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ORIGINAL RESEARCH ARTICLE

Expression Signature of Immune-Related MicroRNAs in Autoimmune Skin Disease: Psoriasis and Vitiligo Insights

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Abstract

Background Psoriasis and vitiligo are both chronic, skin-specific diseases classified as autoimmune diseases due to the involvement of several biochemical pathways in their pathogenesis, similar to those altered in other autoimmune diseases. The role of miRNAs in regulating skin autoimmune function has yet to be fully characterized.

Aim The aim of this study was to assess the expression profile of a panel of 11 circulating immune-related miRNAs in patients with autoimmune skin diseases, specifically psoriasis and vitiligo, and correlate their expression signature with the clinicopathological features of the diseases.

Subjects and Methods Relative gene expression quantification for 11 immune-related circulating miRNAs in plasma was done for 300 subjects—100 patients with psoriasis, 100 patients with vitiligo and 100 normal healthy volunteers—followed by different modalities of bioinformatics analysis for the results.

Results The expression levels of all the studied immune-related miRNAs were elevated in both autoimmune skin disorders, with much higher levels of expression in psoriasis than in vitiligo patients. There was a significant correlation between most of the studied miRNAs, suggesting shared target genes and/or pathways. Moreover, all the studied miRNAs showed significant results as biomarkers for autoimmune skin disease, with miRNA-145 being the best candidate. Regarding the clinicopathological data, miRNA-7, miRNA-9, miRNA-145, miRNA-148a, and miRNA-148b were positively correlated with age. All the miRNAs were inversely correlated with obesity and disease duration.

Conclusion This study highlights the critical role of miRNAs in skin-specific autoimmune diseases that proved to be potential biomarkers for autoimmune skin disorders, warranting their exploration as therapeutic targets.

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1 Introduction

Autoimmune diseases are a group of chronic conditions that destroy the body's own healthy tissues through immune mechanisms [1]. Although the primary etiology of most autoimmune diseases remains unspecified, they are known to be associated with a combination of environmental, genetic, and epigenetic factors [2–4]. Skin is affected by several autoimmune diseases, including psoriasis and vitiligo.

Psoriasis and vitiligo are both chronic, skin-specific diseases with evidence of genetic predisposition, which have been classified as autoimmune diseases due to the involvement of several biochemical pathways in their pathogenesis, similar to those altered in other autoimmune diseases [5]. Psoriasis involves epidermal thickening due

Key Points

The panel of 11 miRNAs under study comprising miRNA-7, miRNA-9, miRNA-23b, miRNA-124, miRNA-145, miR148a, miRNA-148b, miRNA-155, miRNA-181a, miRNA-203a, and miRNA-320a could serve as potential biomarkers in autoimmune skin disorders, warranting their exploration as therapeutic targets.

The expression levels of all the studied immune-related miRNAs were elevated in both autoimmune skin disorders, with much higher levels of expression in psoriasis than in vitiligo patients.

There was a significant correlation between most of the studied miRNAs, suggesting shared target genes and/or pathways.

MiRNAs under study were correlated with various clinicopathological features in both psoriasis and vitiligo.

to keratinocyte hyperproliferation [6], while vitiligo is characterized by skin depigmentation due to the selective destruction of melanocytes resulting in depigmented skin patches [7].

Psoriasis and vitiligo share several features. They have many immunological signaling pathways in common, including the Th1 and Th17 pathways and regulatory T cell (Treg) alterations [8–12]. At the genetic level, both diseases are associated with *NALP1* gene polymorphisms [13, 14]. Moreover, there are clinicopathological similarities such as skin patches, neuropeptide involvement, absence of organ-specific autoantibodies, positive family history of cardiovascular disease, and the Koebner phenomenon [15–18].

MicroRNAs (miRNAs) are small (18–25 nucleotides [nt]), highly conserved, non-coding RNA (ncRNA) sequences [19]. Recent advances in molecular medicine have led to the discovery of thousands of miRNAs that are relevant to translational research [20]. The primary function of miRNAs is to block messenger RNA (mRNA) translation into protein via binding to complementary sequences in mRNA. A single miRNA may target multiple genes, and multiple miRNAs may target a single gene [21]. MiRNAs are thus potent modulators of gene expression and regulate up to 60% of protein-coding genes [22].

The role of miRNAs in regulating skin functions has been uncovered but has yet to be fully characterized [23]. Recent research efforts have implicated miRNAs in several skin physiological processes, such as keratinocyte proliferation, melanogenesis, wound healing, and skin ageing [24]. Altered miRNA expression could thus result in skin disease. Since miRNAs are expressed in the skin and biological fluids (e.g., plasma, serum, urine), they could be considered potential non-invasive biomarkers with implications for diagnosis and prognosis and the prediction of therapeutic responses [23]. Recent research efforts have implicated miRNAs in several skin physiological processes, such as keratinocyte proliferation, melanogenesis, wound healing, and skin ageing [24]. Altered miRNA expression could thus result in skin disease. Since miRNAs are expressed in the skin and biological fluids (e.g., plasma, serum, urine), they could be considered potential non-invasive biomarkers with implications for diagnosis and prognosis and the prediction of therapeutic responses.

In this regard, the present study aimed to assess the expression profile of a panel of 11 circulating immune-related miRNAs in patients with the autoimmune skin diseases psoriasis and vitiligo. We also correlated the expression signature with the clinical features of the diseases to shed light on their role in disease pathogenesis and to evaluate their role as disease biomarkers.

2 Subjects and Methods

2.1 Study Population

The current study included 300 subjects. The study subjects were divided into three groups:

(i) Psoriasis patients (Group 1) comprising 100 patients diagnosed with chronic plaque psoriasis of both sexes, aged above 16 years. Immunosuppressed patients or those with serious chronic illnesses or autoimmune diseases were excluded. Psoriasis severity was assessed using Psoriasis Activity Score Index (PASI) score. Psoriasis was rated as mild if the PASI score was < 10, moderate if the PASI score was 10–20 and severe if PASI was > 20.

(ii) Vitiligo patients (Group 2) comprised 100 patients, both males and females, diagnosed with non-segmental vitiligo and assessed by a dermatologist according to the criteria of the Vitiligo Area Severity Index (VASI) and the Vitiligo European Task Force (VETF). Patients with other hypopigmentation disorders were excluded. Patients in both groups were recruited to the Dermatology Clinic. Clinicopathological data were obtained for both groups, including personal history, disease onset age, disease duration, severity, and medication history. The size, site, pattern, and distribution of individual lesions for both groups were examined via detailed dermatological examination.

(iii) Control group (Group 3) comprised 100 healthy volunteers with no history of any autoimmune or chronic diseases.

2.2 The Selection of the Circulating Immune-Related miRNAs Under Study

Selection of the 11 circulating immune-related miRNAs was based on results of the online bioinformatics tools, miR2Disease (http://www.mir2disease.org/) [25] and HMDD (http://www.cuilab.cn/) [26], and available literature [20, 24]. Circulating immune-related miRNAs under study are listed in Supplementary file 1 (see electronic supplementary material [ESM]).

2.3 Blood Sample Collection, miRNA Isolation, and miRNA Quality Assessment

Ethylene diamine tetra-acetic acid (EDTA) anticoagulant vacutainers were used to collect 3 mL of venous blood from each subject. Samples were centrifuged and 100 µL of separated plasma was added to a 500-µL qiazole reagent. Total RNA, including miRNA, was extracted from the plasma-qiazole mixture following the manufacturer's protocol using Qiagen miRNeasy mini kit (Catalog no. 217004, Qiagen, Hilden, Germany). NanoDrop 2000 1C (NanoDrop Tech., Inc. Wilmington, USA) was used to determine miRNA purity and concentration.

2.4 Relative Gene Expression Quantification of the Circulating Immune-Related miRNAs

The isolated miRNA was subjected to two-step relative gene expression quantification. The first step was reverse transcription (RT), where miScript II RT Kit (Catalog no. 218161, Qiagen, Hilden, Germany) was used to generate complementary DNA (cDNA) from isolated miRNA. Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Thermo Fischer, Waltham, USA) was used to carry the RT reaction for 1 hour at 37 °C, followed by brief incubation at 95 °C for inactivating the reaction.

The second step was SYBR Green-based real-time PCR using miScript SYBR Green PCR Kit with a universal reverse primer (Catalog no. 218076, Qiagen, Hilden, Germany) for the circulating immune-related miRNAs under study. Primer sequences for the 11 circulating immune-related miRNAs (miRNA-7, miRNA-9, miRNA-23b, miR124, miRNA-145, miRNA-148a, miRNA-148b, miRNA-155, miRNA-181a, miRNA-203a, and miRNA-320a) are described in Supplementary file 1 with the experiment set up and conditions (see ESM).

2.5 Gene Expression Data Analysis

The 11 circulating immune-related miRNA expression fold changes were calculated for patients' samples relative to the comparable controls and estimated using the Livak method [27].

2.6 Circulating Immune-Related miRNAs Predictive Significance Testing

The discriminative significance of the circulating immune-related miRNAs under study was analyzed using the receiver operating characteristic (ROC) curve to assess the diagnostic accuracy of the transcripts under study as biomarkers in both psoriasis and vitiligo.

2.7 Scoring of Disease Prioritization for Circulating Immune-Related miRNAs in Relation to Autoimmune Skin Disease

Utilizing data from the MalaCards database, the human disease database, relationship analysis for vitiligo and psoriasis was carried out using the GeneAnalytics software gene set option [28]. First, the number of miRNAs under study that matched psoriasis and vitiligo was normalized by the number of miRNAs associated primarily with psoriasis and vitiligo. Then the quality and type of the miRNA–psoriasis or miRNA–vitiligo relations, with each messenger RNA (mRNA) in both diseases as represented in MalaCards, was used to determine the disease matching score parameters. VarElect software [29] was used to prioritize the genes of the circulating immune-related miRNAs under study based on their relevance to the autoimmune skin diseases under study, vitiligo and psoriasis. VarElect score is based in part on the weight each gene currently has in relation to vitiligo and psoriasis based on prior research. The weight associated with autoimmune skin diseases was calculated using the frequency (term frequency) at which the miRNA gene is associated with psoriasis and vitiligo in comparison with all other miRNAs' genes under investigation (inverse document frequency).

2.8 Function and Pathway Enrichment Analysis of the Circulating Immune-Related miRNAs Under Study

The functional enrichment analysis of the 11 circulating immune-related miRNAs under research was performed using the Gene analytics software (https://geneanalytics.genecards.org) [28]. The immune-related circulating miRNAs under study and the gene ontology (GO) concepts that matched them were listed in the order of the matching scores. Higher scores indicated better matches, and the

reported score for each circulating immune-related miRNA is a transformation (log2) of the derived *p*-value. GO, consisting of cellular components, biological processes, and molecular functions terms, was searched for via pathway analysis on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to determine the affected pathways. The pathway enrichment analysis was carried out using the software Database for Annotation Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) [30] and DIANA Tool mirPath v.3. (http://snf-515788.vm.okeanos.grnet.gr/) [31].

2.9 MiRNA Regulatory Network Construction

Using miRTargetLink 2.0 (Version 2.0, available at https://ccb-compute.cs.uni-saarland.de/), the targets of the circulating immune-related miRNAs with statistically significant differential expression were predicted [32]. MiRNA targets that were statistically significant and homogeneous were retained, and negative correlations between miRNA and mRNA pairings were also taken into account.

2.10 Statistical Analysis

The software Statistical Package for the Social Sciences (SPSS) for Windows, version 26.0, was used (IBM Corp., New York, USA). G*Power 3.1.9.2 was used to calculate the sample size and research power. At a total sample size of 300, the power specified for the gene expression study design choice was 90%. While categorical variables were shown as frequencies and percentages, continuous variables were shown as means and standard deviations. The data profile was checked for uniformity and outliers. Mann-Whitney and Student *t* tests were employed as necessary to compare cases and controls. The correlation coefficient was calculated using the Spearman correlation test. When analyzing two-sided *p*-values for correlation, Spearman's rank test was used. Statistical significance was defined as a two-tailed *p*-value = 0.05.

3 Results

3.1 Description of the Study Population

The baseline variables of the 300 study participants are depicted in Table 1. The age of the study participants ranged from 16 to 60 years. The mean age for vitiligo patients was 35.41 ± 15.05 years; for psoriasis patients was 41.8 ± 14.08 years; and for controls was 39.17 ± 11.65 years. When patients were stratified according to age, 42% of vitiligo patients were in the 18–30 years group, while psoriasis patients were more evenly distributed in the different age groups ($p = 0.002$). Regarding special habits, 55%, 57%, and 61% were smokers among controls, vitiligo patients, and psoriasis patients, respectively, with significant differences among the study groups. Obesity was more represented in vitiligo patients than in other study groups ($p < 0.001$). Finally, gender and family history did not show statistically significant differences between psoriasis and vitiligo patients.

3.2 Clinical Features of Autoimmune Skin Disease Patients

Table 2 shows disease features of psoriasis and vitiligo patients. The duration of disease was longer in vitiligo patients compared with psoriasis patients ($p < 0.001$). VASI, Vitiligo Disease Activity Score (VIDA), psoriasis severity, and PASI score showed statistically high significant values ($p < 0.001$) in relation to the diseases.

3.3 Circulating Immune-Related miRNAs Expression Signature in Autoimmune Skin Disease

Figures 1 and 2 display the expression levels of 11 immune-related miRNAs studied in psoriasis and vitiligo patients. The level of expression of all studied miRNAs was significantly higher in psoriasis patients than in vitiligo patients. MiRNA-7 showed the highest expression level in psoriasis (Log_2FC 10.7), followed by miRNA-23b and miRNA-145 (Log_2FC 10.2), miRNA-148b (Log_2FC 9.8), miRNA-181a (Log_2FC 9.0), miRNA-148a (Log_2FC 8.6), miRNA-124 (Log_2FC 8.3), miRNA-203a (Log_2FC 8.1), miRNA-9 (Log_2FC 8.0), miRNA-320a (Log_2FC 6.6), and miRNA-155 (Log_2FC 5.5) in descending order. In vitiligo patients, miRNA-155 was the highest expressed miRNA (Log_2FC 4.1) followed by miRNA-23b and miRNA-124 (Log2FC 3.4), miRNA-9 (Log_2FC 3.1), miRNA-203a (Log_2FC 2.9), miRNA-181a (Log_2FC 2.4), miRNA-320a (Log_2FC 1.7), miRNA-148a (Log_2FC 1.6), miRNA-7 (Log_2FC 0.8), miRNA-148b (Log_2FC 0.7), and miRNA-145 (Log_2FC 0.2) in descending order.

3.4 Predictive Significance of the Circulating Immune-Related miRNAs Under Study in Autoimmune Skin Disease

The 11 studied circulating immune-related miRNAs were examined for being potential biomarkers in autoimmune skin disease using ROC curve analysis (Fig. 3). The area under the curve (AUC) of the miRNAs ranged from 0.620 to 0.985, all being significantly associated with

Table 1 Demographic features of study groups

Variable	Controls ($n = 100$)	Vitiligo ($n = 100$)	Psoriasis ($n = 100$)	p-value
Age (y)[a]	39.17 ± 11.65	35.41 ± 15.05	41.8 ± 14.08	0.474
Age group (y)[b]				
18–30	32 (32)	42 (42)	21 (21)	**0.002***
31–40	24 (24)	25 (25)	25 (25)	
41–50	19 (19)	9 (9)	22 (22)	
51–60	25 (25)	24 (24)	32 (32)	
Gender[b]				
Females	53 (53)	52 (52)	55 (55)	0.77
Males	47 (47)	48 (48)	45 (45)	
moking[b]				
Smoker	55 (55)	57 (57)	61 (61)	**0.013***
Non-smoker	45 (45)	43 (43)	39 (39)	
Obesity[b]				
Obese	48 (48)	56 (56)	27 (27)	**< 0.001***
Non-obese	52 (52)	44 (44)	73 (73)	
Family history[b]				
Positive		18 (24.4)	15 (15)	0.569
Negative		82 (81.1)	85 (85)	

Data are shown as number (percentage) or mean ± standard deviation

*p-value < 0.05 was considered statistically significant

[a]Independent t-test between psoriasis and vitiligo groups (parametric)

[b]Mann-Whitney test between psoriasis and vitiligo groups (non-parametric)

autoimmune skin disease ($p < 0.001$) with miRNA-145 representing the largest (AUC 0.985), followed by miRNA-148b (AUC 0.982), miRNA-7 (AUC 0.979), miRNA-148a (AUC 0.931), miRNA-23b (AUC 0.913), miRNA-9 (AUC 0.881), miRNA-203a (AUC 0.864), miRNA-320a (AUC 0.849), miRNA-181a (AUC 0.848), miRNA-124 (AUC 0.847), and miRNA-155 (AUC 0.620) in descending order.

3.5 Correlation Between the Expression of the Circulating Immune-Related miRNAs and the Clinical Features of Autoimmune Skin Disease

Patients with autoimmune skin diseases showed diverse distributions in the circulating immune-related miRNAs' expression levels. Table 3 summarizes the analysis of the immune-related Spearman's rank correlation of the 11 immune-related miRNAs in autoimmune skin disease. Nearly all of the examined miRNAs showed a significant connection with autoimmune skin disease, with a Spearman correlation coefficient of 0.59 and a two-tailed significance of $p < 0.001$.

When the expression levels of the studied miRNAs were correlated with the clinical features of autoimmune skin disease, miRNA-7, miRNA-9, miRNA-145, miRNA-148a, and miRNA-148b were positively correlated with the age of the patient. In contrast, miRNA-7 was correlated with different age groups. All the studied miRNAs showed an inverse correlation between obesity and disease duration. MiRNA-7 and miRNA-320a showed a significant correlation with age at disease onset. Finally, miRNA-320a was correlated with gender (Table 4).

Several correlations were also found among the clinical features of our study participants, as shown in Table 5, where the patient's age was positively correlated with the age of onset of disease, VASI, and VIDA. Age groups showed significant correlations with VASI. Gender showed a significant correlation with the age of onset of disease. Obesity and family history showed a significant correlation with disease duration. VASI was significantly correlated with the patient's age, age group, obesity, and disease duration, while VIDA was only correlated with the patient's age. Finally, PASI and psoriasis severity showed a significant direct correlation.

Table 2 Clinical features of patients with autoimmune skin disease

Variable	Vitiligo ($n = 100$)	Psoriasis ($n = 100$)	p-value
Duration of disease[a]			
Mean ± SD	9.37 ± 9.73	7.0 ± 6.34	**< 0.001***
Min–max	0.1–39	0.1–30	
Age of onset[a]			
Mean ± SD	16 ± 1.41	14.9 ± 2.83	0.140
Min–max	15–17	21–25	
VIDA			**< 0.001***
Stage −1	0 (0)		
Stage 0	5 (5)		
Stage 1	25 (25)		
Stage 2	33 (33)		
Stage 3	35 (35)		
Stage 4	2 (2)		
Psoriasis severity			
Mild		45 (45)	**0.032***
Moderate		31 (31)	
Severe		24 (24)	
VASI[a]			
Mean ± SD	0.50 ± 0.31		**< 0.001***
Min–max	0.10–1.0		
PASI[a]			
Mean ± SD		13.65 ± 9.02	**< 0.001***
Min–max		1.5–40.5	

Data are shown as mean ± standard deviation or number (percentage)

One sample Chi-square test was used

Max maximum, *Min* minimum, *PASI* Psoriasis Activity Score Index, *SD* standard deviation, *VASI* Vitiligo Area Severity Index, *VIDA* Vitiligo Disease Activity

*p-value < 0.05 was considered statistically significant

[a]Independent t-test between psoriasis and vitiligo groups (parametric)

3.6 Prioritization Score of Circulating Immune-Related miRNA Genes in Relation to Autoimmune Skin Disease

Figure 4 displays the prioritization scores of the circulating immune-related miRNA genes under study classified into two categories, directly and indirectly related to disease, according to detected links between immune-related miRNA genes and both psoriasis and vitiligo. This list is based on the association between these genes and the shared pathways, interaction networks, paralog relations, domain sharing, and the mutual publications of the autoimmune skin diseases under study. Table 6 lists the genes implicated with the indirectly related category of miRNA genes.

3.7 Pathway and Function Enrichment Analysis for the Circulating Immune-Related miRNAs

A pathway enrichment analysis was performed based on annotated gene targets to detect the pathways targeted by the differentially expressed immune-related miRNAs in autoimmune skin disease. MirPath v.3 database was used to perform functional pathway analysis. KEGG enrichment analysis (Fig. 5) revealed the following pathways: extracellular matrix (ECM)-receptor interaction, proteoglycans in cancer, fatty acid biosynthesis, glioma, hippo signaling pathway, adherens junction, pathways in cancer, fatty acid elongation, TGF-β signaling pathway, and transcriptional misregulation in cancer.

The GO biological processes enriched in our analysis were distinctly associated with gene silencing by miRNA, miRNA-mediated inhibition of translation, negative regulation of gene expression, inflammatory response, interleukin (IL)-8 and IL-11 production, microglial cell activation, low-density lipoprotein particle clearance, receptor signaling pathway via JAK-STAT, and positive regulation of macrophage activation as shown (Fig. 6A).

The extracellular space, extracellular vesicles, RNA-induced silencing complex (RISC), extracellular exosomes, apical section of the cell, and perinuclear region of the cytoplasm were all associated with the circulating immune-related miRNAs under study (Fig. 6B). The molecular activities of these miRNAs included mRNA binding involved in posttranscriptional gene silencing, mRNA 3'-UTR binding, high-density lipoprotein particle binding, RNA polymerase II complex binding, and single-stranded RNA binding (Fig. 6C).

3.8 Circulating Immune-Related miRNA-mRNA Regulatory Network in Autoimmune Skin Disease

Our network analysis revealed the connections between the target genes of the circulating immune-related miRNAs under investigation. Initially, 10,962 target genes were identified in the miRNA-target gene network made up of the 11 immune-related miRNAs (Supplementary file 2, see ESM). Then, these were selected to only keep targets that had at least two shared strongly validated targets. Using miRTargetLink 2.0 (https://ccb-web.cs.uni-saarland.de/mirtargetlink/network.php), a final list of 30 target genes was obtained (Fig. 7).

According to our miRNA-mRNA regulatory network analysis results, *STAT3*, *IRS1*, *TGFB2*, *IRS2*, *EGFR*, *MSH3*, *NDUFA4*, *SMAD2*, *RGS5*, *IGF1R*, *STAT1*, *KLF4*, *BCL2*, *CDKN1A*, *CDKN1B*, *MCL1*, *XIAP*, *NRAS*, *CDK6*, *SAMHD1*, *PPP3CA*, *PTEN*, *ETS1*, *MYC*, *ABCC1*, *SMAD3*,

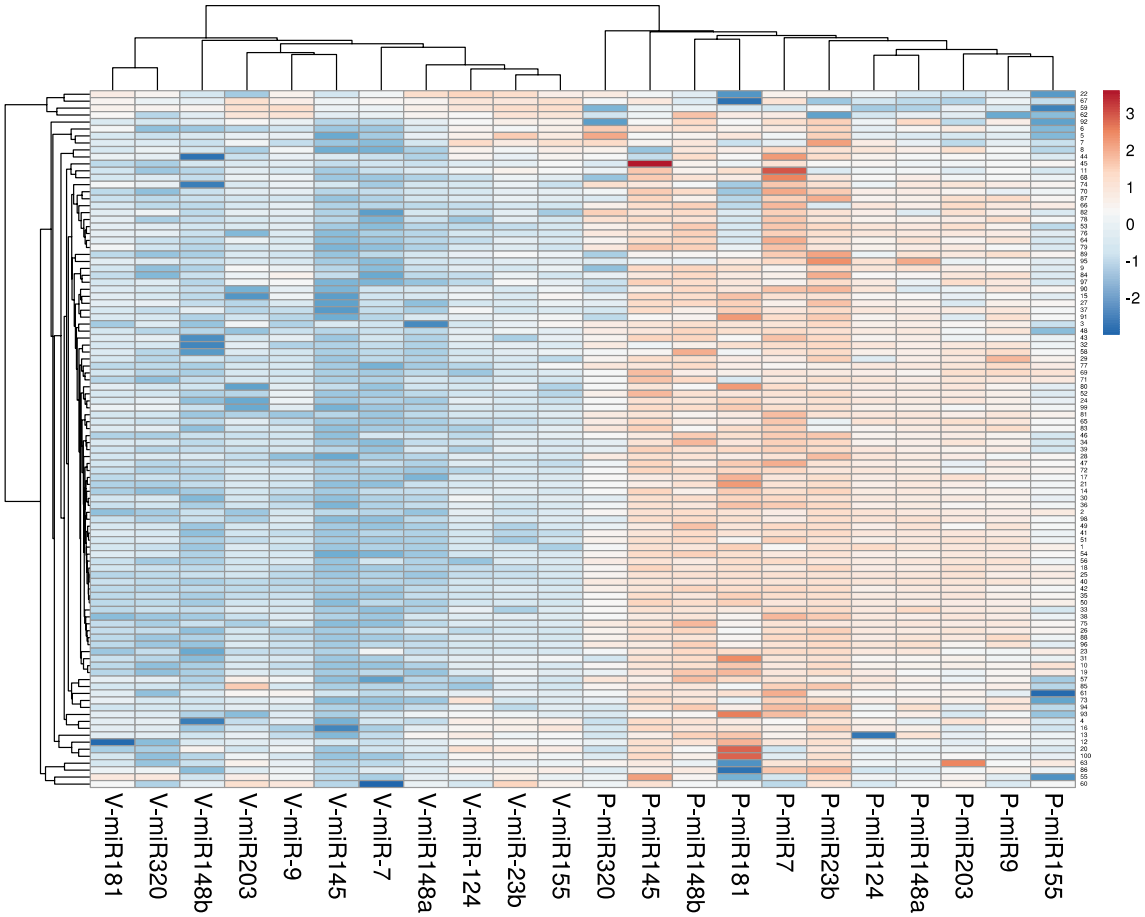

Fig. 1 Expression signature of the circulating immune-related miRNAs under study in autoimmune skin disease. The heatmap illustrates the expression levels (Log2fold change) of the 11 immune-related miRNAs (miRNA-7, miRNA-9, miRNA-23b, miRNA-124, miRNA-145, miRNA-148a, miRNA-148b, miRNA-155, miRNA-181a, miRNA-203a, and miRNA-320a) in autoimmune skin disease patients: vitiligo ($n = 100$) and psoriasis ($n = 100$). Color grades are shown, with highest expression corresponding to deep red and lowest to deep blue. "V" stands for values in vitiligo patients while "P" stands for values in psoriasis patients

BAX, *TGFBR2*, *CCND2*, and *FOS* are the critical genes involved in autoimmune skin disease in relation to the studied immune-related miRNAs.

4 Discussion

Psoriasis and vitiligo are skin-restricted autoimmune diseases with several clinicopathological, immunological, and genetic features [33]. MiRNAs are short gene-regulatory RNA molecules implicated in most physiological and pathological processes, including differentiation, proliferation, migration, and survival [34]. MiRNAs are abundantly expressed in the skin [35] and are involved in skin development, maintenance, and homeostasis through regulating cell proliferation, differentiation, and immune regulation [36]. In addition, they are becoming essential targets for disease diagnosis and treatment [37]. They have been shown to regulate keratinocyte differentiation and proliferation, as well as melanogenesis and development and function of immune cells [38], processes relevant to the pathogenesis of psoriasis and vitiligo, respectively. Dysregulated miRNA expression has been demonstrated in several skin disorders, including the autoimmune skin diseases psoriasis and vitiligo [36, 37].

In this study, we aimed to explore the circulating miRNA repertoire of the autoimmune skin disorders psoriasis and vitiligo and to identify potential biomarkers for the diseases. Eleven immune-related miRNAs were selected to be examined in psoriasis ($n = 100$) and vitiligo ($n = 100$) patients as compared with healthy controls ($n = 100$). The studied miRNAs were miRNA-7, miRNA-9, miRNA-23b, miRNA-124,

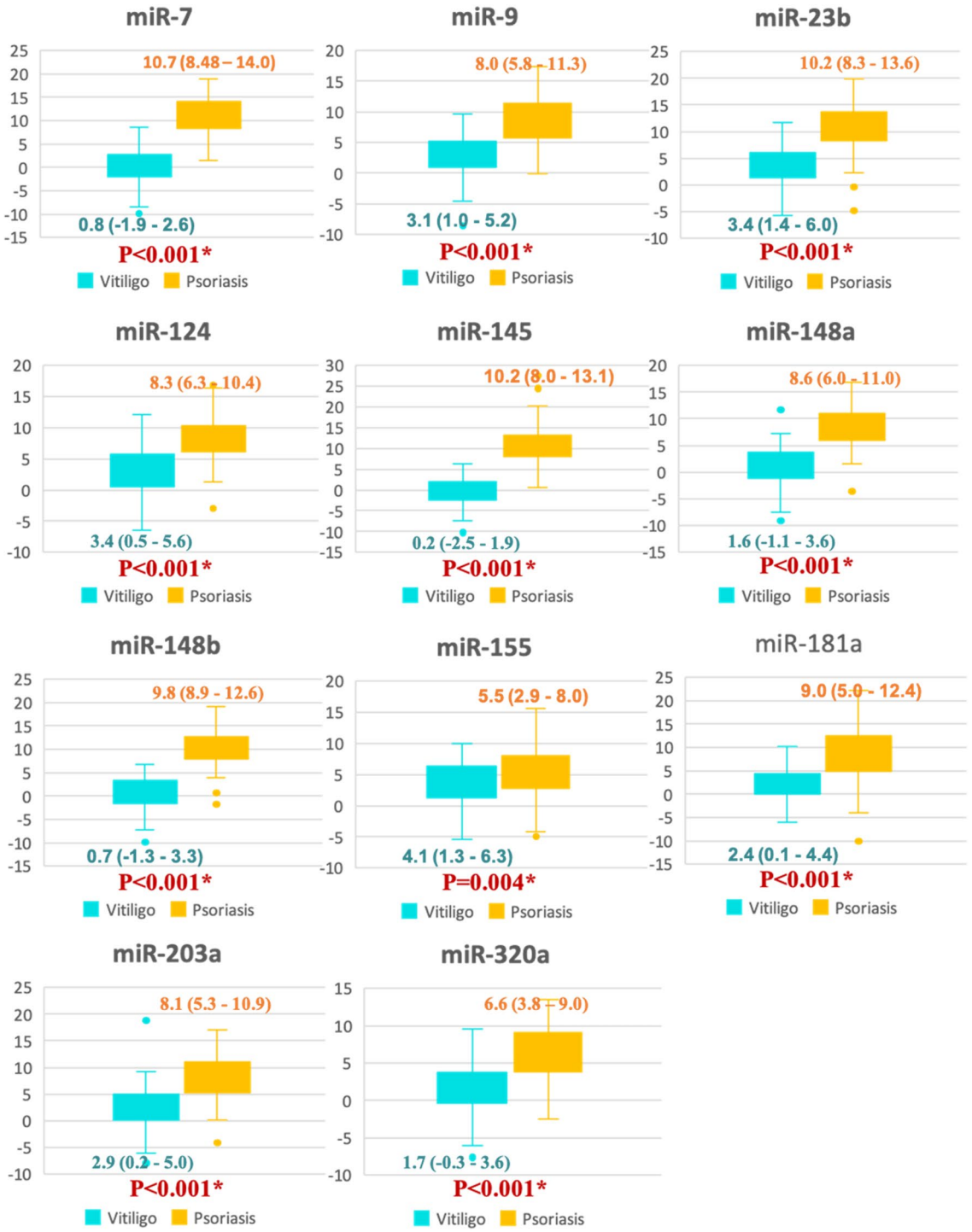

Fig. 2 Relative expression levels of the circulating immune-related miRNAs under study in autoimmune skin disease: vitiligo and psoriasis. Eleven miRNAs were analyzed: miRNA-7, miRNA-9, miRNA-23b, miRNA-124, miRNA-145, miRNA-148a, miRNA-148b, miRNA-155, miRNA-181a, miRNA-203a, and miRNA-320a. SNORD68 and RNU6B were used as endogenous controls. The values are represented as median (Q1 and Q3) using Whiskers and bars. "Mann–Whitney *U* test was used for comparison". *$p < 0.05$ was considered statistically significant

miRNA-145, miR148a, miRNA-148b, miRNA-155, miRNA-181a, miRNA-203a, and miRNA-320a. The expression levels of all the studied immune-related miRNAs were elevated in both autoimmune skin disorders, with much higher levels of expression in psoriasis than in vitiligo patients (Figs 1 and 2).

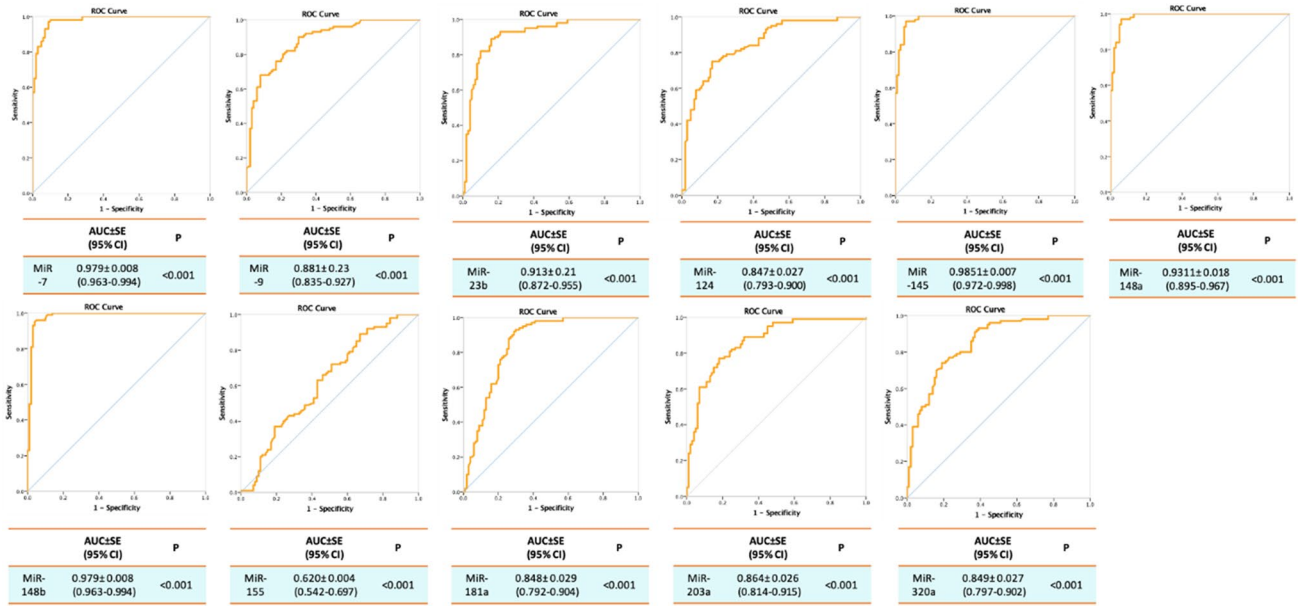

Fig. 3 ROC curve analysis for eleven immune-related miRNAs in autoimmune skin disease. *AUC* area under the curve, *P* *p*-value, *SE* standard error. "AUC: ≤ 0.5 = no discrimination, 0.7–0.8 = acceptable discrimination, 0.8–0.9 = excellent discrimination, > 0.9 = outstanding discrimination"

Table 3 Correlation analysis among the 11 studied circulating immune-related miRNAs

	miR7	miR9	miR23b	miR124	miR145	miR148a	miR148b	miR155	miR181a	miR203a	miR320a
miR7	1.00	0.85**	0.88**	0.85**	0.93**	0.91**	0.91**	0.55*	0.71**	0.81**	0.78**
miR9	0.85**	1.00	0.87**	0.84**	0.88**	0.89**	0.86**	0.67*	0.74**	0.86**	0.80**
miR23b	0.88**	0.87**	1.00	0.85**	0.88**	0.92**	0.88**	0.63*	0.74**	0.83**	0.75**
miR124	0.85**	0.84**	0.85**	1.00	0.83**	0.89**	0.83**	0.71**	0.72**	0.80**	0.78**
miR145	0.93**	0.88**	0.88**	0.83**	1.00	0.91**	0.92**	0.58*	0.73**	0.86**	0.78**
miR148a	0.91**	0.89**	0.92**	0.89**	0.91**	1.00	0.90**	0.63*	0.78**	0.84**	0.80**
miR148b	0.91**	0.86**	0.88**	0.83**	0.92**	0.90**	1.00	0.54*	0.73**	0.83**	0.76**
miR155	0.55*	0.67*	0.63*	0.71**	0.58*	0.63*	0.54*	1.00	0.53*	0.64*	0.57*
miR181a	0.71**	0.74**	0.74**	0.72**	0.73**	0.78**	0.73**	0.53*	1.00	0.66*	0.66*
miR203a	0.81**	0.86**	0.83**	0.80**	0.86**	0.84**	0.83**	0.64*	0.66*	1.00	0.78**
miR320a	0.78**	0.80**	0.75**	0.78**	0.78**	0.80**	0.76**	0.57*	0.66*	0.78**	1.00

Spearman's correlation coefficients are presented for immune-related miRNAs in psoriasis and vitiligo patients

Significant values are highlighted

Color grades are shown, with the highest expression corresponding to deep orange and the lowest to light orange

*Correlation significant at the 0.05 level (2-tailed)

**Correlation significant at the 0.01 level (2-tailed)

Table 4 Correlation of circulating immune-related miRNA expression and clinical features of autoimmune skin disease

	Age	Age group[#]	Gender	Smoking	Obesity	FH	Disease duration	Onset age	VASI	VIDA	PASI	Psoriasis severity
miR7	0.184**	0.162*	0.066	0.052	− 0.323**	− 0.036	− 0.717**	0.253**	0.081	− 0.025	0.022	− 0.002
miR9	0.152*	0.136	0.088	0.048	− 0.250**	− 0.053	− 0.57**	0.216	0.081	− 0.010	0.131	0.100
miR23b	0.125	0.105	0.071	0.110	− 0.294**	− 0.048	− 0.631**	0.201	0.079	0.100	0.115	0.102
miR124	0.109	0.064	0.044	0.057	− 0.186**	− 0.030	− 0.514**	0.183	0.095	− 0.050	0.093	0.069
miR145	0.154*	0.138	0.012	0.046	− 0.296**	− 0.026	− 0.726**	0.243	0.075	0.116	− 0.036	− 0.061
miR148a	0.156*	0.125	0.091	0.036	− 0.278**	− 0.057	− 0.657**	0.234	0.093	0.033	0.000	− 0.030
miR148b	0.179*	0.164	0.046	0.035	− 0.299**	− 0.041	− 0.715**	0.224	− 0.009	− 0.015	− 0.031	− 0.047
miR155	− 0.121	− 0.137	− 0.045	0.083	− 0.183**	0.028	− 0.181*	0.049	0.050	0.037	0.035	0.046
miR181a	0.090	0.056	0.069	0.015	− 0.199**	− 0.050	− 0.507**	0.123	0.096	0.034	0.101	0.149
miR203a	0.041	0.040	0.015	0.015	− 0.302**	− 0.055	− 0.578**	0.127	0.061	0.192	0.126	0.100
miR320a	0.131	0.114	0.145*	0.049	− 0.256**	− 0.067	− 0.511**	0.169*	0.140	0.047	− 0.009	− 0.043

Spearman's correlation coefficients are presented for immune-related miRNAs and clinical features of psoriasis and vitiligo patients

Significant values are highlighted

Color grades are shown, with the highest expression corresponding to orange and the lowest to blue

FH family history, *PASI* Psoriasis Activity Score Index, *VASI* Vitiligo Area Severity Index, *VIDA* Vitiligo Disease Activity Score

[#]Age groups: 18–30, 31–40, 41–50, and 51–60 years

*Correlation significant at the 0.05 level (2-tailed)

**Correlation significant at the 0.01 level (2-tailed)

4.1 Differentially Expressed miRNAs in Vitiligo

MiRNA-155 upregulation has been found in the serum [39] and lesional skin [40] of vitiligo patients. Šahmatova et al. demonstrated that miRNA-155 was stimulated by vitiligo-associated cytokines (*TNF-α*, *IFN-α*, *IFN-γ* and *IL-1β*) in human primary melanocytes and keratinocytes and the upregulated miRNA-155 inhibited the expression of melanogenesis-associated genes (*TYRP1, YWHAE, SDCBP* and *SOX10* in melanocytes, and *YWHAE* in keratinocytes) and altered interferon-regulated genes (*SOCS1, IRF1* and *IFITM1*) in melanocytes and keratinocytes [40]. Moreover, miRNA-155 has been shown to increase the differentiation of Treg cells by stimulating forkhead box P3 (*Foxp3*) transcription [41]. Tregs are suppressive T cells that inhibit autoimmune CD8+ T cells and can damage melanocytes [42]. MiRNA-155 has also been shown to decrease CD8+ T-cell growth and promote melanocyte proliferation via increasing Treg cells [43]. In addition, *IL-1β* stimulated upregulation of miRNA-155 resulted in reduced expression in melanoma cells of the microphthalmia-associated transcription factor (*MIFT*) [44], a melanocyte-specific transcription factor involved in melanocyte survival, proliferation, and differentiation [45]. Therefore, miRNA-155 is a main miRNA in melanocyte and immune cell function, both involved in vitiligo. Treatment with narrowband ultraviolet B (UVB) has been shown to reduce miRNA-155 expression in peripheral mononuclear cells (PMNCs) from active non-segmental vitiligo (NSV) patients [46], making it a potential target for vitiligo treatment. Consistently, our data shows circulating miRNA-155 as being the highest expressed miRNA in vitiligo patients (Log_2FC 5.5) and of moderate discriminative value (AUC 0.620) in autoimmune skin disease.

The miRNA-9 expression has been shown to be increased in the lesional skin [47–49] and serum [49] of vitiligo patients. Raia et al. also demonstrated a positive correlation between circulating miRNA-9 levels with disease extent and VASI score in Egyptian vitiligo patients [49]. MiRNA-9 could target the expression of sirtuin 1 (*SIRT1*), which is known to protect against ageing and other stress-related diseases, implicating a role in destroying melanocytes [47]. Su et al. have also shown that the miRNA-9 upregulation in lesional vitiligo skin was associated with decreased expression of adhesion molecules (*E-cadherin* and *β1 integrin*)

Table 5 Correlation analysis of the different clinical features of the studied autoimmune skin diseases

	Age	Age group[#]	Gender	Smoking	Obesity	FH	Disease duration	Onset age	VASI	VIDA	PASI	Psoriasis severity
Age	1.0	0.963**	0.045	− 0.110	0.101	− 0.106	− 0.132	0.458**	0.246**	− 0.208*	0.026	0.082
Age group	0.963**	1.0	0.047	− 0.125	0.066	− 0.054	− 0.123	0.431**	0.274**	− 0.156	0.027	0.082
Gender	0.045	0.047	1.0	0.027	− 0.056	− 0.109	0.045	0.164*	0.067	− 0.045	0.055	0.032
Smoking	− 0.110	− 0.125	0.027	1.0	0.104	0.015	− 0.084	0.003	− 0.103	0.039	0.131	0.121
Obesity	0.101	0.066	− 0.056	0.104	1.0	− .019	0.266**	0.126	0.274**	− 0.126	0.132	0.072
FH	− 0.106	− 0.054	− 0.109	0.015	− 0.019	1.0	0.233**	− 0.136	0.072	0.128	− 0.016	− 0.016
Disease duration	− 0.132	− 0.123	0.045	− 0.084	0.266**	0.233**	1.0	− 0.371**	0.324**	− 0.151	− 0.016	− 0.016
Onset age	0.458**	0.431**	0.164*	0.003	0.126	− 0.136	− 0.371**	1.0	0.017	− 0.079	− 0.004	0.029
VASI	0.246*	0.274**	0.067	− 0.103	0.274	0.072	0.324**	0.017	1.0	0.141		
VIDA	− 0.208*	− 0.156	− 0.045	0.039	− 0.126	0.128	− 0.151	− 0.079	0.141	1.000		
PASI	0.026	0.027	0.055	0.131	0.132	− 0.016	− 0.016	− 0.004			1.0	0.930**
Psoriasis severity	0.082	0.082	0.032	.121	0.072	− 0.016	− 0.016	0.029			0.930**	1.0

Spearman's correlation coefficients are presented for different clinical features among psoriasis and vitiligo patients

Significant values are highlighted

FH family history, *PASI* Psoriasis Activity Score Index, *VASI* Vitiligo Area Severity Index, *VIDA* Vitiligo Disease Activity Score

[#]Age groups: 18–30, 31–40, 41–50, and 51–60 years

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

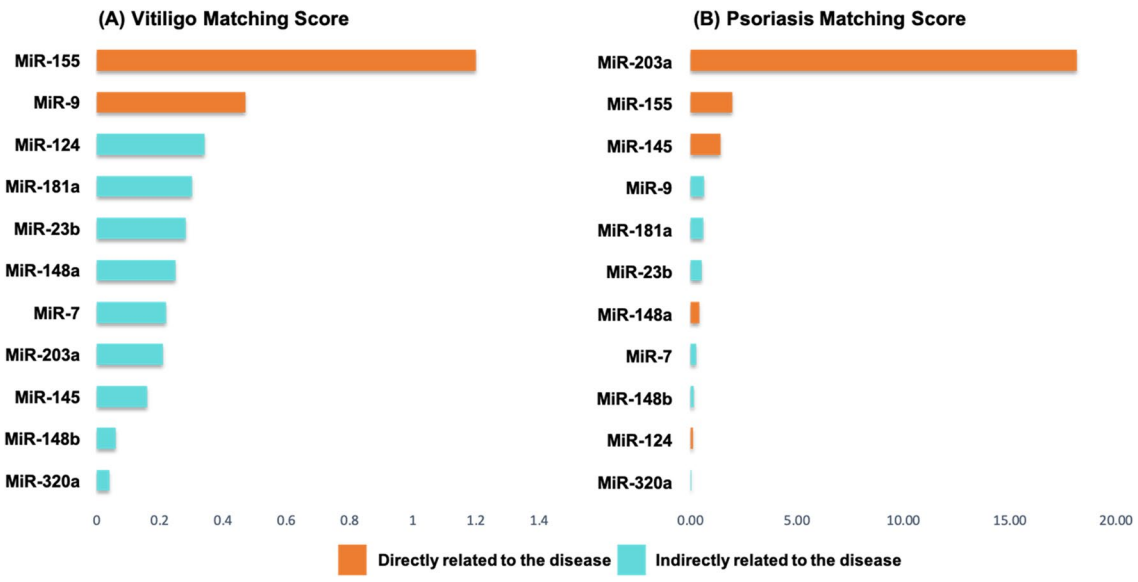

Fig. 4 Prioritization score of eleven circulating immune-related miRNA genes in autoimmune skin disease. **A** Prioritization score for miRNA genes in vitiligo. **B** Prioritization score for miRNA genes in psoriasis. All scores were generated with the VarElect tool (https://ve.genecards.org)

Table 6 List of genes implicated with the indirectly related miRNA genes in autoimmune skin disease

Gene	Implicated genes related to psoriasis	Implicated genes related to vitiligo
MiRNA-7	*DDX58, VDR, IFIH1, NOD2, CXCL8*	*MITF, PRDX5, POMC, FAS, NBN*
MiRNA-9	*TNF,* MIRNA-203a, *IL1B, TNFRSF1A, ADAM17*	
MiRNA-23b	*TNF,* MIRNA-203a, *IL6, IFNG, IL4*	*TNF, IL2, ATM, IFNG, IL6*
MiRNA-124		*TNF, FAS, MSRA, IL1B, TP53*
MiRNA-145		*ATM, TGFBR2, TP53,* MIRNA-21, *ERBB3*
MiRNA-148a		*HLA-C, TP53, VEGFA, MYC*
MiRNA-148b	MIRNA-200a, MIRNA-141, MIRNA-30, MIRNA-LET-7e, *TP53*	*TP53, ITGA5,* MIRNA-200C, MIRNA-25, *MYC*
MiRNA-155		
MiRNA-181a	MIRNA-203a, *TNF, IL6, IL1B, TNFRSF1A*	*KITLG, TNF, FAS, ATM, TPO*
MiRNA-203a		*FAS, ATM, NBN, TP53, STAT3*
MiRNA-320a	*TP53*	*TP53*

and that exposing the normal human keratinocyte cell line HaCaT cells to UVB decreased the expression of miRNA-9 and increased the expression of *IL-10*, *E-cadherin* and *β1 integrin*, thereby antagonizing the miRNA-9 migration-inhibitory effect on pigment cells during UVB-induced repigmentation [48], making miRNA-9 an essential therapeutic target in vitiligo. This is consistent with our findings, where the circulating miRNA-9 was the third highest expressed miRNA in our vitiligo patients (Log_2FC 3.1), is positively correlated with patient age and negatively correlated with obesity and disease duration, and is of excellent discriminative value using ROC curve analysis (AUC 0.881) in autoimmune skin disease.

MiRNA-124 is a tumor suppressor in several tumors, including melanoma [50]. To our knowledge, miRNA-124 has not been examined for vitiligo. However, melanoma and vitiligo have long been considered related diseases [51]. Downregulated miRNA-124 expression has been demonstrated in melanoma cells associated with upregulated *RACK1*. Through *RACK1* degradation, overexpression of miRNA-124 resulted in impaired melanoma cell proliferation, migration, invasion, and increased apoptosis [50]. This may be relevant to vitiligo immunopathogenesis. Our findings have demonstrated that increased circulating miRNA-124 expression levels, being the second highest expressed (Log_2FC 3.4) in vitiligo patients, is positively correlated with age and negatively correlated with obesity and disease duration and is of excellent discriminative value in autoimmune skin disease (AUC 0.847).

The expression level of miRNA-181a has been shown to be increased in PBMCs from active NSGV patients [44]. This is consistent with our current and previous findings in vitiligo patients [52], where it is the fifth highest expressed miRNA in vitiligo (Log_2FC 2.4), is inversely correlated with obesity and disease duration, and is of excellent discrimination value in autoimmune skin disease (AUC 0.848).

Collectively, these data on the role of miRNA-155 and miRNA-9 in vitiligo pathogenesis support our data that these miRNAs are directly related to vitiligo pathogenesis (Fig. 4). Other miRNAs were indirectly related to vitiligo with varying degrees (Table 6). Implicated genes related to melanogenesis (MITF, POMC), oxidative stress defense (PRDX5), apoptosis (FAS, p53, MYC), DNA repair (NBN, p53, and ATM), autoimmunity-related genes (TNF, IL2, IL6, IL1B, IFNG, TGFBR2, HLA-C, and STAT3), and cell adhesion (ITGA5) are closely related to the pathogenesis of vitiligo [53].

4.2 Differentially Expressed miRNAs in Psoriasis

MiRNA-203a is a keratinocyte-derived miRNA involved in the balance of keratinocyte proliferation and differentiation and is considered a marker of keratinocyte differentiation [54]. MiRNA-203a has reportedly been upregulated in psoriasis skin lesions [55–57] and plasma [58]. MiRNA-203a in keratinocytes is believed to inhibit skin immune responses by downregulating pro-inflammatory cytokine genes *TNF-α* and *IL-24* [59]. Moreover, miRNA-203a inhibits the expression of suppressor of cytokine signaling 3 (SOCS-3) with subsequent upregulation of signal transducer and activator of transcription-3 (STAT-3), a transcription factor in keratinocytes that regulates keratinocyte proliferation and differentiation [60]. In addition, miRNA-203 inhibits the expression of liver X receptor-α (*LXR-α*) and peroxisome proliferator-activated receptor-γ (*PPAR-γ*) in psoriatic lesions. In contrast, the overexpression of *LXR-α* and *PPAR-γ* inhibits keratinocyte proliferation, suggesting a role for the miRNA-203a-LXR-α /PPARγ axis in keratinocyte hyperproliferation in psoriasis [61]. In our study, circulating miRNA-203a was upregulated in psoriasis (Log_2FC 8.1). It was significantly higher in psoriasis when compared with vitiligo (Log_2FC 2.9) ($p < 0.001$), reflecting its crucial role

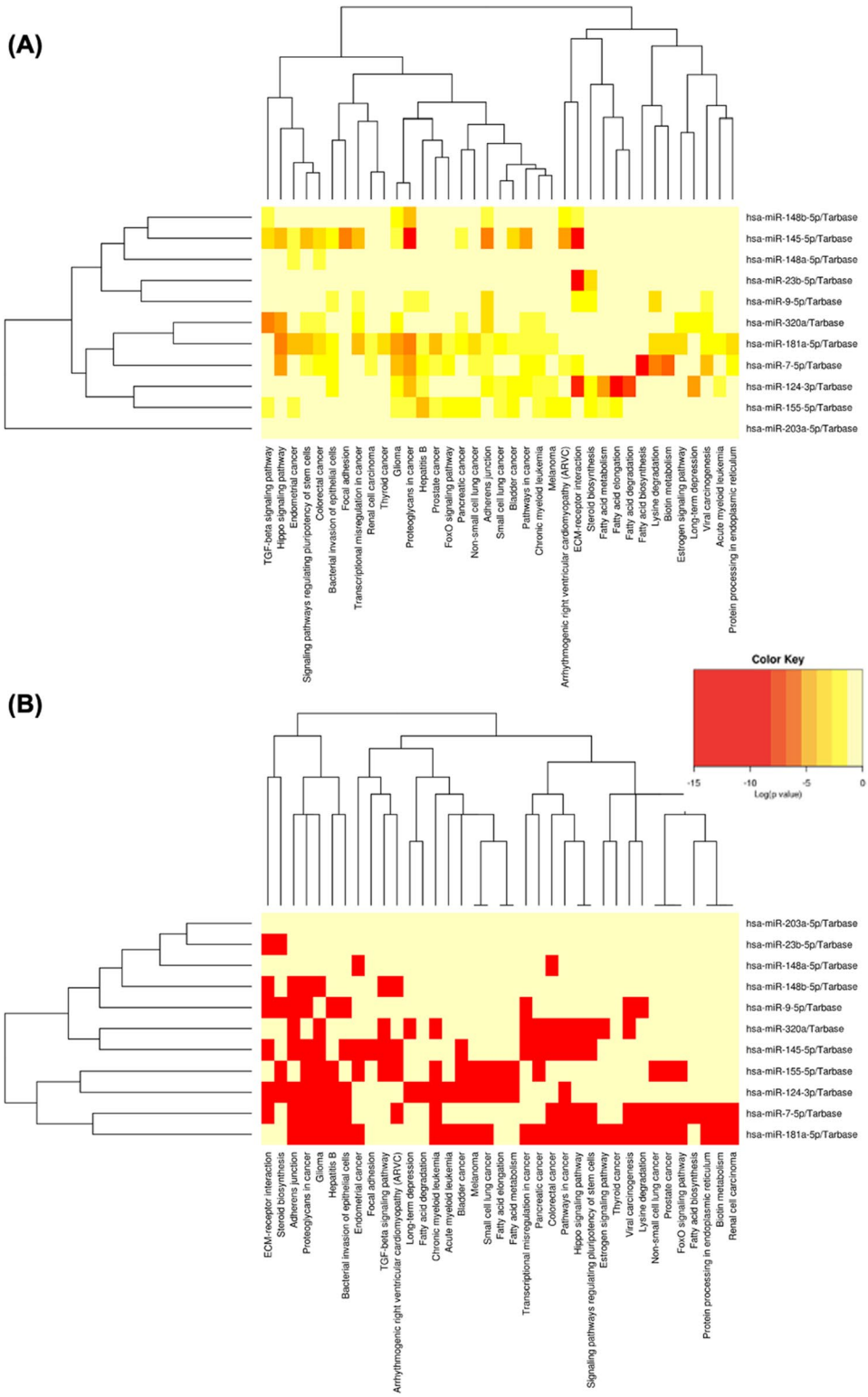

Fig. 5 "KEGG pathways enriched analysis for eleven differentially expressed immune-related miRNAs in autoimmune skin disease using **A** targeted pathways clusters/heatmap and **B** significance clusters/heatmap". *ECM* extracellular matrix, *TG*, transforming growth factor

in keratinocyte differentiation and proliferation and making it a target for psoriasis therapy. MiRNA-203a was inversely correlated with obesity and disease duration and was of excellent discriminative value (AUC 0.864) in autoimmune skin disease.

MiRNA-155 has also been shown to be upregulated in skin lesions [62–64] and PMNCs [66] of psoriasis. The role of miRNA-155 in psoriasis could be explained by promoting keratinocyte proliferation and inhibiting apoptosis through downregulating *PTEN* [62, 66]. Moreover, the knockdown

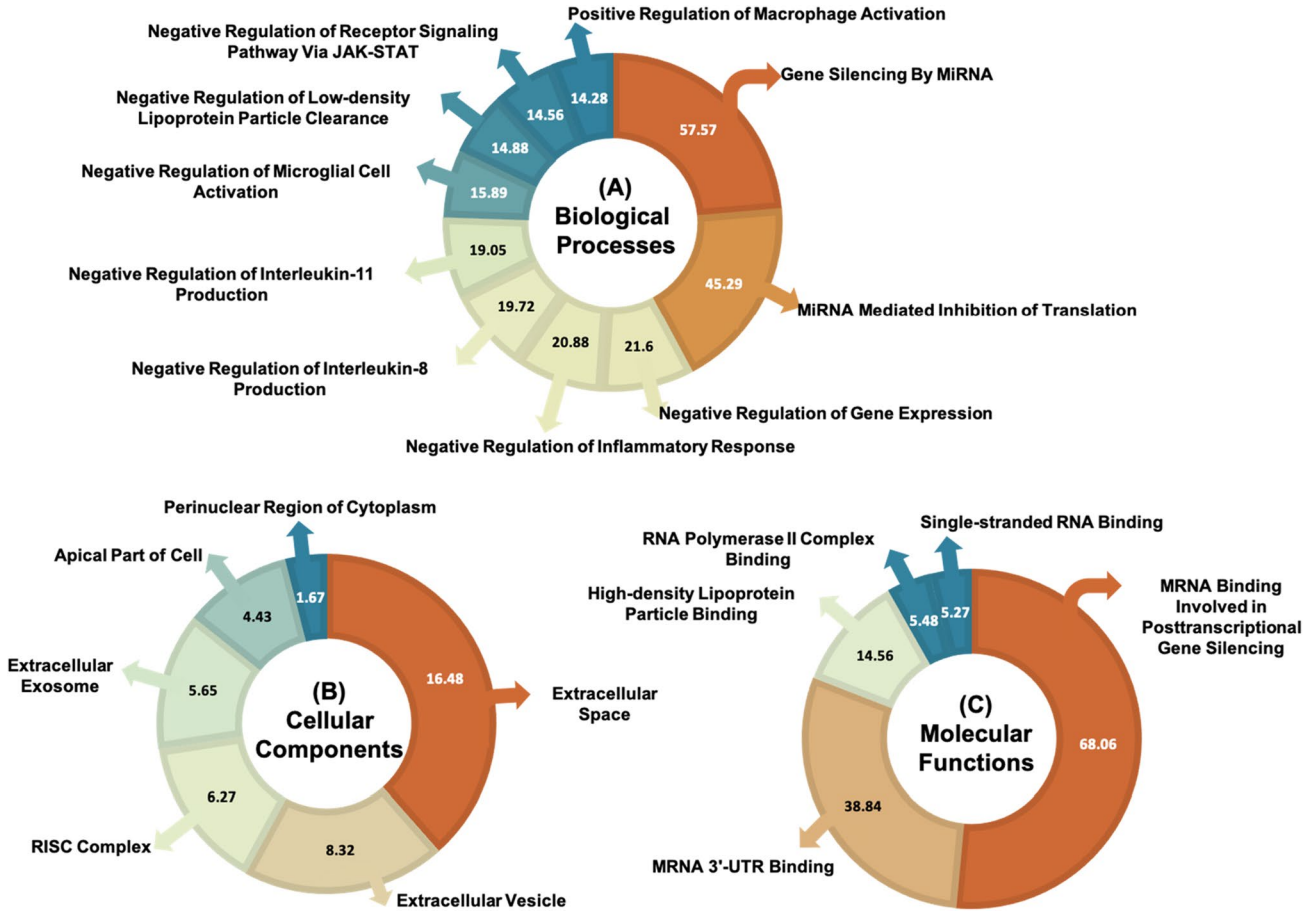

Fig. 6 "Gene ontology matching scores results for eleven immune-related miRNAs in autoimmune skin disease. **A** Biological processes in matching score. **B** Cellular component according to matching score. **C** Molecular function according to matching score. All scores were generated using gene analytics tool. (https://geneanalytics.genecards.org)". *MRNA* messenger RNA, *MiRNA* microRNA, *RISC* RNA-induced silencing complex, *UTR* untranslated region

of miR155 in the human keratinocyte line HaCaT cells significantly increased cells in the G0/G1 phase and decreased those in the G2/M phase [62], promoting cell differentiation rather than proliferation. In our study, circulating miRNA-155 was upregulated in psoriasis (Log_2FC 5.5) and was significantly higher in psoriasis than in vitiligo (Log_2FC 4.1) ($p = 0.004$). According to our findings, miRNA-155 has been identified as one of the top four priority miRNAs in both vitiligo and psoriasis. Taken together, miRNA-155 plays multiple roles in keratinocyte proliferation, apoptosis, melanogenesis, and immune cell regulation, making it a potential therapeutic choice in autoimmune skin disease.

The miRNA-145 circulating level has been shown to be downregulated in psoriasis and could be protective against psoriasis [67, 68]. MiRNA-145 inhibits keratinocyte proliferation by downregulating the *MLK3* gene via regulating *NF-kB* and *STAT-3* [68]. Moreover, miRNA-145 inhibits keratinocyte proliferation and stimulates apoptosis through inhibiting Wnt/β-catenin signaling, which is involved in psoriasis pathogenesis [69], implicating an inhibitory role for miRNA-145 in psoriasis [68]. This is consistent with our finding that miRNA-145 is directly involved in psoriasis pathogenesis and is of the highest predictive value among the 11 immune-related miRNAs under study (AUC 0.985, $p < 0.001$) in autoimmune disease. However, it contrasts with our data on circulating miRNA-145 level, which is significantly upregulated in psoriasis (Log_2FC 10.2) and is significantly higher in psoriasis compared with vitiligo (Log_2FC 0.2) ($p < 0.001$).

MiRNA-9 expression levels were shown to be upregulated in the serum of psoriasis patients and were positively correlated with disease extent and PASI [70] but downregulated in the skin of psoriasis patients [70, 71]. Interestingly, miRNA-9 has been shown to influence Th17 differentiation by inhibiting the expression of negative regulators of Th17 differentiation [72]. The serum data is consistent with our findings, where the circulatory miRNA-9 expression is upregulated in psoriasis patients (Log_2FC 8.0) and

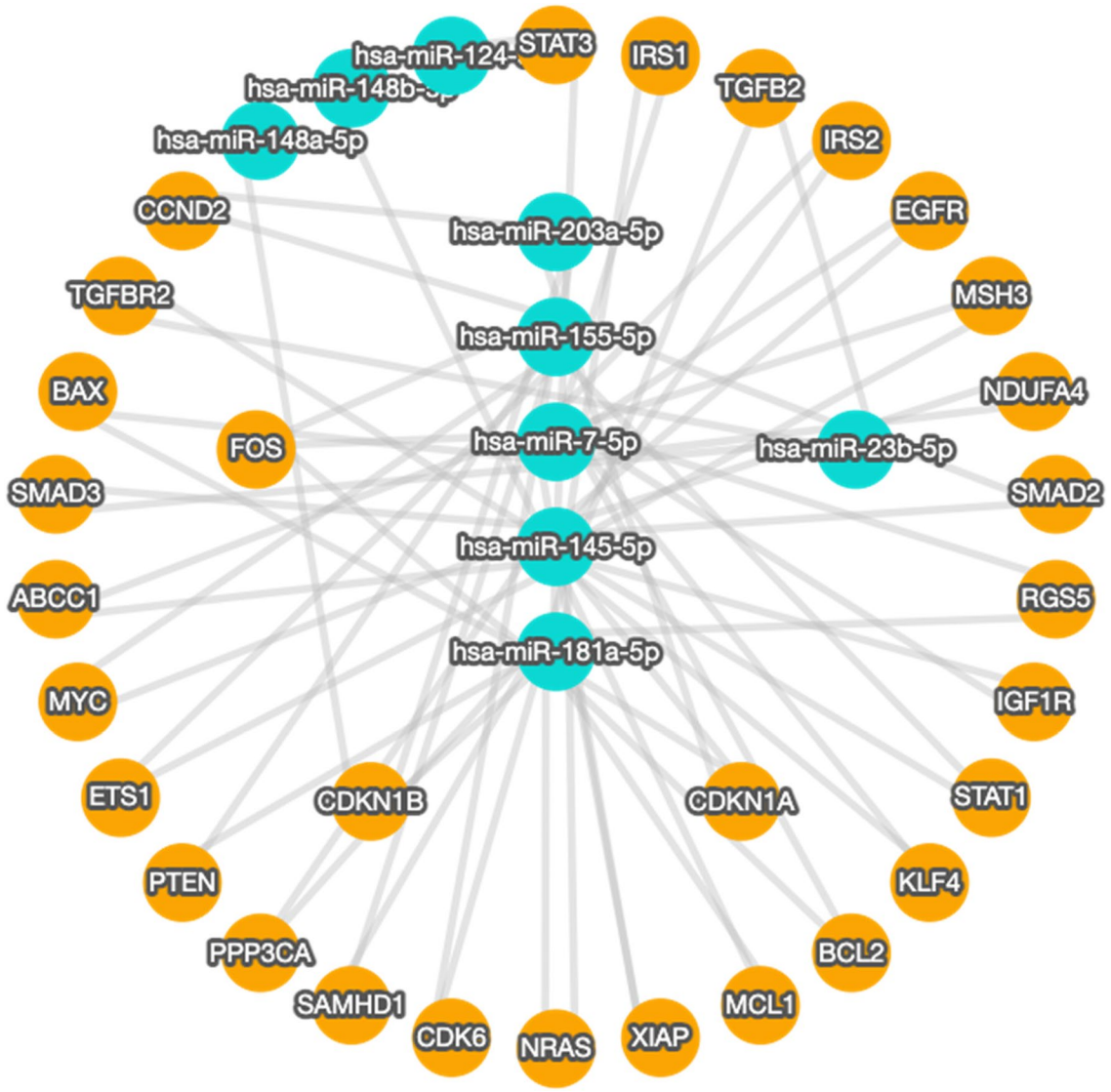

Fig. 7 "MiRNAs-target gene network analysis for eleven immune-related miRNAs in autoimmune skin disease. The targets used were the strongly validated miRNAs in the literature with an additional filter of minimum 2 shared targets using miRTargetLink 2.0 (https://ccb-compute.cs.uni-saarland.de/mirtargetlink2)"

is significantly higher in psoriasis compared with vitiligo (Log_2FC 3.1, $p < 0.001$). When we prioritized the 11 circulating immune-related miRNAs under study, miRNA-9 was in the top four in both psoriasis and vitiligo, directly involved in vitiligo and indirectly in psoriasis (Fig. 4). Moreover, miRNA-203a is one of the genes implicated with miRNA-9 in relation to psoriasis (Table 6); we have already explained its essential role in our previous work [73].

Collectively, this supports our data that miRNA-203 and miRNA-155 are directly involved in psoriasis pathogenesis (Fig. 4). On the other hand, the genes implicated with the miRNAs indirectly involved in psoriasis (Table 6) are related to chemokines (*CXCL8*), cytokines and/or autoimmunity genes (*NOD2, TNF, IL6, TNFRSF1A, IL4, IL1B, IFNG, IFIH1,* and *DDX58*), apoptosis (*Tp53*), and miRNAs associated with psoriasis such as miRNA-203a, miRNA-200a, miRNA-141, miRNA-30 and miRNA-LET-7e [74, 75].

In silico analysis of the 11 immune-related miRNAs in the studied autoimmune skin diseases (Figs 5, 6 and 7) revealed that the KEGG targeted pathways and GO biological processes, as well as the cellular components and target genes, have been consistently shown to be involved in psoriasis and vitiligo pathogenesis [53, 75–78]. Nevertheless, further research is necessary to identify the network of intricate interactions between each of these miRNAs and the molecules/genes/pathways involved in autoimmune disease in general and vitiligo and psoriasis in particular.

Finally, we emphasize that miRNAs are involved in the regulation of various cellular and physiological processes including adipose tissue development and metabolism, insulin secretion and function, and energy balance. Dysregulated miRNA expression, thus, has been incriminated in the pathogenesis of obesity and obesity-related diseases [79–81].

Our findings show that the studied miRNAs were inversely correlated with obesity and disease duration. Interestingly, several miRNAs evaluated in this study have been shown to be involved in obesity-related processes. miRNA-203 is a key regulator of the development of brown adipocytes [82] and is dysregulated in relation to diet [83]. miRNA-155 inhibits adipogenic differentiation and the development of the thermogenic program in brown adipocytes [83] and participates in the amplification of inflammatory status in adipocytes [81]. miRNA-9 regulates the release of insulin in pancreatic β-cells in rat and mouse models [79]. miRNA-124 is involved in pancreatic development, as well as in insulin expression and release [79]. miRNA-320 regulates insulin resistance in insulin-resistant adipocytes [79]. miRNA-148a, normally induced during adipogenesis, is downregulated in cells isolated from mice models of obesity [79]. The expression of miRNA-145 correlates with key metabolic parameters, including fasting plasma glucose, and circulating leptin and adiponectin levels [79], and is involved in adipocyte inflammatory responses via stimulating the expression of TNF-α in adipocytes [81]. Our findings may support the recent suggested connections between obesity and autoimmune disease [84]. Nevertheless, further research is necessary to establish the common signaling and genetic pathways involved.

4.3 Study Limitations

This study is mono-center, so selection bias cannot be disregarded. Moreover, our results are limited by the ethnicity of the study participants. Finally, further in vivo and in vitro research is necessary to examine the molecular relationships uncovered by in silico analysis. Therefore, larger multicenter clinical and functional studies are recommended.

5 Conclusion

This study highlights the critical role of miRNAs in skin-specific autoimmune diseases, namely psoriasis and vitiligo, through examining the expression repertoire of 11 circulatory immune-related miRNAs, which were found to be upregulated in both diseases, more so in psoriasis, and were correlated with various clinicopathological features of both, and proved to be potential biomarkers for autoimmune skin disorders, warranting their exploration as therapeutic targets.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40291-023-00646-1.

Declarations

Ethics approval and consent to participate Committee of Ethics approval no 5091 was granted from the Faculty of Medicine, Suez Canal University, Ismailia, Egypt, and the study was conducted according to the Declaration of Helsinki's guidelines.

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions AHY, EA and TNZ designed the study, TNZ collected the clinical data, SNH collected the patients' samples, AHY, ElA, KRM, FS, and SNH carried out the experiments, AHY, EA, KiRM, FS and SNH analyzed and interpreted the patient data. All authors discussed the results, contributed to the final manuscript and approved it.

Conflicts of interest statement Abdallah HY, Faisal S, Tawfik NZ, Soliman NH, Kishk RM, and Ellawindy A declare that they have no conflicts of interest that might be relevant to the contents of this manuscript.

Code availability Not applicable.

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