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DNA methylation markers in peripheral blood for psoriatic arthritis

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ABSTRACT

Background: The clinical manifestations of psoriatic arthritis (PsA) are highly heterogeneous and no reliable diagnostic biomarkers exist.
Objective: We explored the role of DNA methylation CpG markers in the diagnosis of PsA.
Methods: DNA methylation array was used to screen for differentially methylated sites (DMSs) in the discovery phase (PsA, n = 25; healthy controls [HCs], n = 19; psoriasis vulgaris [PsV], n = 20). In the validation phase, pyrosequencing was used to identify the DMSs in an expanded cohort (PsA, n = 60; HCs, n = 91; PsV, n = 48; rheumatoid arthritis [RA], n = 60). Logistic regression prediction models were established based on the identified DMSs for the diagnosis of PsA.
Results: A total of 17 DMSs differentiating PsA and HCs as well as 11 DMSs differentiating PsA and PsV were screened in the discovery phase. A total of six DMSs (chr14: cg07940072, chr14: 38061320, chr9: cg15734589, chr6: cg12800266, chr3: cg12992827, chr6: cg24500972) differentiating PsA and HCs and two DMSs (chr12: cg16459382, chr2: cg16348668) differentiating PsA and PsV were identified using pyrosequencing. Three logistic regression prediction models were established based on the identified DMSs, which distinguished PsA, RA, PsV, and HCs ($P < 0.001$). The models performed well in differentiating PsA from HCs, RA, and PsV (AUC: 0.858, 0.851, and 0.976, respectively).
Conclusions: The models based on methylated CpG sites are useful for distinguishing patients with PsA from HCs and those with RA or PsV and are a highly sensitive and specific diagnostic biomarker for PsA.

1. Introduction

Psoriatic arthritis (PsA) is an immune-mediated inflammatory skin disease characterized by synovitis, enthesitis, dactylitis, and spondylitis, and is associated with skin and nail lesions [1]. The estimated prevalence of PsA ranges from 0.3 % to 1 % [1,2], and approximately 30 % of psoriasis patients develop PsA [3,4]. Typically, joint symptoms of PsA appear 10 years after the onset of lesions; however, the symptoms can also manifest before or during the onset of lesions [1].

Arthritis is detrimental to the physical and mental health of PsA and is associated with a heavy clinical burden on patients [5,6]. Timely diagnosis and treatment can help prevent long-term damage and disability. However, the diversity of clinical features and nonspecific symptoms of PsA, including skin and nail diseases, mastitis, uveitis, and osteitis, make the diagnosis challenging. To date, there are no reliable clinical diagnostic markers for PsA. Several studies have reported that HLA-B and C molecules and interleukin-13 gene polymorphisms are associated with PsA [7–10]. However, these markers are not currently used to make the diagnosis of PsA. Other potential biomarkers include interleukin-6, CXCL10, and circulating

Abbreviations: PsA, psoriatic arthritis; PsV, psoriasis vulgaris; HCs, healthy controls; RA, rheumatoid arthritis; LASSO, least absolute shrinkage and selector operation; DMSs, differentially methylated sites; PBMCs, peripheral blood mononuclear cells

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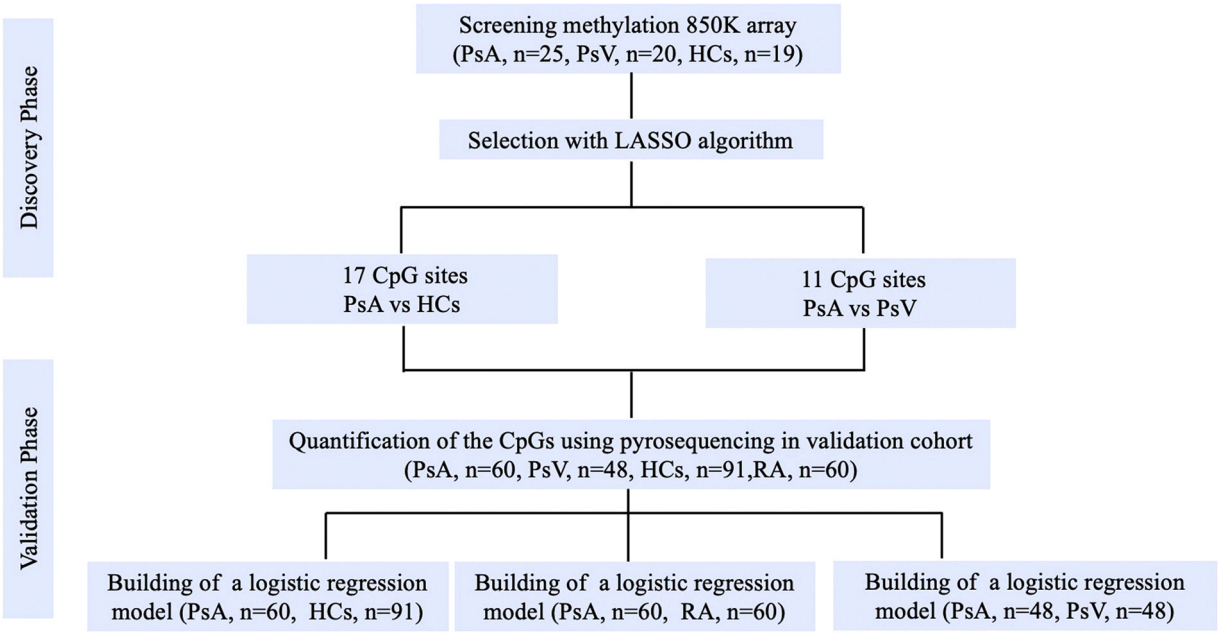

Fig. 1. Workflow chart demonstrating the identification of methylated CpG markers. We used the discovery data set containing 25 psoriatic arthritis (PsA) samples, 20 psoriasis vulgaris (PsV) samples, and 19 healthy controls (HCs) to screen differentially methylated sites (DMSs) using Least Absolute Shrinkage and Selector Operation (LASSO) algorithm. We identified 17 DMSs differentiating PsA patients and HCs, and 11 DMSs differentiating PsA and PsV patients. Then, we identified the DMSs using pyrosequencing and established three logistic regression models based on the identified DMSs to distinguish PsA from HCs and RA and PsV patients. We evaluated the effectiveness of CpG sites-based models as diagnostic markers.

microRNAs in extracellular vesicles, but there is no evidence of their usefulness [11–13].

Importantly, genetics is involved in the pathogenesis of PsA, but the concordance of disease in monozygotic twins is only 35–72 %, suggesting that epigenetic or environmental factors play a crucial role in PsA [14]. DNA methylation is involved in a variety of diseases, including tumor, metabolic, cardiovascular, and inflammatory diseases. Several studies have used DNA methylation sequences as diagnostic or prognostic biomarkers of cancer and autoimmune diseases [15,16]. Our previous research found abnormal DNA methylation in peripheral blood mononuclear cells (PBMCs) and lesions in psoriasis vulgaris (PsV) patients [17,18]. Recent studies have demonstrated that different DNA methylation signatures are found in peripheral blood $CD8^+$ T cells of PsA patients compared to PsV and healthy individuals [19,20], suggesting the involvement of DNA methylation in the pathogenesis of psoriasis and its potential use as diagnostic biomarkers for psoriasis.

This study aimed to identify diagnostic methylation biomarkers for PsA. Accordingly, we performed DNA methylation array in a discovery cohort and identified six CpG sites between PsA and healthy controls (HCs), and two CpG sites between PsA and PsV in a validation cohort. Based on validated CpGs, logistic regression prediction models were established and the application value of the model in the diagnosis for PsA was evaluated by receiver operator characteristic (ROC) curve analysis.

2. Material and methods

2.1. Study design

In the discovery phase, we screened for differentially methylated sites (DMSs) from the peripheral blood among the PsA, PsV, and HC groups using the Illumina 850 K array. In the validation phase, the DMSs were validated in an expanded cohort (PsA, n = 60; PsV, n = 48; rheumatoid arthritis [RA], n = 60; HCs, n = 91) using pyrosequencing. Then, we constructed three logistic regression prediction models for PsA based on validated CpGs. Finally, we evaluated the diagnostic value of the prediction models for PsA by ROC curve analysis (Fig. 1).

2.2. Patient cohorts

Whole blood samples were obtained from PsA, PsV, RA patients, and HCs. PsA was diagnosed based on the CASPAR criteria [21]. RA was diagnosed based on the ACR diagnostic criteria [22]. Peripheral blood collection was approved by the Clinical Research Ethics Committee of the Second Xiangya Hospital, China. Written informed consent was obtained from study participants before sample collection.

2.3. Genome-wide DNA methylation analysis

Genomic DNA was extracted from peripheral blood (Thermo Fisher Scientific, Waltham, MA, USA) and bisulfite converted (Zymo Research, Irvine, CA, USA). Genome-wide DNA methylation was analyzed using the Illumina 850K array. We calculated the mean-difference β-value (Δβ) and *P* value between groups for 746,970 CpG sites. For absolute $\Delta\beta > 0.1$ and $P < 0.05$, we considered the probe to be differentially methylated. The detailed description of data analysis was presented in the online supplementary text. After the first filtering, CpGs were selected using least absolute shrinkage and selector operation (LASSO) algorithm.

The methylation levels of screened CpG sites were measured by pyrosequencing. Bisulfite-modified DNA was amplified using specific primer pairs. Amplification and sequencing primers for CpGs were presented in Tables S1 and S2; PyroMark Gold Q24 reagent was used for pyrosequencing to evaluate DNA methylation levels.

2.4. Cell isolation

PBMCs were separated from whole blood of 10 patients with PsA and PsV, and 10 HCs by density gradient centrifugation. We sorted $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, and $CD14^+$ monocytes by using Miltenyi beads according to the manufacturer's instructions.

2.5. Functional annotation, regulatory enrichment, and pathways

Gene ontology (GO) and KEGG pathway enrichment analyses were performed using the R package cluster profile based on genes enriched by the DMSs between groups [23]; We evaluated whether the associated CpG sites were enriched in cell type-specific regulatory elements using the eFORGE v2.0 online tool [24], including five histone mark categories and 15 Chromatin Hidden Markov Models (ChromHMMs) chromatin states.

2.6. Statistical analysis

We performed a one-way ANOVA or unpaired *t*-test to analyze DNA methylation levels between groups. The clinical diagnosis of PsA, HCs, RA, and PsV was used as the dependent variable (Y), and DNA methylation characteristics were used as the independent variable (X) to establish the logistic regression model. We used ROC curve analysis to calculate the area under curve (AUC) for the efficiency of diagnosis of PsA. A two-sided $P < 0.05$ was considered significant.

3. Results

3.1. Study cohorts

In the discovery cohort, we screened for DMSs from PsA patients (n = 25), PsV patients (n = 20), and HCs (n = 19). In the validation phase, the DMSs were verified in a larger cohort (PsA, n = 60; PsV, n = 48; RA, n = 60; HCs, n = 91).Detailed descriptions of the clinical characteristics of participants were summarized in Table S3.

3.2. Genome-wide DNA methylation analysis

In the discovery phase, we performed a genome-wide DNA methylation study in peripheral blood from PsA and PsV patients, and 19 HCs using the Illumina 850K array. Principal component analysis (PCA) revealed a clear cluster separation between PsA and HCs and between PsA and PsV, indicating distinct methylation patterns associated with disease status (Fig. 2a). There were 571 DMSs between PsA patients and HCs (Fig. 2b, Table S4) (Absolute $\Delta\beta > 0.1$; $P < 0.05$), and 135 DMSs between PsA and PsV patients (Fig. 2c, Table S5) (Absolute $\Delta\beta > 0.1$; adjusted $P < 0.05$).

Next, we performed GO-term enrichment analysis, which revealed distinct functional enrichment of DMSs ($P < 0.05$; Fig. 2d–g). Within the GO biological processes for the genes enriched in significant DMSs between PsA and HCs, cellular response to interferon–γ and interferon–γ–mediated signaling pathways were the most related functions (Fig. 2d). KEGG analysis showed that the genes with DMSs between PsA and HCs were enriched in Th1, Th2, and Th17 cell differentiation, inflammatory bowel disease, and type I diabetes mellitus pathways (Fig. 2e). Within the biological processes for the genes with significant DMSs between PsA and PsV, phosphatidylglycerol metabolism, CDP–diacylglycerol metabolism, cardiolipin metabolism, and CDP–diacylglycerol biosynthesis were the most related functions (Fig. 2f). The associated genes were enriched in glycerophospholipid and glycerolipid metabolism pathways (Fig. 2g).

The regulatory enrichment analysis showed that the PsA/HCs -associated CpG sites were enriched in H3K4me1, H3K4me3, and H3K36Me3 specific histone modifications in blood cells, including monocytes, lymphocytes, and hematopoietic stem cells (Fig. S1a–c, $P < 10^{-5}$). To predict disease-relevant cell types, we investigated the ChromHMM state, which suggested that enhancers and weak gene transcription were associated with DMSs mainly in the blood and mesenchymal cells, and adipose, lung, digestive, thymus cells (Fig. S2, $P < 10^{-5}$). No cell-specific enrichment was observed for PsA/PsV -associated CpG sites.

Next, we performed the LASSO algorithm to screen the most differential CpGs for differentiating between PsA, HCs, and PsV groups. We selected 17 CpGs differentiating PsA and HCs (Table S6, Fig. 3a), and 11 CpGs differentiating PsA and PsV as final markers (Table S7, Fig. 3c). The heatmap (Fig. 3a and c) showed the procedure for the identification of the methylation sites. The methylation levels of the 17 CpGs in PsA were markedly lower than those in HCs (Fig. 3b); in addition, 11 CpGs showed greater methylation levels in PsA compared to PsV samples (Fig. 3d).

3.3. Identification of differentially methylated CpG sites

We analyzed methylation levels of the screened 17 and 11 CpGs on an expanded cohort of samples using pyrosequencing. As a result, six DMSs (chr14:cg07940072, chr14: 38061320, chr9:cg15734589, chr6:cg12800266, chr3:cg12992827, and chr6:cg24500972) between PsA and HCs, and two DMSs (chr12: cg16459382 and chr2: cg16348668) between PsA and PsV were identified. The methylation levels of all six CpGs were lower in blood from PsA patients than HCs ($P < 0.01$, Fig. 4a–f, Table S8). Compared to RA samples, the methylation levels of cg12800266, cg12992827, and cg24500972 were higher in PsA samples ($P < 0.05$, Fig. 4d–f, Table S8). In addition, methylation levels of the two validated DMSs were significantly higher in PsA compared to PsV samples ($P < 0.001$, Fig. 4g–h, Table S9). We also checked the methylation status of neighboring CpGs located within less than 300 bp of the identified CpG sites in the 850 K array data and found that only cg12800266 had two neighboring CpGs within 300 bp among the identified CpGs sites that could distinguish PsA and HCs, and both were significantly hypomethylated ($P < 0.05$, Table S10), which were consistent with the methylation status of cg12800266. And there were three neighboring CpGs of the two identified PsA/PsV-associated CpGs, one of which was significantly hypermethylated, like cg16459382 ($P < 0.05$, Table S10).

3.4. Construction of the logistic regression prediction models

The markers identified by pyrosequencing were used to classify and predict PsA, PsV, RA, and HC samples. We constructed three logistic regression prediction models based on the identified CpG sites to distinguish between PsA and HCs, PsA and RA, and PsA and PsV. The models selected the CpG site of the six CpGs (chr14: 38061326, chr14: 38061320, chr9:cg15734589, chr6:cg12800266, chr3:cg12992827, and chr6:cg24500972) as independent variables (X) to establish the logistic regression model to classify PsA, HCs, and RA samples, and each CpG site of the two CpGs (chr12:cg16459382 and chr2: cg16348668) as independent variables (X) to establish the logistic regression model to classify PsA and PsV samples.

3.5. ROC curve analysis of the logistic regression prediction models

We used ROC curves to estimate the probability of the models to predict PsA. The logistic regression model showed an AUC of 0.858 ± 0.032 ($P < 0.001$) for distinguishing PsA patients from HCs, with a sensitivity of 76.7 % and specificity of 84.6 % (Fig. 5a). In comparison, the logistic regression model showed an AUC of 0.851 ± 0.035 ($P < 0.001$) for distinguishing PsA and RA patients, with a sensitivity of 70% and specificity of 88.3 % (Fig. 5b). The ROC curve analysis for distinguishing between PsA and PsV patients showed that the methylation levels of cg16459382 and cg16348668 had great AUC values of 0.916 ± 0.027 and 0.945 ± 0.023, respectively. The sensitivity and specificity of cg16459382 and cg16348668 methylation levels for PsA were 100 % and 70.8 %, and 87.5 % and 91.5 %, respectively (Fig. 5c). In addition, the logistic regression

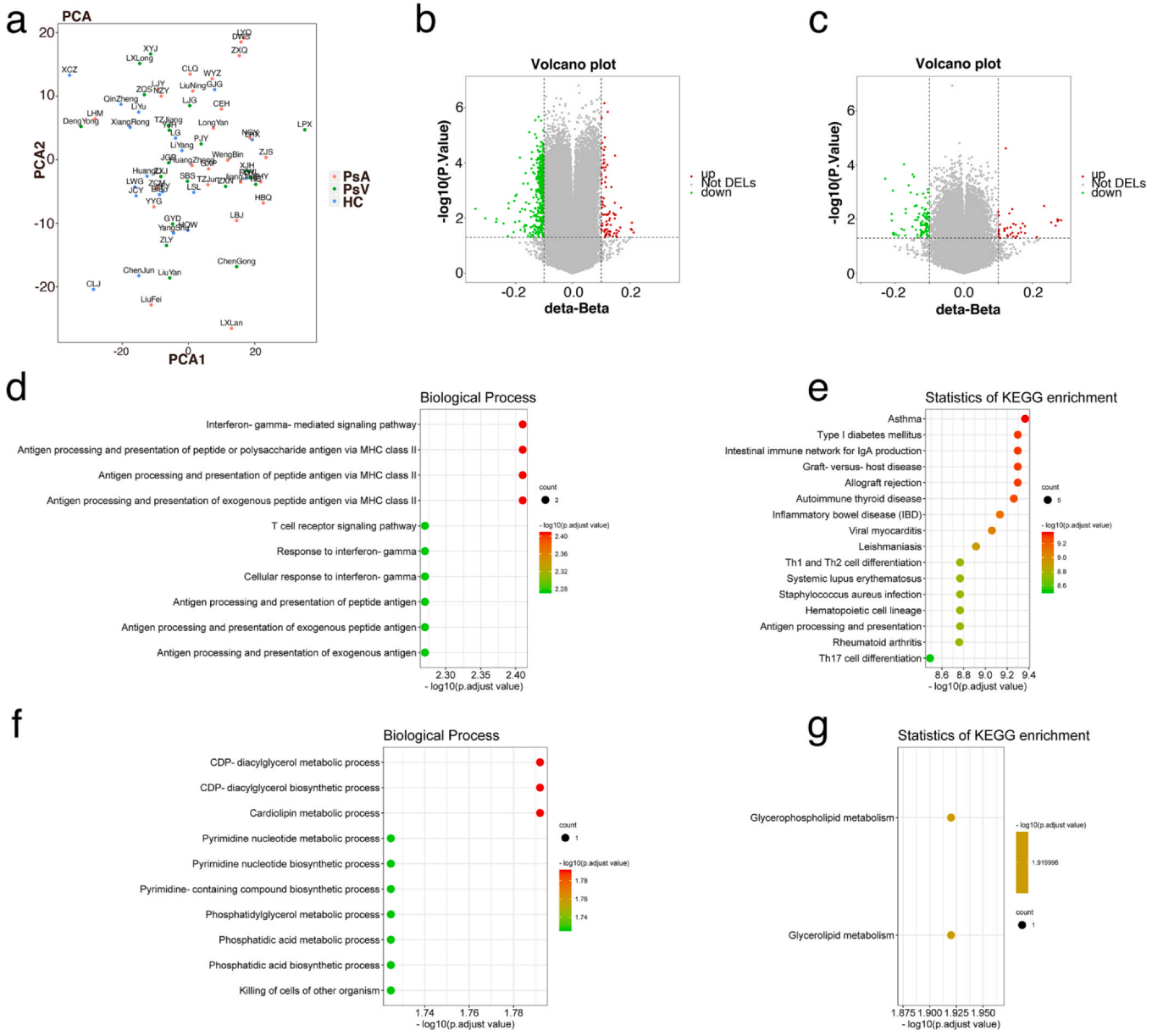

Fig. 2. Differentially methylated CpG sites in patients with psoriatic arthritis (PsA) compared to healthy controls (HCs) and biological processes of differentially methylated genes. PCA showed a clear cluster separation between PsA patients, HCs, and psoriasis vulgaris (PsV) patients (a). (b and c) Volcano plot showing the mean methylation differences between PsA patients and HCs (x-axis) versus log-transformed *P* values (y-axis); between PsA and PsV patients (x-axis) versus log-transformed *P* values (y-axis). (b) 571 CpG sites with an FDR < 0.05 were considered significantly differentially methylated between PsA patients and HCs. (c) 135 CpG sites with an FDR < 0.05 were considered significantly differentially methylated between PsA and PsV patients. Red dots correspond to significantly hypermethylated CpG sites and green dots correspond to significantly hypomethylated CpG sites in the peripheral blood of PsA patients compared to PsV patients and HCs ($P < 0.05$). (d and f) Functional enrichment analysis was performed on the genes associated with significant DMSs between PsA patients and HCs. GO biological processes and KEGG pathways are shown. Significance is indicated as − log10 *P* value. (e and g) Functional enrichment analysis was performed on the genes associated with significant DMSs between PsA and PsV patients. GO biological processes and KEGG pathways are shown. Significance is indicated as − log10 *P* value.

model showed an AUC of 0.976 ± 0.013 ($P < 0.001$) for distinguishing between PsA and PsV patients, with a sensitivity of 95.8 % and specificity of 89.4 % (Fig. 5d).

3.6. DNA methylation of validated CpG sites in peripheral blood cells

$CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, and $CD14^+$ monocytes were positive beads sorted from PBMCs and we observed that the methylation profile of the six CpG sites that could distinguish PsA and HCs in the four types of cells was not completely the same as those in whole blood (Fig. 6a–f), For example, cg15734589, cg12992827, and cg24500972 in $CD8^+$ T cells (Fig. 6c, e, f) and cg12800266 and cg12992827 in $CD19^+$ B cells (Fig. 6d and e) were hypermethylated in PsA compared to HCs. CpG site cg16459382 was significantly hypermethylated in the four types of cells in PsA than that in PsV, which was consistent with the results in whole blood (Fig. 6g, $P < 0.05$). CpG site cg16348668 was hypermethylated only in the $CD19^+$ B cells ($P < 0.05$) and was no difference in the other three types of cells in PsA compared to PsV (Fig. 6h).

4. Discussion

In this study, we used a methylation microarray to identify DMSs in whole blood and used pyrosequencing to identify crucial CpGs. Subsequently, we constructed logistic regression models to predict PsA. We showed that the DMSs in peripheral blood can distinguish

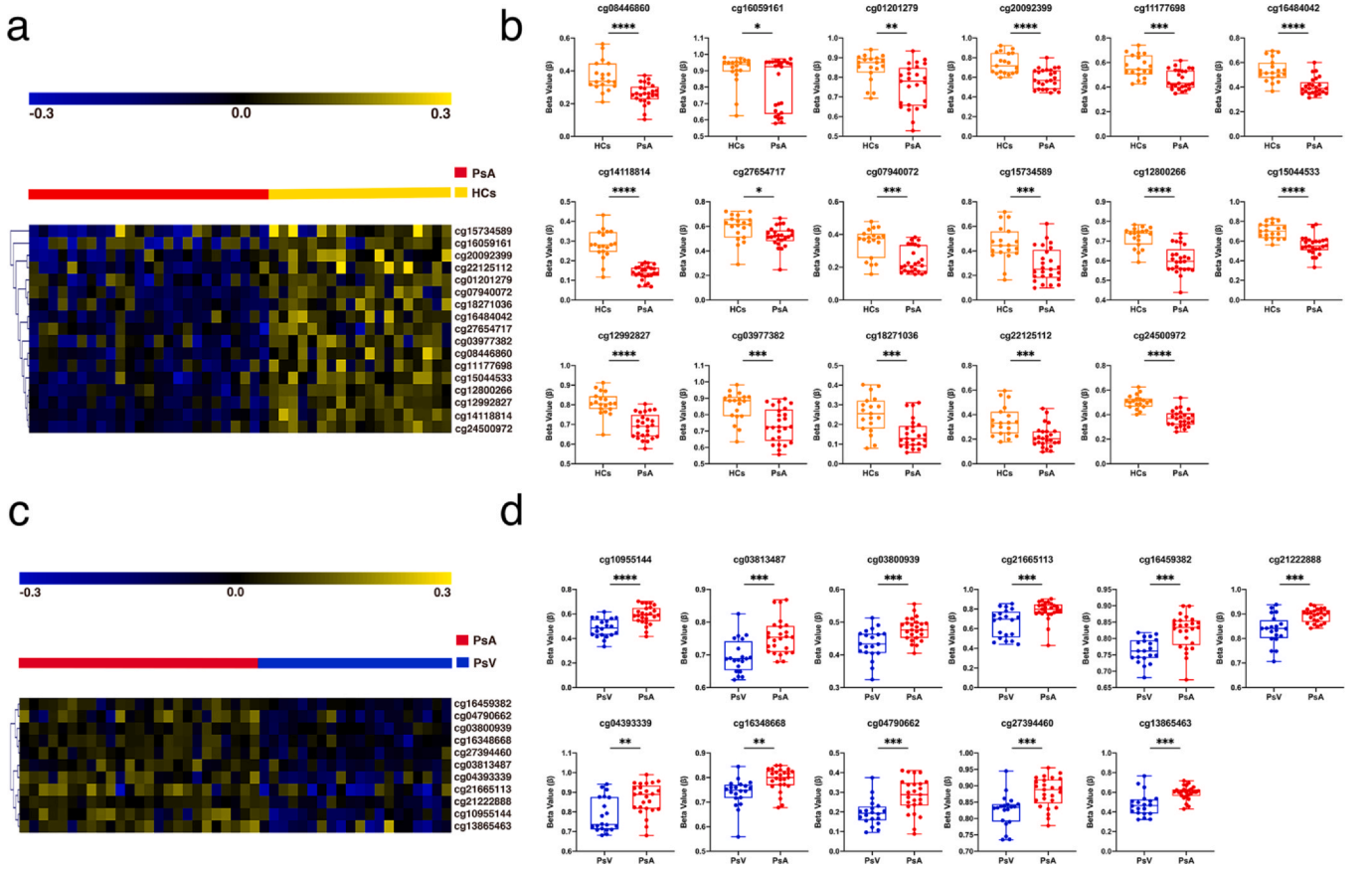

Fig. 3. Heatmap and Tukey boxplots of the differentially methylated sites identified by the least absolute shrinkage and selector operation algorithm in the discovery cohort. There were 17 hypomethylated sites between psoriatic arthritis (PsA) and healthy control (HC) groups (a and b), and 11 hypermethylated sites between PsA and psoriasis vulgaris (PsV) groups (c and d). Red represents PsA samples, blue represents PsV samples, and yellow represents HC samples. Statistical significance for all analyzes were ns, no significance; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

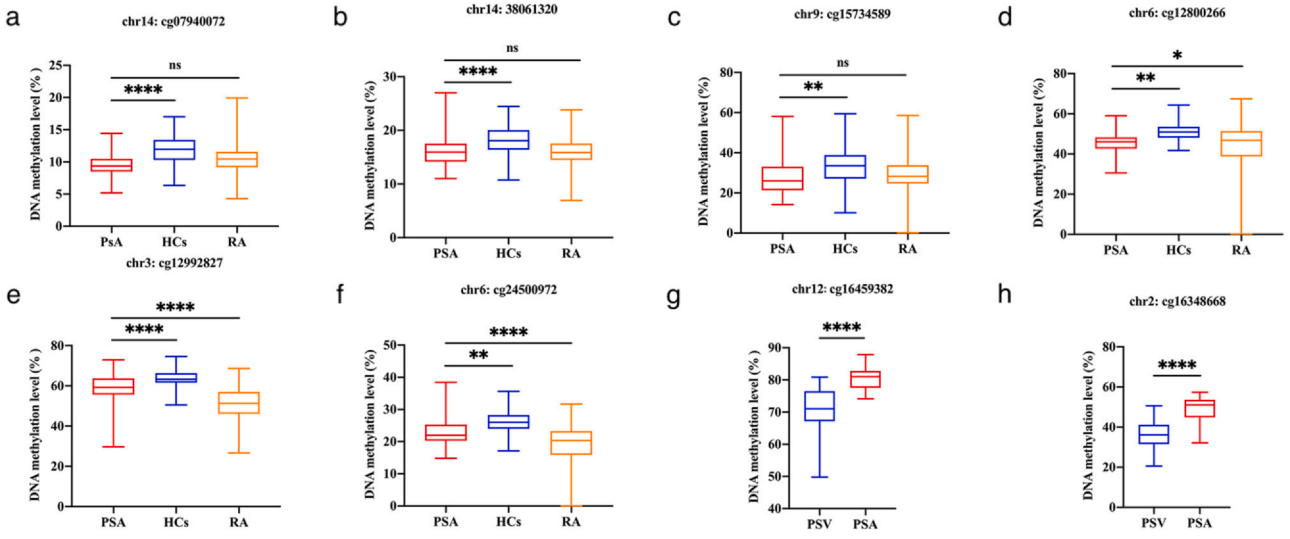

Fig. 4. DNA methylation levels of the six identified differentially methylated sites (DMSs) between psoriatic arthritis (PsA) and healthy controls (HCs), and two DMSs between PsA and psoriasis vulgaris (PsV). (a–f) show six CpGs that were significantly hypomethylated in the peripheral blood of patients with PsA compared to HCs ($P < 0.01$ for all comparisons). (d–f) show three CpGs that were significantly hypermethylated in the peripheral blood of PsA patients compared to patients with rheumatoid arthritis (RA) ($P < 0.05$ for all comparisons). (g-h) show two CpG sites that were significantly hypermethylated in the peripheral blood of PsA patients compared to PsV patients ($P < 0.0001$ for all comparisons). Statistical significance for all analyzes were ns, no significance; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{****}P < 0.0001$.

PsA patients from HCs and PsV and RA patients, suggesting that DNA methylation is a potential diagnostic and predictive biomarker for PsA.

The clinical manifestations of psoriasis precede arthritis by an average of 10 years in most cases and approximately 70 % of patients have no arthritis symptoms at the early stage. Furthermore, joint

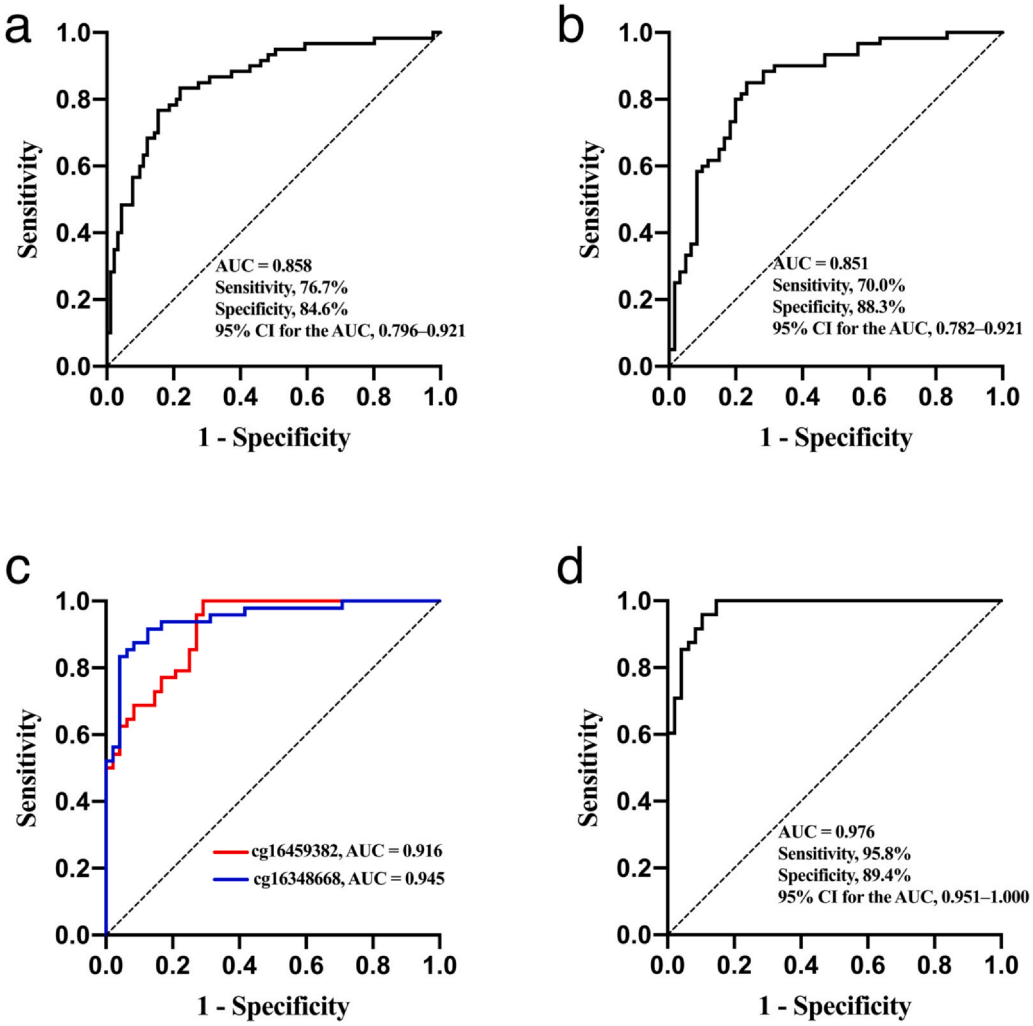

Fig. 5. Receiver operating characteristic (ROC) curves, sensitivities, and specificities of the logistic regression prediction models in the validation cohort. (a–b) show the ROC curves of the logistic regression prediction models based on the six differentially methylated sites (DMSs) in patients with psoriatic arthritis (PsA) compared to healthy controls (HCs) (a) and patients with rheumatoid arthritis (RA) (b). (c–d) show the ROC curves of the DMSs (c) and the logistic regression prediction model (d) based on the two DMSs in patients with PsA compared to those with psoriasis vulgaris (PsV).

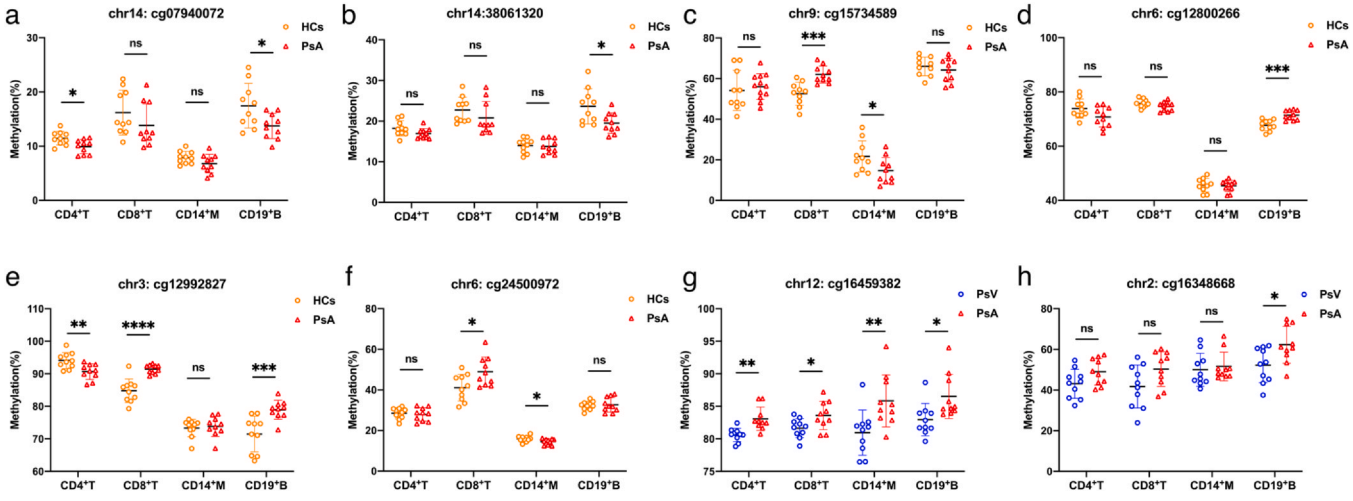

Fig. 6. The comparison of DNA methylation levels for validated CpGs in $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, and $CD14^+$ monocytes from the peripheral blood of 10 patients with psoriatic arthritis, psoriasis vulgaris, and 10 healthy controls. The result showed that the methylation profile of the six CpGs that could distinguish PsA and HCs in the four types of cells was not completely the same as those in whole blood (a–e). One of PsA/PsV-associated CpGs, cg16459382 was significantly hypermethylated in the four types of immune cells in PsA compared to PsV (g, $P < 0.05$). Another PsA/PsV-associated CpG site, cg16348668 was significantly hypermethylated only in the $CD19^+$ B cells (h, $P < 0.05$) and was no difference in the other three types of cells in PsA compared to PsV (h). Statistical significance for all analyzes were ns, no significance; $^*P < 0.05$; $^{**}P < 0.01$.

destruction is irreversible in PsA and there are frequent relapses and joint stiffness, leading to disability. Therefore, early diagnosis of PsA can allow early measures to prevent joint damage.

In previous studies, HLA-B gene [25], HLA-C*06, HLA-B*27 [9], serum biomarkers (including high-sensitivity CRP, osteoprotegerin, CXCL10, interleukin-6, and M2BP) [26–30], and circulating extracellular vesicles (let-7b-5p and miR-30e-5p) [11] were considered potential diagnostic biomarkers for PsA. However, no useful diagnostic biomarkers for PsA are available due to the frequently nonspecific symptoms.

DNA methylation is involved in cancer, RA, systemic lupus erythematosus (SLE), diabetes, and coronary heart disease [31–36]. Several studies have reported DNA methylation in psoriasis. A previous study described significant DMSs in psoriatic lesions compared to uninvolved psoriatic and normal skin using Illumina 27K Beadchip [37]. Other studies [38–40] also used Illumina 450K Beadchip to screen for aberrant DNA methylation in psoriatic lesions and HCs and found skin-specific CpGs that play crucial roles in the pathogenesis of psoriasis. These studies mainly focused on abnormal methylation of psoriasis lesions. In this study, we evaluated the DNA methylation patterns in peripheral blood. A recent study has identified that multiple CpGs in whole blood are differentially methylated in PsV and PsA patients compared to HCs [20], which is consistent with the results of our study. Another study showed that different DNA methylation patterns in peripheral blood $CD8^+$ T cells of PsA patients compared to HCs, which allows the differentiation of PsA from PsV and HCs and reflects the activity of psoriasis[19]. Our study showed different DNA methylation characteristics in peripheral blood of PsA compared to PsV and HCs. However, no correlation was found between DNA methylation and disease activity. A recent genome-wide meta-analysis revealed the genetic differences between PsA and PsV[41]. Genetic mutations may influence DNA methylation modifications, and there is a close relationship between genetic mutations and DNA methylation modifications, which can be further studied in the future.

The key to early diagnosis of PsA based on the methylation patterns is the identification of critical CpGs. Thus, we integrated all critical CpGs identified by pyrosequencing into a single logistic regression model, which improved the diagnostic accuracy of the model over individual CpGs. Our findings showed that the model based on the six CpGs had a sensitivity and specificity to distinguish PsA from HCs and RA of 76.7 % and 84.6 %, and 70 % and 88.3 %, respectively. The logistic regression model based on the two CpGs differentiated between PsA and PsV with a sensitivity of 95.8 % and specificity of 89.4 %, which had better sensitivity and specificity than the previously reported circulating microRNA biomarkers in extracellular vesicles[11]. This logistic regression model is helpful in the differential diagnosis of PsA and PsV and would allow early diagnosis of PsA before joint damage. Timely diagnosis and treatment reduce joint damage, deformities, and comorbidities, thereby leading to improved overall outcomes. Therefore, DNA methylation analysis holds promise as a diagnostic and prognostic tool for the individualized treatment of psoriasis patients.

Peripheral blood contains various kinds of blood cells, some of which may affect the DNA methylation level of whole blood. We refer to some reasons for the DNA methylation change in whole blood cells, though only some cells differ between PsA and HCs. First, it is possible that other cells have no change in number but have changed in function. The DNA methylation we detect in gDNA extracted from whole blood reflects the overall status and function changes of peripheral blood cells. Second, changes in the number of the small proportion of blood cells may affect overall DNA methylation changes of peripheral blood cells. Third, there are some examples of the identification of diagnostic markers of DNA methylation from peripheral blood. For example, previous studies have identified DNA methylation profiles in SLE peripheral blood cells, validated significantly CpGs, and developed the IFI44L methylation diagnostic marker for SLE [15]. In addition, we investigated the DNA methylation changes of four major immune cells in peripheral blood. The validated DMSs from whole blood which reflect the overall status and function changes of blood cells may not be completely consistent with the methylation changes of a particular cell subclass in the blood. The DNA methylation changes in $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, and $CD14^+$ monocytes indicate that psoriatic status affects a wide range of epigenetic processes in immune cells. Furthermore, psoriatic lesions also contain various immune cells, we can investigate DNA methylation changes of the identified CpGs in the immune cells in psoriatic lesions in future studies.

GO analysis revealed the enrichment of the DMSs between PsA and HCs affected distinct pathways, including type I diabetes mellitus, inflammatory bowel disease, viral myocarditis, Th17, Th1, and Th2 cell differentiation, among which Th17 cell has been proven to be one of the key immune cells in the pathogenesis of PsA. Th17 cells are increased in the synovial fluid and circulation of PsA patients [42]. Previous studies have demonstrated that PsA is associated with obesity, diabetes mellitus, hypertension, metabolic syndrome, fatty liver, uveitis, cardiovascular events, and inflammatory bowel disease [4,43,44]. Multiple enriched genes with DMSs are previously reported as being associated with PsA pathogenesis. For example, *HLA*, the gene in which cg27083089 is located, is reported to be the susceptibility gene for PsA and specific HLA susceptibility genes might define sub-phenotypes of PsA. And cg22697477 locates in *RUNX1*, which is the transcription factor that promotes Th 17 cell differentiation and is involved in the IL-23/IL-17 signaling pathway [45]. These results suggest that DNA methylation may play a role in the development of PsA.

GO analysis revealed the enrichment of the DMSs between PsA and PsV affected glycerophospholipid and glycerolipid metabolism pathways. Previous studies have confirmed that dyslipidemia, obesity, and metabolic syndrome are more common in PsA compared to PsV [46,47]. These lipid abnormalities are related to disease activity in psoriasis [46]. The increase in psoriatic lesions and synovial-entheseal complexes promotes insulin resistance and increase the release of TNF-α [48], indicating a relationship between lipid metabolism disorders and skin and joint damage. Studies have reported abnormal fatty acid composition in PsA and PsV patients compared to HCs, while PsA patients had a significantly higher proportion of saturated fatty acids than PsV patients [47]. This suggests a close relationship between PsA and metabolic comorbidities.

The regulatory annotations showed enrichment for H3K4me1, H3K4me3, and H3K36Me3 histone marks overlapping the significant DMSs, which are associated with enhancer, promoter, and transcribed regions, respectively. Further, the significant DMSs were found to be related to the enhancers and weak transcription of chromatin state in blood cells. These results reveal the association between cell-type specific chromatin states and differential DNA methylation in PsA, suggesting interactions between epigenetic processes regulating gene expression.

Although our study demonstrated that DNA methylation can be used as a diagnostic and predictive biomarker, the limitations of this study should be noted. First, the Illumina 850K array mainly reflects DNA methylation of CpG sites near the gene promoter and CpG island regions, accounting for a small fraction of the known 28 million CpGs of the human epigenome. This limitation is somewhat remedied by whole genome bisulfite sequencing, as it covers about 90 % of all the genomic sites, which can better reflect genomic DNA methylation. Second, this was not a prospective study, the changes in methylation patterns in patients who progressed from initial PsV to PsA are unclear. Thus, the specific role of the candidate biomarkers in PsA needs to be further evaluated in prospective studies.

Furthermore, the biological mechanisms underlying the candidate markers are still unknown. Finally, the sample size of our study was relatively small, and prospective, multicenter studies are needed to further validate the diagnostic ability of the CpG-based models.

In conclusion, we built logistic regression models based on CpG sites to distinguish PsA patients from PsV and RA patients, and healthy individuals; accordingly, we demonstrated that DNA methylation was involved in the pathogenesis of PsA and may be a good biomarker for its diagnosis.

CRediT authorship contribution statement

Qianjin Lu: Conceptualization, Methodology, Resources, Writing – review & editing. **Ming Zhao:** Conceptualization, Methodology, Resources, Writing – review & editing. **Min Deng:** Data curation, Investigation, Writing – original draft. **Yuwen Su:** Visualization, Supervision, Resources. **Ruifang Wu:** Software, Validation. **Siying Li:** Software, Validation. **Yanshan Zhu:** Investigation, Data curation. **Guishao Tang:** Investigation, Data curation. **Tian Zhou:** Investigation. **Xiaoli Shi:** Investigation.

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Data Availability statement

The DNA methylation data presented in the study are deposited in the GEO repository, accession no.: GSE200376.

Conflict of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdermsci.2022.11.001.

References

[1] D.D. Gladman, C. Antoni, P. Mease, D.O. Clegg, P. Nash, Psoriatic arthritis: epidemiology, clinical features, course, and outcome, Ann. Rheum. Dis. 64 (2005) 14–17.
[2] P. Sewerin, R. Brinks, M. Schneider, I. Haase, S. Vordenbaeumen, Prevalence and incidence of psoriasis and psoriatic arthritis, Ann. Rheum. Dis. 78 (2) (2019) 286–287.
[3] H. Zachariae, Prevalence of joint disease in patients with psoriasis – implications for therapy, Am. J. Clin. Dermatol. 4 (7) (2003) 441–447.
[4] C.T. Ritchlin, R.A. Colbert, D.D. Gladman, Psoriatic, Arthritis, N. Engl. J. Med. 376 (10) (2017) 957–970.
[5] S.R. Rapp, S.R. Feldman, M.L. Exum, A.B. Fleischer, D.M. Reboussin, Psoriasis causes as much disability as other major medical diseases, J. Am. Acad. Dermatol. 41 (3) (1999) 401–407.
[6] E. McDonough, R. Ayearst, L. Eder, V. Chandran, C.F. Rosen, A. Thavaneswaran, et al., Depression and anxiety in psoriatic disease: prevalence and associated factors, J. Rheumatol. 41 (5) (2014) 887–896.
[7] L. Eder, V. Chandran, F. Pellett, S. Shanmugarajah, C.F. Rosen, S.B. Bull, et al., Differential human leucocyte allele association between psoriasis and psoriatic arthritis: a family-based association study, Ann. Rheum. Dis. 71 (8) (2012) 1361–1365.
[8] V. Chandran, S.B. Bull, F.J. Pellett, R. Ayearst, P. Rahman, D.D. Gladman, Human leukocyte antigen alleles and susceptibility to psoriatic arthritis, Hum. Immunol. 74 (10) (2013) 1333–1338.
[9] R. Winchester, G. Minevich, V. Steshenko, B. Kirby, D. Kane, D.A. Greenberg, et al., HLA associations reveal genetic heterogeneity in psoriatic arthritis and in the psoriasis phenotype, Arthritis Rheumatol. 64 (4) (2012) 1134–1144.
[10] L. Eder, V. Chandran, F. Pellett, R. Pollock, S. Shanmugarajah, C.F. Rosen, et al., IL13 gene polymorphism is a marker for psoriatic arthritis among psoriasis patients, Ann. Rheum. Dis. 70 (9) (2011) 1594–1598.
[11] L. Pasquali, A. Svedbom, A. Srivastava, E. Rosen, U. Lindqvist, M. Stahle, et al., Circulating microRNAs in extracellular vesicles as potential biomarkers for psoriatic arthritis in patients with psoriasis, J. Eur. Acad. Dermatol. Venereol. 34 (6) (2020) 1248–1256.
[12] V. Chandran, R.J. Cook, J. Edwin, H. Shen, F.J. Pellett, S. Shanmugarajah, et al., Soluble biomarkers differentiate patients with psoriatic arthritis from those with psoriasis without arthritis, Rheumatology 49 (7) (2010) 1399–1405.
[13] F. Abji, R.A. Pollock, K. Liang, V. Chandran, D.D. Gladman, CXCL10 is a possible biomarker for the development of psoriatic arthritis among patients with psoriasis, Arthritis Rheumatol. 68 (12) (2016) 2911–2916.
[14] O.B. Pedersen, A.J. Svendsen, L. Ejstrup, A. Skytthe, P. Junker, On the heritability of psoriatic arthritis. Disease concordance among monozygotic and dizygotic twins, Ann. Rheum. Dis. 67 (10) (2008) 1417–1421.
[15] M. Zhao, Y. Zhou, B. Zhu, M. Wan, T. Jiang, Q. Tan, et al., IFI44L promoter methylation as a blood biomarker for systemic lupus erythematosus, Ann. Rheum. Dis. 75 (11) (2016) 1998–2006.
[16] J. Qiu, B. Peng, Y. Tang, Y. Qian, P. Guo, M. Li, et al., CpG methylation signature predicts recurrence in early-stage hepatocellular carcinoma: results from a multicenter study, J. Clin. Oncol. 35 (7) (2017) 734 (+).
[17] P. Zhang, Y. Su, H. Chen, M. Zhao, Q. Lu, Abnormal DNA methylation in skin lesions and PBMCs of patients with psoriasis vulgaris, J. Dermatol. Sci. 60 (1) (2010) 40–42.
[18] P. Zhang, M. Zhao, G.P. Liang, G.L. Yin, D. Huang, F.X. Su, et al., Whole-genome DNA methylation in skin lesions from patients with psoriasis vulgaris, J. Autoimmun. 41 (2013) 17–24.
[19] A. Charras, J. Garau, S.R. Hofmann, E. Carlsson, C. Cereda, S. Russ, et al., DNA methylation patterns in CD8 T cells discern psoriasis from psoriatic arthritis and correlate with cutaneous disease activity, Front. Cell Dev. Biol. 9 (2021) 746145.
[20] M. Vecellio, E.M. Paraboschi, A. Ceribelli, N. Isailovic, F. Motta, G. Cardamone, et al., DNA methylation signature in monozygotic twins discordant for psoriatic disease, Front. Cell Dev. Biol. 9 (2021) 778677.
[21] W. Taylor, D. Gladman, P. Helliwell, A. Marchesoni, P. Mease, H. Mielants, et al., Classification criteria for psoriatic arthritis – development of new criteria from a large international study, Arthritis Rheumatol. 54 (8) (2006) 2665–2673.
[22] F.C. Arnett, S.M. Edworthy, D.A. Bloch, D.J. McShane, J.F. Fries, N.S. Cooper, et al., The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis, Arthritis Rheumatol. 31 (3) (1988) 315–324.
[23] G. Yu, L.-G. Wang, Y. Han, Q.-Y. He, clusterProfiler: an R package for comparing biological themes among gene clusters, OMICS 16 (5) (2012) 284–287.
[24] C.E. Breeze, D.S. Paul, J. van Dongen, L.M. Butcher, J.C. Ambrose, J.E. Barrett, et al., eFORGE: a tool for identifying cell type-specific signal in epigenomic data, Cell Rep. 17 (8) (2016) 2137–2150.
[25] Y. Okada, B. Han, L.C. Tsoi, P.E. Stuart, E. Ellinghaus, T. Tejasvi, et al., Fine mapping major histocompatibility complex associations in psoriasis and its clinical subtypes, Am. J. Hum. Genet. 95 (2) (2014) 162–172.
[26] V. Chandran, R.J. Cook, J. Edwin, H. Shen, F.J. Pellett, S. Shanmugarajah, et al., Soluble biomarkers differentiate patients with psoriatic arthritis from those with psoriasis without arthritis, Rheumatology 49 (7) (2010) 1399–1405.
[27] B. Strober, C. Teller, P. Yamauchi, J.L. Miller, M. Hooper, Y.C. Yang, et al., Effects of etanercept on C-reactive protein levels in psoriasis and psoriatic arthritis, Br. J. Dermatol. 159 (2) (2008) 322–330.
[28] G.M. Alenius, C. Eriksson, S. Rantapää Dahlqvist, Interleukin-6 and soluble interleukin-2 receptor alpha-markers of inflammation in patients with psoriatic arthritis? Clin. Exp. Rheumatol. 27 (1) (2009) 120–123.
[29] F. Abji, R.A. Pollock, K. Liang, V. Chandran, D.D. Gladman, Brief report: CXCL10 is a possible biomarker for the development of psoriatic arthritis among patients with psoriasis, Arthritis Rheumatol. 68 (12) (2016) 2911–2916.
[30] D. Cretu, L. Gao, K. Liang, A. Soosaipillai, E.P. Diamandis, V. Chandran, Differentiating psoriatic arthritis from psoriasis without psoriatic arthritis using novel serum biomarkers, Arthritis Care Res. 70 (3) (2018) 454–461.
[31] K.D. Robertson, DNA methylation and human disease, Nat. Rev. Genet. 6 (8) (2005) 597–610.
[32] M. Klutstein, D. Nejman, R. Greenfield, H. Cedar, DNA methylation in cancer and aging, Cancer Res. 76 (12) (2016) 3446–3450.
[33] G. Agha, M.M. Mendelson, C.K. Ward-Caviness, R. Joehanes, T. Huan, R. Gondalia, et al., Blood leukocyte DNA methylation predicts risk of future myocardial infarction and coronary heart disease a longitudinal study of 11 461 participants from population-based cohorts, Circulation 140 (8) (2019) 645–657.
[34] A. Bansal, S.E. Pinney, DNA methylation and its role in the pathogenesis of diabetes, Pediatr. Diabetes 18 (3) (2017) 167–177.
[35] A. Cribbs, M. Feldmann, U. Oppermann, Towards an understanding of the role of DNA methylation in rheumatoid arthritis: therapeutic and diagnostic implications, Ther. Adv. Musculoskelet. Dis. 7 (5) (2015) 206–219.
[36] C.M. Hedrich, K. Maebert, T. Rauen, G.C. Tsokos, DNA methylation in systemic lupus erythematosus, Epigenomics 9 (4) (2017) 505–525.

[37] E.D.O. Roberson, Y. Liu, C. Ryan, C.E. Joyce, S. Duan, L. Cao, et al., A subset of methylated CpG sites differentiate psoriatic from normal skin, J. Invest. Dermatol. 132 (3) (2012) 583–592.
[38] F.S. Zhou, W.J. Wang, C.B. Shen, H. Li, X.B. Zuo, X.D. Zheng, et al., Epigenome-wide association analysis identified nine skin DNA methylation loci for psoriasis, J. Invest. Dermatol. 136 (4) (2016) 779–787.
[39] X. Gu, E. Nylander, P.J. Coates, R. Fahraeus, K. Nylander, Correlation between reversal of DNA methylation and clinical symptoms in psoriatic epidermis following narrow-band UVB phototherapy, J. Invest. Dermatol. 135 (8) (2015) 2077–2083.
[40] D. Verma, A.K. Ekman, C.B. Eding, C. Enerback, Genome-wide DNA methylation profiling identifies differential methylation in uninvolved psoriatic epidermis, J. Invest. Dermatol. 138 (5) (2018) 1088–1093.
[41] M. Soomro, M. Stadler, N. Dand, J. Bluett, D. Jadon, F. Jalali-Najafabadi, et al., Comparative genetic analysis of psoriatic arthritis and psoriasis for the discovery of genetic risk factors and risk prediction modeling, Arthritis Rheumatol. 74 (9) (2022) 1535–1543.
[42] B. Menon, N.J. Gullick, G.J. Walter, M. Rajasekhar, T. Garrood, H.G. Evans, et al., Interleukin-17+CD8+ T cells are enriched in the joints of patients with psoriatic arthritis and correlate with disease activity and joint damage progression, Arthritis Rheumatol. 66 (5) (2014) 1272–1281.
[43] A. Ogdie, S. Schwartzman, M.E. Husni, Recognizing and managing comorbidities in psoriatic arthritis, Curr. Opin. Rheuma 27 (2) (2015) 118–126.
[44] L.M. Perez-Chada, J.F. Merola, Comorbidities associated with psoriatic arthritis: review and update, Clin. Immunol. 214 (2020) 108397.
[45] D.J. Veale, U. Fearon, The pathogenesis of psoriatic arthritis, Lancet 391 (10136) (2018) 2273–2284.
[46] R.J. de Souza, A. Mente, A. Maroleanu, A.I. Cozma, V. Ha, T. Kishibe, et al., Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies, BMJ 351 (2015) h3978.
[47] H. Mysliwiec, E. Harasim-Symbor, A. Baran, M. Szterling-Jaworowska, A.J. Milewska, A. Chabowski, et al., Abnormal serum fatty acid profile in psoriatic arthritis, Arch. Med. Sci. 15 (6) (2019) 1407–1414.
[48] B.B. Davidovici, N. Sattar, J.C. Prinz, P.C. Jörg, L. Puig, P. Emery, et al., Psoriasis and systemic inflammatory diseases: potential mechanistic links between skin disease and co-morbid conditions, J. Invest. Dermatol. 130 (7) (2010) 1785–1796.