, potentially disrupting immune pathways pivotal to RA [30, 31]. The PTPN22 gene is another primary player in RA risk. Genetic changes here are connected to both seropositive and seronegative RA types. These modifications seemingly interfere with T cell operations, which are crucial in autoimmune conditions like RA [2, 32]. The STAT3 gene is also highlighted as a significant genetic contributor to RA risk. Certain genetic alterations, especially those at specific positions, correlate. with a higher risk of seropositive RA. Studies indicate these changes impact cell signals vital for T cell activation, underscoring their significance in RA emergence [2, 33].

The MBL and DPB1 genes, too, have been identified as impacting RA susceptibility. MBL gene mutations increase the risk, while DPB1 gene variations seem protective against RA [34, 35]. A mosaic of principal genes, entwined in immune modulation, T cell operations, and signaling pathways, shapes RA susceptibility. Unraveling these genetic underpinnings is pivotal for a deeper understanding of RA and tailoring treatments. Summarize the significant genes and mechanisms in RA in **Table 1**.

Table 1. Major genes implicated in RA and mechanisms

Gene	Association with RA	Mechanism	References
HLA-DRB1 (Human Leukocyte Antigen-DRB1)	The HLA-DRB1 locus is consistently associated with RA. Specific alleles, known as the "shared epitope" alleles, are linked to an increased risk of developing RA.	HLA molecules are essential in the immune system as they present antigens to T-cells. Specific HLA-DRB1 alleles may present arthritis-related antigens, leading to autoimmunity.	[36, 37]
PTPN22 (Protein Tyrosine Phosphatase Non-Receptor Type 22)	A specific variant (R620W) of this gene, which encodes a lymphoid-specific phosphatase that negatively regulates T-cell activation, is associated with increased RA risk in various populations.	The risk variant may disrupt the normal regulation of T-cell responses, promoting autoimmunity.	[38, 39]
STAT4 (Signal Transducer and Activator of Transcription 4)	Polymorphisms in the STAT4 gene, which is involved in the signaling pathways of various cytokines, including interleukin-12 (IL-12) and IL-23, have been linked to increased susceptibility to RA.	Variants in STAT4 may influence the differentiation of T helper cells, skewing immune responses and promoting autoimmunity.	[40, 41]
TRAF1/C5 Region (TNF Receptor-Associated Factor 1/Complement Component 5)	Variants in the TRAF1/C5 region, which play roles in immune responses and inflammation, are associated with RA susceptibility.	Altered function or expression of these genes may modulate inflammatory pathways, contributing	[42, 43]

Others (CTLA4, FCRL3, IRF5)	Numerous other genes, including CTLA4 (Cytotoxic T-Lymphocyte Associated Protein 4), FCRL3 (Fc Receptor-Like 3), and IRF5 (Interferon Regulatory Factor 5), have more modest associations with RA.	The exact mechanisms by which these genes influence RA susceptibility are still under investigation.	[28, 44]

The role of genetics in RA prognosis

Genetics plays a pivotal role in forecasting RA prognosis, with many genetic risk factors pinpointed as crucial in the onset and progression of RA. Notably, shared epitope alleles in the MHC class II region make individuals more likely to develop ACPA-positive RA, a marker that can precede clinical symptoms [7, 45]. Apart from these alleles, genome-wide analyses have illuminated SNPs in multiple genes that increase the risk of RA. Smoking is one of the environmental factors that most strongly supports RA susceptibility [45, 46]. The pathophysiology of RA depends on a balance of cell death mechanisms in addition to genetics. Dysregulated apoptosis and autophagy, which eliminate unnecessary or hazardous cells, have been linked to RA [47, 48]. Neutrophil extracellular traps, or NETs, play dual roles: defending against pathogens and potentially exacerbating tissue harm when overly active. Other programmed cell death pathways, like necroptosis and pyroptosis, further contribute to RA's complex causal factors, offering potential treatment avenues [48-50]. Summarize genetic risk factors for RA in **Table 2**.

Table 2. Genetic risk factors for RA

Factor	Role in RA Prognosis	Description	References
Shared Epitope Alleles	Genetic Risk Factor	Residing in the MHC class II region, these alleles predispose individuals to develop anti-citrullinated protein antibody (ACPA)-positive RA, which can be detected years before clinical symptoms appear.	[45, 46]
Genetic Susceptibility Genes	Genetic Risk Factor	GWAS have identified SNPs in genes such as TRAF1, STAT4, CTLA4, IRF5, CCR6, PTPN22, IL23R, and PADI4 associated with an increased risk of developing RA. These SNPs may contribute to the immune dysregulation and inflammation observed in RA.	[51]
Apoptosis	Cell Death Pathway	A programmed cell death pathway implicated in autoimmune diseases, including RA. Dysregulation of apoptosis can lead to the persistence of damaged or unwanted cells.	[47, 48, 50]
Autophagy	Cell Death Pathway	In RA, autophagy is a cellular process that degrades and recycles damaged or unnecessary cellular components. Dysregulation can lead to the accumulation of damaged proteins and organelles, contributing to inflammation and tissue damage.	[47, 49, 50]
NETosis	Cell Death Pathway	Activated neutrophils release neutrophil extracellular traps (NETs) during NETosis, contributing to RA pathogenesis. NETs can cause tissue damage when excessively or persistently released, contributing to synovial inflammation and joint destruction.	[47, 52]
Necroptosis	Cell Death Pathway	Regulated necrosis mediated by RIPKs implicated in RA pathogenesis.	[47, 53]
Pyroptosis	Cell Death Pathway	An inflammatory form of programmed cell death mediated by caspase-1 activation implicated in RA pathogenesis.	[47, 54]

Table 3 shows that various genetic polymorphisms have been implicated in conferring susceptibility to RA. Specifically, shared epitope alleles residing in the MHC class II region are strongly associated with predisposition to ACPA-positive RA, with these risk alleles detectable years before the clinical onset of the disease. Additionally, multiple SNPs identified in genes such as TRAF1, STAT4, CTLA4, IRF5, CCR6, PTPN22, IL23R, and PADI4 have been linked to a heightened risk for developing RA, likely due to effects on immune system dysregulation and inflammatory pathways that may promote the autoimmune features of RA pathogenesis [46, 48, 51].

Table 3. Polymorphisms influencing RA disease risk

Genetic Factor	Role/Impact	Source/Location	Associated Mechanism
Shared epitope alleles	Predisposition to ACPA- positive RA	MHC class II region	Detection before clinical symptoms
TRAF1 SNP	Increased RA risk	Genome-wide study	Immune dysregulation & inflammation
STAT4 SNP	Increased RA risk	Genome-wide study	Immune dysregulation & inflammation
CTLA4 SNP	Increased RA risk	Genome-wide study	Immune dysregulation & inflammation
IRF5 SNP	Increased RA risk	Genome-wide study	Immune dysregulation & inflammation
CCR6 SNP	Increased RA risk	Genome-wide study	Immune dysregulation & inflammation
PTPN22 SNP	Increased RA risk	Genome-wide study	Immune dysregulation & inflammation
IL23R SNP	Increased RA risk	Genome-wide study	Immune dysregulation & inflammation
PADI4 SNP	Increased RA risk	Genome-wide study	Immune dysregulation & inflammation

MTX Pharmacogenomics

Mechanism of MTX action at the genetic level

MTX is a commonly prescribed medication for conditions like RA, psoriasis, and cancer. It's vital to comprehend how MTX works to use it most effectively and innovate treatment methods [55, 56]. MTX primarily influences inflammation in two ways: by facilitating the release of adenosine and halting transmethylation processes. Adenosine, a natural anti-inflammatory agent, is believed to be behind MTX's anti-inflammatory capabilities [55, 57]. Adenosine's role points out that MTX boosts external adenosine levels by impacting internal folate metabolism and activating adenosine receptors [58, 59]. Recognizing adenosine's significance can help craft new treatments for inflammation-related conditions [57, 58].

MTX's pharmacological dynamics are also influenced by its pharmacokinetic characteristics. A study by Maksimovic *et al*. delved into MTX's molecular workings and pharmacokinetics in patients with chronic sarcoidosis who took the drug orally. According to their research, polyglutamation modifies the pharmacokinetic and pharmacodynamic properties of MTX, extending its half-life [60]. In addition to stimulating the release of adenosine, MTX also regulates pathways associated with inflammation. Zhao *et al*. conducted a review that examined the possible drug-related mechanisms that could explain MTX's effectiveness in treating RA. These mechanisms included its function as a folate antagonist and its support of the immune system [55]. These functions collectively bolster MTX's value in RA management.

Yet, it's essential to note MTX's potential harmful effects. Ezhilarasan studied the molecular toxicological processes associated with MTX-caused liver damage. The research illustrated that built-up internal MTX-polyglutamate can induce liver issues, from oxidative stress to cell death. Such knowledge is crucial for vigilantly overseeing liver health during MTX administration [61]. Additionally, recent research points to the gut microbiome's role in shaping the therapeutic response to MTX. Yan *et al*. delved into how MTX affects RA's gut microbiome, uncovering that variance in gut microbes might influence MTX's absorption and ensuing effectiveness [62]. This insight underscores the need to account for individual microbiota variations when fine-tuning MTX treatments.

Genes influencing MTX metabolism

The reaction to MTX therapy varies significantly among individuals; some patients might face side effects or inadequate therapeutic benefits. This variability has spurred investigations into the genetic elements that could shape how MTX is metabolized and its subsequent impact. A noteworthy area of exploration is genes linked to folate metabolism, given that MTX functions by blocking dihydrofolate reductase and affecting nucleotide production [63, 64]. Various research endeavors have explored the connection between folate metabolism-related gene polymorphisms and the reaction to MTX [63-65].

The TYMS gene, which dictates the production of the enzyme thymidylate synthase involved in folate metabolism, has been a central point in multiple studies. One piece of research pinpointed a 6 bp deletion in the TYMS gene's 3' untranslated region (UTR), linking it to suboptimal results in MTX-treated patients [65, 66]. Another piece of research associated TYMS polymorphisms with a reduced reaction to MTX in acute lymphoblastic leukemia cases [65, 66].

Other genes, like methylenetetrahydrofolate reductase (MTHFR) and ATIC, pivotal for MTX metabolism, have also been under the research lens. Polymorphisms like C677T and A1298C in the MTHFR gene appear to modify the reaction to MTX, although some studies present divergent findings [67, 68]. Beyond genes directly tied to folate metabolism, other genetic factors might sway MTX's therapeutic response. As an illustration, gene variations in drug transport mechanisms might change how MTX is taken up or expelled from cells [67, 68]. Investigations indicate that drug transporter polymorphisms, such as those in ABCB1 and ABCC2, can influence MTX pharmacokinetics and therapeutic results [68] **(Table 4)**.

Table 4. Impact of gene polymorphisms on MTX metabolism and efficacy

Gene	Role in MTX Metabolism	Description
TYMS (Thymidylate Synthase)	Folate Metabolism	This gene regulates the production of the enzyme thymidylate synthase, which is involved in folate metabolism. Polymorphisms in TYMS, including a 6-bp deletion in the gene's 3' untranslated region (UTR), have been linked to suboptimal responses to MTX therapy.
MTHFR (Methylenetetrahydrofolate Reductase)	Folate Metabolism	This gene is crucial for MTX metabolism. Polymorphisms (C677T and A1298C) in the MTHFR gene may alter responses to MTX, although study findings vary.
ATIC (5-Aminoimidazole-4-Carboxamide Ribonucleotide Formyltransferase/IMP Cyclohydrolase)	Folate Metabolism	This gene is also pivotal for MTX metabolism, but further study is needed to assess the impact of its polymorphisms on MTX therapy responses.
ABCB1 (ATP Binding Cassette Subfamily B Member 1)	Drug Transport	Variations in this gene involved in drug transport can affect how MTX is absorbed into or expelled from cells. These polymorphisms may influence MTX pharmacokinetics and therapeutic outcomes.
ABCC2 (ATP Binding Cassette Subfamily C Member 2)	Drug Transport	Like ABCB1, polymorphisms in ABCC2, another gene involved in drug transport, may affect MTX absorption, expulsion, pharmacokinetics, and therapeutic outcomes.

Genetic variations impacting MTX responsiveness

Genetic differences that influence the effectiveness of MTX are vital when treating conditions like Crohn's disease, rheumatoid arthritis, psoriasis, acute lymphoblastic leukemia, and more. Various genes linked to the MTX response have been researched. For instance, genes like NOD2, IL23R, and ATG16L1 have strong proven links to Crohn's disease [1]. For rheumatoid arthritis, multiple genetic variations have been found that heighten disease risk, including those in HLA class II genes [2]. When observing MTX reactions in RA patients specifically, the focus has been on genes connected to the folate and adenosine pathways, namely reduced folate carrier 1 (RFC1), ABC transporters, TYMS, and MTHFR [3]. These genes are implicated in MTX transport into and out of cells, thymidine production, and indirect MTHFR enzyme activity inhibition. Changes in these genes can correlate with differing responses or side effects to MTX [3, 6]. Moreover, other studies have explored the link between HLA-Cw1 and psoriasis risk [4], PNPLA3 changes and NAFLD onset [5], and genetic variations related to microRNA and MTX response in Chinese RA patients [9]. Recognizing these genetic markers could help predict how a patient might react to treatment and inform therapeutic decisions. However, deeper investigation is essential to comprehend the basis of these links and confirm their practical application. Furthermore, tailoring treatments based on individual genetic makeup could enhance results for MTX therapy patients.

Polymorphisms associated with therapeutic response

Genetic variations linked to MTX reactions in RA patients have been studied extensively. Smolen *et al*. (2018) outlined the vital genetic differences tied to proteins influencing MTX's action and efficacy [1]. RFC1 aids in ferrying folate and MTX into cells. Among the most analyzed, its 80G>A variant seems to enhance RA's responsiveness to MTX [1]. ABC transporters expel MTX from cells, with the ABCB1 3435 C/T variation showing positive responses to MTX therapy [1]. TYMS, critical for thymidine production, has genetic variations that might reduce MTX's effectiveness and raise its toxicity [1]. Notably, the prevalent MTHFR gene SNPs, C677T, and A1298C, hint at a decline in MTX efficacy and a rise in toxicity [1]. Padyukov *et al*. (2022) explored

the pharmacogenetics of treating RA with MTX [2]. Their findings indicated inconclusive results from pharmacogenetic studies on varying MTX reactions among RA patients. Nevertheless, they delved into the molecular aspects of the folate and adenosine pathways, which might be reasons for therapy failures [2]. Mir *et al*. (2022) researched the treatment modalities for blastic plasmacytoid dendritic cell neoplasm (BPDCN) patients [3]. They classified chemotherapy approaches into five categories. They observed that patients undergoing AML-like, ALL-like, or Aspa-MTX treatments had prolonged survival compared to those on CHOP-like or NOS therapies [3].

Alivernini *et al*. (2019) studied the connection between microRNA-related genetic changes and MTX's clinical reaction in Chinese RA patients [4]. Their research pinpointed a significant link between miRNA-5189 and rs562929801 and MTX clinical response [4]. Guo *et al*. (2018) scrutinized data on SNPs in genes connected to low-dose MTX transport proteins and their potential role in predicting MTX outcomes [5]. Their discoveries raised the prospect of pharmacogenomic testing-based, more individualized RA patient therapies. In 2018, Bullock *et al*. conducted a meta-analysis on the relationship between genetic variants in TYMS and the responsiveness or side effects of MTX in individuals with RA [6]. Their work found no strong tie between TYMS changes and MTX therapy unresponsiveness but noted some toxicity connections in specific genetic configurations [6].

Romão *et al*. (2021) discussed the expanding roles of the BAFF/APRIL system beyond B cell biology [7]. Gene variations like RFC1, ABCB1, TYMS, and MTHFR correlate with MTX's effectiveness and side effects. However, individual variant impacts seem minimal, requiring more validation. The growing emphasis on detailed gene understanding and recognizing variant distributions by ethnicity underscores the value of personalized medicine. This approach helps decipher varying MTX responses at an individual level.

Polymorphisms associated with drug resistance

A study conducted by Valiev *et al*. [16] revealed that inherent genetic differences in genes linked to the MTX pathway impacted the drug's pharmacokinetics, side effects, and outcomes in all childhood patients undergoing MTX treatment. Significant links were found between single nucleotide variations (SNPs) in genes responsible for MTX polyglutamylation, cellular intake, and expulsion mechanisms and the drug's effectiveness. Another study by de Bianchi *et al*. [13] discovered that polymorphisms in the ABCB1 and ABCC3 genes were related to the initial year's MTX response in JIA patients. Those with a variation in ABCB1 rs1045642 were more likely to benefit from MTX than those with different genotypes. Likewise, those with a variation in ABCC3 rs4793665 had a better chance of MTX response. However, those with a SLC19A1 rs1051266 variation had reduced odds of reacting favorably to MTX. Wang *et al*. [11] investigated MTX-induced toxicity and found that nearly all JIA patients who didn't respond to MTX exhibited heterozygosity in the C3435T SNP of the ABCB1 gene. This indicates that this specific SNP might serve as a predictor for MTX ineffectiveness in JIA patients. Additionally, various studies have delved into the connection between genetic variations and particular side effects stemming from MTX treatment. For instance, Zafari *et al*. [9] identified that SNPs in the MDR1 and ABCC2 genes correlated with gastrointestinal complications and liver toxicity, respectively, in RA patients on MTX.

Gene variations linked to MTX's metabolism, transportation, and cellular activities have been demonstrated to shape treatment results. These insights hint at the potential of tailoring treatment strategies rooted in pharmacogenetic traits, potentially enhancing MTX therapy.

Clinical implications of MTX dosage based on genetic profiles

There's a growing interest in understanding how genetic profiles can guide MTX dosing. Numerous studies have delved into the relationship between these profiles and MTX dosage across diverse groups of patients. Lima *et al*. (2014) reported that gene variations in MTHFR were linked to MTX-related nausea in juvenile idiopathic arthritis patients. Likewise, Zaruma-Torres *et al*. (2015) found connections between ABCB1 and ABCC5 genetic variations and MTX side effects in Mexican children with acute lymphoblastic leukemia. Beyond these specific genetic variations, some research has also explored more general pharmacogenetic factors that could impact MTX's outcomes. For instance, Ulrich *et al*. (2001) looked into the HLA-G 14-bp variation in allo-HSCT following a brief MTX regimen for graft-versus-host disease prevention, discovering that those with reduced HLA-G activity faced a heightened risk of acute GVHD. Moreover, a 2015 review by Cheung *et al*. assessed cognitive outcomes in childhood acute lymphoblastic leukemia survivors who underwent MTX treatments. While treatments focused on chemotherapy seemed to be less detrimental to cognitive functions than cranial radiation therapy, survivors nonetheless exhibited mental challenges, especially in areas like attention and executive

functions. In summary, while it seems that genetic makeup could influence MTX outcomes and side effects, the quality of the evidence varies, with some studies being observational or having limited participants. There's also inconsistency between the genetic variations under research and the patient groups analyzed.

Potential for new therapeutic targets based on genetic insights

Various research efforts have investigated the genetic aspects influencing MTX's effectiveness and side effects in RA patients. One study pinpointed genetic variations in genes related to folate processing, such as RFC1 and TYMS, as potential influencers on MTX response. Another research highlighted SNPs in genes associated with adenosine pathways, like ADORA2A, which might affect MTX's efficacy for RA sufferers. A meta-analysis also indicated a potential link between SNPs in genes linked to drug transport, like MDR1, and MTX's response and toxicity in RA patients. The intense study has focused on how MTX works in treating RA. A specific review delved into multiple theories regarding its effectiveness in RA, touching on factors like folate antagonism, adenosine pathways, ROS production, changes in cytokine dynamics, and polyamine suppression. The adenosine pathway is primarily recognized as the main reason for MTX's effectiveness in RA. MTX is believed to boost adenosine levels, triggering internal sequences that usher in an anti-inflammatory environment. Another review centered on the progression of osteosarcoma treatment strategies. Although it didn't specifically tackle genetic perspectives on MTX therapy, it underscored the pressing need for innovative approaches to improve outcomes for osteosarcoma patients. It explored potential therapeutic targets derived from genomic characteristics and treatment reactions.

CONCLUSION

RA is a multifaceted autoimmune disorder influenced by genetic, environmental, and hormonal factors. MTX, a cornerstone in the treatment arsenal for RA, has shown varied efficacy and side-effect profiles across patients, leading to research endeavors aiming to pinpoint the underlying genetic factors. Our comprehensive review journeyed through the genetic landscape of RA and the intricate web of genes and polymorphisms influencing the MTX response.

We unearthed significant associations between genetic polymorphisms, especially in genes central to MTX metabolism and transport, and the variability in drug response. Genetic variations in genes like RFC1, TYMS, MTHFR, ABCB1, and ABCC2, among others, have emerged as potential determinants of MTX efficacy and toxicity. The wide potential of personalized medicine has been shown by this in-depth examination of the genetic foundations. Therapeutic approaches might be customized to each patient's genetic composition, maximizing treatment responses and reducing side effects. Moreover, the review highlighted the complexities surrounding RA's etiology, emphasizing the pivotal role of genes such as HLA class II, IL23R, PTPN22, STAT3, MBL, and DPB1 in disease susceptibility. The intricate dance of genes, immune modulation, T cell operations, and signaling pathways paints a compelling picture of RA's genetic backdrop. Unraveling these genetic intricacies is not just academically intriguing but also promises more targeted and effective therapeutic interventions in the future. Lastly, the interplay of genetics and MTX pharmacogenomics underscores the importance of continued research in this domain. As we gather more insights into the genetic factors modulating MTX's therapeutic outcomes, the horizon brightens for more tailored, effective, and safer treatment modalities for RA patients worldwide.

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