Systematic druggable genome-wide Mendelian randomization identifies novel therapeutic targets or repurposing opportunities for rheumatoid arthritis

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Article

Keywords: Rheumatoid arthritis, Drug target, Expression quantitative trait loci, Mendelian randomization

Posted Date: October 18th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3438026/v1

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Additional Declarations: No competing interests reported.
Abstract

Background Rheumatoid arthritis (RA) is a common autoimmune inflammatory disease. Currently, a complete cure for RA is still unavailable. Mendelian randomization (MR) has emerged as a valuable tool for identifying potential therapeutic targets or drug repurposing opportunities for certain diseases. Therefore, our aim was to identify novel effective targets or drug repurposing opportunities for RA and analyze their mechanisms and potential side effects.

Methods A MR integrating the identified druggable genes was used to evaluate the causal effects of druggable gene cis-expression quantitative trait loci (cis-eQTLs) on RA, while additional RA cohort was employed for validation. Colocalization analysis was performed to determine the probability of shared causal variants between the identified targets and RA. The protein-protein interaction network analysis was conducted to explore associations between the identified druggable genes and current RA drug targets. The MR and colocalization analyses were used to assess the potential side effects of the identified targets in RA treatment.

Results Nine druggable genes (TYK2, PTTPN22, ATP2A1, APOM, RXRB, NOTCH4, HLA-DRA, CCR6, and CTLA4) showed significant MR results in both the training cohort (p<1.99E-05) and validation cohort (p<0.0025). Colocalization analysis indicated that cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and RA (PPH4.abf=0.98), as well as C-C motif chemokine receptor 6 (CCR6) and RA (PPH4.abf=0.99), shared the same causal variant. Hence, these two genes were identified as the final therapeutic targets. Furthermore, CTLA4 and CCR6 interacted with the current RA drug targets. Subsequent MR analysis revealed that genetically proxied activation of CTLA4 and inhibition of CCR6 might decrease the risk of hypothyroidism but increase the risk of malignant skin neoplasm. Additionally, genetically proxied activation of CTLA4 may also reduce the risk of type 1 diabetes.

Conclusions This study supports the idea that targeting the activation of CTLA4 and the inhibition of CCR6 may reduce the risk of RA with fewer side effects, and highlights the potential of CTLA4 and CCR6 as promising druggable targets for RA treatment.

Background

Rheumatoid arthritis (RA) is a common, chronic autoimmune inflammatory disease characterized by symmetrical inflammation of multiple joints[1]. It manifests with joint pain, swelling, and morning stiffness. As the disease progresses, it may cause joint deformities, functional impairments, and even disability[1]. Apart from joint symptoms, RA often presents with extra-articular manifestations, such as vasculitis, synovitis, peripheral neuropathy, and Felty's syndrome[1]. Globally, the incidence of RA ranges between 0.5% and 1.0%, with females being affected at a rate approximately three times higher than males, particularly middle-aged women[2, 3]. RA can also affect children and the elderly[2, 3]. The exact mechanism of RA is not fully understood, but it is believed to involve genetics, environment, lifestyle, and autoimmune response[4]. The main pathological features of RA include chronic inflammation proliferation of synovial membrane, massive infiltration of inflammatory cells in the joint, formation of synovial pannus, and erosion of cartilage and bone[5].

Currently, a complete cure of RA is still not possible[6]. The treatment of RA involves both pharmacologic and non-pharmacologic approaches. Commonly used RA medications include nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease-modifying anti-rheumatic drugs (DMARDs), and biologics[6]. Although these medications can effectively relieve symptoms and improve the condition of RA, long-term use may lead to various side effects, including liver and kidney damage, gastrointestinal symptoms, infections, cardiovascular complications, and malignancies[6, 7]. Therefore, it is necessary to explore effective drugs with less side effects for RA treatment.

Mendelian randomization (MR) is a powerful method that uses genetic instrumental variables as proxies to explore causal relationships[8]. Compared to observational studies, MR can avoid the influence of confounding factors. In drug target MR analysis, cis-expression quantitative trait loci (cis-eQTL) or cis-protein quantitative trait loci (pQTL) are considered regulatory factors of gene expression and commonly used as proxies to explore causal relationships between genes and diseases, which can be used to identify potential drug targets[8, 9]. Nelson et al.[10] found that genetically associated protein drug targets are more likely to be approved for market than non-genetically associated ones, suggesting that MR is an effective strategy in drug target screening. Currently, MR method combining disease genome-wide association studies (GWAS) data and eQTL or pQTL to identify potential therapeutic targets or drug repurposing opportunities has been widely used in various diseases, such as COVID-19, stroke, Alzheimer's disease, aortic aneurysm, multiple sclerosis, and more[9, 11–14]. However, there is still limited research exploring the genomic evidence of potential therapeutic targets for RA.
In this study, we employed MR analysis and colocalization analysis using RA GWAS data and eQTL data to identify potential therapeutic targets for RA and provide novel therapeutic strategies. Additionally, we further explored the associations between these identified target genes and 11 common cardiovascular risk factors, as well as 32 potential diseases related to RA treatment, in order to assess the safety of drug applications targeting these genes.

Methods

Study design

In this study, our objective was to identify potential therapeutic targets for RA. The study design is depicted in Fig. 1. Firstly, we extracted druggable genes located on autosomes from a previous study[15] and selected cis-eQTL data associated with these genes from the eQTLGen Consortium database as instrumental variables[16]. Secondly, we conducted a two-sample MR analysis and colocalization analysis to identify potential druggable genes for RA using GWAS data from the FinnGen database, and this was further validated in an additional cohort[17]. Thirdly, we examined the interactions between the identified genes and the targets of current RA drugs to evaluate the clinical potential of these novel targets and gain a deeper understanding of the underlying mechanisms of new RA drug targets. Finally, we conducted a safety evaluation of drug applications targeting the final therapeutic target genes.

Selection of cis-eQTL data related to druggable genes as instrumental variables

The data on druggable genes were obtained from a previous study by Finan et al.[15], where they identified 4479 potential target genes for drug development. For our study, we only included the 4317 druggable genes located on autosomes. The cis-eQTLs of these 4317 target genes were obtained from the eQTLGen Consortium and eQTL meta-analysis of peripheral blood from 31,684 individuals[16]. To ensure the robustness of our analysis, we only included cis-eQTLs that met the following rigorous criteria: (i) demonstrating a genome-wide significant association ($p < 5.0E-08$); (ii) exhibiting independent association (pairwise linkage disequilibrium (LD) $r^2 < 0.001$ within a 10000 kb distance); (iii) displaying robust strength (F-statistic > 10); and (iv) exhibiting greater variance than the RA trait (Steiger p-value greater than 0.05) (Supplementary Table 1).

Outcome data

GWAS summary statistics of RA. For the training cohort, the GWAS data for RA were obtained from individuals of European ancestry in the FinnGen biobank (https://r9.finnngen.fi/pheno/M13_RHEUMA (accessed on 16 May 2023, R9 release))[18], including 12555 cases and 240862 controls (Supplementary Table 2). For the validation cohort, the GWAS data were obtained from a previously published paper, which included 14361 cases and 43923 controls of European ancestry (Supplementary Table 2)[17].

GWAS data of common cardiovascular risk factors and potential diseases related to RA treatment. To evaluate drug safety, we analyzed the correlation between the identified target genes and 11 common cardiovascular risk factors. These factors included blood glucose-related data like fasting glucose, fasting insulin, and Hba1c; blood lipid-related data including total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, apolipoprotein B, and apolipoprotein A1; and blood pressure-related data such as diastolic blood pressure and systolic blood pressure. Additionally, we evaluated the associations between the identified target genes and 32 potential diseases related to RA treatment. These diseases included thrombocytopenia, heart diseases such as coronary artery disease, acute myocardial infarction, and heart failure; strokes including any stroke, lacunar stroke, cardioembolic ischemic stroke, ischemic stroke (small-vessel), and ischemic stroke (large artery atherosclerosis); chronic kidney disease; gastrointestinal ulcers such as duodenal ulcer and gastric ulcer; osteoporosis; osteonecrosis; hypothyroidism; rash and other nonspecific skin eruptions; type 1 and type 2 diabetes; and 13 types of malignant tumors (Supplementary Table 3).

Statistics

Mendelian randomization. The MR analysis was conducted using the ‘TwoSampleMR’ R package (https://github.com/MRCIEU/TwoSampleMR). When only one cis-eQTL was available, we utilized the Wald ratio method for MR analysis. For two or more cis-eQTLs were present, we employed the random effect inverse variance weighted (IVW) MR method and followed it with sensitivity analysis[9]. Bonferroni correction was employed to adjust for multiple testing. For the training cohort, a p-value threshold of $p < 1.99E-05$ (0.05/2516) was considered statistically significant, while a p-value between 1.99E-05 and 0.05 was considered suggestive. Target genes identified with statistical significance were then validated in the validation cohort. A p-value less
than 0.0025 (0.05/20) was considered statistically significant, and a p-value between 0.0025 and 0.05 was considered suggestive. Additionally, we employed the Steiger filtering to detect the direction of correlation between target genes and RA in the two independent cohorts[19]. A p-value less than 0.05 considered statistically significant.

Colocalization analysis. Genes with statistically significant MR results in both cohorts were selected for further colocalization analysis using the "coloc" package with default parameters (https://github.com/chr1swallace/coloc). The focus of this study was to evaluate the posterior probability of hypothesis 4 (PPH4), which suggests that both the druggable genes and RA are associated with the region through shared variants. Colocalization was considered significant if the posterior probability was greater than 0.80 (PPH4 > 0.80).

Subsequently, genes exhibiting strong colocalization with RA were identified as final therapeutic targets and further investigated. The LocusZoom online website was used to visualize the colocalization analysis results (http://locuszoom.org/)[20].

Phenome-wide scan. To investigate whether the instrumental variables of the final therapeutic target genes exhibit horizontal pleiotropy, we conducted a phenome-wide scan of GWAS for these SNPs to identify potential associations with other risk factors that may affect the risk of RA. Using a genome-wide association threshold of a p-value less than 5.0E-08, we searched for proteins, gene expression, traits, and diseases associated with any known risk factors of RA on Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk/).

Drug safety evaluation

We employed MR analysis, Steiger filtering, and colocalization analysis to explore the relationship between the final therapeutic targets identified above and 11 common cardiovascular risk factors, as well as 32 potential diseases related to RA treatment. In the MR analysis, a p-value less than 1.16E-03 (0.05/43) was considered statistically significant, while a p-value between 1.16E-03 and 0.05 was considered suggestive. A p-value threshold of less than 0.05 was employed in Steiger filtering analysis, and a PPH4.abf threshold of greater than 0.80 was applied in the colocalization analysis.

Protein–protein interaction network

By exploring the interactions between the identified target genes and targets of current RA drugs, we can evaluate the potential clinical applications of new targets and gain a better understanding of the mechanisms underlying new drug targets for RA. To achieve this, we constructed a protein-protein interaction (PPI) network using the final therapeutic targets identified above and the targets of current RA drugs. These medications, which are commonly used in the treatment of RA, were selected from two recent reviews and encompass 37 drugs[6, 21]. The corresponding targets for the 37 drugs were obtained from the Drugbank database (https://www.drugbank.ca) (Supplementary Table 4)[22]. All PPI analyses were performed using the online database STRING (version 11.5), with a minimum required interaction score of 0.40[23]. Furthermore, we also searched for current approved drugs or small molecule compounds targeting the final therapeutic target genes in the Drugbank database.

Results

Screening potential druggable genes for RA in the training cohort

Following the inclusion criteria for cis-eQTL proxy target genes in our study, we ultimately identified 2516 druggable genes as exposure. In the training cohort, MR analysis indicated that 235 druggable genes were suggestively associated with RA risk (p < 0.05), while 20 of these genes exhibited a significant association with RA risk at Bonferroni significance (p < 1.99E-05, Supplementary Table 5). No heterogeneity or horizontal pleiotropy was detected for these significant druggable genes (p > 0.05, Supplementary Table 6). Steiger filtering confirmed the directionality of the causal association from the druggable genes to RA (p < 0.05, Supplementary Table 7).

Validation of significant druggable genes in the validation cohort

To enhance the reliability of our findings, we conducted a validation analysis using another RA cohort (N = 58284). Our MR analysis revealed that, at Bonferroni significance (P < 0.0025), 11 out of the 20 druggable genes showed a significant association with RA risk. These genes include tyrosine kinase 2 (TYK2), protein tyrosine phosphatase non-receptor type 22 (PTPN22), ATPase sarcoplasmic/endoplasmic reticulum Ca²⁺ transporting 1 (ATP2A1), retinoid X receptor beta (RXRB), notch 4 (NOTCH4), apolipoprotein M (APOM), major histocompatibility complex, class II, DR alpha (HLA-DRA), euchromatic histone-lysine N-methyltransferase 2 (EHMT2), EGF-like-domain multiple 8 (EGFL8), C-C motif chemokine receptor 6 (CCR6), and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) (Supplementary Fig. 1 and Supplementary Table 8). Importantly, most of these 11 significant druggable genes exhibited the same effect direction as observed in the training cohort, except for EHMT2, which displayed an opposing effect direction (Supplementary Table 5 and Supplementary Table 8). Furthermore, Steiger filtering revealed that majority of these 11 significant genes demonstrated a positive
causal relationship with RA, with the exception of EHMT2 and EGFL8, which exhibited a reverse causal relationship (Supplementary Table 9). As a result, we identified a total of nine potential druggable genes, including TYK2, PTPN22, ATP2A1, APOM, RXRB, NOTCH4, HLA-DRA, CCR6 and CTLA4 (Table 1).

Table 1
The MR results of the nine druggable genes in the two independent cohorts

<table>
<thead>
<tr>
<th>Gene</th>
<th>FinnGen cohort</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method</td>
<td>SNPs Beta OR (95% CI)</td>
</tr>
<tr>
<td>TYK2</td>
<td>Wald ratio</td>
<td>1 -0.24 0.79 (0.73–0.86)</td>
</tr>
<tr>
<td>PTPN22</td>
<td>Wald ratio</td>
<td>1 0.49 1.62 (1.49–1.77)</td>
</tr>
<tr>
<td>ATP2A1</td>
<td>Wald ratio</td>
<td>1 0.44 1.55 (1.27–1.88)</td>
</tr>
<tr>
<td>RXRB</td>
<td>Wald ratio</td>
<td>1 0.66 1.93 (1.47–2.56)</td>
</tr>
<tr>
<td>NOTCH4</td>
<td>Wald ratio</td>
<td>1 0.60 1.82 (1.61–2.04)</td>
</tr>
<tr>
<td>APOM</td>
<td>Wald ratio</td>
<td>1 0.78 2.18 (1.77–2.69)</td>
</tr>
<tr>
<td>CCR6</td>
<td>IVW</td>
<td>2 0.24 1.27 (1.17–1.38)</td>
</tr>
<tr>
<td>CTLA4</td>
<td>IVW</td>
<td>2 -0.50 0.61 (0.54–0.69)</td>
</tr>
<tr>
<td>HLA-DRA</td>
<td>Wald ratio</td>
<td>1 2.30 9.98 (7.53–13.21)</td>
</tr>
</tbody>
</table>

SNP, single nucleotide polymorphism; CI, confidence interval; OR, odds ratio; IVW, inverse variance weighted; TYK2, tyrosine kinase 2; PTPN22, protein tyrosine phosphatase non-receptor type 22; ATP2A1, ATPase sarcoplasmic/endoplasmic reticulum Ca^2+ transporting 1; RXRB, retinoid X receptor beta; NOTCH4, notch 4; APOM, apolipoprotein M; CCR6, C-C motif chemokine receptor 6; CTLA4, cytotoxic T-lymphocyte-associated protein 4; HLA-DRA, major histocompatibility complex, class II, DR alpha.

Colocalization analysis and phenome-wide scan

To determine the probability of the nine identified druggable genes sharing the same causal variant with RA, we performed a colocalization analysis. The results strongly suggested that CTLA4 and RA (PP.H4.abf = 0.98, Fig. 2), as well as CCR6 and RA (PP.H4.abf = 0.99, Fig. 2), share the same causal variant (Supplementary Table 10). Therefore, we considered these two genes as the final therapeutic targets. Additionally, the findings of the phenome-wide scan indicated that the instrumental variables of CTLA4 (rs13030124
and rs148849825) and CCR6 (rs3093025 and rs6928890) did not show strong correlation with other risk factors that may affect the risk of RA (Supplementary Table 11).

**Association between the final therapeutic target genes and targets of current RA drugs**

We constructed a PPI network using the final therapeutic targets, CTLA4 and CCR6, and the 55 targets of current RA medications (Supplementary Table 4). The results showed that CTLA4 exhibited the most reliable interactions (curated databases or experimentally determined) with tyrosine-protein kinase JAK1 (JAK1), tyrosine-protein kinase JAK2 (JAK2), tyrosine-protein kinase JAK3 (JAK3), TYK2, T-lymphocyte activation antigen CD86 (CD86), T-lymphocyte activation antigen CD80 (CD80), and inhibitor of nuclear factor kappa-B kinase subunit beta (IKKB), while CCR6 had the most reliable interactions with JAK2 and JAK3 (Fig. 3 and Supplementary Fig. 2). Among these target genes, JAK1, JAK2, JAK3, and TYK2 are target genes of Upadacitinib, Tofacitinib, or Baricitinib, and CD86 and CD80 are target genes of Abatacept, while IKKB is a target gene of Sulfasalazine and Auranofin. Additionally, CTLA4 was found to be associated with other target genes, including toll-like receptor 7 (TLR7), lymphotranscript-alpha (LTA), tumor necrosis factor (TNF), prostaglandin G/H synthase 2 (PTGS2), aryl hydrocarbon receptor (AHR), mitogen-activated protein kinase 3 (MAPK3), and interleukin-6 receptor subunit alpha (IL6R) (through text mining or co-expression) (Supplementary Fig. 2). Similarly, CCR6 was found to be associated with other target genes, such as AHR, TNF, CD86, CD80, TLR7, toll-like receptor 9 (TLR9), and IL6R (through text mining or co-expression) (Supplementary Fig. 2). We also searched the Drugbank database for approved drugs or small molecule compounds targeting the final therapeutic target genes. Three CTLA4 inhibitors were identified (Ipilimumab, Tremelimumab, and Abatacept), while no drugs targeting CCR6 were found (Supplementary Table 12).

**CTLA4**

The MR beta-coefficient for CTLA4 was negative in both cohorts (Table 1), indicating that lower expression of CTLA4 is associated with an increased risk of RA. Therefore, CTLA4 agonists may represent a novel therapy for treating RA. The assessment of drug safety is also crucial during drug development. Hence, we investigate the relationship between CTLA4 and 11 common cardiovascular risk factors, as well as 32 potential diseases related to RA treatment.

The MR results showed that the expression of CTLA4 was significantly negatively correlated with the risk of hypothyroidism (OR = 0.98, 95% CI: 0.98–0.99, p = 1.45E-30) and type 1 diabetes (OR = 0.59, 95% CI: 0.49–0.73, p = 3.86E-07), and significantly positively correlated with the risk of malignant neoplasm of skin (OR = 1.01, 95% CI: 1.01–1.02, p = 1.01E-09) (Supplementary Table 13). Based on the findings, it suggests that CTLA4 agonists could potentially lower the risk of hypothyroidism and type 1 diabetes, but may also increase the risk of malignant skin neoplasm (Fig. 4). Additionally, CTLA4 displayed a suggestive positive correlation with apolipoprotein A1 (OR = 1.02, 95% CI: 1.00-1.04, p = 0.04), HbA1C (OR = 1.01, 95% CI: 1.00-1.03, p = 4.94E-02), triglycerides (OR = 1.02, 95% CI: 1.00-1.04, p = 0.02), and the risk of malignant neoplasm of prostate (OR = 1.00, 95% CI: 1.00-1.01, p = 0.04) and male genital organs (OR = 1.00, 95% CI: 1.00-1.01, p = 0.03) (Supplementary Table 13, and Fig. 4). No heterogeneity was found (p > 0.05, Supplementary Table 14), and the results of the Steiger filtering analysis confirmed the directionality of the causal association from CTLA4 to the risk factors or diseases (p < 0.05, Supplementary Table 15). Our further colocalization analysis revealed that CTLA4 and type 1 diabetes, as well as CTLA4 and hypothyroidism, shared the same causal variant (PPH4.abf > 0.80, Supplementary Table 10).

Currently, there are three clinically-approved drugs targeting CTLA4, namely Ipilimumab, Tremelimumab, and Abatacept. Among them, Ipilimumab and Tremelimumab are inhibitors of CTLA4, while Abatacept functions as an analogue of CTLA4 (Supplementary Table 12). Abatacept has already been approved for the treatment of RA.

**CCR6**

The MR beta-coefficient of CCR6 was positive in both cohorts (Table 1), indicating that higher CCR6 expression is associated with an increased risk of RA. Therefore, CCR6 antagonists may represent a novel and effective therapeutic strategy for RA treatment.

To further assess the safety profile of CCR6 antagonists, we conducted MR analysis and colocalization analysis. The results revealed a significant positive correlation between CCR6 expression and the risk of hypothyroidism (OR = 1.01, 95% CI: 1.00-1.01, p = 6.08E-09), as well as a significant negative correlation with the risk of malignant skin neoplasm (OR = 0.99, 95% CI: 0.99-1.00, p = 3.60E-07) (Supplementary Table 16). Based on these findings, it can be suggested that CCR6 antagonists may have the potential to reduce the risk of hypothyroidism and increase the risk of malignant skin neoplasms (Fig. 5). Moreover, CCR6 expression showed suggestive positive
correlations with the risk of total cholesterol (OR = 1.04, 95%CI: 1.00-1.07, p = 0.03), thrombocytopenia (OR = 1.47, 95%CI: 1.09–1.98, p = 0.01), and type 2 diabetes (OR = 1.09, 95%CI: 1.02–1.17, p = 0.02), while it was suggestively negatively correlated with HDL (OR = 0.98, 95%CI: 0.97-1.00, p = 0.01). (Supplementary Table 16). There was no observed heterogeneity (p > 0.05, Supplementary Table 14), and the results of the Steiger filtering analysis confirmed the directionality of the causal association between CCR6 and the identified risk factors or diseases (p < 0.05, Supplementary Table 15).

Discussion

In this study, our objective was to identify potential drug targets for the treatment of RA by integrating RA GWAS data, the drug genome data, and eQTL data, using two-sample MR analysis and colocalization analysis. As a result, we finally identified two promising drug targets for RA: CTLA4 and CCR6. Our findings suggest that decreased CTLA4 expression and increased CCR6 expression are associated with a higher risk of RA. Hence, CTLA4 agonists and CCR6 antagonists may serve as potential therapeutic strategies for RA treatment. Moreover, we also investigated the safety profile of targeting these two genes.

Up to now, there is still no complete cure for RA[6]. It’s crucial to explore new therapeutic strategies for RA treatment. Accumulating evidence suggests that integrating disease GWAS data with eQTL data or pQTL data using MR analysis is an effective and efficient approach for identifying potential treatment targets[9–14]. In this study, we employed MR analysis to integrate RA GWAS data and eQTL data, resulting in the identification of CTLA4 and CCR6 as novel promising target genes for RA. Despite the pathogenesis of RA is not yet fully understood, existing studies have demonstrated that over-activation of T cells plays a crucial role in its development[4, 24]. Full activation of T cells requires signals from the T-cell receptor (TCR) upon binding to antigens presented by major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs), as well as co-stimulatory signals originating from the interaction between CD28 protein on T cells and CD80/86 protein on APCs[25–27]. CTLA4, a cell surface receptor expressed on activated T cells, competes with CD28 for binding to CD80/CD86 molecules on APCs[26, 28]. This interaction attenuates the costimulatory signal and inhibits further T cell activation[26, 28]. As a result, the balance between CD28 and CTLA4 on T cells influences the degree and duration of T cell activation[26, 28]. Therefore, interventions targeting CD28 inhibition, CTLA4 activation, or disruption of the CD28-CD80/CD86 interaction could be potential directions for novel drug development in RA.

Consistent with the aforementioned theory, our study revealed a negative MR beta-coefficient for CTLA4 (Table 1), indicating that lower CTLA4 expression is associated with a higher risk of RA. These findings suggest the potential of CTLA4 agonists as a novel therapeutic approach for RA. Currently, three CTLA4-targeting drugs have gained FDA approval, namely Ipilimumab, Tremelimumab, and Abatacept. Ipilimumab and Tremelimumab, as monoclonal antibodies, obstruct the interaction between CTLA4 and CD80/CD86, resulting in the inhibition of CTLA4's negative regulatory effect on T cell activation and the promotion of T cell activation, proliferation, and cytotoxicity[29]. These agents have emerged as promising immunotherapies for cancer treatment[29]. Klocke et al.[30] found that CTLA4 deficiency led to severe exacerbation of disease and greater joint damage in a collagen-induced arthritis (CIA) model, highlighting the crucial role of CTLA4 in suppressing arthritis inflammation. Of particular interest is Abatacept, a selective co-stimulatory modulator primarily used for the treatment of RA with an inadequate response to anti-TNFα therapy[31]. Abatacept consists mainly of the extracellular structural domain of CTLA4 and, similar to CTLA4, competes with its ligands CD80/CD86 for binding, thereby inhibiting the transmission of co-stimulatory signals necessary for optimal T cell activation and suppressing T cell activation[31]. In addition, the PPI analysis results revealed interactions between CTLA4 and other RA drug targets, including JAK1, JAK2, JAK3, TYK2, and IKBKB. These findings provide insights into the potential mechanisms of action of CTLA4 in RA, and also indirectly validate the reliability of using MR analysis for screening candidate target genes. Despite the availability of Abatacept, a CTLA4 analogue, for RA treatment, CTLA4 agonists are still lacking. Hence, the development of CTLA4 agonists remains promising for RA treatment.

We investigated the safety of CTLA4 agonists and found that they can decrease the risk of hypothyroidism and type 1 diabetes. However, there is a potential increase in the risk of malignant skin neoplasms. Further colocalization analysis revealed that CTLA4 and type 1 diabetes, as well as CTLA4 and hypothyroidism, shared causal variants (HH.P4 > 0.8), suggesting that decreased CTLA4 expression may contribute to the development of these conditions.

This is consistent with previous studies that found CTLA4 may reduce the risk of hypothyroidism and type 1 diabetes by inhibiting autoimmune attacks through the suppression of T cell hyperactivation and slowing down the destruction of islet β cells and thyroid tissue[32–35]. Although no relevant research exists on the relationship between CTLA4 agonists and malignant skin neoplasms, studies have found that inhibition of CTLA4 promotes T cell activation, proliferation, and enhances anti-tumour immunity[36]. Additionally, CTLA4 antagonists may reduce regulatory T cells (Tregs) and increase Th17 cells, thereby altering the Treg/Th17 balance and exhibiting...
anti-tumor effects[37–40]. Therefore, we speculate that CTLA4 agonists may increase the risk of malignant skin neoplasm by inhibiting T cell activation and proliferation and altering the Treg/Th17 balance. However, the reason why this specific risk appears to be specific to malignant skin neoplasm and not other malignancies remains unclear and requires further research.

CCR6, a G-protein coupled receptor (GPCR), is another promising target gene that is involved in the regulation of immune cell migration and inflammatory responses in RA by interacting with its ligand cysteine–cysteine motif chemokine ligand 20 (CCL20). It plays a key role in the pathogenesis of RA[41, 42]. GWAS studies have shown an association between the CCR6 gene and susceptibility loci in RA patients[43–45], and it is considered a risk factor in Asian and European populations[46]. However, in African Americans, CCR6 may have a protective role[43]. The CCR6/CCL20 axis is responsible for recruiting immune cells, such as T cells, monocytes, immature dendritic cells, and neutrophils, to the inflamed joints and synovial tissue in RA[47, 48]. Increased expression of CCL20 has been observed in the peripheral blood and synovial fluid of RA patients[47, 49], promoting local inflammation by facilitating the migration of CCR6+Th17 cells to the joints[49]. Studies have shown that treatment with CCR6 monoclonal antibodies significantly reduces arthritis severity in RA mice and inhibits the migration of Th17 cells to the joints[49]. Therefore, targeted inhibition of CCR6 or CCL20 may emerge as a novel strategy for RA treatment.

In our study, we found a positive MR beta-coefficient for CCR6, indicating that high expression of CCR6 is associated with an increased risk of RA and that CCR6 antagonists could serve as a new therapeutic strategy for RA, which is consistent with the findings mentioned above. We also investigated the safety profile of CCR6 antagonists and observed a significant positive correlation between CCR6 expression and the risk of hypothyroidism, as well as a significant negative correlation with the risk of skin malignant neoplasms. These results suggest that CCR6 antagonists may reduce the risk of hypothyroidism, they might also increase the risk of malignant skin neoplasms.

Hypothyroidism is a condition characterized by reduced thyroid function and is commonly observed in advanced stages of Hashimoto's thyroiditis, where lymphocyte infiltration leads to extensive destruction of thyroid follicles and eventual hypothyroidism[50]. Increasing evidence indicates that hypothyroidism is closely associated with local infiltration of Th17 cells[51]. Studies have demonstrated a significant increase in the proportion of Th17 cells in the peripheral blood and thyroid tissue of patients with Hashimoto's thyroiditis[51]. These elevated Th17 cells within the thyroid tissue secrete excessive amounts of IL-17, which subsequently triggers an immune inflammatory response and destruction of thyroid follicles[51]. Based on these findings, we speculate that CCR6 antagonists may potentially reduce the risk of hypothyroidism by inhibiting the expression of CCR6 on the surface of Th17 cells and blocking their migration to the thyroid.

No studies have been conducted on the effects of CCR6 antagonists on malignant skin neoplasm, but the existing studies suggest that in certain tumours, the CCL20-CCR6 axis may directly promote cancer progression by enhancing the migration and proliferation of cancer cells, and indirectly remodel the tumour microenvironment through immune cell control[52, 53]. Thus, disrupting the CCL20-CCR6 interaction may be a promising strategy for cancer treatment. This is inconsistent with our findings, we found that CCR6 antagonists actually increase the risk of malignant skin neoplasm. We speculate that CCR6 antagonists decrease the proportion of Th17 cells in local tissues, disrupt the Treg/Th17 balance, and subsequently reduce anti-tumor activity, resulting in the development of malignant skin neoplasms[37, 53]. These results indicate that the development of drugs targeting the disruption of the CCL20-CCR6 axis for the treatment of RA is undoubtedly promising. Although small molecule compounds that inhibit CCR6 expression already exist, they have not yet been clinically applied, and further research is still required[54, 55].

This study has several limitations. Firstly, it should be noted that this study utilized MR analysis as a simulation of randomized controlled trials (RCTs), but it is not true RCTs and clinical trials are needed to assess the efficacy and safety of the final therapeutic targets. Secondly, the evaluation of side effects in this study was limited to common cardiovascular risk factors and potential diseases related to RA treatment. Future research should also focus on systemic side effects. Thirdly, the participants in this study were of European ancestry, thus the generalizability of the findings to other ethnic groups is uncertain. Fourthly, only eQTL data were used in this study and were not further validated in pQTL data (no available data), despite the fact that most drug targets are proteins. Finally, although we attempted to explain the mechanisms of these targets in RA by analyzing the interaction between the final therapeutic target genes and current RA drug targets using PPI analysis, it is important to note that the results are only suggestive and further experimental validation of the specific mechanisms is required.

Conclusions
In summary, this study identified CTLA4 and CCR6 as promising drug targets for RA treatment, and that targeting the activation of CTLA4 and the inhibition of CCR6 may potentially reduce the risk of RA with fewer side effects. However, their efficacy and safety in preventing RA need to be further evaluated in adequately powered RCTs.

**Abbreviations**

RA
Rheumatoid arthritis
NSAIDs
Nonsteroidal anti-inflammatory drugs
DMARDs
Disease-modifying anti-rheumatic drugs
MR
Mendelian randomization
eQTL
Expression quantitative trait loci
pQTL
Protein quantitative trait loci
GWAS
Genome-wide association studies
HDL
High-density lipoprotein
LDL
Low-density lipoprotein
IVW
Inverse variance weighted
PPI
Protein-protein interaction
TYK2
Tyrosine kinase 2
PTPN22
Protein tyrosine phosphatase non-receptor type 22
ATP2A1
ATPase sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) transporting 1
RXRB
Retinoid X receptor beta
NOTCH4
Notch 4
APOM
Apolipoprotein M
HLA-DRA
Major histocompatibility complex, class II, DR alpha
EHMT2
Euchromatic histone-lysine N-methyltransferase 2
EGFL8
EGF-like-domain multiple 8
CCR6
C-C motif chemokine receptor 6
CTLA4
Cytotoxic T-lymphocyte-associated protein 4
JAK1
Tyrosine-protein kinase JAK1
JAK2
Tyrosine-protein kinase JAK2
JAK3
Tyrosine-protein kinase JAK3
CD86
T-lymphocyte activation antigen CD86
CD80
T-lymphocyte activation antigen CD80
IKBKB
Inhibitor of nuclear factor kappa-B kinase subunit beta
TLR7
Toll-like receptor 7
LTA
Lymphotixin-alpha
TNF
Tumor necrosis factor
PTGS2
Prostaglandin G/H synthase 2
AHR
Aryl hydrocarbon receptor
MAPK3
Mitogen-activated protein kinase 3
IL6R
Interleukin-6 receptor subunit alpha
TLR9
Toll-like receptor 9
TCR
T-cell receptor
MHC
Major histocompatibility complex
APCs
Antigen-presenting cells
CIA
Collagen-induced arthritis
Tregs
Regulatory T cells
GPCR
G-protein coupled receptor
CCL20
Cysteine–cysteine motif chemokine ligand 20
RCTs
Randomized controlled trials
CI
Confidence interval
OR
Odds ratio.

Declarations

Acknowledgments

We want to acknowledge the eQTLGen Consortium, the participants and investigators of the FinnGen study and Ha's study.
Authors’ contributions

All authors have read and approved the final version of the manuscript. The study concept and design were developed by PX and YSC. Data acquisition, analysis, and interpretation were conducted by YSC, QLY, and XYW. YSC drafted the initial manuscript and provided the funding. PX supervised and reviewed the manuscript. XYW and QLY verified all data.

Funding

This study was supported by the China Postdoctoral Science Foundation (No. 2020M673454), the Foundation of the Natural Science Basic Research of Shaanxi Province of China (2021JQ-924), and the Fundamental Research Funds for the Central Universities (xzy012021078).

Data availability statement

The data on druggable genes were obtained from a previous study by Finan et al [15]. The cis-eQTLs data obtained from the eQTLGen Consortium (https://www.eqtlgen.org/). GWAS summary statistics for RA training cohort can be downloaded from the FinnGen consortium (https://r9.finnngen.fi/phenofield/M13_RHEUMA). GWAS summary statistics for RA validation cohort can be obtained from a previously published paper [17]. GWAS summary statistics for common cardiovascular risk factors and potential diseases related to RA treatment can be downloaded from the ieu open GWAS project (www.gwas.mrcieu.ac.uk/).

Ethics approval and consent to participate

This study involved a secondary analysis of publicly available data, hence, there was no need to obtain informed consent or seek ethical approval.

Consent for publication

Not applicable.

Competing interests

There are no conflicts of interest to declare.

References


Figures
Figure 1

Study design for identification of the therapeutic targets for RA
Figure 2

Regional association plots of the CTLA4 and CCR6 loci.

The variants rs13030124 and rs3093025 were used to proxy the expression of CTLA4 and CCR6, respectively. (a) Manhattan plot of the rs13030124 variant and its 500 kb region on both sides in peripheral blood from the eQTLGen Consortium. (b) Manhattan plot of the rs13030124 variant and its 500 kb region on both sides in RA. (c) Manhattan plot of the rs3093025 variant and its 500 kb region on both sides in peripheral blood from the eQTLGen Consortium. (d) Manhattan plot of the rs3093025 variant and its 500 kb region on both sides in RA.
CTLA4 and CCR6 were identified as the final therapeutic targets in this study, while the other genes represented the targets of current RA drugs that exhibited the most reliable interactions (curated databases or experimentally determined) with CTLA4 and/or CCR6.
The results of the side effects evaluation in RA treatment with genetically-proxied CTLA4 agonists.

The correlation between genetically-proxied CTLA4 agonists and 11 common cardiovascular risk factors, as well as 32 potential diseases related to RA treatment, was evaluated through MR analysis, and a forest plot was used for visualization. CI, confidence interval; OR, odds ratio.
Figure 5

The results of the side effects evaluation in RA treatment with genetically-proxied CCR6 antagonists.

The MR analysis was used to evaluate the correlation between genetically-proxied CCR6 antagonists and 11 common cardiovascular risk factors, as well as 32 potential diseases related to RA treatment. The results were visualized with a forest plot. CI, confidence interval; OR, odds ratio.

Supplementary Files

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