

Candidate pharmacogenetic signals at CCL2 and ITPA associated with JAK inhibitor response in Taiwanese rheumatoid arthritis

T.-Y. Hsieh^{1,2}, I.-C. Chen³, T.-S. Wu⁴, S.-M. Lo³, C.-M. Kao^{3,5},
Y.-J. Chen^{3,5,6}, W.-N. Huang^{5,6,7}, F.-C. Liu⁸, Y.-M. Chen^{3,5,6,7,9,10}

¹Department of Medical Education, Taichung Veterans General Hospital, Taichung, Taiwan;

²Faculty of Medicine, National Defense Medical Center, Taipei, Taiwan;

³Department of Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan;

⁴Department of Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan;

⁵Division of Allergy, Immunology, and Rheumatology, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; ⁶Faculty of Medicine, National Yang-Ming Chiao Tung University, Taipei, Taiwan; ⁷Department of Post-Baccalaureate Medicine, College of Medicine, National Chung Hsing University, Taichung, Taiwan; ⁸Rheumatology/Immunology and Allergy, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; ⁹Graduate Institute of Clinical Medicine, College of Medicine, National Chung Hsing University, Taichung, Taiwan; ¹⁰Precision Medicine Research Center, College of Medicine, National Chung Hsing University, Taichung, Taiwan.

Abstract Objective

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by persistent synovial inflammation. Janus kinase inhibitors (JAKi) are effective targeted therapies for RA, yet clinical responses vary. We aimed to identify genetic predictors of JAKi effectiveness in Taiwanese patients with RA.

Methods

In this retrospective study, 275 RA patients treated with tofacitinib, baricitinib, or upadacitinib in the Taiwan Precision Medicine Initiative (TPMI) were recruited and classified as Effective or Non-effective. Fifty-eight candidate single-nucleotide polymorphisms (SNPs) were evaluated using existing Taiwan Biobank v2 array data. We integrated cell-type-specific eQTL resources (scQTLbase) and single-cell RNA sequencing (scRNA-seq) to support gene-level associations.

Results

In this cohort (Effective, n=217; Non-effective, n=58), CCL2 rs1024611/rs4586 and ITPA rs1127354 were the top nominal candidates (all $p < 0.05$). In scQTLbase (GRCh38), rs1024611 associated with higher CCL2 in dendritic cells, and rs1127354 with higher ITPA in CD14⁺ monocytes. scRNA-seq showed higher CCL2 in Non-effective patients ($p < 0.001$), predominantly across M0/M1/M2 monocytes. ITPA was higher in Effective patients ($p = 0.018$), most notably in M2 monocytes ($p < 0.001$), with elevated ITPA also observed in M1 monocytes of Non-effective patients ($p = 0.004$).

Conclusion

Our data prioritise CCL2 and ITPA as candidate pharmacogenetic signals for JAKi response. Given the use of a persistence-based effectiveness endpoint and the exploratory nature of secondary EULAR-based outcome assessments, independent replication using standardised ACR/EULAR outcomes is warranted.

Key words

rheumatoid arthritis, JAK inhibitors, pharmacogenetics, SNP, scRNA-seq

Ting-Yu Hsieh, MD
 I-Chieh Chen, PhD
 Ting-Shuan Wu, PhD
 Sheng-Min Lo, PhD
 Chung-Mao Kao, MD
 Yen-Ju Chen, MD, PhD
 Wen-Nan Huang, MD, PhD
 Feng-Cheng Liu, MD, PhD
 Yi-Ming Chen, MD, PhD

Please address correspondence to:
 Yi-Ming Chen

Division of Allergy, Immunology,
 and Rheumatology,
 Department of Internal Medicine,
 Taichung Veterans General Hospital,
 1650, Taiwan Boulevard Sect. 4,
 407 Taichung, Taiwan
 E-mail: ymchen1@vghtc.gov.tw

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Competing interests: none declared.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily affects the small joints, leading to persistent inflammation, pain, and progressive structural damage (1). Without timely intervention, RA may cause severe joint deformities and functional impairment (2). RA inflammation is primarily driven by the clonal expansion of autoreactive cells, particularly T cells and B cells, along with a reduction in regulatory T cells (Treg) (3). B cells differentiate into plasma cells, producing anti-citrullinated peptide antibodies (ACPAs) and rheumatoid factor (RF), which serve as key serological markers of RA. Autoreactive cells infiltrate the synovial membrane, where monocytes release pro-inflammatory cytokines, including interleukin-1 (IL-1), tumour necrosis factor-alpha (TNF), and interleukin-6 (IL-6), thereby amplifying the inflammatory response and exacerbating the pathogenesis of RA (4, 5). Janus kinase inhibitors (JAKi) are approved targeted therapies for RA. Janus kinases (JAKs) are tyrosine kinase proteins that play a key role in mediating intracellular signalling through the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway (6). Recent pathogenesis reviews have highlighted JAK1 as a clinically relevant therapeutic target, with transcriptomic studies demonstrating that JAK1-selective inhibition suppresses key inflammatory programs in synovial fibroblasts (7). Cytokines that activate JAK/STAT pathway members are key regulators of lymphocyte development and maintain T-cell activation and differentiation (8, 9). Tofacitinib, which was the first JAKi approved for RA in 2012, has been shown to provide early and sustained improvements in signs and symptoms (10). Following this, baricitinib and upadacitinib received FDA and EMA approvals between 2017 and 2019, further expanding the clinical applications of JAKi in RA treatment (11, 12).

Nevertheless, a substantial subset of patients does not achieve clinically meaningful improvement with JAKi (6). Previous studies have reported an overall cumulative drug retention rate (DRR)

of 51.3% for JAKi, with no significant differences observed among different JAKi agents (13). Another study reported that after 12 months of treatment, the DRR of JAKi was 51.4%, with most patients discontinuing due to insufficient efficacy (14). Although the JAK/STAT pathway plays a crucial role in the pathogenesis of RA, the specific genetic variants that directly influence JAKi treatment efficacy remain unclear. In European RA populations, variations in the *TYK2* gene have been associated with JAKi treatment outcomes (15). Treat-to-target strategies are widely adopted; however, they may contribute to overtreatment in inadequate responders, and robust predictors of JAKi response are not yet available (16, 17). To address this unmet clinical need, this study aimed to identify genetic predictors of JAKi effectiveness in patients with RA.

Materials and methods

Study design and participants

This retrospective case-control study utilised data from the TPMI database, which was established to collect comprehensive clinical and genetic data from one million individuals. Patients diagnosed with RA were enrolled based on the 2010 classification criteria of the American College of Rheumatology (ACR) and the European Alliance of Associations for Rheumatology (EULAR). A total of 275 RA participants were categorised into two groups based on their response to the index JAKi (defined as the first JAKi initiated: tofacitinib, baricitinib, or upadacitinib). A favourable clinical response (Effective group) was operationally defined as uninterrupted use of the index JAKi for more than 6 months (180 consecutive days), accompanied by a reduction in DAS28-ESR when available. Patients who discontinued or switched therapy within 180 days due to inadequate response were classified as Non-effective. Patients who discontinued treatment after 180 days were classified according to available clinical data.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki (2013) for research involving human subjects. The

protocol was reviewed and approved by the Institutional Review Board of Taichung Veterans General Hospital, Taiwan (IRB no. SF19153A). Written informed consent was obtained from all participants prior to enrollment.

DNA purification and genotyping

Candidate SNPs were selected based on prior evidence from the literature, including previously reported associations with rheumatoid arthritis susceptibility, immune and inflammatory pathways, or treatment response to disease-modifying anti-rheumatic drugs (DMARDs), including JAKi. Literature searches were performed in PubMed using keywords such as “rheumatoid arthritis”, “SNP”, “pharmacogenomics”, “JAKi response”, “CCL2 polymorphism”, and “ITPA variant”, and relevant variants were cross-referenced with the GWAS Catalogue. Additional selection criteria included functional relevance supported by expression quantitative trait locus (eQTL) data or mechanistic studies, availability on the Taiwan Biobank version 2 (TWBv2) array, and sufficient minor allele frequency in East Asian populations. As outlined in Supplementary Table S1, candidate genes included conventional synthetic DMARDs (csDMARDs)-related genes (18), genes related to immunity and inflammation (19, 20), JAK/STAT signalling pathway genes (15, 21, 22), and genes related to JAKi (23–26). A total of 58 candidate SNPs were analysed using pre-existing TWBv2 genotyping data, which are tailored to the Han Chinese population in Taiwan, as shown in Supplementary Table S2 (27, 28). Blood samples were collected for transcriptomic and single-cell RNA sequencing experiments; however, no new genotyping was performed. Stringent quality control procedures were applied, excluding SNPs with a call rate <95%, a minor allele frequency (MAF) <0.01, a single allele occurrence within the cohort, or significant deviation from Hardy-Weinberg equilibrium ($p < 1 \times 10^{-5}$). Quality control and downstream analyses were conducted using PLINK version 1.9.29. For rare variant genotyping and cluster normalisation, the *adnorm* package

(Thermo Fisher Scientific, Santa Clara, CA, USA) and the *axiomBestPractices-1.2.4* workflow with the *do-rare-het-adjustment* command were applied 30.

eQTL analysis

To evaluate the functional impact of the index SNPs, we queried *scQTLbase* for previously reported single-cell cis-eQTLs in peripheral blood mononuclear cell (PBMC)-related cell types, extracting effect sizes, standard errors, and *p*-values (31). Significance was defined according to the thresholds reported by each source dataset (typically FDR <0.05). Because our cohort lacked paired genotype-expression data, we did not perform *de novo* eQTL mapping. Effect directions follow effect-allele coding in the original studies.

scRNA-seq and data analysis

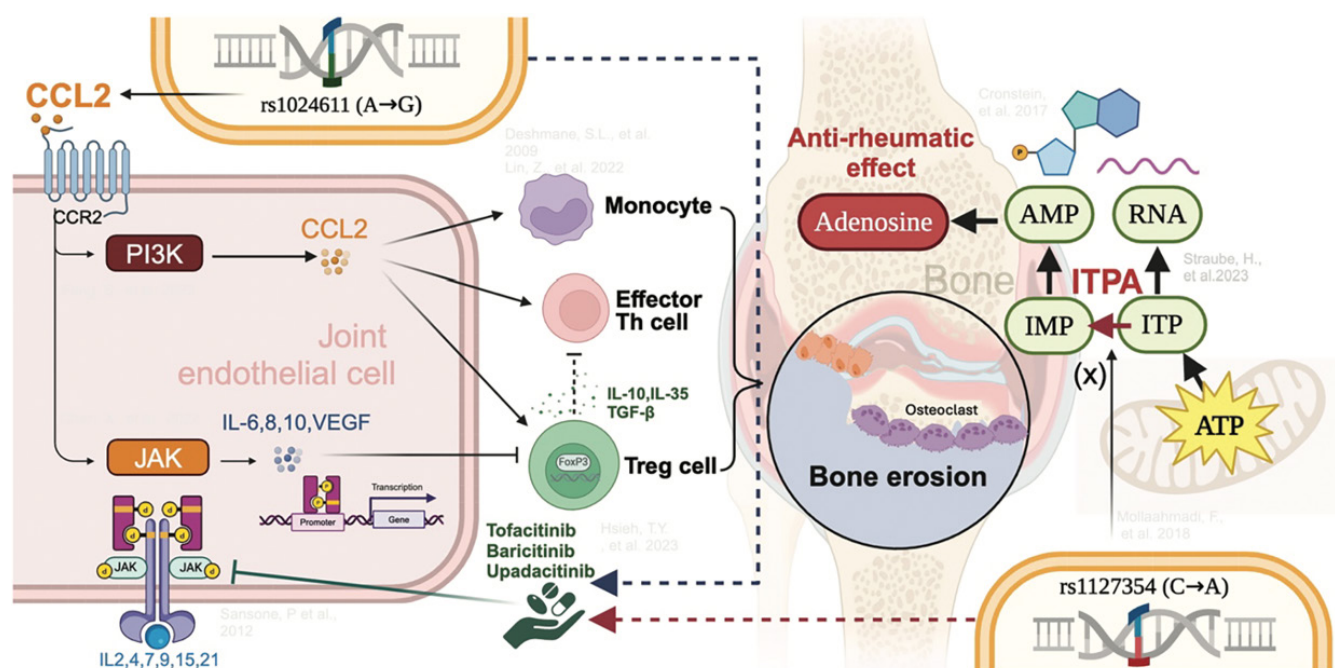
For the scRNA-seq analysis, a subset of patients was selected from the overall cohort based on the availability of high-quality peripheral blood samples and sufficient cell viability at the time of sample processing. Patients were selected to represent both effective and non-effective JAKi response groups. Due to logistical constraints and sample availability, the scRNA-seq cohort was not randomly selected from the entire study population. Whole blood from 13 RA patients was collected for scRNA-seq, with 8 patients receiving baricitinib and 5 patients receiving tofacitinib. Patients were categorised into two groups: 1. the Effective group, defined as those who demonstrated a favourable response to JAKi therapy and maintained for more than 180 consecutive days; 2. the Non-effective group, consisting of patients who exhibited a poor response to JAKi treatment and subsequently switched to other biologic disease-modifying anti-rheumatic drugs (bDMARDs). Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood within 2 hours of collection using Ficoll-Paque density gradient centrifugation. Cell viability was assessed with trypan blue staining, and only samples with >90% viability proceeded to scRNA-seq, which was performed using the 10x Genomics Chromium Next GEM Single Cell 3' Reagent Kits

v. 3.1. Approximately 10,000 cells per sample were loaded onto the Chromium Controller, and libraries were prepared following the manufacturer's protocol. Sequencing was performed on an Illumina NovaSeq 6000 platform with a target depth of 20,000 reads per cell. Raw sequencing data were processed with Cell Ranger (v. 6.0.1) and aligned to the GRCh38 human reference genome. Quality control excluded cells with fewer than 200 detected genes or with over 20% mitochondrial gene content. Data normalisation, dimensionality reduction, clustering, and cell type annotation were conducted using the Seurat package (v. 4.0.1) in R, based on canonical marker gene expression. Differential gene expression (DGE) analysis between these groups was performed using Seurat's 'FindMarkers' function to identify genes associated with therapeutic efficacy.

Cell clusters were further identified using the Seurat v4 package based on the expression of predefined biomarkers. Distinct immune cell types were annotated, including B cells (CD19), CD4⁺ T cells (CD4, CCR7, IL7R), CD8⁺ T cells (CD8A, NKG7), dendritic cells (ITGAX, CST3), monocytes (CD14, MS4A7, ITGAM, CST3), and natural killer (NK) cells (NCAM1, NKG7). M0 (undifferentiated monocytes) were further identified by the expression of MEF2A; M1 (pro-inflammatory monocytes) were defined by the upregulation of NFKB2, IRF1, STAT1, and IRF9, which are associated with inflammatory responses; M2 (anti-inflammatory monocytes) were characterised by high expression of CSF2, IL10RA, ID2, and TIMP1 (32). Uniform Manifold Approximation and Projection (UMAP) was applied for dimensionality reduction and visualisation. Differential expression analysis was conducted to validate cell-type classification and to assess functional differences between clusters.

Statistical analysis

Normality of continuous variables was assessed using the Shapiro-Wilk test and visual inspection of histograms and Q-Q plots. Comparisons between groups were performed using Student's



Graphical abstract. Real-world and single-cell analyses identify *CCL2* and *ITPA* variants as candidate predictors of JAK inhibitor response in rheumatoid arthritis, suggesting opportunities for personalised treatment in under-represented East Asian patients.

t-test for approximately normally distributed variables (DAS28-ESR) and the Mann-Whitney U-test for non-normally distributed variables (age, BMI, disease duration, CRP, and ESR). Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. To evaluate the association between candidate genetic variants and JAKi effectiveness, univariable logistic regression analyses were first conducted for each single nucleotide polymorphism. Subsequently, multivariable logistic regression models were applied to adjust for potential confounders. Age and sex were included *a priori* in all adjusted models. In additional multivariable analyses, clinically relevant variables that differed between the effective and non-effective groups, including concomitant methotrexate use and index JAKi type, were further incorporated as covariates. Statistical analyses were performed using standard statistical software. Continuous variables were summarised as mean $\pm$ standard deviation and compared using Student's t-test, while categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate. Results are presented as odds ratios with corresponding 95% confidence intervals. All statisti-

cal tests were two-sided, and a p -value <0.05 was considered statistically significant. The graphical abstract/schematics were created with BioRender. Missingness was assessed for pre-specified key clinical variables, including age, sex, disease duration, body mass index, smoking status, serological markers (RF and ACPA), disease activity indices (DAS28-ESR, CRP, and ESR), concomitant csDMARD use, and index JAK inhibitor type. For all key variables, the proportion of missing data was $<5\%$. Genotype data were complete for all included participants. Given the low level of missingness, all analyses were performed using a complete-case approach.

Results

Clinical characteristics and

SNP associations with JAKi response

Among 275 patients, 217 (78.9%) were Effective and 58 (21.1%) Non-effective. Mean age was similar between groups. Carriage of rs1024611 and rs4586 was more frequent in the Effective group, whereas rs1127354 was more frequent in the Non-effective group. Methotrexate use was higher in Effective patients ($p=0.013$), while sulfasalazine, leflunomide, and hydroxychloroquine were comparable. Tofacitinib exposure was

more common in Effective patients ($p=0.025$); baricitinib and upadacitinib did not differ significantly (Table I). In regression analyses, rs1024611 and rs4586 associated with increased effectiveness (OR >1 , $p<0.05$), while rs1127354 associated with lower odds of effectiveness (OR <1 , $p<0.05$). In dominant models (carrier vs. non-carrier), these associations were statistically significant and remained directionally consistent after adjustment for age and sex, as well as after further adjustment for age, sex, concomitant methotrexate use, and index JAKi type. In genotype-specific models, effect directions were generally consistent with the dominant models; however, statistical significance was attenuated after full adjustment (Table II). Linkage disequilibrium analysis demonstrated that rs1024611 and rs4586 were in near-complete linkage disequilibrium ($r^2=0.996$, $D'=0.998$), indicating that these variants represent the same association signal at the *CCL2* locus.

Among the 58 patients classified as Non-effective, 36 (62.1%) discontinued the index JAK inhibitor during follow-up. Based on medical chart review, the most common reason for discontinuation was ineffectiveness, accounting for 18 patients (50.0%), followed by

Table I. Baseline clinical and demographic characteristics.

Variable	Effective (n=217)		Non-effective (n=58)		p-value
	n	%	n	%	
Age, mean $\pm$ SD (years)	60.8 $\pm$ 13.2		59.1 $\pm$ 13.3		0.392
Sex, n (%)					0.443
Female	178	82.0	45	77.6	
Male	39	18.0	13	22.4	
Duration of RA, mean $\pm$ SD (years)	9.6 $\pm$ 6.9		8.4 $\pm$ 7.0		0.217
BMI, mean $\pm$ SD (kg/m ²)	24.5 $\pm$ 4.3		24.2 $\pm$ 3.6		0.685
Smoking, n (%)	30	13.8	10	17.2	0.512
[†] Alcohol use, n (%)	12	5.5	0	0	0.128
Comorbidities, n (%)					
Hypertension	65	30.0	21	36.2	0.362
Type 2 diabetes mellitus	43	19.8	8	13.8	0.295
Chronic kidney disease	59	27.2	9	15.5	0.067
Coronary artery disease [†]	17	7.8	3	5.2	0.776
SNP carriers (heterozygous or homozygous), n (%)					
rs1024611	170	78.7	36	63.2	0.015*
rs1127354	75	34.6	30	52.6	0.013*
rs4586	170	78.3	37	63.8	0.023*
Lab data					
RF positivity ($\geq$ 30 U/mL), n (%)	121	55.8	26	44.8	0.171
ACPA positivity ($>$ 10 U/mL), n (%)	101	46.5	19	32.8	0.463
DAS28-ESR, mean $\pm$ SD	3.8 $\pm$ 1.3		4.1 $\pm$ 1.3		0.238
CRP (mg/dL), mean $\pm$ SD	1.1 $\pm$ 1.7		1.5 $\pm$ 1.9		0.031*
ESR (mm/h), mean $\pm$ SD	31.3 $\pm$ 24.5		36.1 $\pm$ 30.8		0.407
Concomitant csDMARDs, n (%)					
Methotrexate [†]	210	96.8	51	88.0	0.013*
Sulfasalazine	120	55.3	26	44.8	0.156
Leflunomide	97	44.7	27	46.6	0.801
Hydroxychloroquine [†]	205	94.5	52	89.7	0.229
Index and/or ever use of JAK inhibitors, n (%) [#]					
Tofacitinib	150	69.1	31	53.4	0.025*
Baricitinib	59	27.2	20	34.5	0.276
Upadacitinib	25	11.5	10	17.2	0.246

Data are presented as mean $\pm$ standard deviation for continuous variables and n (%) for categorical variables. Comparisons between groups were performed using Student's t-test for approximately normally distributed variables (DAS28-ESR) and the Mann-Whitney U-test for non-normally distributed variables (age, BMI, disease duration, CRP, and ESR). Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. [†] Fisher's exact test. The significance is indicated as * $p < 0.05$, ** $p < 0.01$.

[#] For patients exposed to multiple JAK inhibitors, the 'index JAKi' was the first initiated; counts reflect ever-use during follow-up and can exceed the number of unique participants.

adverse events in 9 patients (25.0%), infection in 8 patients (22.2%), and malignancy in 1 patient (2.8%).

eQTL analysis

Minor allele frequencies were 0.444 (rs1024611), 0.174 (rs1127354), and 0.274 (rs4586). On GRCh38, rs1024611 (A>G) and rs4586 (T>C) lie within the *CCL2* locus on chr17, whereas rs1127354 (C>A) maps to *ITPA* on chr20. In scQTLbase, rs1024611 associated with higher *CCL2* in dendritic cells ($\beta = 4.39$, $p = 1.14 \times 10^{-5}$), rs4586 with higher *CCL8* in CD8⁺ T cells ($\beta = 3.69$, $p = 2.25 \times 10^{-4}$), and rs1127354

with higher *ITPA* in CD14⁺ monocytes ($\beta = 0.16$, $p = 3.26 \times 10^{-4}$) (Table III).

scRNA-seq and gene expression analysis

Demographic features of the 13 scRNA-seq patients (Effective n=10; Non-effective n=3) are summarised in Supplementary Table S3. Pseudobulk analysis showed higher *CCL2* in Non-effective patients ($p = 2.02 \times 10^{-30}$) and higher *ITPA* in Effective patients ($p = 0.018$) (Fig. 1A). Cell-type composition is shown in Fig. 1B (Supplementary Fig. S1). At single-cell resolution, *CCL2* expression was enriched

in monocytes and upregulated in Non-effective patients ($p = 1.65 \times 10^{-13}$), whereas *ITPA* expression was higher in monocytes from Effective patients ($p = 0.002$) (Fig. 1C).

Monocyte clusters identified by unsupervised clustering were subsequently mapped to functional M0/M1/M2 phenotypes based on canonical marker expression. The expression patterns of *CCL2* and *ITPA* were further analysed between the Non-effective and Effective groups across different monocyte subtypes (Fig. 2A). *CCL2* expression was significantly increased in clusters 0, 3, and 10 of the Non-effective group ($p = 0.002$; $p = 8.61 \times 10^{-4}$; $p = 0.005$). Furthermore, higher *ITPA* expression was observed in the Effective group within clusters 0 and 5 ($p = 4.03 \times 10^{-8}$ and $p = 0.032$), whereas clusters 1 and 6 exhibited higher expression in the Non-effective group ($p = 0.033$ and $p = 0.003$) (Fig. 2B-C). *CCL2* expression was significantly higher in M0, M1, and M2 in the Non-effective group compared to the Effective group ($p = 3.23 \times 10^{-8}$; $p = 0.028$; $p = 1.57 \times 10^{-6}$). In contrast, *ITPA* expression was significantly higher in M1 of the Non-effective group ($p = 0.004$) and in M2 of the Effective group ($p = 1.28 \times 10^{-8}$), while no significant difference was observed in M0 (Fig. 3A-B).

Discussion

To our knowledge, this is the first study in Taiwanese patients to integrate germline SNP analyses with public single-cell eQTL resources and peripheral-blood scRNA-seq to explore real-world JAKi effectiveness. In dominant genetic models (carrier vs. non-carrier), *CCL2* locus variants rs1024611/rs4586 were associated with increased odds of effectiveness, whereas *ITPA* rs1127354 was associated with lower odds of effectiveness; these associations remained directionally consistent and statistically significant after multivariable adjustment for age, sex, concomitant methotrexate (MTX) use, and index JAKi type. In genotype-specific models, effect directions were generally consistent; however, statistical significance was attenuated after full adjustment, likely reflecting limited num-

Table II. Genetic association with therapeutic efficacy of JAK inhibitors.

	OR	95% CI		p-value
Dominant model (any variant allele vs. reference genotype)				
Unadjusted				
rs1024611	2.16	1.15	4.04	0.016*
rs1127354	0.48	0.26	0.86	0.014*
rs4586	2.05	1.10	3.84	0.024*
Adjusted for age and sex				
rs1024611	2.08	1.10	3.92	0.024*
rs1127354	0.48	0.27	0.87	0.016*
rs4586	1.99	1.06	3.73	0.033*
Adjusted for age, gender, concomitant MTX use, and index JAK inhibitor [#]				
rs1024611	2.02	1.04	3.90	0.037*
rs1127354	0.53	0.29	0.97	0.041*
rs4586	1.94	1.00	3.74	0.049*
Genotype-specific model (per genotype vs. reference)				
Unadjusted				
rs1024611(A>G)				
AG AA	2.17	1.11	4.26	0.024*
GG AA	2.12	0.92	4.86	0.077
rs1127354 (C>A)				
CA CC	0.51	0.27	0.93	0.028*
AA CC	0.29	0.08	1.08	0.065
rs4586 (T>C)				
TC TT	2.05	1.05	3.98	0.036*
CC TT	2.07	0.90	4.75	0.086
Age- and sex-adjusted				
rs1024611(A>G)				
AG AA	2.09	1.06	4.12	0.033*
GG AA	2.05	0.89	4.73	0.092
rs1127354 (C>A)				
CA CC	0.51	0.28	0.95	0.033*
AA CC	0.29	0.08	1.09	0.068
rs4586 (T>C)				
TC TT	1.97	1.01	3.87	0.048*
CC TT	2.01	0.88	4.63	0.100
Adjusted for age, gender, concomitant MTX use, and index JAK inhibitor [#]				
rs1024611(A>G)				
AG AA	2.02	1.00	4.09	0.051
GG AA	2.02	0.86	4.74	0.107
rs1127354 (C>A)				
CA CC	0.557	0.30	1.04	0.066
AA CC	0.346	0.08	1.42	0.141
rs4586 (T>C)				
TC TT	1.92	0.95	3.87	0.069
CC TT	1.98	0.84	4.65	0.119

Dominant model compares any variant allele vs. reference genotype. Genotype-specific model contrasts each genotype to the reference. Outcome coded as Effective = 1, Non-effective = 0. OR (odds ratio), 95% CI (95% confidence interval). [#]Index JAK inhibitor type (tofacitinib vs. baricitinib/upadacitinib). Logistic regression was used for analysis.

The significance is indicated as * $p < 0.05$, ** $p < 0.01$.

bers within individual genotype strata. Prior work suggests that JAKi-modulated epigenetic targets overlap RA risk loci (rs6074022) and may influence treatment response (33). Our data suggest additional loci that may modulate response in Han Chinese; independent validation is required. Integrating public single-cell eQTL resources with our

results, the rs1024611 effectiveness-associated allele was linked to higher *CCL2* expression in myeloid lineages, consistent with a positive association between the *CCL2* promoter variant rs1024611 and treatment effectiveness in our cohort. Functional studies demonstrate allelic expression imbalance at rs1024611, favouring the G-bearing

haplotype in LPS-stimulated monocytes and indicating context-dependent cis-regulation of *CCL2* (34). However, in our peripheral-blood scRNA-seq, *CCL2* levels were higher in Non-effective patients. This apparent discordance is consistent with prior observations that distinguish within-cell genetic inducibility from between-cell disease-state or compositional effects observed cross-sectionally. rs1024611 primarily modulates inducibility of *CCL2* upon stimulation in monocytes (within-cell effect), whereas cross-sectional scRNA-seq reflects contemporaneous inflammation and cell-type composition (between-cell effect) (35).

Given the near-complete linkage disequilibrium between rs1024611 and rs4586, these variants are unlikely to represent independent genetic effects but instead reflect a shared regulatory signal within the *CCL2* locus (34, 35). Accordingly, the genetic associations should be interpreted at the locus level rather than as distinct SNP-specific effects. Although rs1024611 is a well-characterised functional variant associated with inducible *CCL2* transcription (36, 37), our eQTL analyses indicated that rs4586 preferentially maps to *CCL8* rather than *CCL2*. Despite residing within the *CCL2* locus, single-cell eQTL signals consistently point to *CCL8* regulation in specific immune lineages, suggesting that genetic effects at this locus may involve a broader chemokine regulatory region encompassing both *CCL2* and *CCL8* rather than a *CCL2*-exclusive mechanism (31). As a CCR2-engaging chemokine, *CCL8* promotes monocyte trafficking and effector T-cell recruitment, providing a biologically plausible mechanism by which variation at this locus could modulate JAKi response (34, 36). Together, these findings support a model in which rs1024611 and rs4586 tag a shared regulatory haplotype with pleiotropic effects on multiple chemokine genes. Distinguishing whether rs4586 represents a partially independent functional effect acting through *CCL8* will require fine-mapping and functional validation in larger cohorts.

CCL2 is a key chemokine involved in the recruitment of effector T cells, B

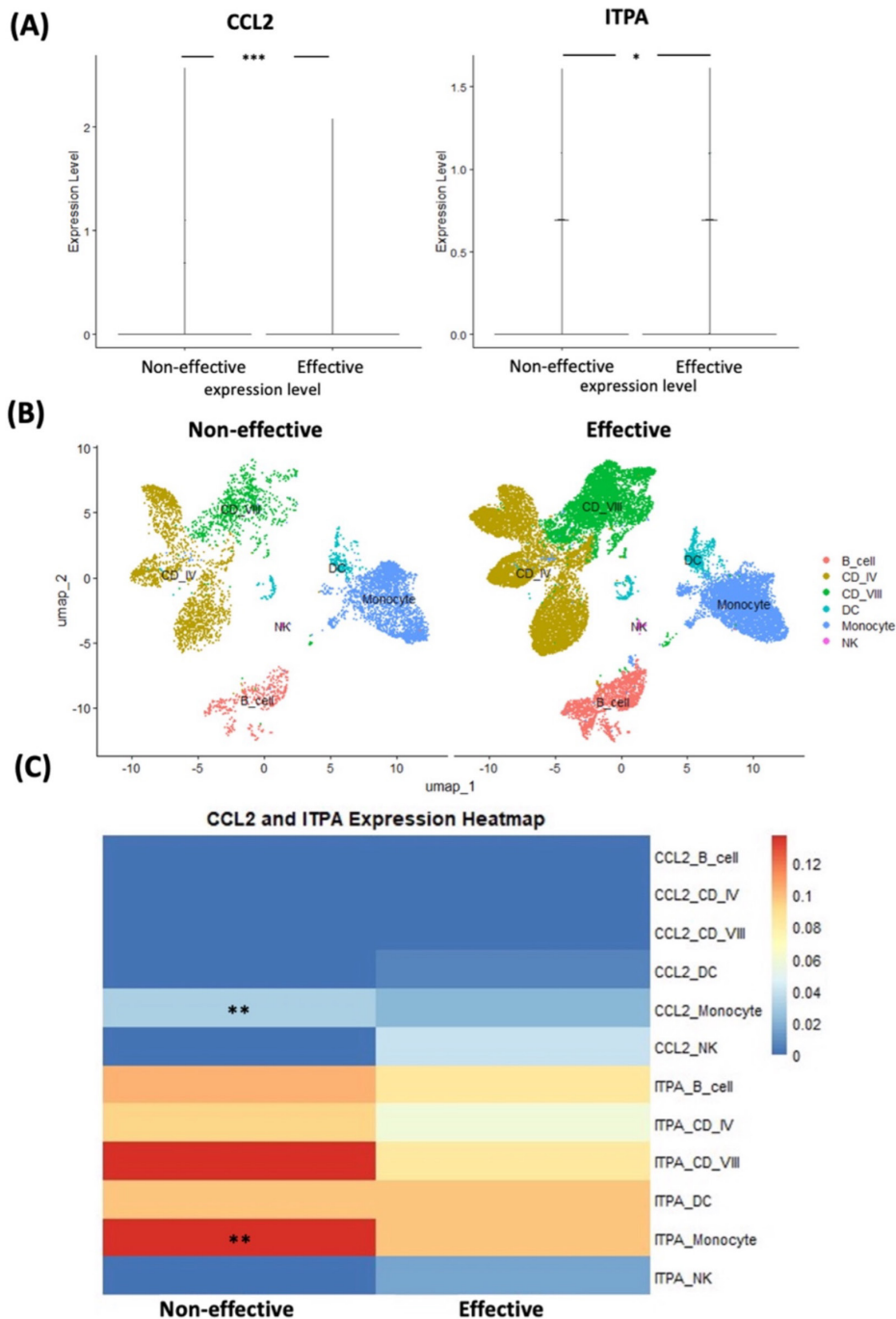


Fig. 1. CCL2 and ITPA expression across diverse immune cells in scRNA-seq

A: Pseudo-bulk RNA-seq expression profiles of CCL2 and ITPA in patients with Effective vs. Non-effective responses to JAK inhibitor (JAKi) therapy.

B: UMAP clustering of immune cell populations based on single-cell RNA sequencing (scRNA-seq) data. Distinct cell types are annotated, including B cells, CD4⁺ T cells, CD8⁺ T cells, dendritic cells (DCs), monocytes, and natural killer (NK) cells.

C: Box plots showing the expression levels of CCL2 and ITPA across immune cell subsets in the Effective vs. Non-effective groups.

Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

cells, and monocytes, while inhibiting Treg in the synovial tissue, thereby playing a critical role in osteoclastogenesis and the progression of RA (37, 38). After being secreted by immune cells, CCL2 binds to CCR2 on joint endothelial cells, activating the PI3K pathway and promoting further CCL2 secretion (39). Additionally, the CCL2/CCR2 signalling axis can induce activation of the JAK/STAT pathway (40, 41). These processes create a sustained pro-inflammatory environment, potentially

reducing the therapeutic effectiveness of JAKi. In our cohort, the number of homozygous carriers for the identified SNPs was relatively small (*e.g.* 11 for rs1024611, 4 for rs1127354, and 11 for rs4586 in the non-effective group). This limited sample size reduces the statistical power to detect robust associations, although the observed effect directions were consistent with the overall findings. Taken together, our results suggest that CCL2 (rs1024611/rs4586) and ITPA (rs1127354) may serve as candi-

date pharmacogenetic markers to anticipate real-world JAKi effectiveness and persistence in RA. While these signals remain hypothesis-generating, they are potentially actionable for patient-level risk-benefit discussions and for stratification in future clinical trials, particularly in East Asian populations.

In patients treated with csDMARDs alone, B cells remain predominant within the synovial microenvironment. By contrast, those receiving JAKi therapy exhibit elevated expression of

Table III. Detailed SNP information related to JAK inhibitors (GRCh38).

Closest gene (HGNC symbol; Ensembl ID)				
rsid	chr	Closest gene (symbol; Ensembl ID)	Ref.	Alt.
rs1024611	17	CCL2 (ENSG00000108691)	A	G
rs1127354	20	ITPA (ENSG00000117222)	C	A
rs4586	17	CCL2 (ENSG00000108691)	T	C

Cell-type-specific eQTL (scQTLbase)					
rsid	gene	Locus (GRCh38)	β (SE)	Cell type	<i>p</i> -value
rs1024611	CCL2	chr17:34,252,769–34,252,770	4.39 (1.00)	dendritic cells	1.14E-05 ***
rs1127354	ITPA	chr20:3,213,196–3,213,197	0.16 (0.04)	CD14 ⁺ monocytes	3.26E-04***
rs4586	CCL8	chr17:34,256,250–34,256,251	3.69 (1.00)	CD8 ⁺ T cells	2.25E-04***

All coordinates in this table are GRCh38 (hg38). rsid (dbSNP identifier); gene (eGene, expression target); Locus (GRCh38 coordinates); β (SE) (regression coefficient and standard error as reported by the source study); Cell (annotated cell type; stimulation/condition follows the source label). Effects and allele coding follow the original study as reported in scQTLbase. The significance is indicated as * p <0.05, ** p <0.01, *** p <0.001.

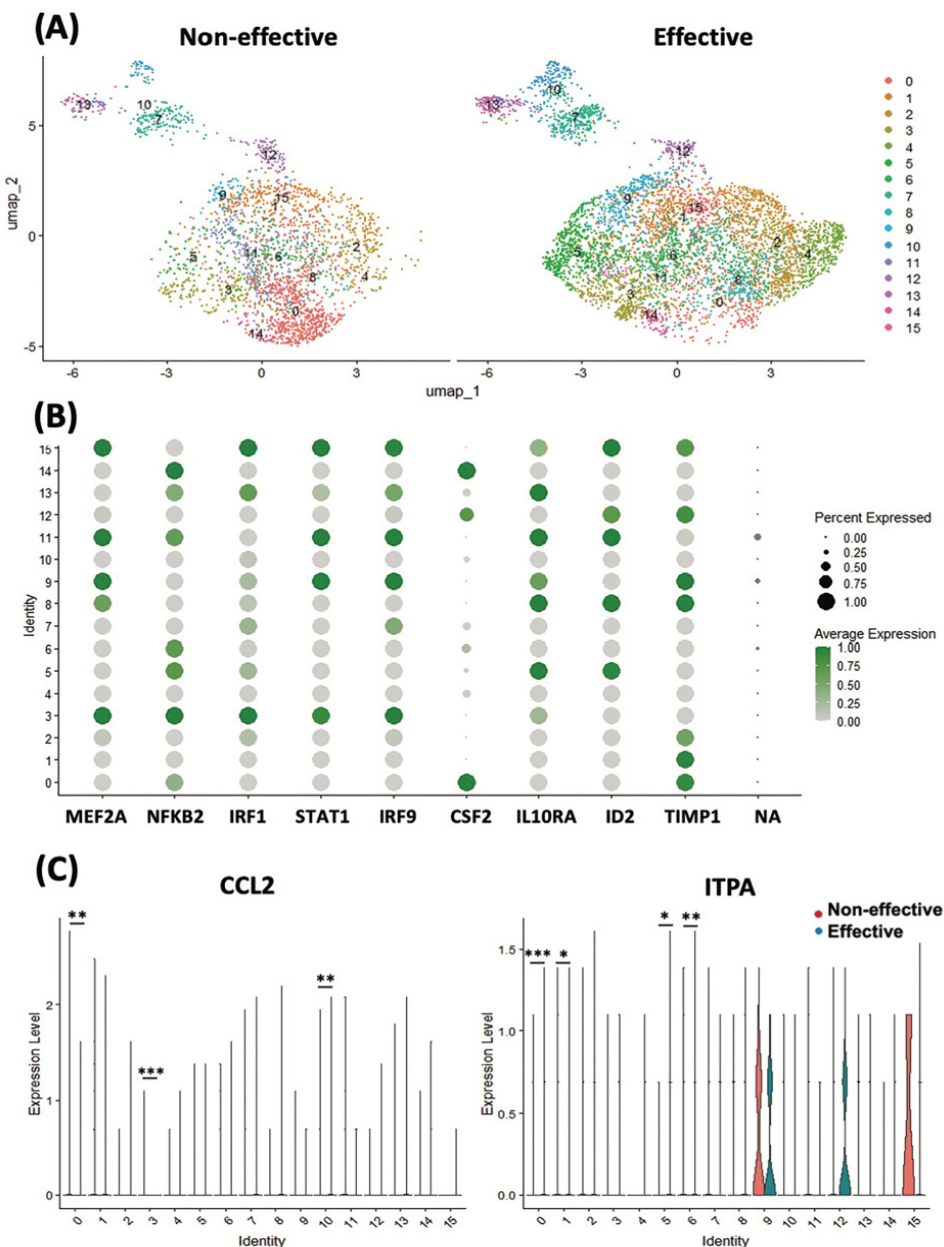


Fig. 2. Monocyte subtype landscape and immune-gene expression by JAKi response.

A: UMAP visualisation of monocyte subtypes clustered by transcriptomic signatures from Effective and Non-effective JAKi response groups. Each colour denotes a distinct monocyte cluster. **B:** Dot plot displaying expression of selected immune-related genes across monocyte clusters. Dot size indicates the percentage of cells expressing each gene, and colour intensity reflects average expression level. Monocyte subsets are defined by canonical markers: M0 (MEF2A), M1 (NFKB2, IRF1, STAT1, IRF9), and M2 (CSF2, IL10RA, ID2, TIMP1).

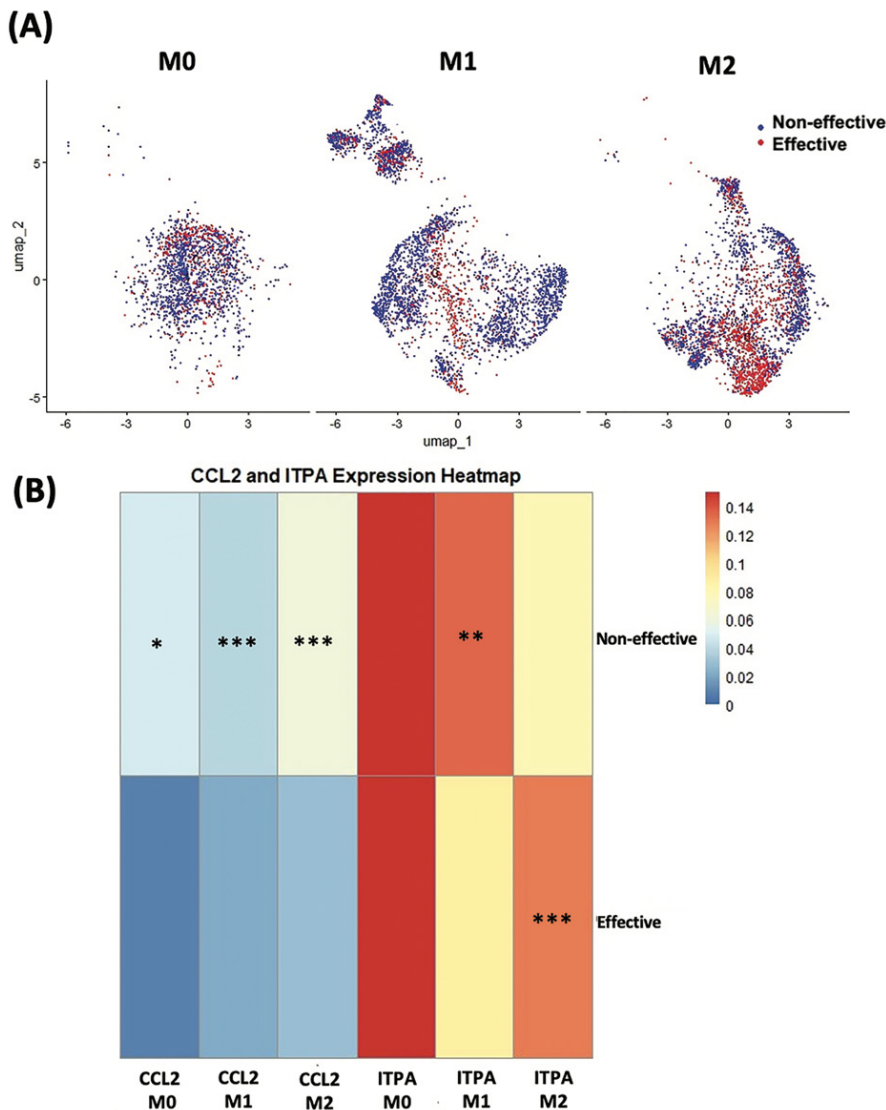


Fig. 3. Monocyte subtype distribution and CCL2/ITPA expression according to JAK inhibitor response. **A:** Uniform Manifold Approximation and Projection (UMAP) visualisation of monocyte subsets (M0, M1, and M2) derived from single-cell RNA sequencing data, coloured by treatment response (Effective vs. Non-effective). **B:** Heatmap showing scaled expression levels of CCL2 and ITPA across monocyte subtypes (M0, M1, M2) in the Effective and Non-effective groups. Colour intensity represents relative gene expression levels. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the endothelial-fibroblast-myeloid cell-type abundance phenotypes (CTAP) interaction pathway, indicating a shift in the inflammatory landscape from lymphoid-driven to stromal-myeloid-dominated crosstalk (42). Overexpression of *CCL2* promoted monocyte polarisation, leading to increased secretion of inflammatory cytokines and further exacerbating RA-related inflammation (43). Recent evidence has also emphasised that macrophage activation in RA exists along a dynamic continuum rather than a binary M1/M2 classification, with substantial plasticity influenced by cy-

tokine signalling and therapeutic interventions (44). Elevated *CCL2* expression across M0, M1, and M2 subsets in the Non-effective group suggested widespread activation of both pro- and anti-inflammatory phenotypes. This pattern highlighted the potential role of *CCL2* in sustaining an inflammatory microenvironment that impaired the therapeutic efficacy of JAKi and diminished the anti-inflammatory functions of M2 monocytes.

The *ITPA* gene encodes inosine triphosphate pyrophosphatase (ITPase), an enzyme that plays a key role in purine

metabolism and immune regulation (45). ITPase catalyses the conversion of inosine triphosphate (ITP) to inosine monophosphate (IMP), thereby influencing the synthesis of AMP and RNA (46). AMP-derived adenosine has well-established anti-inflammatory properties, including inhibition of osteoclastogenesis and modulation of immune responses in the context of RA (47). The rs1127354 (p.Pro32Thr) variant is a loss-of-function allele that reduces ITPase activity and perturbs nucleotide homeostasis with pharmacologic consequences (48, 49). In our JAKi-focused analysis, rs1127354 was associated with lower odds of effectiveness. Yet eQTLs and scRNA-seq showed higher *ITPA* transcripts in Effective patients, particularly in M2 monocytes, suggesting that transcriptional up-regulation may compensate for reduced catalytic function. eQTL analysis showed that rs1127354 correlated with higher *ITPA* mRNA expression, and scRNA-seq data further revealed elevated ITPA transcript levels in monocytes of patients in the Effective group. This apparent discrepancy likely reflects a disconnect between mRNA expression levels and functional enzymatic activity, as reduced ITPase activity in carriers of the variant does not necessarily imply reduced transcription.

scRNA-seq should be interpreted as preliminary and hypothesis-generating, providing supportive biological context rather than validation of the genetic associations. The results revealed that *ITPA* expression was particularly elevated in M2 monocytes among patients who responded effectively to JAKi. This aligns with recent studies highlighting the critical role of the M1/M2 macrophage balance in RA pathogenesis, where a predominance of pro-inflammatory M1 monocytes drives disease progression (50). Given the anti-inflammatory nature of M2 monocytes, this finding further supports the role of ITPase in maintaining immune homeostasis and enhancing JAKi-mediated immunosuppression (51). In contrast, the Non-effective group exhibited a significantly higher proportion of pro-inflammatory M1 monocytes, reinforcing their role in JAKi resistance.

Notably, the upregulation of *ITPA* in M1 monocytes among Non-effective patients might reflect a compensatory response to inflammatory stress. While ITPase was not directly involved in the JAK/STAT signalling pathway, its role in preserving nucleotide balance and promoting M2 monocyte polarisation may indirectly support JAKi-mediated immunomodulatory effects. Together, these findings suggest that the cell-type context of *ITPA* expression, rather than its absolute level, may be critical for therapeutic response.

If prospectively validated, *ITPA* and *CCL2* locus variants could serve as promising candidates for patient stratification in clinical practice. From a translational perspective, strategies targeting the CCR2/CCL2 axis may enhance JAKi responses, though such approaches will require confirmation in clinical trials. Several important limitations should be acknowledged. First, the marked imbalance between the Effective and Non-effective groups (217 vs. 58 patients) substantially limits its statistical power, particularly for analyses focused on non-responders, and may introduce instability or bias in regression estimates. Given the limited sample size, this study was primarily powered to detect moderate-to-large genetic effects, and smaller effect sizes may not have been adequately captured; accordingly, all findings should be interpreted as hypothesis-generating. Second, treatment effectiveness was defined using a persistence-based outcome (>180 consecutive days of uninterrupted JAK inhibitor use), which does not fully capture standardised clinical response measures such as EULAR response or DAS28 remission. Although patients who discontinued or switched therapy within 180 days due to inadequate response were classified as Non-effective, classification of patients who discontinued treatment after 180 days relied on retrospective clinical records. As a result, misclassification of clinical response cannot be fully excluded, particularly among patients who discontinued treatment after 180 days for reasons other than inefficacy. Furthermore, although reasons for JAK inhibitor discontinuation were avail-

able from medical chart review, these were not incorporated into the primary outcome definition. Accordingly, the present findings should be interpreted with caution. Third, although multi-variable models were applied, residual confounding cannot be fully excluded in this retrospective observational design. Fourth, the scRNA-seq analyses were conducted in a small, non-randomly selected subset of patients and should therefore be interpreted as exploratory and hypothesis-generating rather than confirmatory. Finally, after correction for multiple testing, the observed genetic associations did not reach study-wide significance, emphasising the need for validation in larger, independent cohorts.

Conclusion

Based on integrated genetic, single-cell eQTL, and transcriptomic evidence, we prioritise *CCL2* and *ITPA* as pharmacogenetic candidates for JAKi response in Taiwanese patients. Our results provide preliminary insights into genetic factors potentially influencing JAKi response, which require confirmation in future studies.

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