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Identification of potential predictive biomarkers during JAK-inhibitor therapies in rheumatoid arthritis

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Abstract

Background Targeted synthetic therapeutics, such as Janus kinase (JAK) inhibitors, have opened up a new platform for the treatment of various diseases, including rheumatoid arthritis (RA). As with all drugs, the efficacy of different medicine may vary from patient to patient, and there may be different side effects if the expected effect is not achieved. In addition to the uncertain success of treatments, they also place a heavy burden on the health care system, making the identification of potential predictive biomarkers for drug optimisation highly valuable.

Methods In order to identify potential predictive biomarkers, 28 patients with RA were recruited and blood samples were taken twice–before medical treatment and after 6 months of continuous therapy. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples and after RNA isolation, RNA sequencing was performed using high throughput sequencing technology to generate global gene expression data. Validation of target genes was conducted using real-time quantitative PCR methods.

Results After data analyses we examined the gene expression changes between the two sampling time points and responder versus non-responder groups. 225 genes showed significantly different expression between T6 and T0 samples, while 60 and 66 genes showed differential expression between the responder and non-responder patients at T0 and T6 sampling points, respectively. 13 differentially expressed genes were common between two time points and showed the same direction in regulation.

Conclusions Based on our results, several RA-relevant genes were identified, as a result of the JAK-inhibitor treatments in comparison of T6 versus T0 samples. At both time points, the differentially expressed genes between responder and non-responder groups could separate the samples, however, the separation was not clear. The identified 13 common genes could also partially separate the responder and non-responder groups from each other.

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These sets of genes could be the source of potential biomarkers, which could help predict the responsiveness of patients to JAK inhibitor therapy.

Keywords Rheumatoid arthritis, Janus kinase, JAK inhibitors, Gene expression, RNA-sequencing, Response, Prediction

Introduction

Rheumatoid arthritis (RA), one of the most common chronic systemic autoimmune disorders with a relatively high prevalence among the population worldwide, presents a difficult challenge for both society and the health care system [1–3]. The molecular mechanisms underlying the pathomechanism of RA can be associated with a highly complex inflammatory network that causes primarily joint and bone destruction, and, in serious cases, internal organs may be affected as well [1, 3–5]. The extending inflammatory processes result in severe pain, injuries, various comorbidities and relatively high morbidity rates [6, 7]. In addition to genetic factors, there are risk factors for the development of the disease including age, sex, environmental factors (e.g. viral infections), smoking, dietary habits and obesity [8, 9].

As far as the therapeutic agents of RA are concerned, the first line treatment includes glucocorticoids and conventional synthetic disease-modifying antirheumatic drugs (csDMARDs, e.g. methotrexate (MTX)) [6, 7]. In the past few decades biological (bDMARDs) and targeted synthetic therapies (tsDMARDs) have been developed and approved by the U.S. Food and Drug Administration (FDA; USA), which have enabled a new approach in treating RA [10, 11]. Biological agents include various antibodies and soluble receptors (e.g. tumour necrosis factor-α (TNF-α) inhibitors), while various JAK inhibitors form the group of targeted synthetics. JAKs are key components in the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signalling pathway and include four members, namely JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2) [12, 13]. These molecules constitutively bind to their appropriate domains in the intracellular area of their cytokine receptors [12, 14]. Each JAK interacts then with its corresponding STATs, resulting in the activation of different signalling pathways and, hence, different immunological mechanisms as well. On this basis, different drugs have been created that target different JAKs with different specificity – tofacitinib is selective for JAK1 and JAK3 [12, 13, 15], filgotinib and upadacitinib primarily target JAK1 [12, 13, 16], while baricitinib blocks the pathways involving JAK1 and JAK2 [9, 12–14]. A common feature of all these JAK-inhibitors is that they target either the kinase or the pseudokinase domain of JAKs, and by the blocking of their autophosphorylation both the activation of the signalling pathway and their rate of action may be decreased [12]. This regulatory effect can reduce the degree of inflammation and help the patient to enter a sustained remission phase, as a consequence of a potential reduction in the levels of various inflammatory mediators.

Compared to conventional therapies, bDMARD and tsDMARD drugs seem to provide higher efficacy [10, 17], although a significant proportion of patients show little/no response to the treatment and may even develop different side effects and allergic reactions [18]. In order to confidently assess the efficacy of each drug before starting therapies, various studies have been carried out to identify potential biomarkers. Numerous studies have also been conducted in recent decades that examine the molecular genetic aspects of RA (e.g., single nucleotide polymorphisms (SNPs)) [19–21]. Unlike traditional genetic methods, these studies generally provide deeper insight into the investigation of various diseases, especially when the conditions under investigation can be traced back to more complex genetic and environmental factors [19]. Even though a number of proteins; e.g. anti-citrullinated peptide antibody (ACPA), mitogen-activated protein (MAP) kinase, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), activator protein-1 (AP-1), matrix metalloproteinase 3 (MMP3) [22, 23] and polymorphisms affecting genes (e.g. TNFα, *PTPN22*, *ICAM-1*) have been identified that may prognose the responses of patients to medical treatments [22, 24], no certain predictive biomarker has been found so far. It would also be beneficial to investigate and identify potential genomic markers to establish a predictive methodology for treatments in RA [25]. Several studies have been performed on bDMARD, mainly TNFα and interleukin-6 blocker therapies, to identify biomarkers of responsibility to the treatment based on PBMC gene expression profiles [26–31].

Although there are several projects which examined the gene expression changes upon JAK inhibitor treatments or during JAK inhibitor therapies, most of these studies examine the gene expression changes in the synovial fluid and synovial fibroblasts after the selected treatments [32–34]. Moreover, only few studies were found which examined the changes of gene expression patterns in PBMCs before and after JAK inhibitor therapy [35, 36], but there are no studies on identification of responsiveness related genes. In our exploratory study our aims were to fill this scientific gap in the knowledge of using JAK inhibitors in RA based on changes of gene expression patterns of PMBCs and to determine a set of genes that would allow to distinguish between responder (R) and non-responder (NR) patients. Later the identified genes can be used as predictive biomarkers of responsiveness to

JAK inhibitor therapy, with a move towards personalised medicine.

Methods

Patient recruitment

In our study 28 active RA patients between the ages of 33 and 71, including 24 women and 4 men were recruited by the Department of Rheumatology, Faculty of Medicine, University of Debrecen. The inclusion criteria were (1) no previous tsDMARD therapy; (2) previously unsuccessful csDMARD therapy; (3) balanced medical treatment with MTX (to a maximum extent of 20 mg/week), prednisolone and/or NSAIDs for at least four weeks prior to sampling (4) active disease status according to the disease activity score of 28 joints ((DAS28) > 3.2). The exclusion criteria were (1) current malignancies; (2) pregnancy or breastfeeding; (3) active infectious disease(s); and (4) other inflammatory joint disease(s). Continuous health monitoring, clinical data collection and the sampling were also conducted at the Department of Rheumatology. Patients received three types of JAK inhibitor therapies, which were baricitinib (10 female patients), tofacitinib (12 patients; 4 male and 8 female) or upadacitinib (7 female patients). One female patient initially received tofacitinib treatment but was switched to baricitinib treatment due to medical decision. Some patients may have received supplementary treatments e.g. MTX or other DMARDs or low-dose corticosteroid based on medical recommendations. DAS28 values for each patient fell within the intervals 3.2–6.83 at baseline, sampling time 0 (T0) and 1.15–4.54 six months after starting the therapy (T6). Responder and non-responder status were determined based on the change of the DAS28 values. According to the internationally accepted recommendations a patient was considered as responder for a medication if the DAS28 value has decreased below 3.2. The clinical data of the patients are shown in Table 1. The study was approved by the Hungarian Scientific Research Council Ethical Committee (approval No. IV/2958-1/2022/EKU). Assessments were carried out according to the Declaration of Helsinki and its amendments. All participants gave oral and written consent to use their samples and data.

Table 1 Clinical data of patients

Parameters	Responders	Non-responders
Patients	15	13
Male	1	3
Female	14	10
Age (years)	49.2 ± 8.8	56.6 ± 11.3
Range	33–64	34–71
Duration of RA (years)	12.2 ± 8.0	15.3 ± 6.3
Range	3–29	3–24
CRP (T0)	7.9 ± 12.1	7.3 ± 8.6
Range	0.1–47.5	0.4–32.4
CRP (T6)	5.0 ± 8.6	6.5 ± 5.9
Range	0.4–33.8	1–18.9
DAS28 (T0)	4.59 ± 0.86	5.11 ± 0.94
Range	3.20–5.83	3.93–6.83
DAS28 (T6)	2.07 ± 0.48	3.93 ± 0.48
Range	1.15 – 2.73	3.20–4.54
JAK inhibitors		
Baricitinib (Male/Female)	0/6	0/4*
Tofacitinib (Male/Female)	1/5	3/3*
Upadacitinib (Male/Female)	0/3	0/4
Supplementary treatments		
Medrol (T0; Male/Female)	0/5	3/6
Medrol (T6; Male/Female)	0/3	2/3
Metothrexate (T0; Male/Female)	1/8	2/1
Metothrexate (T6; Male/Female)	1/7	2/2
DMARD (T0; Male/Female)	1/2	2/6
DMARD (T6; Male/Female)	1/2	2/5

Age, Duration of RA, CRP and DAS28 data are presented as means ± SD and ranges. T0 and T6 represent the two sampling time points. DMARD includes leflunomide and/or sulfasalazine supplementary treatments

*One patient started her inhibitor therapy on tofacitinib and then changed to baricitinib owing to the treating physician

Isolation of peripheral blood mononuclear cells and total RNA

10 ml of venous blood samples were collected into Vacutainer K_2EDTA blood collection tubes by the Department of Rheumatology, Faculty of Medicine, University of Debrecen. Samplings were performed twice during the study at T0 (before the treatment) and T6 (six months after continuous treatment). Maximum 2 h after blood collection, PBMCs were separated by Ficoll gradient centrifugation. Cell lysis was performed by Trizol reagent and then total RNA were isolated. The concentration was determined by UV photometry using DeNovix (DeNovix Inc., Wilmington, DE, USA). The quality of RNAs was checked on 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) using RNA 6000 Nano Assay kit.

RNA sequencing (RNA-Seq) and bioinformatic methods

RNA-Seq libraries were generated by using MGIEasy RNA Library Prep Set kit (MGI, Shenzen, China) according to the manufacturer's protocol. Libraries were sequenced on MGI DNBSeq G400 instrument, in single-end 100 bp sequencing. Raw reads were then aligned to GRCh38 human reference genome using HiSat2 aligner and BAM files were generated. StrandNGS 4.0 was used for the downstream analyses. Normalisation and quantification were done by using the integrated DEseq algorithm of StrandNGS. To determine the differentially expressed genes (DEGs) between different time points and responder versus non-responder groups, Mann–Whitney statistical test was performed, $p < 0.05$ was considered as significant difference.

Pathway analyses

CytoScape v3.4 software with ClueGo v2.3.5. application was used for identifying over- represented Gene ontology (GO) terms. Two-sided hypergeometric test was performed, the list of DEGs was tested against GO Biological process databases.

Real-Time Quantitative Polymerase Chain Reaction

To validate RNA-Seq data, real-time quantitative polymerase chain reaction (RT-qPCR) method was used. cDNA was generated from 1 µg of RNA using High-Capacity cDNA Reverse Transcription Kit (Thermo Fischer Scientific, Waltham, MA, USA). Then relative gene expression levels of selected genes were determined by using TaqMan assays (Thermo Fischer Scientific) (Supplementary Table 1). TaqMan Gene Expression Master Mix 2X was used for the reactions and the measurements were executed on QuantStudio 12KFlex (Thermo Fischer Scientific) instrument.

Results

Global gene expression analyses

We recruited 28 active RA patients, 24 women and 4 men, who underwent JAK inhibitor therapy. Based on the clinical outcome, 15 responder and 13 non-responder patients were identified. As a further isolation step, PMBC separated from blood samples of each sampling time points and total RNA was isolated. To obtain whole gene expression data, RNA-Seq was performed using high throughput sequencing technology. 27–65 millions of reads were generated in single read 100 bp sequencing with Q30 > 94% base calling accuracy. Raw data were aligned to the GRCh38 human reference genome, the alignment percentage of the reads was > 95%, and downstream analyses were performed in StrandNGS 4.0 software.

Effect of the JAK inhibitor therapy after six months (T6 versus T0)

225 DEGs were identified between T6 and T0 time points, 66 genes were downregulated and 159 genes were upregulated (Supplementary Table 2). Hierarchical cluster analysis was executed, and we found that these 225 genes partially separate the T6 and T0 samples from each other (Fig. 1). To investigate the biological function and of these genes GO analysis was performed and overrepresented GO terms were identified, which may be related to the pathomechanism of RA. In case of the downregulated genes *BCL2* and *IL2RA* play role in lymphocyte homeostasis and B cell proliferation, while several genes such as *CCR1, CCR2, CISH, FCGR1A, FCGR1B, GBP1, IFI6, IFIT2, IFIT3, ISG15, MX1, OSM, SOCS1, SOCS2, SOCS3, STAT1* belong to inflammation related pathways, including cytokine and interferon-gamma-mediated signalling pathways. Some of these genes, like *SOCS1/2/3* and *STAT1*, also play significant role in regulation of JAK-STAT cascade (Fig. 2A and Supplementary Table 3).

GO analysis revealed that the 159 upregulated genes play role in immune related pathways such as leukocyte and lymphocyte proliferation and TNF production (*AHR, ATM, CCDC88B, CLU, IKZF3, NFATC2, SPN, THBS1*). Some of the genes are involved in metabolic biological processes, like lipid transport (*ABCA2, ABCA7, ABCC3, BMP6, ITGB3, LRP1*), and in tissue remodelling related pathways, such as endothelial cell proliferation (*ALOX12, PTPN23, SPARC*), blood coagulation and vasculature development (*ADGRG1, AHR, ALOX12, ARID1A, DGKQ, GP1BA, GP1BB, GP9, ITGA2B, SMOX, SPARC, STAB1, TCF7L2, VSIG2, ZMIZ1*) (Fig. 2B and Supplementary Table 4).

Identification of responsiveness related genes

After comparing the two sampling time points, we determined which patients were considered responders and non-responders along clinical parameters. Based on changes in DAS28 values, 15 patients were considered responders, and 13 patients were classified as non-responders. We analysed the difference of gene expression patterns between R and NR patients separately in the two sampling time points.

At T0 60 DEGs were identified in the R vs NR comparison, from which 37 genes were down- and 23 were upregulated (Supplementary Table 5). When we examined the differences between R and NR samples at T6, 66 DEGs were found, 24 down- and 42 upregulated (Supplementary Table 6).

We further analysed these genes sets. Hierarchical cluster analyses were performed and the results of T0 and T6 time points are demonstrated on heatmaps (Fig. 3A and B, respectively). Both gene sets at T0 and T6 time points did not give clear separation between R and NR samples based on the clustering. Using principal component analysis (PCA) we further tested the separator capability of the gene sets and found that although PCA also did not separate perfectly R and NR samples from each other, but the separation had much higher degree (Fig. 3C and D).

Comparing the two gene sets containing 60 genes at T0 and 66 at T6, only 13 overlapping DEGs were found: *ADAM9*, *ADK*, *CD36*, *FAM198B*, *HOTAIRM1-1*, *HOXB2*, *NOG*, *PLA2G7*, *PRSS57*, *RNASE2*, *RPSAP47*, *RPS4XP3* and *RPS6P25*.All of the overlapping genes showed the same direction of regulation at both time points, 8 down- and 5 up-regulated (Supplementary Table 7). PCA analyses of the common 13 genes showed that this gene set could also partially separate the R and NR groups from each other at T0 and T6 time points (Fig. 4A and B, respectively), increasing their potential in response-based categorization.

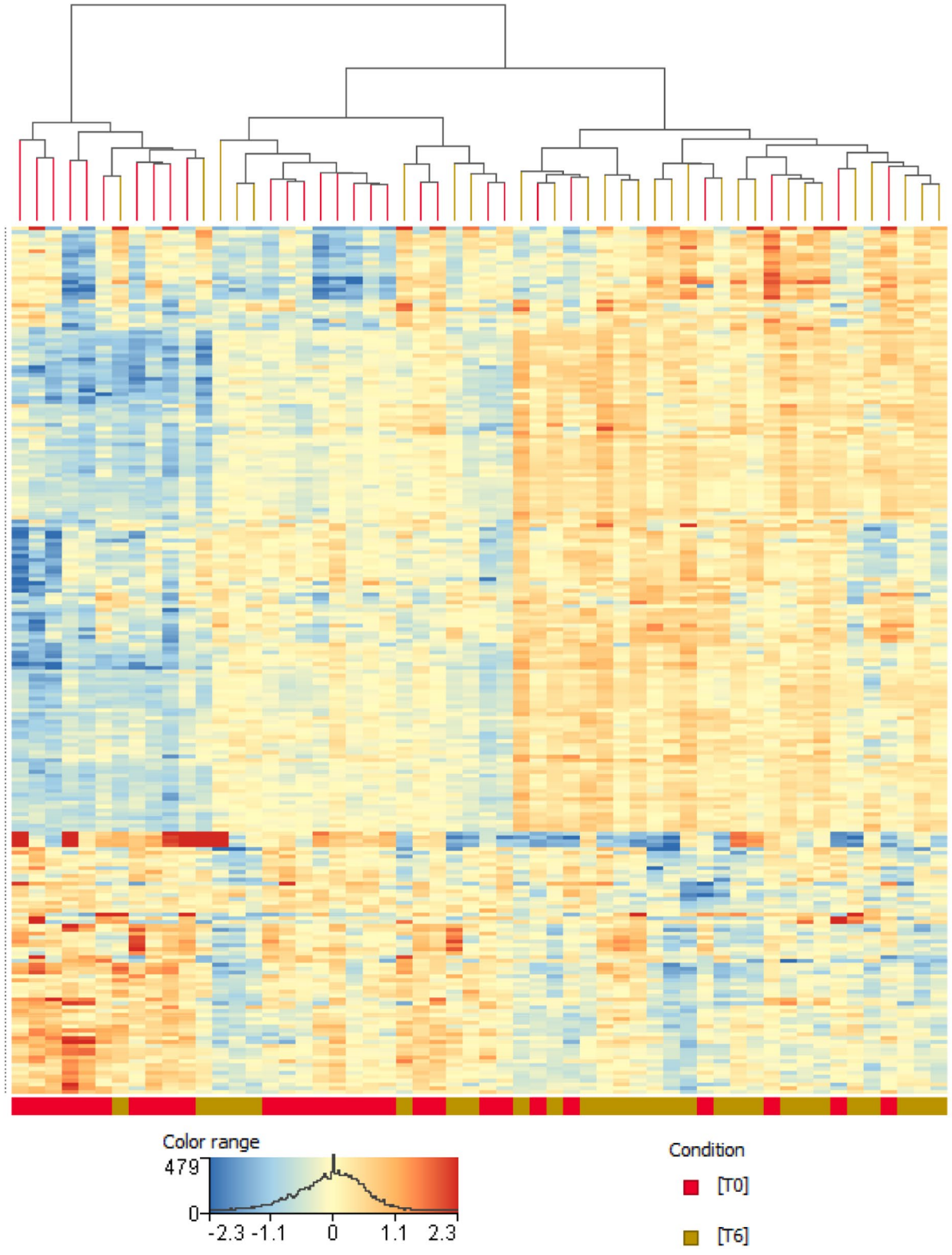

Fig. 1 Hierarchical cluster analysis of DEGs between T6 and T0 samples. Red colour represents patients at T0, while green colour represents patients at T6. Each row represents a gene that was found to be significant between the two time points

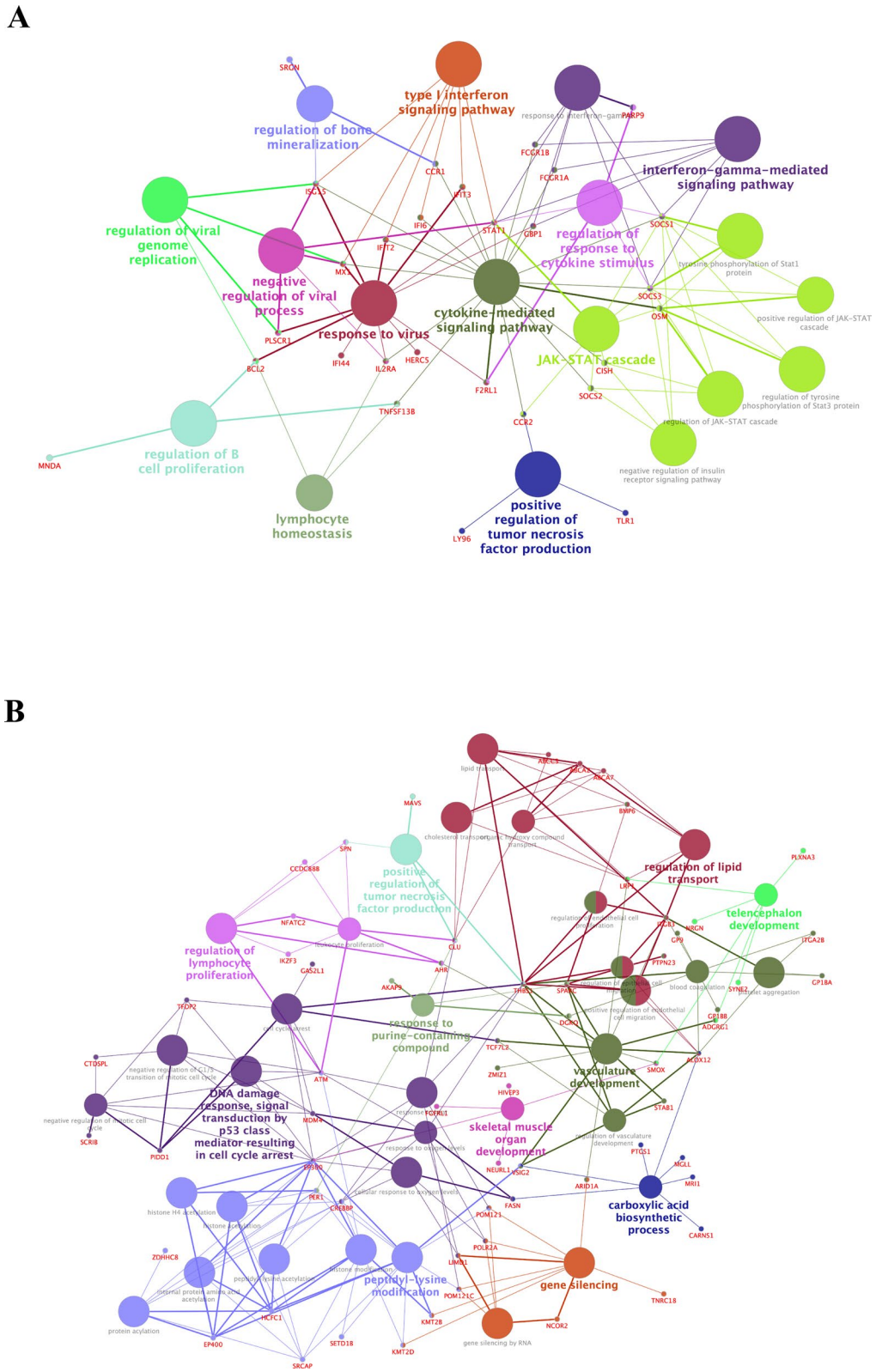

Fig. 2 GO analysis of DEGs between T6 and T0 samples. The overrepresented GO categories and key genes are presented of downregulated (**A**) and upregulated (**B**) DEGs from the comparison of T6 and T0 samples

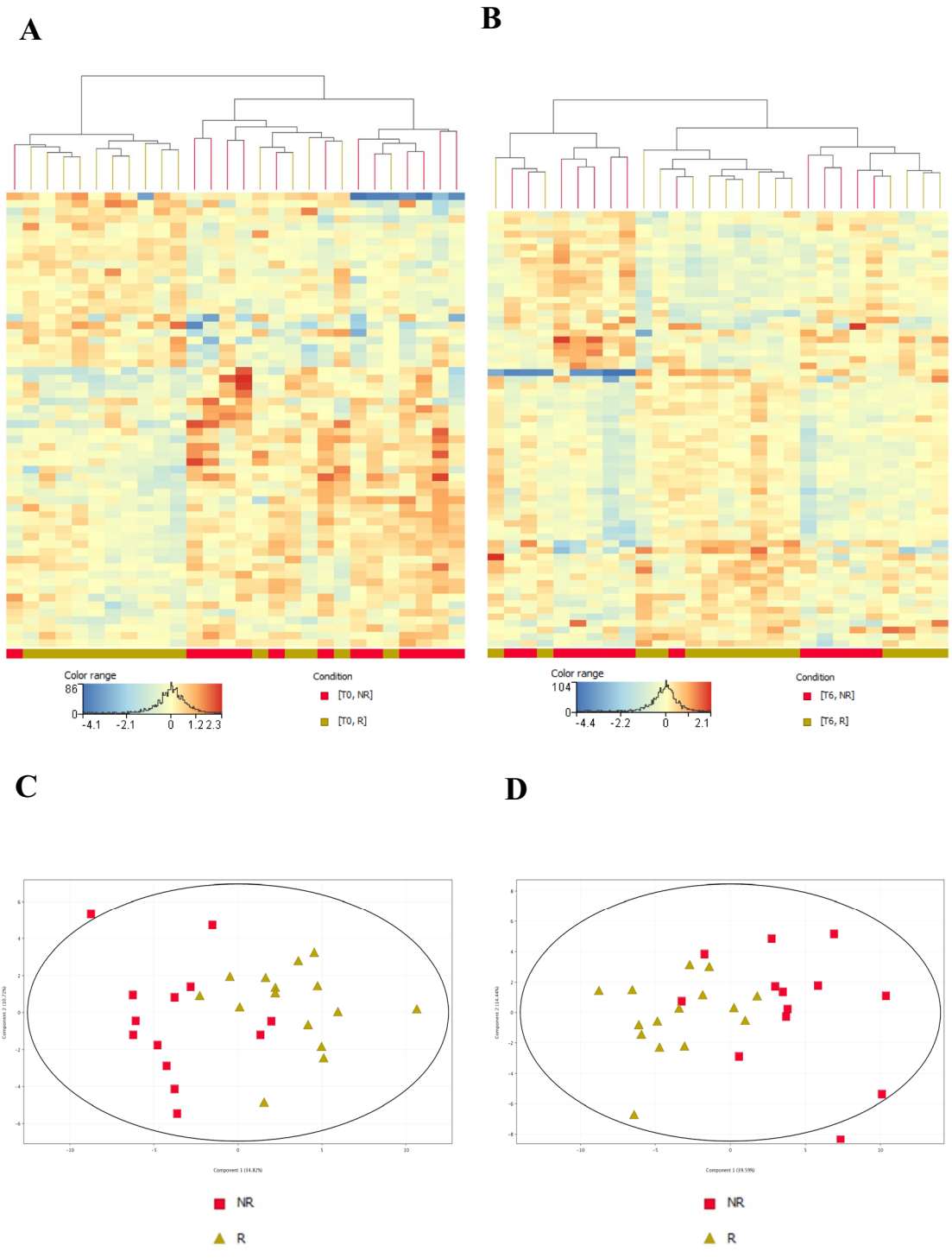

Fig. 3 Hierarchical cluster and principal component analyses of R and NR samples based on responsiveness related DEGs. Heatmaps show the clustering of R and NR samples at T0 (**A**) and T6 (**B**) timepoints when only DEGs are plotted. PCA was performed using the DEGs of R versus NR comparisons at T0 (**C**) and T6 (**D**) timepoints. PCA plots represents the relationship of the individual samples to each other. Green colour represents the R and red colour the NR samples

Validation of genes by RT-qPCR

To confirm our results some of the genes were chosen for RT-qPCR measurements. 9 genes were selected, 5 of the 13 common genes (*ADK, ADAM9, CD36, NOG* and *PLA2G7*) and other 4 of the genes from the comparison of R vs. NR groups at T0 time point (*MARCO, MS4A4A, PLA2G4A* and *SPARC*). For normalising our data, four reference genes (*RPLP0, GAPDH, HPRT1* and *PPIA*)

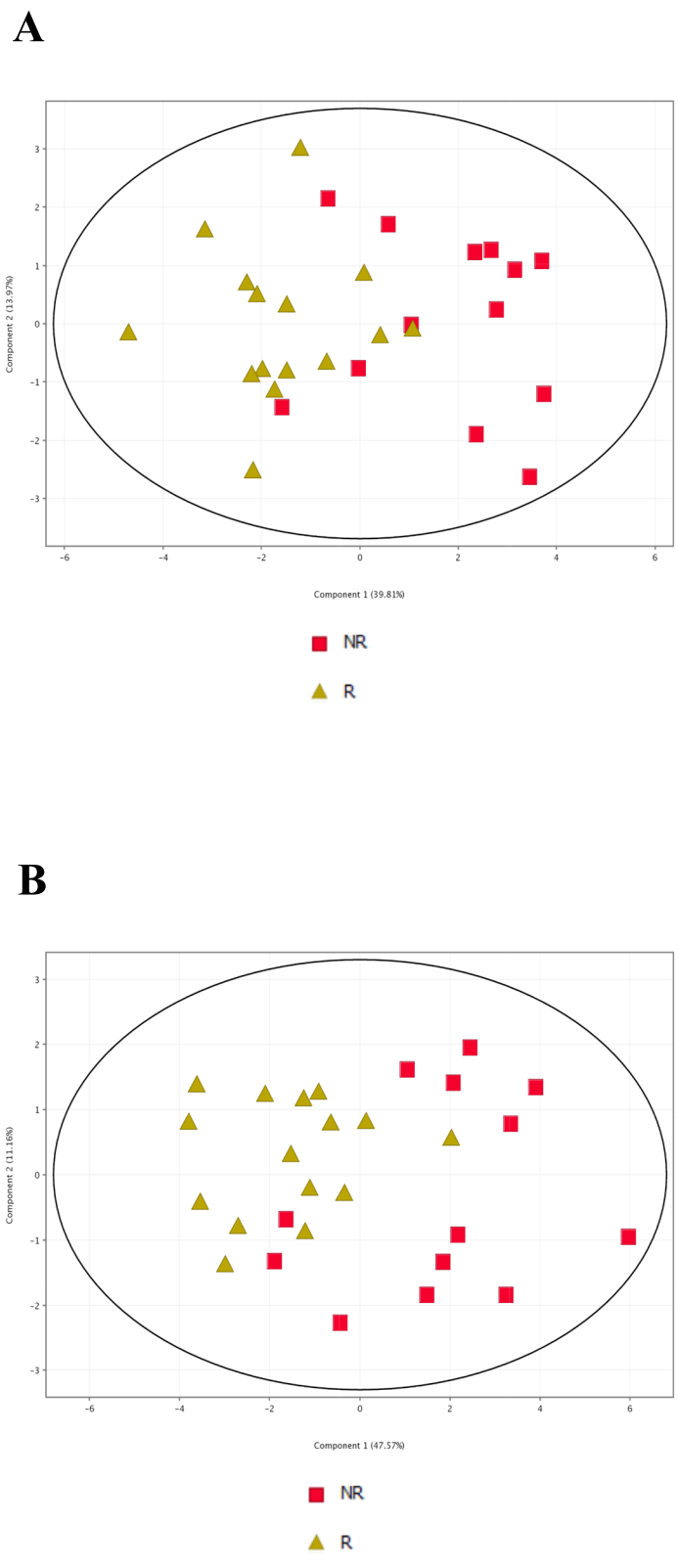

Fig. 4 PCA analysis of common responsiveness related DEGs. 13 genes were overlapped between lists of DEGs from R vs NR comparisons at T0 and T6 timepoints. PCA were performed using these genes and plots shows the relationship of R and NR samples to each other at T0 (**A**) and T6 (**B**) timepoints. Green colour represents the R and red colour the NR samples

were tested. An online application, RefFinder (https://www.ciidirsinaloa.com.mx/RefFinder-master/) [37], which uses the algorithms of geNorm, Normfinder, BestKeeper and delta Ct methods, was used to find the best reference gene for normalising our RT-qPCR data. After the calculation, the *HPRT1* showed the highest stability on our data set (Table 2) and it was used to generate normalised gene expression values.

All of the 9 genes were measured with samples of both time points. After the analysis of the results of T0 samples, *ADK, CD36 PLA2G7* and *MARCO* genes showed significant differences between the R and NR groups, and the direction of the regulation was also the same as we obtained in the RNA-Seq analysis (Fig. 5A). In the case of *ADAM9, MAS4A4A, NOG, PLA2G4A* and *SPARC* we failed to detect significant differences between the two groups during the RT-qPCR measurements, but the direction of the regulation was the same as in the RNA-Seq results (data not shown).

We also analysed the results of the T6 samples and found that 4 genes from the common gene list (*ADAM9, ADK, CD36* and *PLA2G7*) confirmed our RNA-Seq results (Fig. 5B), however, there was no significant difference in the case of *NOG*, only the direction of regulation was the same (data not shown). Interestingly, *MARCO* also showed significant difference between R and NR groups after the analysis, however it was not a DEG in the RNA-Seq analysis at T6.

Discussion

The aim of our study was to investigate gene expression changes in patients with RA receiving JAK inhibitor therapies, and to identify genes that could serve as predictive biomarkers for estimating a patient's response to these medicines. Our study included 28 active RA patients (4 men and 24 women) who received JAK inhibitor therapies: baricitinib (10 patients), tofacitinib (12 patients) and upadacitinib (7 patients), and based on decision of treating physician, one patient changed from tofacitinib to baricitinib therapy. Venous blood samples were collected from the patients before the start of treatment (T0) and after six months of continuous therapy (T6), then PBMCs were separated and RNA was isolated. To obtain global gene expression data, we performed RNA-Seq of T0-T6 sample pairs of the recruited 28 RA patients. Patients were categorised into R and NR groups according to the changes of their DAS28 scores during the therapy.

Table 2 Analysis of normalizing genes using RefFinder

Ranking order (1–Best; 4–Worst)				
Method	**1**	**2**	**3**	**4**
Delta CT	HPRT1	PPIA	GAPDH	RPLP0
BestKeeper	HPRT1	PPIA	RPLP0	GAPDH
Normfinder	HPRT1	PPIA	GAPDH	RPLP0
Genorm	HPRT1	PPIA	GAPDH	RPLP0
Recommended comprehensive ranking	HPRT1	PPIA	GAPDH	RPLP0

Based on the results of the comparative methods used by the RefFinder software, the *HPRT1* gene proved to be the most stable normalizing candidate on the sample sets examined

First, we investigated the effect of the therapy at the gene expression level comparing the gene expression patterns of T6 and T0 samples to determine the effect of the treatments. 225 genes, 66 down- and 159 upregulated, showed differential expression between the two time points. Several of the downregulated genes, such as *TNFSF13B, TNFSF10, GBP1, BCL2A1* and *CMPK2* play a role in the pathogenesis of RA [38–42]. GO pathway analyses revealed that several downregulated genes like *STAT1*, *SOCS1*, *SOCS2*, *SOCS3* and *CISH* are involved in the regulation of JAK/STAT cascade and interferon-gamma-mediated signalling pathway, [43]. We identified several RA-relevant genes among the 159 upregulated genes, such as *MDM4*, which, may reduce symptom manifestation by participating in the modulation processes of the Circ-FAM120A/miR-671-5p/MDM4 axis [44]. The pathway analysis showed that the upregulated genes play role in various biological processes, such as vasculature development, lymphocyte proliferation, DNA damage response and lipid transport. Overviewing the results of gene expression changes caused by JAK inhibitor treatments at time point T6, we can conclude that our results correlate with the expected outcome of therapy, which leads to the alleviation of inflammatory symptoms. The expected reduction in inflammatory processes is also supported by downward trends in patients' DAS28 values.

After categorizing patients into R and NR groups, we identified which genes might be related to the responsiveness and determined the differentially expressed genes between R and NR at both time points. At T0 60 DEGs were found between R and NR groups, 37 down- and 23 up-regulated. Some of them like *RNASE2, SPARC, NOG* and *MS4A4A* were reported to have influence on RA pathogenesis [45–48]. Some genes, such as *MARCO*, *CCR7*, *IGF1R, PLA2G7* or *COL18A1*, have also been identified in the literature as potential therapeutic or diagnostic biomarkers in RA [49–52]. Others play role in inflammatory and immune processes, coagulation and lipid metabolism such as *PLA2G4A, HOTAIRM1-1*, *CD36, CD163* and *ADK* [53–57]. At T6 we also investigated which genes showed significant differences between R and NR groups and 66 DEGs were found. These genes have various functions, based on the literature *BACH1*, *YOD1, CCR2, CREB5, NDRG2* and *PLD4* are involved in RA pathomechanism [58–64].

13 DEGs (*ADAM9, ADK, CD36, FAM198B, HOTAIRM1-1, HOXB2, NOG, PLA2G7, PRSS57,*

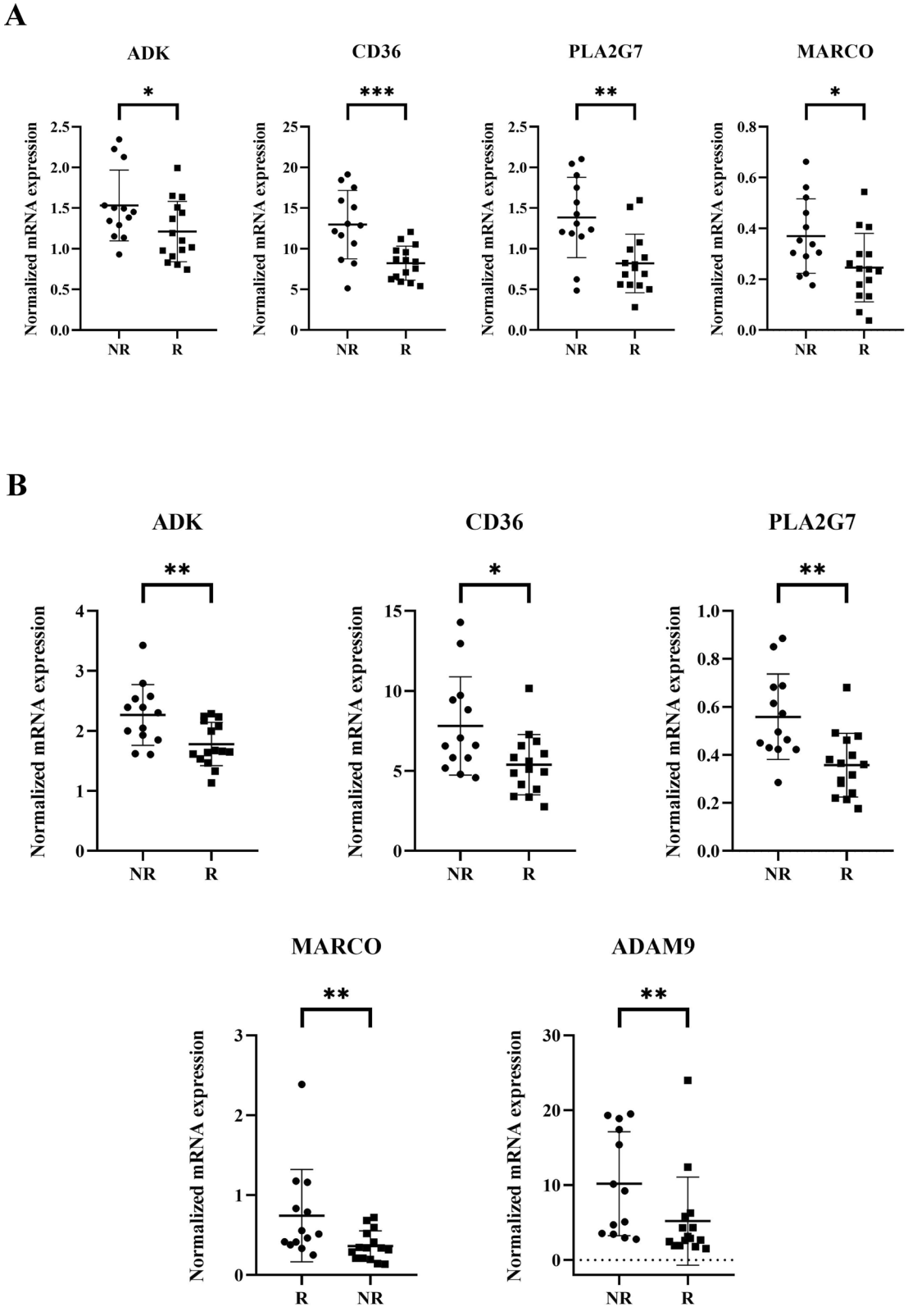

Fig. 5 RT-qPCR validated responsiveness related genes. Panels show the results of RT-qPCR validated genes at T0 and T6 timepoints (**A** and **B**, respectively). Relative expression levels are shown normalized to *HPRT1*. Significance levels represent: *p < 0.05; **p < 0.01; ***p < 0.001

RNASE2, RPS4XP3, RPS6P25 and *RPSAP47*) were common between the responsiveness related gene sets at T0 and T6 timepoints. PCA showed that this set of genes could also separate the R and NR samples from each other, however, the separation is not perfect.

5 genes from the common gene list and other 4 from T0 R vs NR DEGs were selected for RT-qPCR measurements to confirm our RNA-Seq results, all of which were reported to be RA-related according to the literature. After the analysis we found that *ADK, CD36*, *MARCO* and *PLA2G7* showed the same expression profile at both time points as in the RNA-Seq analysis. *ADAM9* was significant only at T6 time points. The other four genes (*MS4A4A, PLA2G4A, NOG* and *SPARC*) did not show significant differences based on the RT-qPCR measurements, but the direction of their regulation was similar to the RNA-Seq results. If succeeding in identifying and validating these and other potential response-specific genes in larger patient cohorts, integrating them into a complex prediction algorithm would enable us to take further steps towards personalized medicine, facilitating more effective optimization of therapeutic strategies in RA.

Conclusion

Analysis of RNA-Seq data resulted to identify several RA-relevant genes in comparison of T6 versus T0, showing that genes expression changes caused by JAK inhibitor treatments potentially lead to the relief of inflammation symptoms. This was further confirmed by GO analyses, which revealed that the identified genes play role in lymphocyte proliferation, inflammatory and JAK/STAT signalling pathways. In the case the responsiveness related genes some of the results of the high throughput sequencing analyses were confirmed by RT-qPCR. The 13 genes which were common at both time points in the comparison of R and NR groups and especially the RT-qPCR validated 4 genes could be potential biomarkers of responsiveness to JAK inhibitor therapy. As in our experimental cohort only 28 patients were examined, therefore further experiments and validation using larger independent patient cohort is needed to confirm our findings and finalize a gene set, which can be used for prediction of responsiveness.

Abbreviations

ACPA	Anti-citrullinated peptide antibody
ADK	Adenosine kinase
AP-1	Activator protein 1
DAS28	Disease activity score of 28 joints
DEG	Differentially expressed gene
DMARD	Disease-modifying antirheumatic drug
ERK	Extracellular signal-regulated kinase
FDA	U.S. Food and Drug Administration
GO	Gene ontology
JAK	Janus kinase
JNK	C-Jun N-terminal kinase
MAP	Mitogen-activated protein
MMP-3	Matrix metalloproteinase 3
NR	Non-responder
PBMC	Peripheral blood mononuclear cell
R	Responder
RA	Rheumatoid arthritis
RT-qPCR	Real-time quantitative polymerase chain reaction
SNP	Single nucleotide polymorphism
STAT	Signal transducer and activator of transcription
T0	Sampling time 0
T6	Sampling time 6
TNF	Tumor necrosis factor
TYK2	Tyrosine kinase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40246-025-00897-5.

Additional file 1: Supplementary Table 1. List of genes of TaqMan qPCR assays. Description of data: Gene lists of TaqMan qPCR assays.

Additional file 2: Supplementary Table 2. DEGs at T6 as opposed to T0. Description of data: List of DEGS (T6 vs. T0).

Additional file 3: Supplementary Table 3. GO result table of downregulated DEGS of T6 vs. T0 comparison. Description of data: GO analysis results of downregulated DEGs (T6 vs. T0).

Additional file 4: Supplementary Table 4. GO result table of upregulated DEGS of T6 vs. T0 comparison. Description of data: GO analysis results of upregulated DEGs (T6 vs. T0).

Additional file 5: Supplementary Table 5. DEGs at T0 in terms of responsiveness. Description of data: List of DEGs at T0 in terms of responsiveness.

Additional file 6: Supplementary Table 6. DEGs at T6 in terms of responsiveness. Description of data: List of DEGs at T6 in terms of responsiveness.

Additional file 7: Supplementary Table 7. Common DEGs at T0 and T6 in terms of responsiveness. Description of data: List of common DEGs at T0 and T6 in terms of responsiveness.

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Author contributions

J.R. processed the samples, performed the experiments and prepared the manuscript., D.Cs. and M.B. organised the sample collection and provided the clinical data of the patients. Sz.Sz., Á.H., N.B., E.V., S.Sz., G.Sz., Zs.P., Zs.Gy. and B.L. organized the patients' recruitment and reviewed the manuscript. Zs. H-Sz. and F.T. processed the samples. K.J. was project administration and reviewed the manuscript. Z.Sz. supervised and reviewed the manuscript. Sz.P. supervised, analysed the data and prepared the manuscript.

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Data availability

Data supporting the study findings are available by the corresponding authors upon reasonable request. Raw sequencing data sets of the RNA-Seq analyses are accessible in the NCBI SRA database under PRJNA1322084 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1322084). Reviewer link: https:/dataview.ncbi.nlm.nih.gov/object/PRJNA1322084?reviewer=qmukfqu5lqlb07htrkdnh6uqgn. https://dataview.ncbi.nlm.nih.gov/object/PRJNA1322084?reviewer=qmukfqu5lqlb07htrkdnh6uqgn

Declarations

Ethics approval and consent to participate
Not applicable.

Institutional review board statement
The study was approved by the Hungarian Scientific Research Council Ethical Committee (approval No. IV/2958- 1 /2022/EKU). Assessments were carried out according to the Declaration of Helsinki and its amendments.

Consent for publication
No data that may be able to identify any single patient are accessible.

Competing interests
J.R., J.K., Z.Sz and Sz.P. are involved in currently applied patent related to the content of the manuscript.

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