RESEARCH ARTICLE

MicroRNA profiling of psoriatic skin identifies 11 miRNAs associated with disease severity

Åshild Ø. Solvin MD[1] | Konika Chawla PhD[1,2,3] | Lene C. Olsen PhD[1,2,3] | Siv Anita Hegre PhD[1,2,3] | Kjersti Danielsen MD, PhD[4] | Marita Jenssen MD, PhD[4] | Anne-Sofie Furberg MD, PhD[5,6] | Marit Saunes MD, PhD[7] | Kristian Hveem MD, PhD[1,8] | Pål Sætrom PhD[1,2,3,9] | Mari Løset MD, PhD[1,7]

[1]K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

[2]Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

[3]Bioinformatics Core Facility, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

[4]Department of Dermatology, University Hospital of North Norway, Tromsø, Norway

[5]Faculty of Health Sciences and Social Care, Molde University College, Molde, Norway

[6]Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway

[7]Department of Dermatology, Clinic of Orthopaedy, Rheumatology and Dermatology, St. Olavs Hospital Trondheim University Hospital, Trondheim, Norway

[8]HUNT Research Centre, Department of Public Health and Nursing, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

[9]Department of Computer Science, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

Correspondence
Mari Løset, K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, NTNU, Norwegian University of Science and Technology, Postboks 8905, N-7491 Trondheim, Norway.
Email: mari.loset@ntnu.no; mari.loset@stolav.no

Abstract

MicroRNAs (miRNAs) are small non-coding RNAs that have emerged as central regulators of gene expression and powerful biomarkers of disease. Much is yet unknown about their role in psoriasis pathology. To globally characterize the miRNAome of psoriatic skin, skin biopsies were collected from psoriatic cases ($n = 75$) and non-psoriatic controls ($n = 46$) and RNA sequenced. Count data were meta-analysed with a previously published dataset (cases, $n = 24$, controls, $n = 20$), increasing the number of psoriatic cases fourfold from previously published studies. Differential gene expression analyses were performed comparing lesional psoriatic (PP), non-lesional psoriatic (PN) and control (NN) skin. Further, functional enrichment and cell-specific analyses were performed. Across all contrasts, we identified 439 significantly differentially expressed miRNAs (DEMs), of which 85 were novel for psoriasis and 11 were related to disease severity. Meta-analyses identified 20 DEMs between PN and NN, suggesting an inherent change in the constitution of all skin in psoriasis. By integrating the miRNA transcriptome with mRNA target interactions, we identified several functionally enriched terms, including "thyroid hormone signalling," "insulin resistance" and various infectious diseases. Cell-specific expression analyses revealed that the upregulated DEMs were enriched in epithelial and immune cells. This study provides the most

comprehensive overview of the miRNAome in psoriatic skin to date and identifies a miRNA signature related to psoriasis severity. Our results may represent molecular links between psoriasis and related comorbidities and have outlined potential directions for future functional studies to identify biomarkers and treatment targets.

KEYWORDS
biopsy, inflammation, microRNAs, psoriasis, RNA-seq

1 | INTRODUCTION

Psoriasis is a complex disease, triggered by environmental factors in genetically susceptible individuals. The prevalence is substantially higher in the Nordic countries[1-3] compared to global estimates.[4] Genomic studies have established psoriasis as a Th-17/IL-23-dominated disease[5] and guided development of novel treatments.[5] However, the exact pathophysiological mechanisms are unknown, and the regulatory mechanisms of psoriasis genetics have not yet been fully explained.

MicroRNAs (miRNAs) are non-coding RNAs that contribute to post-transcriptional downregulation of gene expression by repressing or degrading their target messenger RNAs (mRNAs).[6] More than 60% of human mRNAs contain evolutionary conserved miRNA target sites, suggesting that miRNAs can have a profound impact on cellular states and disease phenotypes.[7] The first miRNAs associated with psoriasis were published in 2007.[8] With increasing sample sizes, advancing technologies and analytical methods, several differentially expressed miRNAs (DEMs) have since been identified in psoriasis.[8-15] Targeted characterization has shown that many of these DEMs play central roles in regulating cellular functions, including regulatory roles of miR-125b in epidermal differentiation[16] and miR-21 in T-cell apoptosis.[17] Further, several DEMs have been shown to have central roles in psoriasis pathophysiology, including miR-31 which increases production of tumor necrosis factor (TNF)[18] and miR-21 which leads to chronic inflammation in patients with psoriasis.[18] In addition, specific miRNAs show promise as sensitive biomarkers for psoriasis disease activity, prognosis and treatment response,[19] as well as targets for epigenetic-modifying therapies.[20] Examples include miR-146a whose levels correlate with increasing psoriasis severity.[21] However, previous studies have involved a relatively small number of skin samples, some did not include a control group and the majority have employed microarrays rather than next-generation sequencing. For the study with the largest reported sample size to date (psoriatic cases, n = 24),[11] we found the reproducibility of the reported miRNAs unclear, most likely due to their choice of statistical approach, employing a chi-square goodness-of-fit test.

To comprehensively profile the psoriasis miRNAome, we used high-resolution RNA sequencing (RNA-seq) of a large number of lesional psoriatic (PP), non-lesional psoriatic (PN) and control (NN) skin from 121 individuals (cases, n = 75; controls, n = 46). To test the reproducibility of our results and to increase the power to detect novel DEMs, we did a robust statistical re-analysis of the largest dataset published to date (GSE31037)[11] and meta-analysed this with our data. The meta-analysis included 99 psoriatic cases and 66 non-psoriatic controls, increasing the number of psoriatic cases fourfold from previously published studies on miRNAs in psoriasis.

2 | MATERIALS AND METHODS

2.1 | Participants and skin samples

Participants were adults of European descent. Cases had psoriasis vulgaris diagnosed by a dermatologist and underwent a wash-out period without any topical or systemic-/phototherapy for 2 and 4 weeks prior to participation, respectively. Controls did not have psoriasis or any other inflammatory skin disease, nor any first-degree relatives with psoriasis. Participants were excluded if they had used tanning beds 4 weeks prior to participation. Disease severity was assessed using Psoriasis Area and Severity Index (PASI)[22] by a dermatologist or specially trained MD/PhD Candidate. From cases, two 4 mm skin biopsies were collected: one PP and one PN. PP was collected from the outer rim of a plaque, in the area which appeared most inflamed macroscopically. From controls, one 4 mm skin biopsy NN was collected. When possible, the PN and NN samples were taken from a sun-protected area on the buttock and PN was taken at least 10 cm away from any visible plaque. Biopsies were snap frozen in liquid nitrogen bedside and later stored at −80°C. Written informed consent was obtained from all participants. The study was approved by the Regional Committee for Medical and Health Research Ethics in Mid-Norway (27396) and conducted according to the Declaration of Helsinki principles.

2.2 | Total RNA extraction, library preparation and RNA sequencing

Skin biopsies were homogenized using Precellys homogenizer (Bertin Technologies), and RNA was extracted using mirVana Isolation Kit (Applied Biosystems). To mitigate potential batch effects, samples were RNA isolated and sequenced in a randomized order. Sequencing libraries were prepared using the NEXTflex small RNA-seq kit v3 (Bioo Scientific/PerkinElmer). Fragments/libraries were run on a Labchip GX (Caliper/PerkinElmer), for quality control and

quantitation. Individual libraries were normalized and pooled. The library pool was later purified with the QIAquick PCR Purification Kit (Qiagen AB) and evaluated on Bioanalyzer (Agilent Technologies) according to instructions. The mean RNA integrity number (RIN) across all samples was 8.2 (standard deviation 1.2). We did not apply a specific RIN value cut-off threshold, as miRNA expression is highly stable despite tissue degradation.[23,24] The pool of libraries was quantified with the KAPA Library Quantification Kit (Roche) and subject to clustering by a cBot Cluster Generation System on a HiSeq4000 flow cell (Illumina, Inc.). Single-end read sequencing was performed on an Illumina HiSeq4000 instrument, in accordance with the manufacturer's instructions (Illumina, Inc.).

2.3 | Differential expression analysis

Adapter sequences were removed from the raw data using cutadapt,[25] and reads with Phred quality score of less than 33 were filtered out using fastx_collapser. The processed reads were aligned to the human genome GRCh38, and htseq-count[26] was used to annotate the reads to distinct gene types in GRCh38.92 and RNAcentral-9.0 (Figure S1). The reads were then mapped to human miRNAs in the miRBase database v22[27] with the miraligner tool.[28] The count matrix was prepared with number of reads mapping to each miRNA per sample and was transformed using the voom algorithm from the limma package of Bioconductor.[29,30] MicroRNAs with an average count per million across all samples of less than one were filtered out. This normalized count matrix of 526 expressed miRNAs is deposited in the Gene Expression Omnibus (GEO) repository (GSE174763). We identified DEMs in our samples (henceforth; the NTNU dataset) across three contrasts (PP versus NN, PP versus PN and PN versus NN) using a linear model adjusted for sex, age and BMI. The Benjamini-Hochberg method[31] was used to control the false discovery rate (FDR), and miRNAs with FDR <0.05 were considered significantly differentially expressed. We reanalysed the hitherto largest published miRNA dataset in psoriatic skin (NCBI GEO accession number: GSE31037) (henceforth; the Joyce dataset).[11] To enable comparison of statistical approaches, we applied the same statistical approach on the Joyce dataset as on the NTNU dataset, before combining the two datasets for a meta-analysis. This meta-analysis included an additional factor estimating expression differences between the two datasets in the linear model. To determine whether any miRNAs were related to psoriasis severity, cases were separated into PASI <10 and PASI ≥10 before differential expression analysis.

2.4 | Quantitative RT-PCR (qRT-PCR)

For technical validation of the RNA-seq data, we did quantitative RT-PCR (qRT-PCR) on four selected DEMs: miR-664b-3p, miR-103a-3p, miR-374a-5p and miR-10a-5p. The four DEMs were prioritized based on novelty, association with PASI, fold change (FC), FDR values and manual literature searches. Total RNA was reverse transcribed using TaqMan Advanced miRNA cDNA synthesis kit (Applied Biosystems, A28007) followed by qRT-PCR using TaqMan Fast Advanced master mix (Applied Biosystems, 4444557) and TaqMan Advanced miRNA assays (Applied Biosystems, A25576, Assay IDs 479148_mir, 478253_mir, 478238_mir and 479241_mir). Quantification was done using the Step One Real-time PCR system (Applied Biosystems). The relative expression of miRNAs was calculated using the ddCt method[32] with let-7b-5p (Assay ID 478576_mir) as an endogenous control. let-7b-5p was chosen as an endogenous control as it was not differentially expressed in the RNA-seq data, was highly expressed and had low variation across all samples. The results were presented as $\log_2FC$ values ± standard error of mean (SEM). Differences between sample groups were estimated using the two-tailed Student's *t* test, assuming equal variances. A value of $p < 0.05$ was considered statistically significant. Plots were made using the R packages gglot2 and ggpubr.

2.5 | Target prediction and functional enrichment analysis

Target genes for the up- and downregulated DEMs with FDR <0.05 and average RPM >100 were identified using the TargetScan database.[33] Functional enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) terms was performed using the gprofiler2 package in R.[34] DEMs associated with psoriasis severity were analysed for enrichment of Reactome terms[35] using the online tool miRnet (www.mirnet.ca).[36,37]

2.6 | Cell and tissue-specific expression analysis

The FANTOM5 atlas[38] was used to determine which cells express the top 10 up- and downregulated DEMs by FC in the meta-analysis, both overall and after PASI stratification. The FANTOM5 miRNA atlas is based on a comprehensive range of human samples, and each miRNA has been annotated as cell type enriched and depleted based on its expression profile in different cell types.[38] FANTOM5 data were also used to do cell-specific functional enrichment analysis. Specifically, for each cell type among a set of selected FANTOM5 cell types, DEMs with RPM >100 for that cell type were identified and target prediction and KEGG enrichment analyses were then done for these DEMs.

3 | RESULTS

A total of 75 psoriatic cases and 46 controls were enrolled in the study (cases: mean age 52.7, 44% women; controls: mean age 38.9, 50% women); clinical characteristics are given in Table S1. We isolated and sequenced small RNAs from the samples and the resulting libraries were highly enriched for miRNAs (Figure S1). Individual

participant and sample characteristics are presented in Table S2. Multidimensional scaling of the miRNA expression profiles revealed overlap between PN and NN, with PP clearly separated from PN and NN (Figure 1A). For comparison, we reanalysed the small RNA sequencing data from Joyce et al.[11] Despite major differences in the overall miRNA expression profiles between the two datasets, both showed consistent separation between PP and non-lesional (PN, NN) samples (Figure 1A).

Focusing on annotated DEMs, we then identified DEMs between the three sample groups. In PP/NN, we identified 375 DEMs in the NTNU dataset, 231 DEMs in the Joyce dataset and 395 DEMs in the meta-analysis (FDR <0.05, abs(log$_2$FC) >0) (Table 1, Figure 1B,C, Tables S3–S5). In PP/PN, we identified 415, 225 and 401 DEMs in the NTNU, Joyce and meta-analysis datasets, respectively. In PN/NN, we identified 4 DEMs in the NTNU dataset and none in the Joyce dataset. However, when the data were pooled for the meta-analysis, 20 DEMs were identified (Figure 1D,E, Table S5). Table 2A–C shows the top 10 DEMs for each contrast in the meta-analysis. Technical

validation by qRT-PCR analysis of four miRNAs (miR-664b-3p, miR-103a-3p, miR-374a-5p and miR-10a-5p) showed a high degree of correlation with the RNA-seq data (Pearson's correlation coefficient $r = 0.997$, $p = 0.003$; Figure S2).

The reproducibility of our results was further high as (1) the shared DEMs in the NTNU and Joyce datasets were highly correlated (Pearson's correlation coefficient $r = 0.89$ for PP/NN, $r = 0.87$ for PP/PN) and (2) these shared DEMs (Figure 1F) where largely consistently expressed, as only 4 of 155 DEMs has opposite log$_2$FC signs between the two datasets. Intersecting the DEMs showed substantial overlap between datasets (Figure 1F). A total of 28 DEMs in PP/NN were exclusively identified in the meta-analysis, and many of these were among the DEMs with the lowest log$_2$FC (Figure S3, Table S6). To identify annotated miRNAs which had not previously been associated with psoriasis, we compared our results from the meta-analysis to previously published studies.[8–12,14,15,21,39,40] In total, we identified 85 miRNAs novel for psoriasis (Table S7), corresponding to 19.4% of DEMs identified in the meta-analysis.
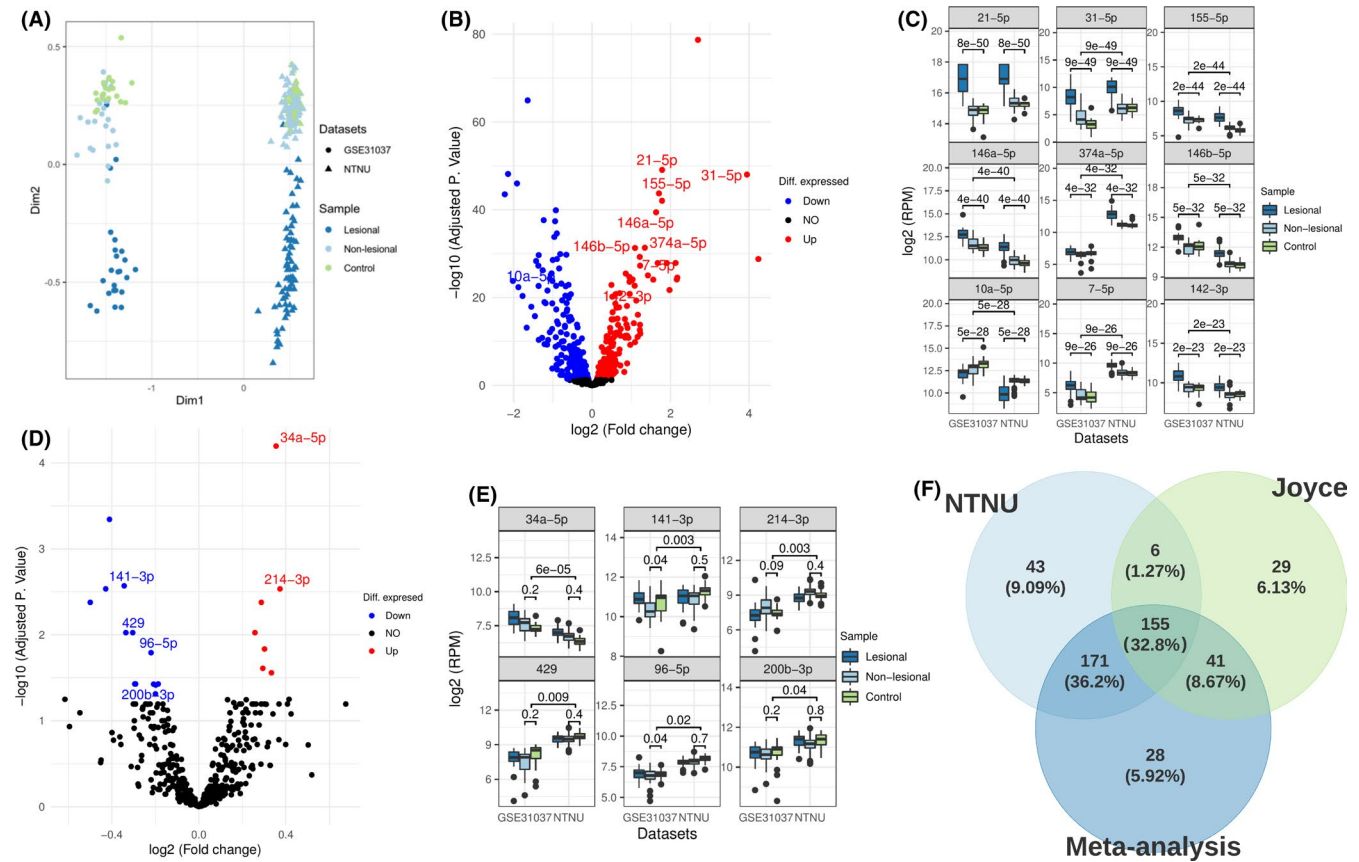

FIGURE 1 **Differentially expressed miRNAs across the three contrasts lesional psoriatic (PP), non-lesional psoriatic (PN) and control (NN) skin**. Multidimensional scaling (MDS) plot of lesional (PP), non-lesional (PN) and control (NN) samples in the NTNU (triangles) and Joyce (GSE31037, circles) data. Differences between the two datasets are the prime source of variation in the combined data (Dim1). Differences between PP samples and PN and NN samples are the second dominant source of variation (Dim2), and these differences are consistent between the NTNU and Joyce data **(A)**. Volcano plot of miRNAs in PP/NN (meta-analysis) **(B)**. Box plot of expression levels of the nine most highly expressed DEMs in PP/NN **(C)**. Volcano plot of miRNAs in PN/NN (meta-analysis) **(D)**. Box plot of expression levels of the six DEMs in PN/NN (meta-analysis) with reads per million (RPM) >100 in the NTNU and Joyce (GSE31037) datasets with *p*-values given for both separate and meta-analysis. These DEMs had small, but consistent expression differences between PN and NN across the datasets **(E)**. Venn diagram showing overlap of the DEMs between datasets for PP/NN at FDR <0.05. A total of 155 (33%) DEMs were identified in all three datasets **(F)**

Contrast	Number of differentially expressed miRNAs		
	NTNU	Joyce	Meta-analysis
PP/NN	375	231	395
PP/PN	415	225	401
PN/NN	4	0	20

Abbreviations: NN, healthy control skin; PN, non-lesional psoriatic skin; PP, lesional psoriatic skin.

To gain biological insight into the processes influenced by the identified DEMs, we performed target prediction analysis to identify genes which are possible targets of the DEMs. Targets of the miRNAs with average expression ≥100 reads per million (RPM) and FDR <0.05 for PP/NN and PN/NN in the meta-analysed data set were identified using TargetScan.[33] This threshold was chosen as most miRNAs below this threshold show no functional activity.[41,42] Results from PP/PN are not presented as they were similar to PP/NN. Predicted targets in PP/NN were enriched for several terms (Figure 2A), including "thyroid hormone signalling pathway," "insulin resistance," "human papillomavirus infection" and "melanogenesis." The most significantly enriched term for PP/NN and PN/NN was "microRNAs in cancer." To evaluate which cells that express the top 10 up- and downregulated DEMs by FC in PP/NN and PN/NN, we extracted cell-specific data from the FANTOM5 atlas.[38] In PP/NN, DEMs were enriched in fibroblasts and epithelial cells, as well as in immune cells including monocytes and leukocytes (Figure 2B). In PN/NN, several of the DEMs were enriched in epithelial and endo-epithelial cells (Figure 2B). Cell-specific KEGG enrichment analysis showed that the enriched terms for each cell type were largely overlapping with the enriched terms for non–cell-specific DEMs (Figure 2A,C). There was no clear separation of enriched terms between the different cell types.

To identify a severity-specific miRNA signature, we dichotomized the cases into PASI <10 and PASI ≥10. We chose this threshold as PASI is a widely used instrument for clinical severity assessment.[43] Furthermore, PASI ≥10 often requires second-line treatments including phototherapy or systemic therapy.[44] For this analysis, we compared PP in PASI ≥10 to PP in PASI <10, as well as PN in PASI ≥10 compared to PN in PASI <10. Multidimensional scaling of the PP and PN samples showed non-lesional samples separated from lesional samples and a greater spread for PASI <10 than PASI ≥10 samples (Figure 3A). In total, we identified 11 DEMs, of which five were dysregulated in PP skin and six were dysregulated in PN skin (Figure 3B–E). In PP skin, all five miRNAs were upregulated, while in PN skin, four miRNAs were upregulated and two were downregulated (Table S8). The targets of the upregulated DEMs in PP and PN skin were enriched for inflammatory pathways including "IL1-signalling," "transforming growth factor beta (TGFβ)-receptor complex," "toll-like receptor (TLR) cascades" and "signalling by interleukins." (Table S9). The targets of the downregulated DEMs were

enriched in mRNA processing pathways including splicing and translation (Table S9). miR-374a and miR-146a were both upregulated in PP skin, and these were enriched in myeloid leukocytes and hematopoietic cells (Figure 3F). All DEMs in PN were depleted in leukocytes and hematopoietic cells.

4 | DISCUSSION

In this study, we explored the miRNAome in psoriatic skin by analysing RNA-seq data from 99 psoriatic cases and 66 controls, increasing the number of psoriatic cases fourfold from previous work. By improving statistical power due to a large sample, we identified 439 DEMs, of which 85 were novel for psoriasis and 11 were related to psoriasis severity. Further, we identified 20 DEMs between psoriatic non-lesional (PN) and healthy control skin (NN). This suggests an inherent change in all psoriatic skin, supporting the previously shown "pre-inflammatory" signature outside established plaques for protein-coding RNAs.[45] We identified enrichment of terms representing potential disease pathology (eg "Wnt-" and "Ras signalling") and established or emerging comorbidities (eg "insulin resistance" and "thyroid hormone signalling").

Expression levels of several previously identified DEMs in psoriatic skin, including miR-21, miR-31 and miR-146a,[21] were reproduced in this study. miR-21, overexpressed in PP/NN, regulates keratinocyte proliferation and differentiation,[19] and inhibition of this miRNA has been shown to ameliorate psoriasis pathology in a murine model.[46] miR-31 has been shown to increase keratinocyte proliferation and differentiation[21,47] and modulates production of inflammatory cytokines in keratinocytes.[48] miR-146a has been shown to modulate innate immune response through regulation of the NF-kB pathway[49] and is positively correlated with PASI and serum IL-17 levels.[21,50] Further, miR-146a-deficient mice exposed to imiquimod have more severe inflammation than their mir-146a-positive counterparts, and delivery of the miRNA leads to improvement of the skin inflammation.[51] miR-128a, also highly upregulated in PP/NN, is a regulator in Wnt- and IL-23 signalling and is upregulated in peripheral blood neutrophils of patients with diffuse cutaneous systemic sclerosis.[52] miR-128a also targets genes involved in insulin signalling and may mediate some of the increased risk of insulin resistance and obesity in psoriasis.[53] miR-197 was downregulated in accordance with previously published works.[8,54] This miRNA has been shown to regulate signalling by IL-17[55] and IL-22,[56] important inflammatory mediators in psoriasis. Among the DEMs novel for psoriasis, miR-103a was upregulated. Increased serum levels of this miRNA have been suggested to predict development of rheumatoid arthritis, an inflammatory disease of the joints.[57]

The DEMs identified in PN/NN reflect inherent changes in all psoriatic skin and could thereby play important roles in disease pathology. As psoriasis is no longer thought of as a localized skin disease, but rather a systemic inflammatory disorder,[58] alterations in macroscopically non-inflamed skin may represent effects of systemic immune system dysregulation. Two of the upregulated

miRNA	$\log_2$FC	p-value	FDR	$\log_2$ average expression
A)				
miR-31-3p	4.238	7×10^{-31}	2×10^{-29}	0.147
miR-31-5p	3.948	9×10^{-51}	9×10^{-49}	6.980
miR-21-3p	2.697	4×10^{-82}	2×10^{-79}	5.294
miR-6510-3p	−2.227	5×10^{-46}	3×10^{-44}	4.296
miR-1268a	2.171	2×10^{-26}	3×10^{-25}	0.486
miR-1268b	2.157	6×10^{-26}	8×10^{-25}	0.509
miR-885-5p	−2.144	5×10^{-51}	7×10^{-49}	1.406
miR-940	2.129	6×10^{-30}	1×10^{-28}	−0.235
miR-3617-5p	−2.020	1×10^{-25}	2×10^{-24}	−0.280
miR-33b-3p	1.972	2×10^{-23}	2×10^{-22}	0.023
B)				
miR-31-5p	3.799	1×10^{-57}	2×10^{-55}	6.980
miR-31-3p	3.719	8×10^{-33}	2×10^{-31}	0.147
miR-21-3p	2.603	6×10^{-91}	3×10^{-88}	5.294
miR-3617-5p	−2.068	2×10^{-30}	3×10^{-29}	−0.280
miR-135b-5p	1.949	8×10^{-59}	1×10^{-56}	5.553
miR-33b-3p	1.944	2×10^{-30}	3×10^{-29}	0.023
miR-940	1.913	4×10^{-34}	9×10^{-33}	−0.235
miR-4517	1.906	6×10^{-38}	2×10^{-36}	0.005
miR-664b-3p	−1.899	3×10^{-54}	3×10^{-52}	2.203
miR-885-5p	−1.853	2×10^{-46}	1×10^{-44}	1.406
C)				
miR-6510-3p	−0.499	5×10^{-5}	0.004	4.296
miR-598-3p	−0.428	2×10^{-5}	0.003	3.370
miR-450a-5p	−0.411	2×10^{-6}	5×10^{-4}	4.601
miR-214-3p	0.373	3×10^{-5}	0.003	8.638
miR-34a-5p	0.355	1×10^{-7}	6×10^{-5}	6.972
miR-141-3p	−0.343	2×10^{-5}	0.003	10.921
miR-429	−0.336	2×10^{-4}	0.009	9.072
miR-574-5p	0.334	0.001	0.028	5.132
miR-542-3p	−0.304	2×10^{-4}	0.009	5.725
miR-485-3p	0.302	3×10^{-4}	0.015	2.531

Abbreviations: FC, fold change; NN, healthy control skin; PN, non-lesional psoriatic skin; PP, lesional psoriatic skin.

FIGURE 2 **Functional enrichment and cell-specific expression analyses for differentially expressed miRNAs (DEMs) PP/NN and PN/NN in the meta-analysis.** Dot plot of the most significantly enriched KEGG pathways among the targets of up- and downregulated DEMs **(A)**. Primary cell expression analysis using the FANTOM5 atlas for the top 10 DEMs. The miRNAs with an asterisk* were analysed based on pre-miRNA, as the mature miRNA was not annotated **(B)**. Enrichment analysis of DEMs by cell type showed a large overlap of enriched terms for up- and downregulated DEMs. Colours on the bottom bar indicate cell type, and colour on second bottom bar indicates direction of DEM regulation. Cell types on the x-axis are clustered based on the similarity in the KEGG terms. Certain terms were only enriched for up- and downregulated DEMs, including "epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance," "thyroid hormone signalling pathway" and "T-cell receptor signalling pathway." Terms enriched in leukocytes were more significant as a group than the other cell types **(C)**. Abbreviations: Wnt, wingless-related integration site; Rap1, Ras-related protein 1; PI3K-Akt, phosphoinositide-3 kinase-akt; mTOR, mechanistic target of rapamycin; MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor; TGF, transforming growth factor; JAK-STAT, janus kinase-signal transducer and activator of transcription; FoxO, forkhead box transcription factor class O; ECM, extracellular matrix; cAMP, cyclic adenosine monophosphate; AMPK, AMP-activated protein kinase; AGE-RAGE, advanced glycation end products-receptor for advanced glycation end products; CD, cluster of differentiation; OR, odds ratio; miRNA, microRNA; GAG, glycosaminoglycan
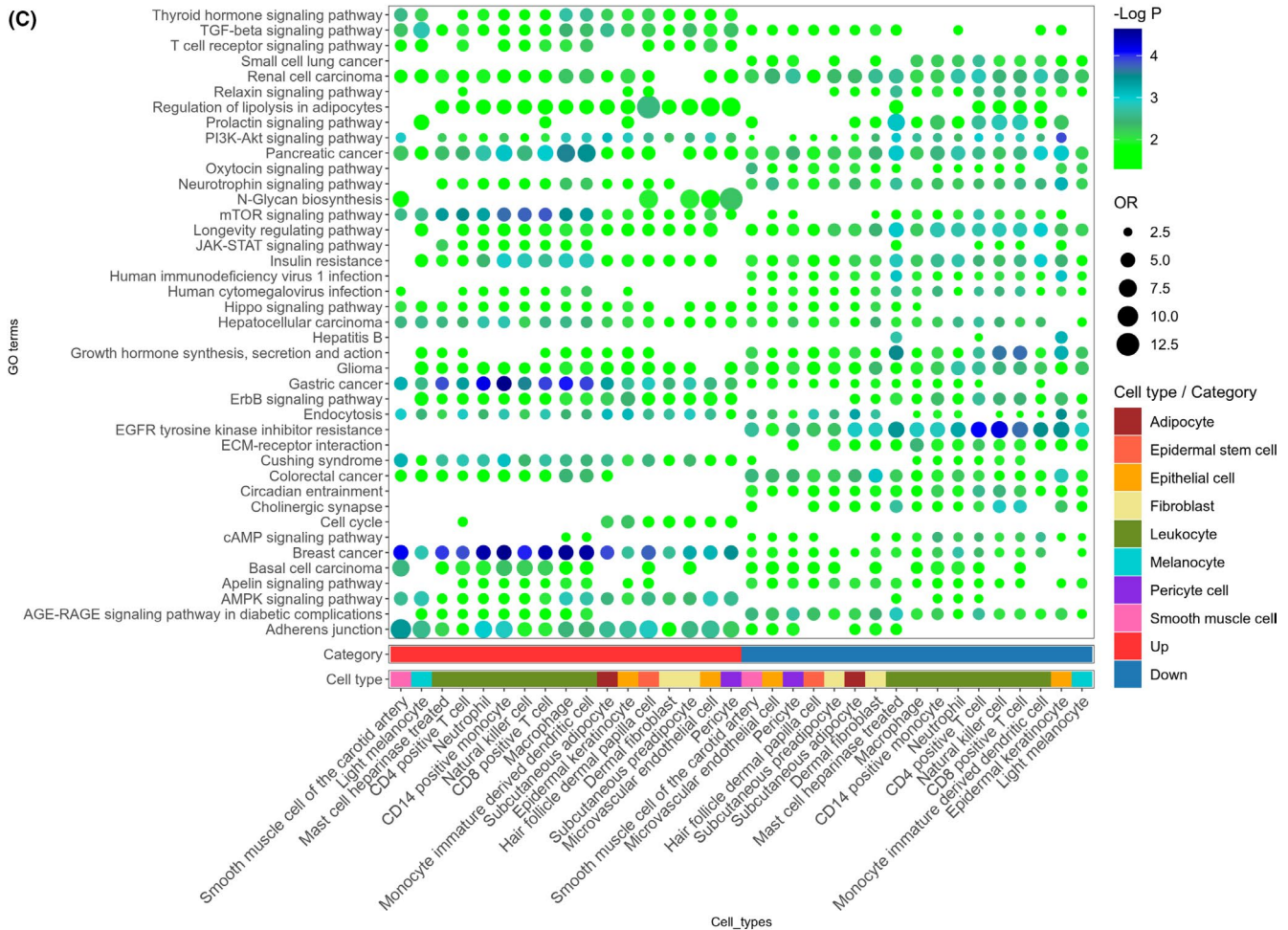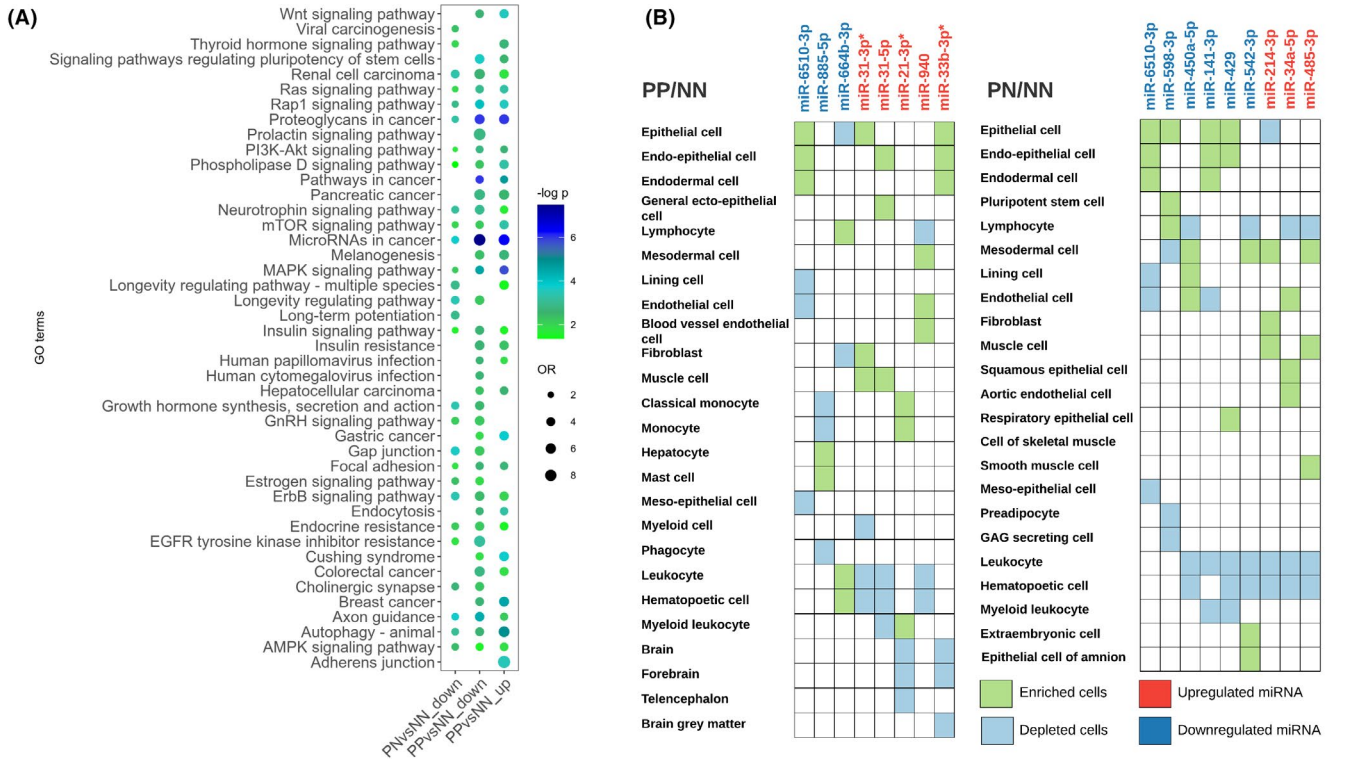
(A)

(B)

(C)

(A) PCA plot showing Dim1 vs Dim2 with PASI scores (PASI < 10, PASI > 10) and Sample types (Lesional, Non-lesional).

(B) Volcano plot of −log10 (Adjusted P. Value) vs log2 (Fold change); Diff. expressed: NO, Up. Labeled: 374a-5p, 10a-5p, 146a-5p, 202-5p, 10a-3p.

(C) Boxplots of log2 (RPM) for 10a-3p, 10a-5p, 146a-5p, 202-5p, 374a-5p by PASI>10, PASI<10, Control; Sample: Lesional, Non-lesional, Control.

(D) Volcano plot of −log10 (Adjusted P. Value) vs log2 (Fold change); Diff. expressed: Down, NO, Up. Labeled: 196b-5p, 196a-5p, 641, 615-3p, 508-3p, 509-3p.

(E) Boxplots of log2 (RPM) for 196a-5p, 196b-5p, 508-3p, 509-3p, 615-3p, 641 by PASI>10, PASI<10, Control; Sample: Lesional, Non-lesional, Control.

(F) Heatmap of Enriched cells / Depleted cells and Upregulated miRNA / Downregulated miRNA for miRNAs across cell types (PP, PN).

FIGURE 3 Differentially expressed miRNAs (DEMs) associated with disease severity identified by comparing PASI≥10 to PASI<10 in the NTNU dataset. Multidimensional scaling plot of PP and PN skin in PASI ≥10 and PASI <10 skin samples (A). Volcano plot of DEMs in PP skin comparing PASI ≥10 to PASI <10 samples (B). Box plot of expression levels of the five DEMs identified in PP skin (C). Volcano plot of DEMs in PN skin comparing PASI ≥10 to PASI <10 samples (D). Box plot of expression levels of the six DEMs identified in PN skin (E). Primary cell expression analysis using the FANTOM5-atlas for the DEMs identified in PASI ≥10 to PASI <10 samples. The miRNAs with an asterisk* were analysed based on pre-miRNA, as the mature miRNA was not annotated (F). Abbreviations: PASI, Psoriasis Area and Severity index; GAG, glycosaminoglycan; miRNA, microRNA

miRNAs, miR-34a and miR-214, target common genes (Figure S4) involved in cell cycle regulation and apoptosis. miR-34a is increased in the serum of patients with type 2 diabetes[59] and has also been shown to promote keratinocyte inflammation.[60] Interestingly, three of the downregulated DEMs in PN/NN (miR-429, miR-200b and miR-141) are part of the miR-200 family. This miRNA family is highly expressed in skin and is central for skin homeostatic functions.[61] miR-511-5p, a novel miRNA for psoriasis identified in PN/NN, has been suggested to influence differentiation and activation of myeloid cells and is also affected by endogenous levels of glucocorticoids,[62] a well-known inductor of the components of metabolic syndrome, including insulin resistance, dyslipidaemia and obesity.[63] Our PN/NN results support the finding of an inflammatory and proliferative response in skin outside of established plaques.[45]

Enrichment analysis identified terms involved in well-known disturbed functions in psoriasis pathology, including functions crucial for epidermal- and stem cell behaviour and development ("Wnt-" and "Ras signalling").[64,65] Further, some of the identified pathways may represent shared mechanisms for psoriasis and related comorbidities, including thyroid hormone signalling and insulin resistance. Increased levels of thyroid hormones have been identified in individuals with psoriasis,[66] and altered levels of thyroid hormones lead to increased levels of epidermal growth factor, driving keratinocyte proliferation and differentiation.[18,67] miR-142-3p/-5p and miR-146a are upregulated in Hashimoto's thyroiditis[68] and were also upregulated in PP/NN. Insulin resistance, a prominent feature of obesity, metabolic syndrome and diabetes is also seen in patients with psoriasis,[18] and an association between insulin resistance and chronic systemic inflammation has long been recognized.[69] Suggested mechanisms include the induction of proinflammatory cytokines in adipose tissue[70] and proinflammatory cytokines shared between diabetes and psoriasis.[18] Decreased serum levels of miR-126, miR-197, miR-320 and miR-486 have been highlighted in type 2 diabetes,[71] and these were also downregulated in PP/NN. In PN/NN, the targets were enriched for pathways important for proliferation and inflammation, including "MAPK-signalling" and "ErbB-signalling," demonstrating changes in all skin of psoriatic patients. The mTOR signalling pathway was enriched in all contrasts. This pathway has been demonstrated to be important in wound healing and is affected by the miR-99 family.[72] miR-99a has been shown to regulate expression of *IGF-1R* and drives differentiation of keratinocytes in psoriatic skin.[54] Further, the downregulated miRNAs in PN/NN and PP/NN were enriched for "axon guidance." Axonal function has been recognized as an important factor in pruritus,[73] a symptom of psoriasis often overlooked. The target prediction analyses are based on

statistically enriched sequence patterns in 3′ UTRs for genes that are down- and upregulated in experiments where individual miRNAs are, respectively, up- and downregulated. We acknowledge the limitations applying this method, as the relationship between miRNA expression and mRNA repression is not linear and depends on a variety of factors including sequence complementarity and alternative polyadenylation.[33,74] As such, the in vivo effects of endogenous miRNAs are difficult to predict reliably and predicted biological enrichments should be interpreted with caution.

In this study, we obtained full-thickness skin biopsies, containing a mixture of cell types, including keratinocytes, fibroblasts and immune cells. By extracting data from the FANTOM5 atlas,[38] we aimed to dissect the cell-specific origin of the DEMs. The top DEMs in PP/NN were enriched in cell types important for skin structure (epithelial cells and fibroblasts) and immune cells (monocytes and leukocytes). We further aimed to elucidate the functions of the DEMs by cell type. Insulin resistance was enriched for almost all cell types, supporting the hypothesis of shared inflammatory pathways as a tie between psoriasis and diabetes.[53] Insulin resistance is also a risk factor for metabolic syndrome and cardiovascular disease, and systemic inflammation has been proposed as a link between psoriasis and these diseases.[53] We acknowledge the limitations of using the FANTOM5 atlas as it is based on a limited set of non-inflamed skin samples. Further, our cell-specific analyses focused on highly expressed miRNAs, which may downplay effects of miRNAs in rare cell types.

Patients with severe psoriasis show a higher risk of comorbidities,[75] and the underlying molecular mechanisms may differ. We identified 11 DEMs comparing samples from cases with PASI ≥10 to PASI <10. In lesional skin (PP), miR-202-5p was upregulated in PASI≥10 samples. This miRNA has been shown to increase proliferation and induce apoptosis-resistance in cancer cells by targeting phosphatase and tensin homolog (*PTEN*).[76] miR-146a was also upregulated in PP. This miRNA is increased in skin and peripheral blood mononuclear cells (PBMC) of patients with psoriasis and is positively correlated with PASI and levels of interleukin-17.[21,50] Overexpression of miR-146a has been shown to suppress inflammation in keratinocytes and miR-146a-deficient mice treated with imiquimod have more severe psoriasiform lesions,[51] suggesting that this miRNA is overexpressed in response to increasing skin inflammation. In non-lesional skin (PN), miR-508-3p was upregulated in PASI ≥10 samples. This miRNA is upregulated in placental tissue in women with gestational diabetes.[77] The DEMs which were positively associated with PASI ≥10 were enriched for inflammatory pathways including interleukin and TLR-signalling. The DEMs which were negatively associated with PASI ≥10 were enriched for pathways involved in mRNA processing, such

as splicing and translation. This suggests that the upregulated DEMs reflect or respond to changes in immune function, while the downregulated DEMs are involved in basic cellular functions. To determine whether these changes are a cause or a consequence of increasing disease severity, functional studies are needed. Cell-specific expression of the severity associated DEMs identified miRNAs enriched in both leukocytes and hematopoietic cells. Hematopoietic cells have been implicated in regulation of proliferating skin tissues during an early inflammatory phase.[78] We propose the DEMs associated with disease severity as candidates for future studies as biomarkers as they have the potential to monitor disease activity, identify individuals with subclinical inflammation, predicting comorbidities and the prediction of future need of systemic anti-psoriatic therapy.

A major strength of this study is the large number of cases and controls that improved power to detect new DEMs associated with psoriasis. Further, our study provides insight into the effect of applying different statistical methods on gene expression data. The Joyce data (GSE31037) were originally analysed using a Pearson's chi-squared goodness-of-fit test on the normalized total reads from all PP, PN and NN skin.[11] To the best of our knowledge, this approach is not beneficial to identify significant expression differences between sample groups, as it ignores individual variation within each sample group, and thereby inflates the significance of highly expressed genes with higher variation compared to genes expressed at lower levels.[30] We therefore reanalysed the Joyce dataset which reduced the number of DEMs from the originally reported 317 to 257 (FDR <0.05, FC >1.4).

In conclusion, this study provides comprehensive insights into the miRNAome of psoriatic skin. By including a large sample of psoriatic cases and controls, we have identified 11 miRNAs associated with disease severity and expanded the number of psoriasis-associated miRNAs by 85. We identified 20 DEMs in PN/NN, representing inherent alterations in the miRNAome of all skin of psoriatic patients. This suggests systemic involvement and may represent shared molecular mechanisms of the systemic inflammation of psoriasis. Our study outlines future directions for targeted functional follow-up studies to explore the causal effects of the DEMs. Increased knowledge of the psoriasis transcriptome can prove crucial in understanding disease pathology, which can catalyse breakthroughs in the development of biomarkers for diagnostic purposes, predicting comorbidities and treatment response, as well as identifying treatment targets.

CONFLICT OF INTEREST
None declared.

AUTHOR CONTRIBUTIONS
ML, KH, MS and PS conceived the study concept and managed the project. ÅØS, ML and MS collected the skin samples at St. Olavs hospital, Trondheim University Hospital, Trondheim, Norway. MJ and KD managed and performed the skin sample collection at the University Hospital of North Norway, Tromsø, Norway. SAH planned and performed the qRT-PCR experiments with support from ÅØS, PS, LCO, KC and ML. ÅØS, KC, LCO and PS performed the statistical analyses. ÅØS conducted the literature review with support from ML. ÅØS drafted the initial version of the manuscript. All authors were involved in the interpretation of the data and contributed to and approved the final version of the manuscript.

ORCID
Åshild Ø. Solvin https://orcid.org/0000-0003-3209-3558
Konika Chawla https://orcid.org/0000-0002-8380-0315
Lene C. Olsen https://orcid.org/0000-0002-5869-4952
Siv Anita Hegre https://orcid.org/0000-0003-1122-4045
Kjersti Danielsen https://orcid.org/0000-0002-6862-4632
Anne-Sofie Furberg https://orcid.org/0000-0002-6311-166X
Marit Saunes https://orcid.org/0000-0001-5177-9390
Kristian Hveem https://orcid.org/0000-0001-8157-9744
Pål Sætrom https://orcid.org/0000-0001-8142-7441
Mari Løset https://orcid.org/0000-0003-3736-6551

REFERENCES
1. Danielsen K, Olsen AO, Wilsgaard T, Furberg AS. Is the prevalence of psoriasis increasing? A 30-year follow-up of a population-based cohort. *Br J Dermatol.* 2013;168(6):1303-1310. https://doi.org/10.1111/bjd.12230

2. Jensen P, Thyssen JP, Zachariae C, Hansen PR, Linneberg A, Skov L. Cardiovascular risk factors in subjects with psoriasis: a cross-sectional general population study. *Int J Dermatol*. 2013;52(6):681-683. https://doi.org/10.1111/j.1365-4632.2011.05408.x

3. Modalsli EH, Snekvik I, Asvold BO, Romundstad PR, Naldi L, Saunes M. Validity of self-reported psoriasis in a general population: the HUNT study, Norway. *J Invest Dermatol*. 2016;136(1):323-325. https://doi.org/10.1038/jid.2015.386

4. Michalek IM, Loring B, John SM. A systematic review of worldwide epidemiology of psoriasis. *J Eur Acad Dermatol Venereol*. 2017;31(2):205-212. https://doi.org/10.1111/jdv.13854

5. Nair RP, Duffin KC, Helms C, et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet*. 2009;41(2):199-204. https://doi.org/10.1038/ng.311

6. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281-297. https://doi.org/10.1016/s0092-8674(04)00045-5

7. Friedman RC, Farh KK-H, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*. 2009;19(1):92-105. https://doi.org/10.1101/gr.082701.108

8. Sonkoly E, Wei T, Janson PC, et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? *PLoS One*. 2007;2(7):e610. https://doi.org/10.1371/journal.pone.0000610

9. Zibert JR, Lovendorf MB, Litman T, Olsen J, Kaczkowski B, Skov L. MicroRNAs and potential target interactions in psoriasis. *J Dermatol Sci*. 2010;58(3):177-185. https://doi.org/10.1016/j.jdermsci.2010.03.004

10. Lovendorf MB, Mitsui H, Zibert JR, et al. Laser capture microdissection followed by next-generation sequencing identifies disease-related microRNAs in psoriatic skin that reflect systemic microRNA changes in psoriasis. *Exp Dermatol*. 2015;24(3):187-193. https://doi.org/10.1111/exd.12604

11. Joyce CE, Zhou X, Xia J, et al. Deep sequencing of small RNAs from human skin reveals major alterations in the psoriasis miRNAome. *Hum Mol Genet*. 2011;20(20):4025-4040. https://doi.org/10.1093/hmg/ddr331

12. Delic D, Wolk K, Schmid R, et al. Integrated microRNA/mRNA expression profiling of the skin of psoriasis patients. *J Dermatol Sci*. 2020;97(1):9-20. https://doi.org/10.1016/j.jdermsci.2019.11.003

13. Chicharro P, Rodríguez-Jiménez P, Llamas-Velasco M, et al. Expression of miR-135b in psoriatic skin and its association with disease improvement. *Cells*. 2020;9(7):1603. https://doi.org/10.3390/cells9071603

14. Srivastava A, Meisgen F, Pasquali L, et al. Next-generation sequencing identifies the keratinocyte-specific miRNA signature of psoriasis. *J Invest Dermatol*. 2019;139(12):2547-2550.e12. https://doi.org/10.1016/j.jid.2019.05.019

15. Ichihara A, Jinnin M, Yamane K, et al. microRNA-mediated keratinocyte hyperproliferation in psoriasis vulgaris. *Br J Dermatol*. 2011;165(5):1003-1010. https://doi.org/10.1111/j.1365-2133.2011.10497.x

16. Xu N, Brodin P, Wei T, et al. MiR-125b, a MicroRNA downregulated in psoriasis, modulates keratinocyte proliferation by targeting FGFR2. *J Invest Dermatol*. 2011;131(7):1521-1529. https://doi.org/10.1038/jid.2011.55

17. Meisgen F, Xu N, Wei T, et al. MiR-21 is up-regulated in psoriasis and suppresses T cell apoptosis. *Exp Dermatol*. 2012;21(4):312-314. https://doi.org/10.1111/j.1600-0625.2012.01462.x

18. Srivastava AK, Chand Yadav T, Khera HK, et al. Insights into interplay of immunopathophysiological events and molecular mechanistic cascades in psoriasis and its associated comorbidities. *J Autoimmun*. 2021;118:102614. https://doi.org/10.1016/j.jaut.2021.102614

19. Liu Q, Wu DH, Han L, et al. Roles of microRNAs in psoriasis: immunological functions and potential biomarkers. *Exp Dermatol*. 2017;26(4):359-367. https://doi.org/10.1111/exd.13249

20. Yi JZ, McGee JS. Epigenetic-modifying therapies: an emerging avenue for the treatment of inflammatory skin diseases. *Exp Dermatol*. 2021;30(8):1167-1176. https://doi.org/10.1111/exd.14334

21. Hawkes JE, Nguyen GH, Fujita M, et al. microRNAs in psoriasis. *J Invest Dermatol*. 2016;136(2):365-371. https://doi.org/10.1038/jid.2015.409

22. Fredriksson T, Pettersson U. Severe psoriasis–oral therapy with a new retinoid. *Dermatologica*. 1978;157(4):238-244. https://doi.org/10.1159/000250839

23. Jung M, Schaefer A, Steiner I, et al. Robust microRNA stability in degraded RNA preparations from human tissue and cell samples. *Clin Chem*. 2010;56(6):998-1006. https://doi.org/10.1373/clinchem.2009.141580

24. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci*. 2008;105(30):10513-10518. https://doi.org/10.1073/pnas.0804549105

25. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. Next generation sequencing; small RNA; microRNA; adapter removal. *EMBnet.journal*. 2011;17(1):3. https://doi.org/10.14806/ej.17.1.200

26. Anders S, Pyl PT, Huber W. HTSeq–a Python framework to work with high-throughput sequencing data. *Bioinformatics*. 2015;31(2):166-169. https://doi.org/10.1093/bioinformatics/btu638

27. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res*. 2006;34:D140-D144. https://doi.org/10.1093/nar/gkj112

28. Pantano L, Estivill X, Martí E. SeqBuster, a bioinformatic tool for the processing and analysis of small RNAs datasets, reveals ubiquitous miRNA modifications in human embryonic cells. *Nucleic Acids Res*. 2010;38(5):e34. https://doi.org/10.1093/nar/gkp1127

29. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. https://doi.org/10.1093/nar/gkv007

30. Law CW, Chen Y, Shi W, Smyth GK. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol*. 2014;15(2):R29. https://doi.org/10.1186/gb-2014-15-2-r29

31. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc Ser B (Methodol)*. 1995;57(1):289-300.

32. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25(4):402-408. https://doi.org/10.1006/meth.2001.1262

33. Agarwal V, Bell GW, Nam J-W, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *Elife*. 2015;4:e05005. https://doi.org/10.7554/eLife.05005

34. Reimand J, Kull M, Peterson H, Hansen J, Vilo J. g:Profiler—a web-based toolset for functional profiling of gene lists from large-scale experiments. *Nucleic Acids Res*. 2007;35:W193-W200. https://doi.org/10.1093/nar/gkm226

35. Fabregat A, Sidiropoulos K, Viteri G, et al. Reactome pathway analysis: a high-performance in-memory approach. *BMC Bioinformatics*. 2017;18(1):142. https://doi.org/10.1186/s12859-017-1559-2

36. Fan Y, Siklenka K, Arora SK, Ribeiro P, Kimmins S, Xia J. miRNet - dissecting miRNA-target interactions and functional associations through network-based visual analysis. *Nucleic Acids Res*. 2016;44(W1):W135-W141. https://doi.org/10.1093/nar/gkw288

37. Chang L, Zhou G, Soufan O, Xia J. miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. *Nucleic Acids Res*. 2020;48(W1):W244-W251. https://doi.org/10.1093/nar/gkaa467

38. Lizio M, Harshbarger J, Shimoji H, et al. Gateways to the FANTOM5 promoter level mammalian expression atlas. *Genome Biol.* 2015;16(1):22. https://doi.org/10.1186/s13059-014-0560-6

39. Nedoszytko B, Szczerkowska-Dobosz A, Stawczyk-Macieja M, et al. Pathogenesis of psoriasis in the "omic" era. Part II. Genetic, genomic and epigenetic changes in psoriasis. *Postepy Dermatol Alergol.* 2020;37(3):283-298. https://doi.org/10.5114/ada.2020.96243

40. Xiuli Y, Honglin W. miRNAs flowing up and down: the concerto of psoriasis. *Front Med (Lausanne).* 2021;8:646796. https://doi.org/10.3389/fmed.2021.646796

41. Mullokandov G, Baccarini A, Ruzo A, et al. High-throughput assessment of microRNA activity and function using microRNA sensor and decoy libraries. *Nat Methods.* 2012;9(8):840-846. https://doi.org/10.1038/nmeth.2078

42. Bosson AD, Zamudio JR, Sharp PA. Endogenous miRNA and target concentrations determine susceptibility to potential ceRNA competition. *Mol Cell.* 2014;56(3):347-359. https://doi.org/10.1016/j.molcel.2014.09.018

43. Schmitt J, Wozel G. The psoriasis area and severity index is the adequate criterion to define severity in chronic plaque-type psoriasis. *Dermatology.* 2005;210(3):194-199. https://doi.org/10.1159/000083509

44. Boehncke WH, Schon MP. Psoriasis. *Lancet (London, England).* 2015;386(9997):983-994. https://doi.org/10.1016/s0140-6736(14)61909-7

45. Tsoi LC, Rodriguez E, Degenhardt F, et al. Atopic dermatitis is an IL-13-dominant disease with greater molecular heterogeneity compared to psoriasis. *J Invest Dermatol.* 2019;139(7):1480-1489. https://doi.org/10.1016/j.jid.2018.12.018

46. Guinea-Viniegra J, Jiménez M, Schonthaler HB, et al. Targeting miR-21 to treat psoriasis. *Sci Transl Med.* 2014;6(225):225re1. https://doi.org/10.1126/scitranslmed.3008089

47. Peng H, Kaplan N, Hamanaka RB, et al. microRNA-31/factor-inhibiting hypoxia-inducible factor 1 nexus regulates keratinocyte differentiation. *Proc Natl Acad Sci USA.* 2012;109(35):14030-14034. https://doi.org/10.1073/pnas.1111292109

48. Xu N, Meisgen F, Butler LM, et al. MicroRNA-31 is overexpressed in psoriasis and modulates inflammatory cytokine and chemokine production in keratinocytes via targeting serine/threonine kinase 40. *J Immunol.* 2013;190(2):678-688. https://doi.org/10.4049/jimmunol.1202695

49. Taganov KD, Boldin MP, Chang K-J, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA.* 2006;103(33):12481-12486. https://doi.org/10.1073/pnas.0605298103

50. Xia P, Fang X, Zhang ZH, et al. Dysregulation of miRNA146a versus IRAK1 induces IL-17 persistence in the psoriatic skin lesions. *Immunol Lett.* 2012;148(2):151-162. https://doi.org/10.1016/j.imlet.2012.09.004

51. Srivastava A, Nikamo P, Lohcharoenkal W, et al. MicroRNA-146a suppresses IL-17-mediated skin inflammation and is genetically associated with psoriasis. *J Allergy Clin Immunol.* 2017;139(2):550-561. https://doi.org/10.1016/j.jaci.2016.07.025

52. Li L, Zuo X, Liu D, Luo H, Zhu H. The profiles of miRNAs and lncRNAs in peripheral blood neutrophils exosomes of diffuse cutaneous systemic sclerosis. *J Dermatol Sci.* 2020;98(2):88-97. https://doi.org/10.1016/j.jdermsci.2020.02.009

53. Takeshita J, Grewal S, Langan SM, et al. Psoriasis and comorbid diseases: epidemiology. *J Am Acad Dermatol.* 2017;76(3):377-390. https://doi.org/10.1016/j.jaad.2016.07.064

54. Lerman G, Avivi C, Mardoukh C, et al. MiRNA expression in psoriatic skin: reciprocal regulation of hsa-miR-99a and IGF-1R. *PLoS One.* 2011;6(6):e20916. https://doi.org/10.1371/journal.pone.0020916

55. Elharrar E, Masalha M, Lerman G, et al. Positive-negative feedback loop between MiR-197 and IL-17A signaling in human keratinocytes. *Immunome Res.* 2016;12(1):1.

56. Lerman G, Sharon M, Leibowitz-Amit R, Sidi Y, Avni D. The cross-talk between IL-22 signaling and miR-197 in human keratinocytes. *PLoS One.* 2014;9(9):e107467. https://doi.org/10.1371/journal.pone.0107467

57. Evangelatos G, Fragoulis GE, Koulouri V, Lambrou GI. MicroRNAs in rheumatoid arthritis: From pathogenesis to clinical impact. *Autoimmun Rev.* 2019;18(11):102391. https://doi.org/10.1016/j.autrev.2019.102391

58. Korman NJ. Management of psoriasis as a systemic disease: what is the evidence? *Br J Dermatol.* 2020;182(4):840-848. https://doi.org/10.1111/bjd.18245

59. Zhu H, Leung SW. Identification of microRNA biomarkers in type 2 diabetes: a meta-analysis of controlled profiling studies. *Diabetologia.* 2015;58(5):900-911. https://doi.org/10.1007/s00125-015-3510-2

60. Wu J, Li X, Li D, et al. MicroRNA-34 family enhances wound inflammation by targeting LGR4. *J Invest Dermat.* 2020;140(2):465-476.e11. https://doi.org/10.1016/j.jid.2019.07.694

61. Hoefert JE, Bjerke GA, Wang D, Yi R. The microRNA-200 family coordinately regulates cell adhesion and proliferation in hair morphogenesis. *J Cell Biol.* 2018;217(6):2185-2204. https://doi.org/10.1083/jcb.201708173

62. Clayton SA, Jones SW, Kurowska-Stolarska M, Clark AR. The role of microRNAs in glucocorticoid action. *J Biol Chem.* 2018;293(6):1865-1874. https://doi.org/10.1074/jbc.R117.000366

63. Walker BR. Glucocorticoids and cardiovascular disease. *Eur J Endocrinol.* 2007;157(5):545-559. https://doi.org/10.1530/eje-07-0455

64. Veltri A, Lang C, Lien WH. Concise review: wnt signaling pathways in skin development and epidermal stem cells. *Stem Cells.* 2018;36(1):22-35. https://doi.org/10.1002/stem.2723

65. Drosten M, Lechuga CG, Barbacid M. Ras signaling is essential for skin development. *Oncogene.* 2014;33(22):2857-2865. https://doi.org/10.1038/onc.2013.254

66. Ulker G, Müzeyyen G, Ilhan K, Erkan A. Autoimmune thyroid disorders in patients with psoriasis. *Eur J Dermatol.* 2009;19(3):221-223. https://doi.org/10.1684/ejd.2009.0632

67. Alidrisi HA, Al Hamdi K, Mansour AA. Is there any association between psoriasis and hashimoto's thyroiditis? *Cureus.* 2019;11(3):e4269. https://doi.org/10.7759/cureus.4269

68. Zhu J, Zhang Y, Zhang W, et al. MicroRNA-142-5p contributes to hashimoto's thyroiditis by targeting CLDN1. *J Transl Med.* 2016;14(1):166. https://doi.org/10.1186/s12967-016-0917-6

69. Bruun JM, Verdich C, Toubro S, Astrup A, Richelsen B. Association between measures of insulin sensitivity and circulating levels of interleukin-8, interleukin-6 and tumor necrosis factor-alpha. Effect of weight loss in obese men. *Eur J Endocrinol.* 2003;148(5):535-542.

70. Zhou T, Hu Z, Yang S, Sun L, Yu Z, Wang G. Role of adaptive and innate immunity in type 2 diabetes mellitus. *J Diabetes Res.* 2018;2018:1-9.

71. Zampetaki A, Kiechl S, Drozdov I, et al. Plasma MicroRNA profiling reveals loss of endothelial MiR-126 and other microRNAs in type 2 diabetes. *Circ Res.* 2010;107(6):810-817. https://doi.org/10.1161/CIRCRESAHA.110.226357

72. Jin Y, Tymen SD, Chen D, et al. MicroRNA-99 family targets AKT/mTOR signaling pathway in dermal wound healing. *PLoS One.* 2013;8(5):e64434. https://doi.org/10.1371/journal.pone.0064434

73. Elias MS, Wright SC, Nicholson WV, et al. Functional and proteomic analysis of a full thickness filaggrin-deficient skin organoid model. *Wellcome Open Res.* 2019;4:134. https://doi.org/10.12688/wellcomeopenres.15405.2

74. Afonso-Grunz F, Müller S. Principles of miRNA–mRNA interactions: beyond sequence complementarity. *Cell Mol Life Sci*. 2015;72(16):3127-3141. https://doi.org/10.1007/s00018-015-1922-2

75. Oliveira Mde F, Rocha Bde O, Duarte GV. Psoriasis: classical and emerging comorbidities. *An Bras Dermatol*. 2015;90(1):9-20. https://doi.org/10.1590/abd1806-4841.20153038

76. Liu T, Guo J, Zhang X. MiR-202-5p/PTEN mediates doxorubicin-resistance of breast cancer cells via PI3K/Akt signaling pathway. *Cancer Biol Ther*. 2019;20(7):989-998. https://doi.org/10.1080/15384047.2019.1591674

77. Li J, Song L, Zhou L, et al. A microRNA signature in gestational diabetes mellitus associated with risk of macrosomia. *Cell Physiol Biochem*. 2015;37(1):243-252. https://doi.org/10.1159/000430349

78. Kim WB, Jerome D, Yeung J. Diagnosis and management of psoriasis. *Can Fam Physician*. 2017;63(4):278-285.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Supplementary Tables
Supplementary Figures