Systematic analysis of the molecular mechanisms of methotrexate therapy for rheumatoid arthritis using text mining

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Abstract

Objective

The purpose of this study was to determine the expression of related genes in patients with rheumatoid arthritis (RA) treated with methotrexate (MTX), to identify hub genes, and to systematically analyse the functions, pathways, and networks of these genes.

Methods

The PubMed identifiers (PMIDs) of relevant publications were obtained from the PubMed database, and gene data were extracted from these documents using the text mining software PubTator. The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to obtain enriched Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway information. In addition, the STRING database was used to construct a protein-protein interaction (PPI) network. Genes with which at least 10 other genes interacted were identified as hub genes.

Results

A total of 216 genes were identified as being associated with treatment efficacy for MTX, of which 14 pathways exhibited significant correlation ($p<0.05$, FDR<0.05). In addition, the constructed MTX treatment-related network consisted of 267 interactions. Fourteen genes were found to interact with at least 10 other genes ($p<0.05$, FDR<0.05) and identified as hub genes in the PPI network. These genes were JAK1, MAPK1, JUN, AKT1, MAPK14, MAPK8, FGB, FN1, ALB, B2M, IL2RB, GGH, IL2RA, and TP53.

Conclusion

This study will assist in elucidating the molecular mechanisms associated with the treatment efficacy of MTX for RA and provide a scientific rationale for guiding patient medication. However, the relationship between particular genes and the efficacy of MTX treatment for RA patients requires additional investigation.

Key words

rheumatoid arthritis, methotrexate, text mining, hub gene

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that affects individuals of all adults (1), usually causing damage to the surrounding cartilage and bone, with a prevalence of approximately 0.5 to 1% (2). In recent years, with the continuous development of medical technology, the severity of disease in RA patients has been alleviated to some extent, but the incidence rate has not decreased, representing a heavy burden both economically and in terms of quality of life (3).

Although there are many clinical treatments for RA, methotrexate (MTX) is among the most widely used antirheumatic drug in clinical practice (4). However, patients with RA respond in a highly heterogeneous manner to drug therapy. A large proportion of patients either do not respond to MTX or have serious side effects. Therefore, it is of critical importance to judge whether RA patients are suitable for MTX treatment as rapidly as possible. Studies have shown that the efficacy of MTX treatment in RA is associated with genetic polymorphisms (5-7). Pharmacogenomics research could help identify individual heterogeneity and guide clinical use of MTX therapy for each RA patient, which was an innovation in the treatment of RA. Defining personalised MTX treatment for RA patients of specific genotypes would be of great benefit in order to guide the selection of suitable drugs. The biomarkers that predict MTX treatment response would help optimise treatment, avoid ineffective treatments for individual patients, reduce adverse side effects, and reduce the waste of healthcare resources, which had very important practical significance and laid a good foundation for the realisation of precision medicine (8-10).

At present, there are a large number of biomedical publications related to MTX treatment for RA, the rapid increase in their number being responsible for preventing researchers from manually identifying and processing all the relevant information. Text mining tools may solve this problem by automatically identifying and annotating key biological entities from a large portfolio of biomedical literature (11). In this study, data mining was used to analyse genes related to the use of MTX in treating RA in the PubMed database, and combined with bioinformatics methods to identify the central genes and pathways associated with the therapeutic effects of MTX.

Materials and methods

Text mining

PubMed was searched using ("Arthritis, Rheumatoid/drug therapy"[Mesh]) AND "Methotrexate/therapeutic use "[Mesh] as a search term, the species selected as human, extracting the PubMed identity (PMID) for each publication from the establishment of PubMed to November 2019, its abstract exported. Genes and proteins among these abstracts were located and tagged using PubTator (A Biomedical Named Entity Recognizer) (12). The Entrez Gene Database (NCBI's repository for gene-specific information) was used to normalise gene symbols with unified results. The frequency of occurrence of each gene was then calculated and the hypergeometric distribution used to calculate the probability that a particular gene would be collinear with MTX for RA patients, with genes where $p<0.05$ considered related to treatment efficacy of MTX. The disease activity score 28 (DAS28) was used to measure the patient's disease activity status, including 28 tender joint count (TJC), 28 swollen joint count (SJC), the erythrocyte sedimentation rate (ESR) and visual analogue scale (VAS) for general health. The change of DAS28 score before and after MTX treatment was used to judge the efficacy of drugs.

Genetic analysis

Gene ontology (GO) analysis was performed using the database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov), a gene function classification tool. Genes were annotated and classified according to biological processes, molecular functions and cell components (13).

The Kyoto Encyclopedia of Genes and Genomes annotation system is a bioinformatics resource based on the Kyoto

Gene and Genomics Encyclopedia and was used for enrichment annotation analysis of signalling pathways associated with the use of MTX in the treatment of RA (14).

Protein interaction analysis

The STRING database (http://www.string-db.org/) was used to construct a PPI network related to the treatment efficacy of MTX for RA. The interaction score was set at 0.9 (15). Cytoscape was then used to illustrate the PPI network which was then analysed with cytoHubba (a plugin for Cytoscape) (16). Hub genes were defined as those that interacted directly with 10 or more other genes. The threshold for *p*-values and false discovery rates (*FDRs*) was 0.05.

Results

Genetic analysis related to the efficacy of MTX for the treatment of RA

A total of 4732 abstracts were found by searching the literature on PubMed, from which 451 genes were obtained. Eventually, 216 genes were identified as being related to treatment efficacy of MTX for RA through a hypergeometric distribution ($p<0.05$). The most frequently studied genes (>11 times) are listed in Table I. Among these genes, *CRP* (203 times), *MTHFR* (55 times) and *MTX1* (54 times) were mentioned most frequently.

The classification results of the Gene Ontology analysis included mainly biological processes, cell components, and molecular functions, as shown in Figure 1. The results of Biological processes (Fig. 1A) suggest that genes related to the efficacy of MTX for RA were principally involved in the immune response, inflammatory response and drug response. Various cell components, including extracellular space, external side of the plasma membrane, extracellular regions and plasma membrane are shown in Fig. 1B. In terms of molecular function, these genes were mostly related to cytokine activity, protease binding, enzyme binding, protein binding and glycoprotein binding (Fig. 1C) (See the online Supplementary file, Tables S1-S3 for details).

Table I. The 24 most frequently studied genes related to MTX efficacy on RA based on text mining.

Genes	Description	Count	*p*-value
CRP	C-reactive protein	203	4.91E-04
MTHFR	methylenetetrahydrofolate reductase	55	9.23E-27
MTX1	metaxin 1	54	1.27E-07
MMP3	matrix metallopeptidase 3	30	2.01E-02
KRT20	keratin 20	30	<1.00 E-100
IL1B	interleukin 1 beta	28	2.92E-11
ABCB1	ATP binding cassette subfamily B member 1	24	7.20E-11
CD8A	CD8a molecule	23	6.20E-05
IL1A	interleukin 1 alpha	19	2.86E-09
TYMS	thymidylate synthetase	19	6.92E-13
SLC17A5	solute carrier family 17 member 5	19	6.92E-13
PRTN3	proteinase 3	17	1.04E-07
IFNG	interferon gamma	16	5.10E-09
IL2RA	interleukin 2 receptor subunit alpha	16	1.90E-07
IL17A	interleukin 17A	14	3.59E-02
JAK1	Janus kinase 1	14	5.52E-03
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	13	5.82E-05
TNFSF11	TNF superfamily member 11	13	3.41E-05
DHFR	dihydrofolate reductase	12	1.85E-08
FPGS	folylpolyglutamate synthase	12	2.68E-07
IL1RN	interleukin 1 receptor antagonist	12	1.85E-08
SLC19A1	solute carrier family 19 member 1	12	7.79E-07
GGH	gamma-glutamyl hydrolase	12	7.79E-07
ASPRV1	aspartic peptidase retroviral like 1	12	<1.00 E-100

Pathway and protein interaction analysis

Following pathway analysis, 14 pathways were found to be significant ($p<0.05$, *FDR* <0.05). The 10 most significant pathways of genes associated with treatment efficacy of MTX for RA are shown in Figure 2. The results suggest that genes associated with MTX efficacy are primarily involved in cytokine-cytokine receptor interaction, Jak-STAT signalling pathways, osteoclast differentiation and rheumatoid arthritis (See Supplementary Table S4 for details).

For gene interaction analysis, a PPI network was further constructed related to the efficacy of MTX for the treatment of RA. The results included 267 interactions (Fig. 3A). Among these, 14 genes interacted with at least 10 other genes ($p<0.05$, *FDR* <0.05) and so were classified as hub genes related to the efficacy of MTX in the treatment of RA. These genes were *JAK1*, *MAPK1*, *JUN*, *AKT1*, *MAPK14*, *MAPK8*, *FGB*, *FN1*, *ALB*, *B2M*, *IL2RB*, *GGH*, *IL2RA* and *TP53* (Fig. 3B). Among them, *JAK1*, which interacts with 20 other genes exhibited the greatest number of interactions (Fig. 4). Additionally, a Venn diagram (Fig. 5) was used to classify and compare hub genes from the PPI with the top 24 genes (Table I) obtained from text mining, to identify those pathways of genes shared by both text mining and PPI analysis.

Discussion

In the present study, we extracted information from the biological research literature, obtained genetic datasets related to the treatment efficacy of MTX for RA, and consequently identified 14 hub genes based on the PPI network. Among these genes, *JAK1*, *GGH* and *IL2RA* have been extensively studied (Table I), playing important roles in the inflammatory and immune response, being closely related to the efficacy of MTX for the treatment of RA. As for *MAPK1*, *JUN*, *AKT1*, *MAPK14*, *MAPK8*, *FGB*, *FN1*, *ALB*, *B2M*, *IL2RB* and *TP53*, there have been fewer reports, suggesting that they require additional study. To our knowledge, this is the first report about genes related to MTX efficacy for the treatment of RA based on text mining and bioinformatics methods.

In clinical practice, MTX is widely used as a disease-modifying antirheumatic drug for the treatment of RA. However, not all RA patients respond

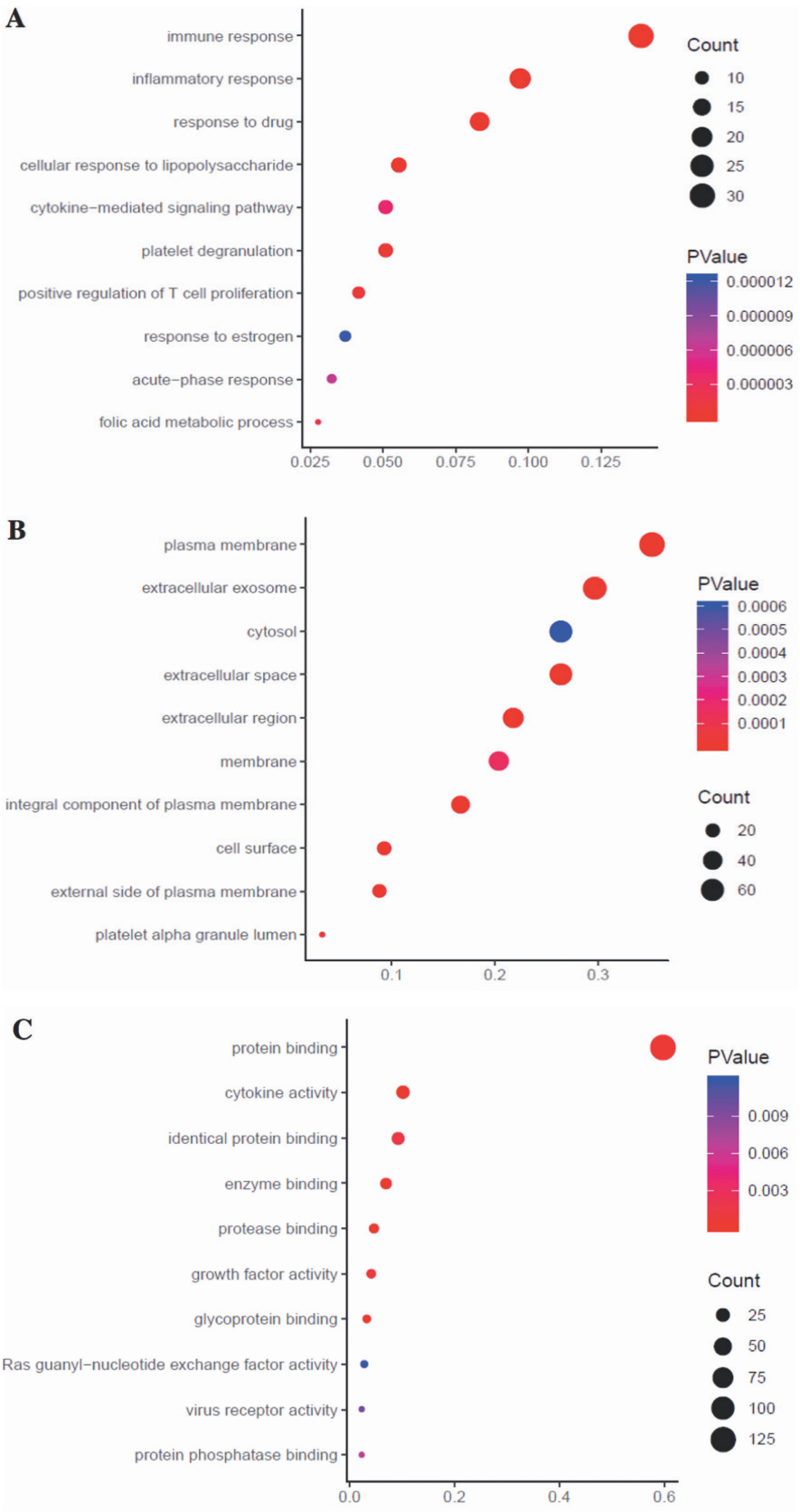

Fig 1. Classification results for biological process, cellular components, and molecular functions by Gene Ontology analysis.
A: Biological process.
B: Cellular component.
C: Molecular function.

well to MTX and may even exhibit serious adverse reactions. If the condition of an RA patient cannot be controlled expeditiously, joint damage or even an increase in disability may result (17). Therefore, it is of great benefit to ascertain at an early stage whether an RA patient is suitable for treatment with MTX. Genetic background plays an important role in drug efficacy. Understanding the genetic factors associated with MTX efficacy may contribute to individualised medication (18). Text mining can assist pinpointing the target content from a large number of publications, provide the data required to identify the genes involved in the treatment efficacy of MTX for RA.

MAPK, *JUN* and *TP53*

Proteins encoded by *MAPK1*, *MAPK8*, and *MAPK14* are the three major members of the mitogen-activated protein kinase (MAPK) family. The protein kinases encoded by these genes are the integration points of various biochemical signals and participate in the regulation of multiple important cellular functions. Among them, the *MAPK8* and *MAPK14*-related pathways are the main MAPK pathways, which are related to chronic inflammatory response (19).

MAPK1 encodes an extracellular signal-regulated kinase (ERK) that is involved in the regulation of cell division. Multiple types of stimulus, including growth factors and cytokines, may activate the ERK pathway (20).

MAPK8 encodes the c-Jun N-terminal kinase (JNK), which is involved in transcriptional regulation. JNK signalling is involved in both endogenous and exogenous apoptotic pathways (21). *JUN* is a target gene of JNK, and JNK can phosphorylate JUN family members. The most studied interaction is the binding of JNK to the DNA binding protein c-Jun, and its subsequent phosphorylation, thereby increasing the latter's transcriptional activity (22). c-Jun is a component of the AP-1 transcriptional complex, which is involved in the regulation of the expression of many cytokines, including pro-apoptotic proteins (20, 23). JNK also directly phosphorylates P53 protein, regulated by the *TP53* gene, which increases the

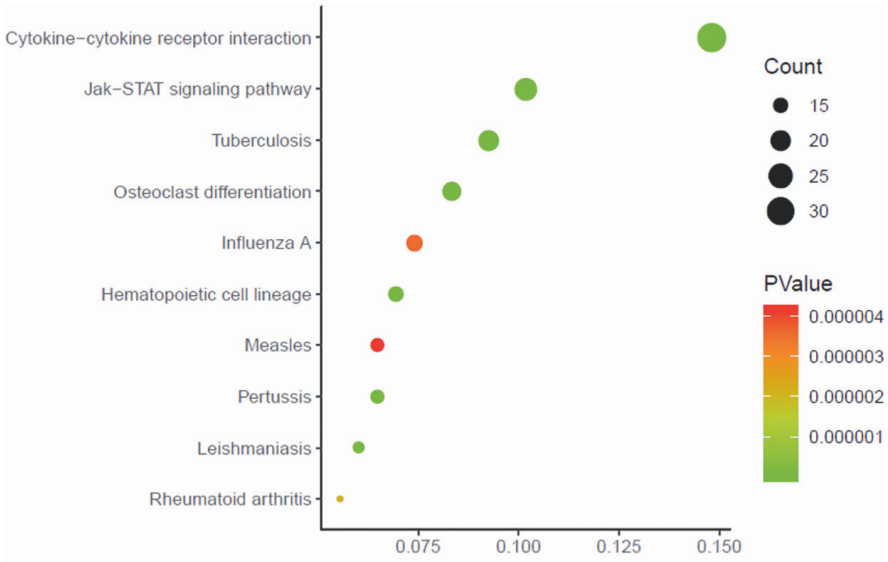

Fig. 2. The 10 most significant pathways associated with MTX efficacy-related genes.

activity of P53. P53 can promote the expression of a variety of pro-apoptotic genes and participate in the apoptotic pathway of JNK activation. P53 is also involved in cell cycle arrest, a key factor affecting cell proliferation (20, 24).

In 2011, Spurlock *et al*. found that MTX, a folic acid antagonist, induced activated T cells to produce reactive oxygen species, increasing JNK activity and *JUN* mRNA expression through a JNK-dependent mechanism, thereby increasing the sensitivity of cells to apoptosis. Additionally, the study also found that low doses of MTX increased the levels of *JUN* in RA patients, possibly related to the activation of the JNK-related pathway, contributing to the efficacy of MTX (25). Spurlock *et al*. also found in 2012 that RA patients had a significant reduction in *TP53* and JNK protein levels compared with healthy controls. JNK protein and *TP53* transcription levels were found to increase in these patients when treated with MTX, which may repair partial defects in the cell cycle checkpoints of RA patients through the JNK pathway, allowing activated RA T cells to apoptose in the face of normal DNA damage, reducing inflammation in RA patients (26). The results of a study by Bergstrom *et al*. support this observation (27). All these observations suggest that JNK-related pathways might affect the efficacy of MTX in RA patients.

The *MAPK14*-encoded p38 kinase cascade is activated by inflammatory cytokines and regulates their expression playing an important role in the immune response and is associated with autoimmune diseases (20). p38 is also essential for the synthesis and release of pro-inflammatory cytokines and is the target of many therapeutic drugs to control inflammatory diseases (28). Studies have shown that MTX can stimulate IL-1β expression, induce phosphorylation of various proteins in the p38-MAPK signalling cascade, thereby promoting the release of IL-8 (29). IL-8 is a neutrophil chemokine that causes their accumulation in the lungs where they eventually trigger inflammation, resulting in RA patients having side effects related to treatment by MTX (30).

In the present study, we found that *MAPK*, *TP53* and *JUN* are involved in multiple signalling pathways, including those of cell differentiation and inflammatory factors. In addition to pathway module analysis of genes related to MTX treatment efficacy, analysis of the biological processes, cell components and molecular functions of GO modules was also performed. *MAPKs*, *JUN* and *TP53* were enriched in processes associated with apoptosis and drug response. *MAPKs* may affect the expression of a variety of cytokines and are the hub of multiple pathways.

IL2R

IL2 is an autocrine product of activated T cells whose receptor *IL2RA* and *IL2RB* are associated with inflammatory diseases in addition to autoimmune diseases (31). Regulatory T cells have a high expression level of *IL2RA*, which may inhibit the autoimmune response and maintain immune homeostasis (32). *IL2RB* encodes a receptor subunit and is expressed in the hematopoietic system. It is also involved in the activation of T cells and NK cell subsets. Single nucleotide polymorphisms of *IL2RA* and *IL2RB* genes have been shown to be associated with susceptibility to RA and to be also associated with the risk of bone erosion in early RA patients, affecting severity of the disease (33-35).

Studies have shown that low-dose MTX can induce apoptosis in activated T cells, thereby exerting immunosuppressive effects on RA patients (36). In addition, Herman *et al*. demonstrated that MTX can down-regulate *IL2R* expression and reduce the expression of the *IL2RA*-related lymphocyte activation marker CD25 in active RA patients, ultimately inhibiting their immune response (37). Furthermore, MTX was also shown to reduce the level of sIL-2R and improve clinical indicators in RA patients (38). Determination of sIL-2R and CD25 expression may allow prediction of the efficacy of MTX (7, 39).

Based on text mining, we found that *IL2RA* and *IL2RB* were involved in multiple pathways, including cytokine-cytokine receptor interaction, the Jak-STAT signalling pathway and hematopoietic cell lineage. The results of GO enrichment indicated that *IL2RA* and *IL2RB* were mainly involved in immune and inflammatory responses, and cytokine-related signalling pathways. The relationship between *IL2RA*, *IL2RB* and MTX require additional investigation.

JAK1

JAK1 is a member of the Janus kinase (JAKs) family, which could bind to the phosphorylation of different isoforms of activators of transcriptional protein (STATs) and mediate the signal transduction of cytokines and growth factor receptors associated with RA pathogenesis (40). JAK/STAT signalling pathway plays an important role in immune regulation and inflammation. Drosoph-

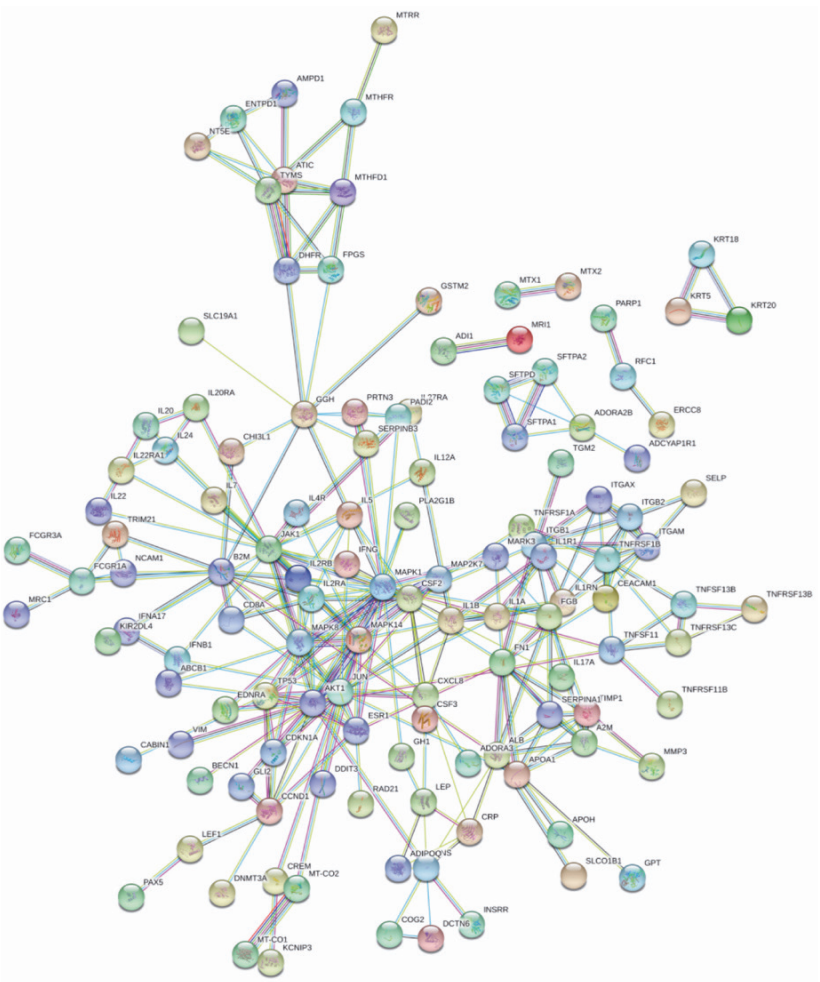

Fig. 3A. Network analysis of MTX efficacy-related genes.

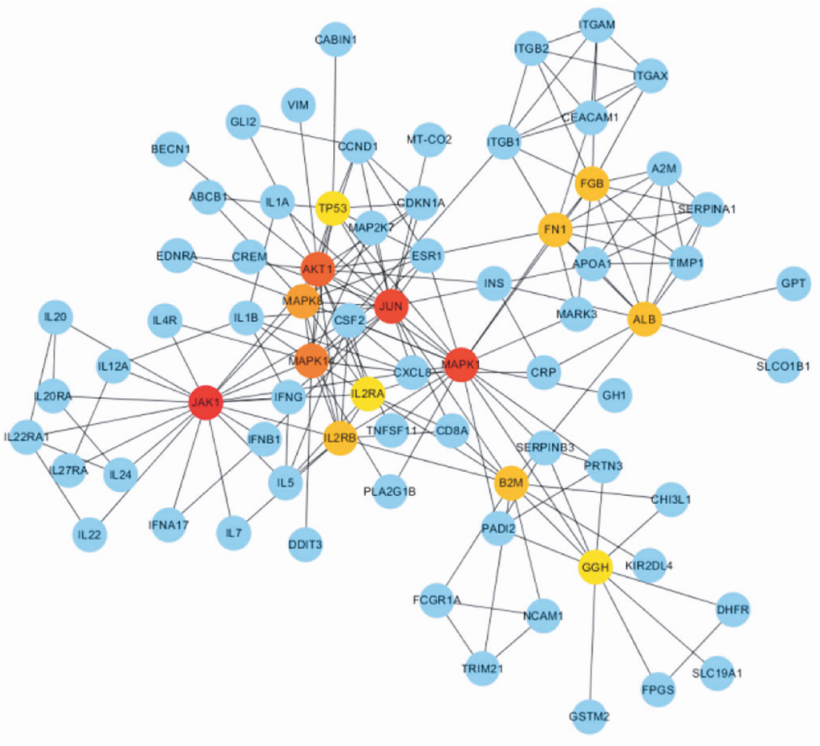

Fig. 3B. MTX efficacy-related hub genes in the network.

ila and human cell line related experiments have shown that the phosphorylation of STATs, the main physiological substrate of JAK kinase, is affected by MTX and decreases in dose-response, thereby inhibiting JAK activity. Low-dose methotrexate can reduce the activity of the JAK/STAT pathway and play an anti-inflammatory and immunosuppressive effect on RA patients (41, 42).

GGH

Gamma-glutamyl hydrolase (GGH) is a lysosomal peptidase, which could convert long-chain polyglutamic acid into short-chain polyglutamic acid and degrade polyglutamic acid (43). MTX that had entered the target cell would be converted into MTX polyglutamates (MTXPGs) for long-term existence, and *GGH* could remove glutamine from MTXPGs and participate in MTX metabolism (44). *GGH* gene polymorphism might change enzyme activity and affect the efficacy of MTX on RA patients (45, 46). In addition, related studies had shown that *GGH* gene polymorphism might be related to MTX hepatic toxicity (47). Analysis of *GGH* alleles and genotypes might help identify RA patients who were not suitable for MTX treatment.

However, for these genes, *AKT1*, *FGB*, *FN1*, *ALB*, and *B2M*, there were few related reports. The A (3) adenosine receptors A3ARs are highly expressed in inflammatory cells and are therapeutic targets for RA. The anti-inflammatory process involved in A3ARs is related to the PI3K/Akt signalling pathway where *AKT1* is located. The experimental model of rats showed the anti-inflammatory effects of A3ARs could inhibit the PI3K/Akt signalling pathway and affect the inhibitory effect of MTX on inflammation (48, 49), but its role in the human body still need further exploration. The fibrinogen encoded by *FGB* is involved in the inflammatory response and is related to the pathogenesis of RA. Different *FGB* genotypes are associated with fibrinogen and CRP levels and CRP is an important indicator of the disease activity of RA patients (50, 51). At present, there is still a lack of research related to the efficacy of FGB and RA drugs, and further research on

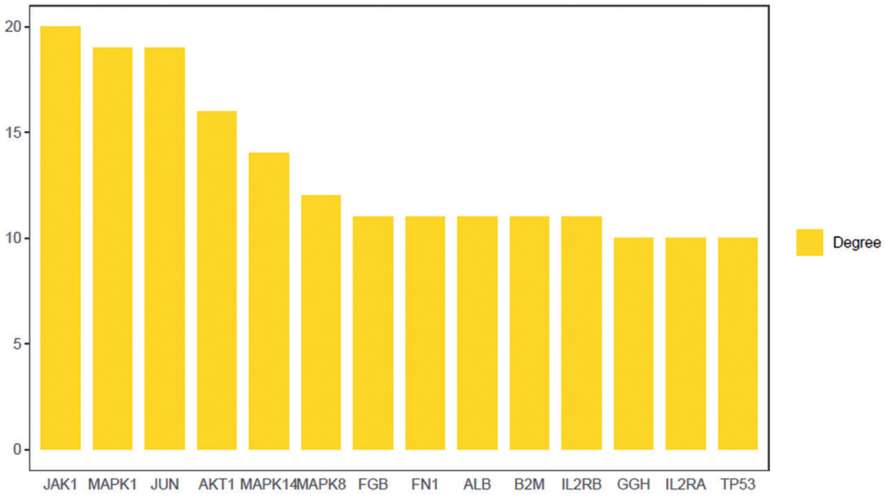

Fig. 4. Top 14 genes' interaction count associated with MTX efficacy.

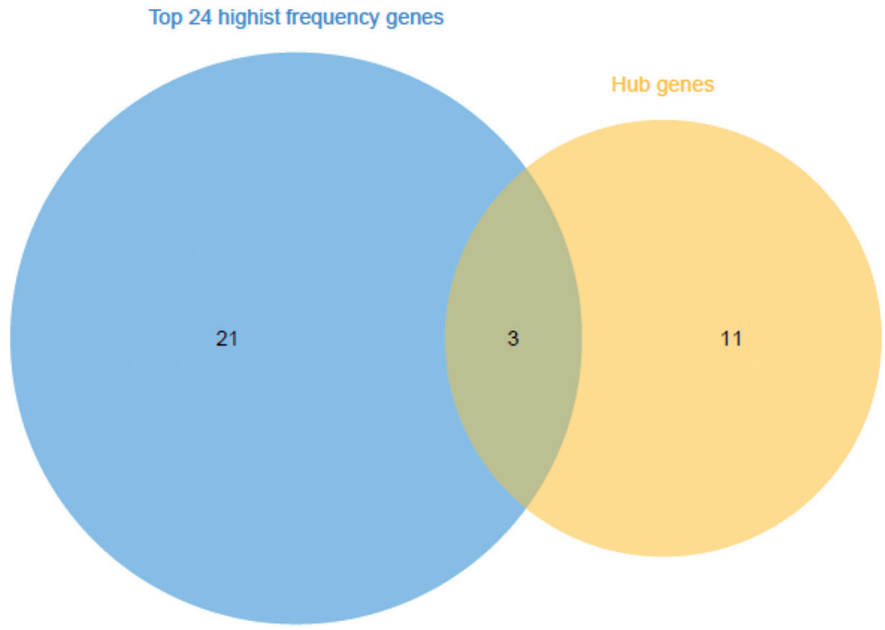

Fig. 5. Similarities and differences between MTX efficacy-related hub genes and the top 24 highest frequency genes.
The top 24 highest frequency genes are *CRP*, *MTHFR*, *MTX1*, *MMP3*, *KRT20*, *IL1B*, *ABCB1*, *CD8A*, *IL1A*, *TYMS*, *SLC17A5*, *PRTN3*, *IFNG*, *IL2RA*, *IL17A*, *JAK1*, *ATIC*, *TNFSF11*, *DHFR*, *FPGS*, *IL1RN*, *SLC19A1*, *GGH* and *ASPRV1*.
Hub genes are *JAK1*, *MAPK1*, *JUN*, *AKT1*, *MAPK14*, *MAPK8*, *FGB*, *FN1*, *ALB*, *B2M*, *IL2RB*, *GGH*, *IL2RA* and *TP5*.
Three genes covered in both groups: *JAK1*, *GGH*, *IL2RA*.

FGB might have new discoveries. The *FN1* gene could produce multiple transcript variants through selective cleavage, encoding different FN isoforms (52). Among them, the FN isoform containing ED-B (B-FN) is rarely expressed in normal tissues but is highly expressed in the vascular membrane of RA, which could be used for the diagnosis of RA (53). In addition, the research made by Connolly *et al*. showed that FN was related to the disease activity of RA, and the application of MTX treatment could reduce the level of FN and alleviate the patient's disease status (54). Albumin encoded by the *ALB* gene exists in a large amount in plasma and is a carrier of a variety of endogenous molecules and exogenous drugs. The synthesis of albumin can be inhibited by the inflammatory response and is a marker of the inflammatory response, which could reflect the inflammation and disease activity of RA patients (55). In addition, related animal models indicated that MTX damage to the liver could cause changes in serum albumin levels (56), and this suggests that the level of albumin might be an indicator of adverse drug effects. *B2M* is the major histocompatibility complex (MHC) class I associated gene, and the MHC region is closely related to the heritability of RA. In 2011, Uddin *et al*. performed RA-related genome-wide analysis and found that the potential copy number variations (CNV) of the B2M gene were also associated with RA (57), and another related study also showed that the concentration of B2M in the serum and synovial fluid of RA patients was higher (58). The above research results imply that B2M might be a target for drug therapy, however, there is still a lack of research in this area and further exploration is still needed.

In summary, the present study systematically analysed genes associated with the efficacy of MTX on RA. The hub gene related to MTX efficacy was identified by gene ontology enrichment, pathway enrichment, and construction of a PPI network. This study may help in understanding the efficacy of MTX in treating RA and contribute to the development of personalised medicine. However, this study only considered the published literature, and the accuracy of gene recognition in natural language requires further improvement. The correlation between particular genes and the treatment efficacy of MTX requires additional elucidation.

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