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Posted Date: 12 May 2025

doi: 10.20944/preprints202505.0857.v1

Keywords: alopecia areata; microRNA; biomarkers; transcriptomics; non-invasive diagnostics; autoimmune skin diseases; machine learning; epigenetics; inflammatory skin disorders; drug repurposing



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Article

Unique Circulating MicroRNA Signatures Distinguish Severe Alopecia Areata from Other Inflammatory Skin Diseases

Running title: Distinct Circulating MicroRNA Profiles in Severe Alopecia Areata

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Abstract: Introduction: Alopecia areata (AA) is an autoimmune disorder characterized by non-scarring hair loss due to immune dysregulation. Despite advances, its precise molecular mechanisms remain unclear. This study investigates plasma miRNA expression profiles in AA patients to identify biological pathways influenced by miRNAs and potential therapeutic targets. **Methods:** Fifty patients with AA were categorized as severe or mild based on SALT scores. Plasma miRNA levels were compared to healthy controls and individuals with other immune-mediated skin diseases. In the discovery phase, 754 miRNAs were analyzed in 20 participants (five severe AA, five mild AA, and ten controls). Key miRNAs identified were then validated in a second cohort of 90 participants, including patients with AA, non-segmental vitiligo, atopic dermatitis (AD), psoriasis (PsO), and healthy controls, using RT-PCR. Machine learning classified patients based on miRNA profiles, and pathway enrichment analysis and drug targeting were conducted to explore therapeutic opportunities. **Results:** Nineteen miRNAs were significantly downregulated in AA, with ten technically and clinically validated for both mild and severe forms. The top four miRNAs with the highest classification potential were miR-130b-3p, miR-296-5p, miR-424-5p, and miR-195-5p. Distinct upregulation patterns were identified in vitiligo, AD, and PsO. Machine learning models showed vital classification accuracy for AA (AUC = 0.94) and PsO (AUC = 0.88), with moderate performance for vitiligo and AD. Pathway enrichment analysis highlighted immune-related pathways, including Interferon-gamma and JAK/STAT signaling. Drug repositioning identified kinase inhibitors showing the most significant promise for reversing miRNA dysregulation. **Conclusion:** This study identifies distinct plasma miRNA profiles in AA, with potential for diagnostic and therapeutic applications. Machine learning validated their predictive solid accuracy, and pathway analysis highlighted key immune pathways in AA. These insights pave the way for future miRNA-based diagnostics and treatments for AA and related conditions.

Keywords: alopecia areata; autoimmune skin diseases; gene regulation; micro RNA; transcriptomic analysis; microarray analysis; validation; computational biology

Plain Language Summary

Alopecia areata (AA) is an autoimmune condition that leads to hair loss, but its underlying biological causes are not fully understood. In this study, we investigated small molecules called microRNAs (miRNAs) in the blood of AA patients to better understand their role in the disease. MiRNAs help regulate gene expression and may be involved in the development and progression of AA.

We examined blood samples from patients with severe and mild AA, as well as individuals with other immune-related skin diseases like vitiligo, psoriasis, and atopic dermatitis, along with healthy controls. Using advanced techniques, we found that 19 miRNAs were significantly reduced in AA, with 10 miRNAs confirmed in both mild and severe cases. These miRNAs could distinguish AA from other skin diseases, suggesting they may play a role in the unique immune processes of AA.

Our study also identified important biological pathways, such as the JAK/STAT and Interferon-gamma signaling pathways, that may be involved in AA. Additionally, we discovered potential drugs, including JAK inhibitors, that could target these pathways and help restore the balance of miRNA expression in AA patients. This research highlights the potential of miRNAs as biomarkers for AA and suggests new treatment options for this challenging condition.

Introduction

Alopecia areata (AA) is an immunomediated inflammatory skin disease that causes hair loss without scarring, usually appearing as isolated or multiple hairless patches on the scalp.¹ With a prevalence of 0.5-2%, an estimated 6.6 million individuals in the U.S. and 147 million worldwide are affected.^{2,3} While mild cases can be managed with topical corticosteroids, approximately 10% of patients progress to more severe forms such as alopecia totalis (total scalp hair loss, AT) or alopecia universalis (loss of all body hair, AU).⁴ Prior research has indicated systemic inflammatory activity in these severe cases,^{5,6} and various treatments targeting these severe stages are currently under clinical trial.^{7,8}

Traditional clinical criteria used to assess AA severity include the Severity of Alopecia Tool (SALT),⁹ which quantifies the extent and density of scalp hair loss; the visual Alopecia Areata Investigator Global Assessment (AA-IGA),¹⁰ based on patient and physician inspections; and the Alopecia Areata Disease Activity Index (ALADIN),¹¹ which utilizes a patented panel of skin-expressed genes to monitor disease activity and classify severity. However, these methods have limitations: SALT and AA-IGA primarily provide a current severity stage without predicting disease progression, while ALADIN, though more diagnostic, requires invasive skin biopsies.

Emerging biomarkers of severity,¹²⁻¹⁴ progression,¹⁵ and treatment response^{16,17} offer a promising alternative. To a lesser extent, microRNAs (miRNAs) have been described emerging with these functions.¹⁸⁻²¹ MiRNAs are small, non-coding RNAs regulate gene expression post-transcriptionally by binding to complementary messenger RNA sequences, often leading to gene silencing. Circulating miRNAs are stable in the bloodstream and can reflect pathophysiological conditions, making them valuable for early diagnosis and monitoring of AA and other diseases.²²

This study profiles circulating miRNAs in alopecia areata (AA) patients to explore associated biological pathways and molecular underpinnings. By analyzing miRNA expression and pathway interactions, we aim to enhance understanding of AA's pathophysiology and identify new therapeutic targets, potentially revolutionizing its clinical management with miRNA-based strategies.

Material and Methods

Inclusion and Exclusion Criteria

The study included patients over 17 years of age with a clinical diagnosis of AA, excluding those with other immunologically mediated diseases, except Hashimoto's thyroiditis, provided that the

patient was in a state of euthyroidism with or without medication. All patients were required to be treatment-naïve for AA, both topically and systemically, or to have undergone a previous washout period. The washout period was two weeks for topical corticosteroids and topical minoxidil, four weeks for intralesional corticosteroids, systemic corticosteroids, cyclosporine, methotrexate, and azathioprine, and three months or more than five times the half-life of the drug for any biological or JAK inhibitors. The control group consisted of individuals who donated blood without associated inflammatory pathology, matching age and sex with the patient group.

Disease Severity Assessment

The severity of AA was evaluated considering the number of hairless plaques, their extent, and the duration of the disease. The extent of the plaques was quantified using the SALT. Based on these criteria, patients were classified into two groups: a) Mild AA: Patients with plaques covering less than 50% of the affected area and a disease duration of less than one year. b) Severe AA: Patients with a disease duration of more than one year, plaques affecting 50% or more of the affected area according to SALT, or exhibiting AT or AU.

Positive controls were enrolled into the validation subanalysis based on the following severity definitions and thresholds: severe atopic dermatitis (AD), Investigator's Global Assessment (IGA) ≥ 3 and/or Eczema Area and Severity Index (EASI) ≥ 12 ; ²³ severe plaque psoriasis (PsO), Physician's Global Assessment (PGA) ≥ 3 and/or Psoriasis Area and Severity Index (PASI) > 10 ; ²⁴ non-segmental vitiligo, Vitiligo Area Scoring Index (VASI) and/or Vitiligo Extent Score (VES) $> 10\%$. ²⁵ These standardized scales and indices ensure consistent and accurate assessment of disease severity across different conditions, facilitating meaningful comparisons and robust analysis.

Sample Collection

After an 8-hour fasting period, a sample of anticoagulated blood (K2-EDTA 5.4 mg) was collected. Blood was centrifuged at 1,800 g for 10 minutes at room temperature to obtain plasma samples. The upper layer phase containing the plasma was carefully collected and stored at -80 °C for further analysis. A Qiagen commercial kit (miRNeasy serum / plasma kit) was used following the manufacturer's instructions for miRNA purification. From 200 μ L of plasma, miRNA was purified and eluted using 14 μ L of RNase-free water. The purified miRNA samples were quantified and stored at -80 °C for future use.

Discovery Phase

In the discovery phase, a cohort of ten AA patients (5 mild, 5 severe) and ten healthy controls was selected. Reverse transcription of 758 miRNAs per sample was performed using the TaqMan Advanced miRNA cDNA Synthesis Kit (Thermo Fisher Scientific, USA). The samples were then analyzed with the TaqMan OpenArray Human MicroRNA Panel, offering a high-throughput platform for comprehensive miRNA profiling.

Validation Phase

Technical Validation

To validate the OpenArray results, real-time PCR (RT-PCR) was performed on the same samples using the QuantStudio 12K Flex system. Data analysis with Thermo Fisher Connect software confirmed the differential expression of specific miRNAs.

Clinical Validation

The validated miRNAs were assessed as circulating biomarkers for disease classification by comparing 30 AA patients to 30 healthy controls and 30 patients with other immune-mediated skin diseases, ten patients for each group: PsO, severe AD, and non-segmental vitiligo. This analysis

aimed to evaluate the specificity and diagnostic potential of the miRNA profile for AA, distinguishing it from other inflammatory skin conditions.

Predictive Model

To develop a predictive model using miRNA expression data, the dataset was split into 70% for training and 30% for testing. A machine learning classifier was trained on the training set, with performance evaluated on the test set using accuracy, AUC (Area Under the ROC Curve), and a confusion matrix. ROC curves were generated for validated miRNAs to assess classification performance, and the top-performing miRNAs were ranked. The best four miRNAs were used to generate ROC curves to distinguish AA patients from controls, with the top miRNA further analyzed for its potential to differentiate AA from other immune diseases, evaluating its specificity as an AA biomarker.

Biological Insights and Pathway Analysis

Pathway Enrichment Analysis

Enrichment analysis of the validated miRNAs was performed using target gene sets across multiple databases, including Gene Ontology (Biological Process and Molecular Function), KEGG pathways, Reactome, and WikiPathways. The analysis, conducted with GeneCodis 4,²⁵ applied a hypergeometric distribution and corrected p-values using the Benjamini-Hochberg false discovery rate (FDR) method, with significance set at $p < 0.05$.

Identification of Drug Targets

Differentially expressed circulating miRNAs in severe AA were cross-referenced with PharmaGKB and LINCS databases to identify potential therapeutic drugs. These databases provide insights into small molecules and their impact on gene expression and pathways, highlighting promising drug candidates for AA treatment based on miRNA-related pathways.

Statistical Analysis

Results of the miRNA expression analysis were obtained in terms of cycle threshold (Ct) values and were subsequently normalized using the formula $\text{miRNA}_{\text{normalized}} = 2^{-(\text{miRNA} - \text{miRNA with the lowest variability})}$. Missing values were estimated at 20% of the minimum expression level. Data underwent quality control and batch corrections. Group differences in miRNA expression were assessed using the Mann-Whitney U and Kruskal-Wallis tests (significance $p < 0.05$), with post hoc comparisons to identify specific group differences. Results were visualized with dendrograms, heatmaps, and miRNA term networks to display relationships between miRNAs and functional terms. Statistical analyses were conducted using R and Python.

Ethics

The project was approved by the Cordoba Provincial Research Ethics Committee, and all patients provided informed consent after reviewing the patient information sheet. Data were anonymized to protect confidentiality, in line with good clinical practice, the Declaration of Helsinki, and the Belmont Report. Anonymization followed EU Regulation 2016/679 and Spain's Organic Law 3/2018 to ensure participant privacy and compliance with data protection standards.

Availability of Data and Reproducibility of Results

Raw data and R scripts used for statistical analysis are available upon reasonable request. Data requests will be reviewed to ensure they comply with ethical and scientific standards.

Results

Population Characteristics

The demographic and clinical characteristics of the population in both the Discovery and Validation phases are shown in Table 1. In the discovery/technical validation phases, there were no significant differences in gender ($p = 0.54$) or age ($p = 0.61$) between the AA severe, AA mild-moderate, and control groups. Similarly, the Clinical validation phase with a larger cohort found no significant differences in gender ($p = 0.76$) or age ($p = 0.45$) across AA severe, AA mild-moderate, control, vitiligo, AD, and PsO groups. Further details are provided in Table S1.

Table 1. Demographic and Clinical Characteristics of the Population in the Discovery and Validation Phases. This table summarizes the demographic and clinical characteristics of the population in both the Discovery and Validation phases, along with the p-values for gender and age comparisons between the groups.

Phase	Group	Severity	n	Gender (M/F)	Age (years)	P value gender (*)	P value age (**)
Discovery/Tech Validation (n=20)	AA	Severe	5	1/4	44.4 (20-61)	0.54	0.61
		Mild-moderate	5	2/3	42.6 (30-64)		
	control	-	10	5/5	36.8 (18-68)		
Clinical Validation (n=90)	AA	Severe	15	7/8	35.9 (23-47)	0.76	0.45
		Mild-moderate	15	6/9	46.2 (18-76)		
	control	-	30	13/17	44.8 (18-76)		
	Vitiligo	-	10	4/6	42.8 (16-74)		
	AD	-	10	7/3	44.8 (27-75)		
	PsO	-	10	7/3	47.6 (23-71)		

AA, Alopecia Areata, AD, Atopic Dermatitis; PsO, psoriasis; * Chi-squared; ** ANOVA.

Discovery Phase

In the discovery phase, 19 miRNAs, including miR-16-5p, miR-296-5p, miR-140-3p, miR-29b-3p, and miR-424-5p, were significantly downregulated in severe AA compared to controls, indicating a shared regulatory mechanism in AA pathogenesis (Table 2). Mild cases showed only three differentially expressed miRNAs: two upregulated (miR-495-3p, miR-655-3p) and one downregulated (miR-495-3p), all with lower fold-change values than in severe AA.

Table 2. Significant Results of Differentially Expressed miRNAs in Severe AA from the Discovery Phase of the Study. In this analysis, a total of 740 probes were evaluated in AA patients (n=10) compared to healthy controls (n=10) using a microarray platform. Each probe represents a unique identifier for a specific miRNA in the

microarray, allowing for the detection of miRNA expression levels. The fold change (FCH) indicates the magnitude of expression change between severe AA cases and healthy controls, with positive values representing upregulation and negative values representing downregulation. The results are sorted by statistical significance (p-value) in descending order.

microRNA	Probe	control	Severe AA	p values	FCH
miR-195-5p	477957	1.023	0.436	0.004	-0.586
miR-93-3p	478209	1.008	0.511	0.004	-0.496
miR-130b-3p	477840	1.021	0.578	0.008	-0.443
miR-21-3p	477973	1.038	0.5	0.008	-0.537
miR-214-3p	477974	1.073	0.4	0.008	-0.672
miR-101-3p	477863	1.062	0.428	0.017	-0.633
miR-153-3p	477922	1.229	0.262	0.017	-0.967
miR-16-5p	477860	1.05	0.457	0.017	-0.592
miR-296-5p	477836	1.01	0.668	0.017	-0.341
miR-325	478025	1.169	0.345	0.017	-0.824
miR-132-3p	477900	1.038	0.536	0.03	-0.502
miR-140-3p	477908	1.049	0.57	0.03	-0.479
miR-19a-3p	479228	1.039	0.5	0.03	-0.539
miR-29b-3p	478369	1.026	0.568	0.03	-0.458
miR-30b-5p	478007	1.055	0.484	0.03	-0.571
miR-30c-5p	478008	1.039	0.525	0.03	-0.514

microRNA	Probe	control	Severe AA	p values	FCH
miR-376a-3p	478240	1.037	0.517	0.03	-0.52
miR-424-5p	478092	1.05	0.543	0.03	-0.506
miR-92a-3p	477827	1.067	0.469	0.03	-0.598

Validation Phase

In the **validation phase (Figure 2)**, we focused on the 19 downregulated miRNAs in severe AA due to their consistent and robust downregulation, providing a clearer molecular profile for validation. Of these, 10 miRNAs were technically validated in both mild and severe AA (e.g., miR-102, miR-130-p, miR-195-5p), while others were specific to mild or severe AA (e.g., miR-296-5p in mild AA, miR-140-3p in severe AA).

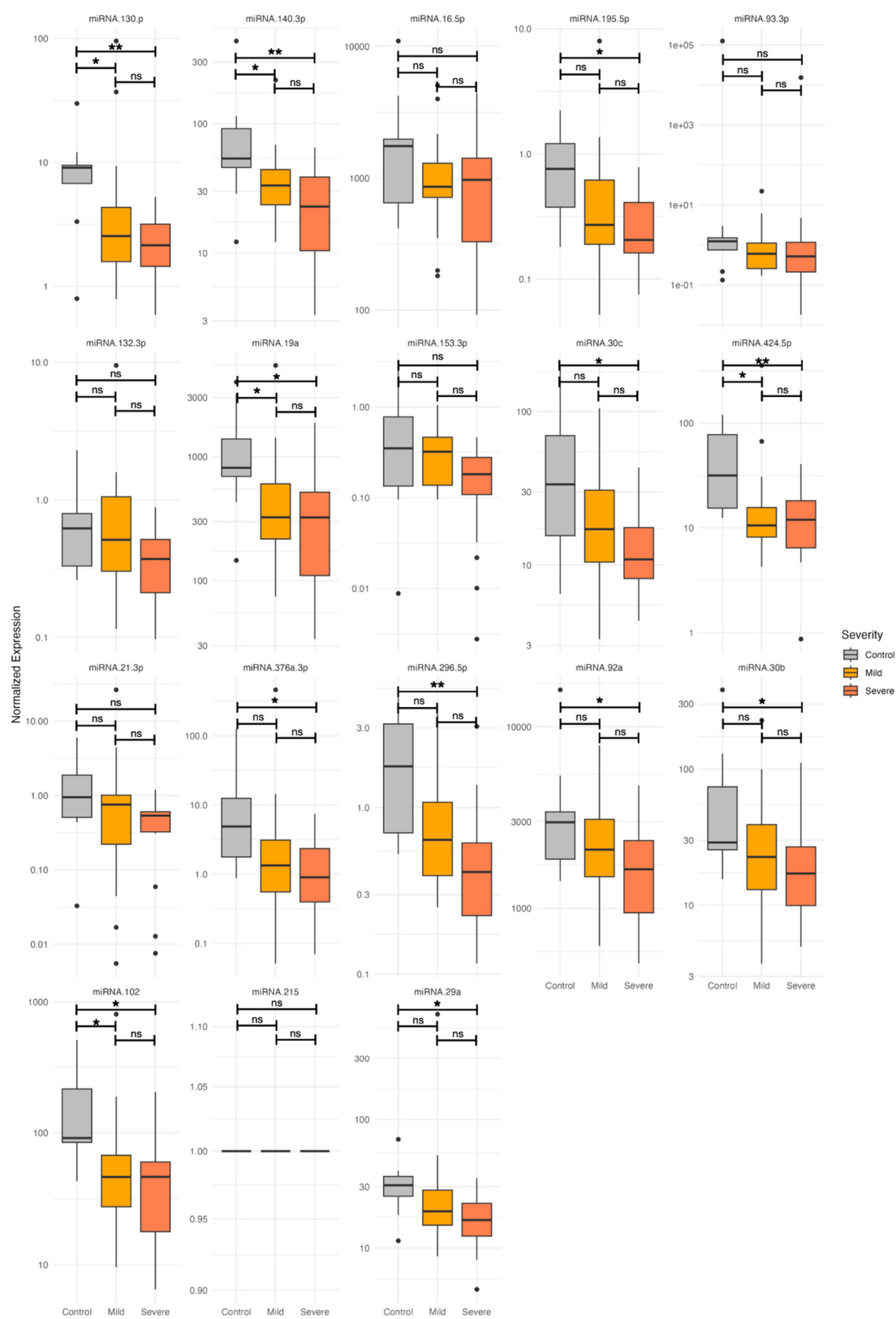


Figure 1. Validation of Down-Regulated miRNAs in Plasma of Alopecia Areata Patients Compared to controls. Technical validation of down-regulated miRNAs in patients with AA compared to controls. Plasma samples from the same patients included in the discovery phase were used for this analysis. Boxplots show the expression levels of each miRNA in control individuals (gray), patients with mild AA (yellow), and patients with severe AA (orange). miR-215 was employed as a normalizer to standardize the expression levels of all other miRNAs. Asterisks indicate statistical significance between groups: *p<0.05; **p<0.01; ***p<0.001; ns, not significant.

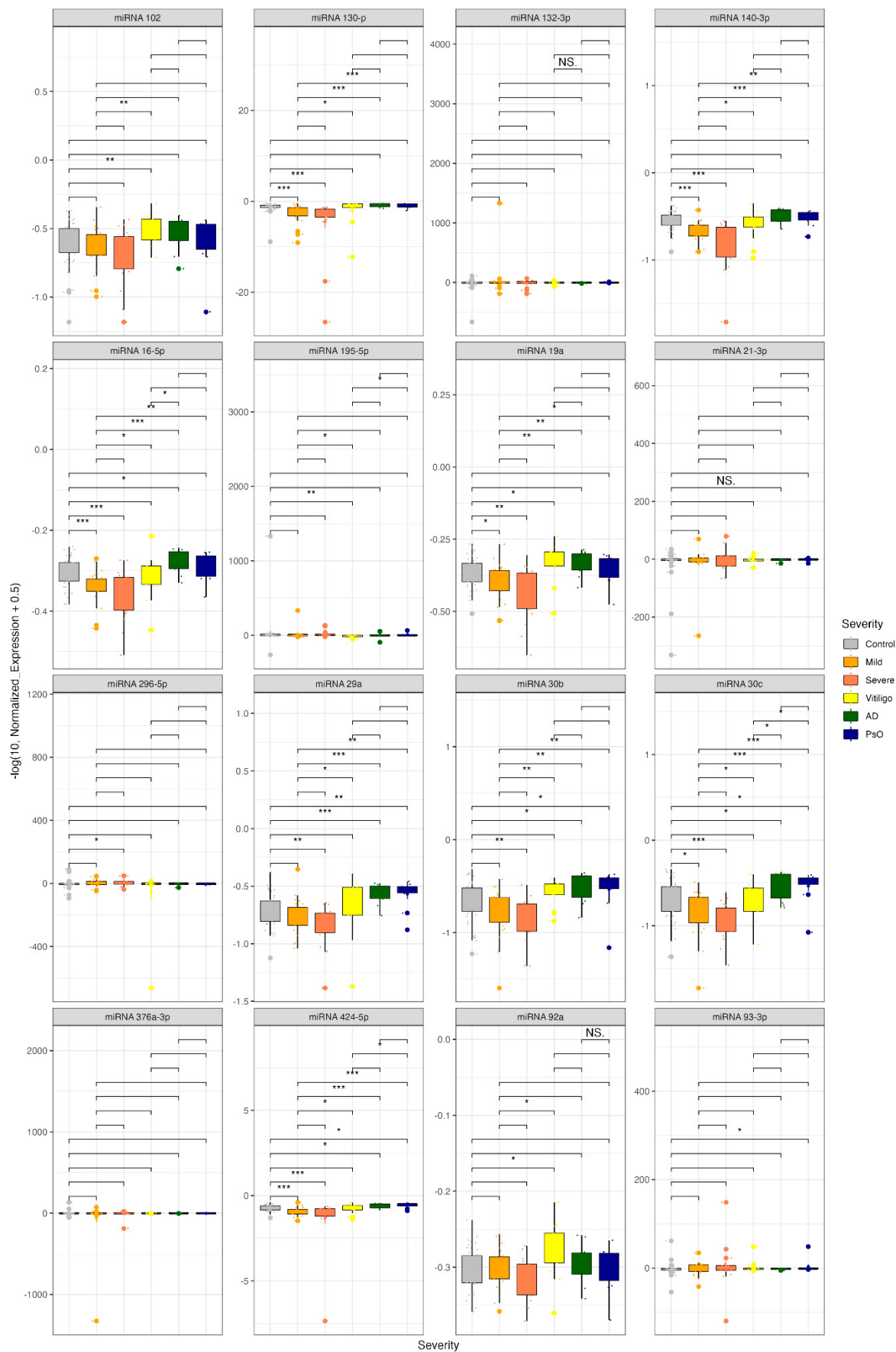


Figure 2. Clinical Validation of miRNA Expression in an Independent Cohort of AA Patients Compared to control Groups and Other Immune-Mediated Inflammatory Skin Diseases. This boxplot presents the clinical validation results of 19 miRNAs in an independent cohort of patients with alopecia areata (AA), using plasma

samples from AA patients (n=30), negative controls (30 healthy individuals), and positive controls with other immune-mediated inflammatory skin diseases, including psoriasis (PsO; n=10), severe atopic dermatitis (AD; n=10), and non-segmental vitiligo (n=10). Unlike the discovery phase, this validation was conducted on a different population of AA patients and controls, adding patients with other skin diseases characterized by systemic inflammation. This analysis aimed to assess the diagnostic utility and specificity of the miRNA profile for AA compared to other inflammatory skin conditions. Expression levels are normalized, and asterisks indicate statistical significance: *p<0.05; **p<0.01; ***p<0.001; ns, not significant.

In clinical validation (Figure 3), all tested miRNAs remained significantly downregulated in AA patients, with some shifting between severity groups. Additionally, certain miRNAs were upregulated in non-segmental vitiligo, AD, and PsO. Co-expression analysis (Figure 4A) revealed strong correlations among miRNAs, suggesting co-regulation, with unique patterns emerging in severe AA.

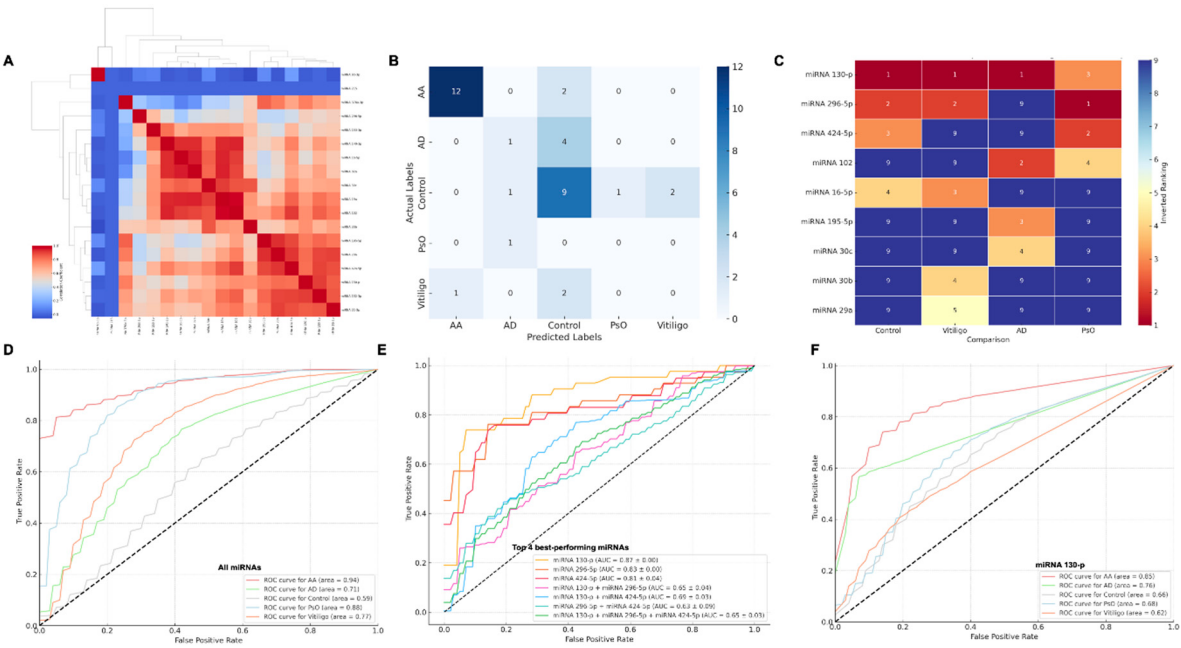


Figure 3. Classification Performance of Validated Circulating miRNAs in Alopecia Areata and Other Immune-Mediated Skin Diseases. Panel A: Correlation heatmap displaying the clustering and correlation of the top-ranked differentially expressed miRNAs across Alopecia Areata (AA), Atopic Dermatitis (AD), Psoriasis (PsO), Vitiligo, and control subjects. Panel B: Confusion matrix summarizing the performance of the machine learning model in classifying the study groups based on miRNA expression profiles. Panel C: Heatmap ranking the top miRNAs by their predictive power across the different conditions. The color gradient represents the rank, with red indicating the top-ranked miRNAs for each comparison. Panel D: Receiver Operating Characteristic (ROC) curve displaying the performance of the machine learning model using all miRNAs in distinguishing AA from other conditions (AUC = 0.94). Panel E: ROC curve comparing the classification performance of the top three miRNAs with the highest diagnostic potential for AA (miR-130p, miR-296-5p, and miR-424-5p) against controls and other conditions. Panel F: ROC curve for miR-130p across AA, AD, PsO, Vitiligo, and control groups, demonstrating its superior classification accuracy (AUC = 0.85 for AA).

Plasma miRNA-Based Predictive Model

We used logistic regression, ROC curve analysis, and bootstrap iterations to evaluate the performance of 10 validated miRNAs. The model, trained on 30% of the samples and tested on the remaining 70%, showed strong performance for AA, correctly classifying 12 of 14 samples (Figure 4B).

ROC analysis revealed high discriminatory power for AA (AUC = 0.94) and PsO (AUC = 0.88), moderate for vitiligo (AUC = 0.77), and lower for controls (AUC = 0.59) (**Figure 4D**). Testing combinations of top miRNAs (miR-130-p, miR-296-5p, miR-424-5p) did not outperform single miRNAs (**Figure 4E**). miR-130-3p alone (AUC = 0.85) showed strong potential as an AA biomarker (**Figure 4F**).

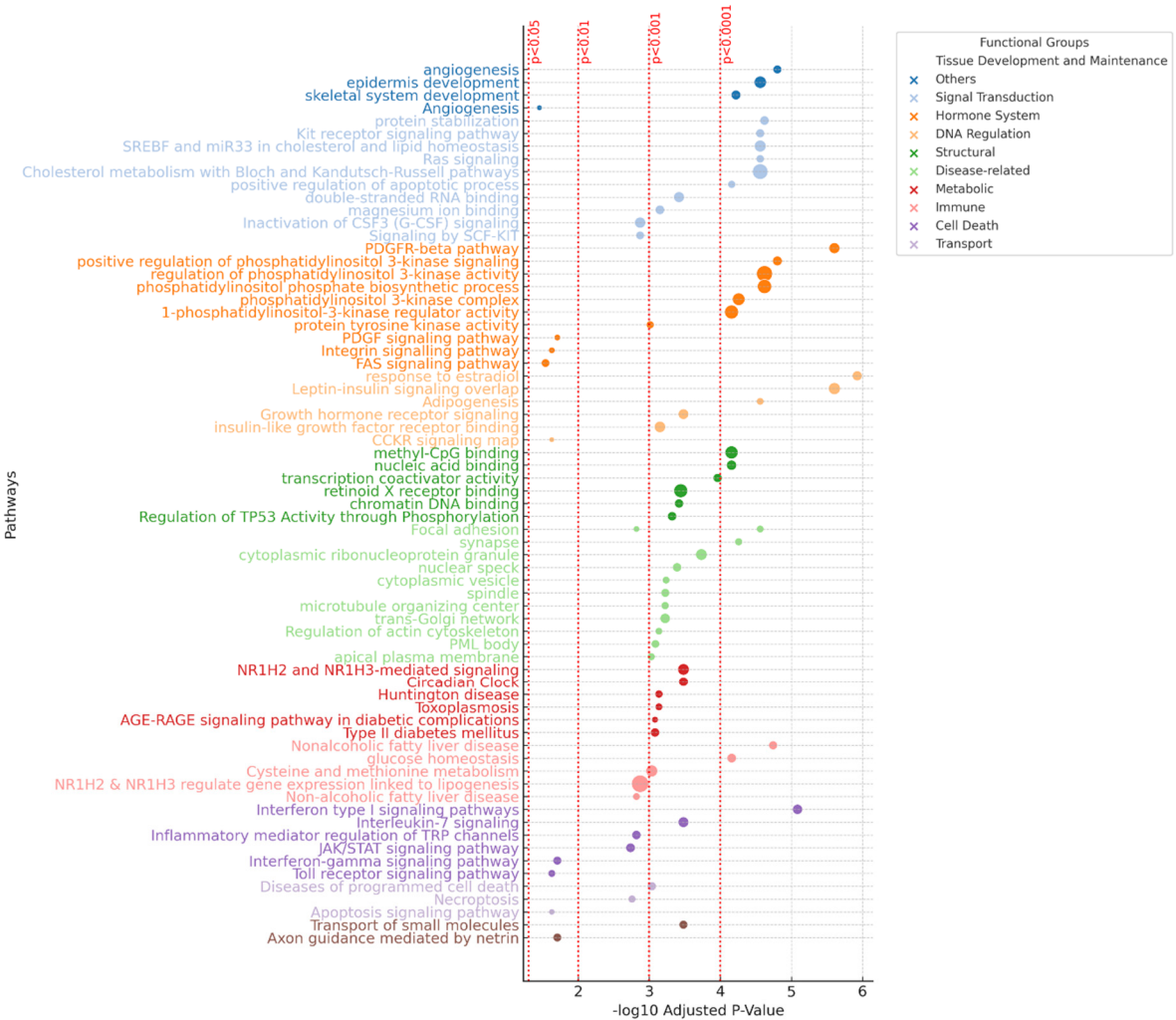


Figure 4. Pathway Enrichment Analysis of Dysregulated Circulating miRNAs in Alopecia Areata. This figure presents the top enriched pathways regulated by dysregulated miRNAs in Alopecia Areata (AA) and other immune-mediated skin diseases. Pathways are ranked in descending order of statistical significance and are categorized into functional groups such as tissue development and maintenance, signal transduction, immune response, metabolic processes, and cell death. The x-axis represents the $-\log_{10}$ of the adjusted p-values for each pathway, highlighting their statistical significance. Horizontal dashed red lines indicate different significance thresholds ($p < 0.05$, $p < 0.01$, $p < 0.001$).

Biological Significance

Pathway Enrichment Analysis

Following the analysis of 9 validated miRNAs, 2,811 enriched pathways across databases, including Panther, KEGG, WikiPathways, Reactome, and GO. Key immune-related pathways included Interferon-gamma, Toll receptor, JAK/STAT, and TRP channel signaling, highlighting immune dysregulation in AA. Metabolic pathways like Cysteine and methionine metabolism, NAFLD, and lipogenesis also showed enrichment, suggesting metabolic involvement. Signal transduction pathways, including PDGF, MAPK, and Hippo signaling, were enriched, emphasizing

their importance in AA. Some pathways, such as Huntington's disease and Type II diabetes, suggest miRNA dysregulation in AA has broader implications (Figure 4).

Drug Targets

Drug targets Analysis of miRNA profiles in AA identified 39 potential therapeutic drugs from LINCS and PharmGKB. Of these, 41% were kinase inhibitors, primarily JAK inhibitors, including sunitinib, pazopanib, semaxanib, sorafenib, dovitinib, tivozanib, axitinib, cediranib, orantinib, motesanib, linifanib, danusertib, ENMD-2076, SU-11652, BMS-536924, Tyrphostin-AG-1295, and PP30. Other identified drugs belonged to groups of antioxidants (12.8%; catechin, curcumin, epicatechin, epigallocatechin, and rhamnetin), epigenetic regulators (7.7%; azacitidine, zebularine), and antimicrobial agents (15.4%; triclosan, procainamide, pyrazinamide, flucytosine, triclabendazole, methyl norlichexanthone). The remaining 23.1% were substances with toxic effects or in very early stages of development, and thus were not considered a priority. These findings highlight promising drug candidates for targeting miRNA dysregulation in AA.

Discussion

This study presents a comprehensive miRNA plasma profiling in patients with severe AA, offering a multi-phase approach that strengthens the reliability of the findings. By including various immune-mediated skin conditions such as PsO, AD, and non-segmental vitiligo, the study underscores the specificity of the miRNA signature to AA. The use of machine learning models provides a modern approach to classify AA based on miRNA expression, demonstrating strong classification power, especially in distinguishing AA from healthy controls. The identification of potential therapeutic targets, particularly JAK inhibitors, based on dysregulated miRNA pathways, offers actionable insights for AA treatment. Additionally, pathway enrichment analysis links the dysregulated miRNAs to immune-related and metabolic pathways, deepening the understanding of AA's pathogenesis.

Several miRNAs identified in our study have been shown to play key roles in skin physiology, particularly in hair follicle biology and keratinocyte function. For instance, **miR-195-5p**, **miR-29a**, and **miR-140-5p** have been shown to regulate hair follicle development, which suggests that their downregulation in severe AA could disrupt normal hair follicle function.²⁷⁻²⁹ Moreover, **miR-130b-3p** and **miR-93-3p** are involved in keratinocyte proliferation and migration, processes essential for maintaining hair follicle integrity and skin barrier function.^{30,31} Further, miRNAs such as **miR-16-5p**, **miR-132-3p**, **miR-29b-3p**, and **miR-376a-3p** have been implicated in wound healing and the transition of epidermal stem cells to mature keratinocytes.³²

In addition to their role in local inflammation and hair follicle biology, several miRNAs dysregulated in severe AA have been linked to systemic inflammatory diseases. For example, **miR-195-5p**, **miR-214-3p**, and **miR-16-5p** are associated with ulcerative colitis and Crohn's disease,³³⁻³⁴ while **miR-16-5p**, miR-19a-3p, miR-132-3p, **miR-140-3p**, miR-92a-3p, and **miR-424-5p** are linked to autoimmune conditions such as osteoarthritis, Sjögren's syndrome, and rheumatoid arthritis.³⁵⁻⁴³ These connections suggest that miRNA dysregulation in severe AA may not be limited to the skin but may also reflect broader systemic immune dysregulation.

Additionally, several of the dysregulated miRNAs have implicated in cardiovascular disease (miR-16-5p, miR-325, miR-132-3p, miR-140-3p, miR-19a-3p, miR-30c-5p, miR-424-5p, miR-92a-3p),⁴⁴⁻⁵² heart failure (miR-30c-5p, miR-424-5p),^{53,54} acute coronary syndrome (miR-140-3p),⁵⁵ metabolic syndrome (miR-132-3p),⁵⁶ and atherosclerosis (miR-325, miR-30c-5p, miR-424-5p).⁵⁷⁻⁵⁹ This potential association highlights the importance of monitoring for cardiovascular risk factors in AA patients, especially those with severe forms of the disease.⁶⁰

Finally, although this study offers a comprehensive miRNA profiling in severe AA, it has certain limitations. The sample size, particularly for patients with other inflammatory skin diseases, is

relatively small, which may affect the generalizability of the results. The study is also limited by its focus on a single time point, lacking longitudinal data to observe how miRNA profiles change over time or with treatment. There may also be potential confounding factors, such as treatment history and disease duration, which were not fully controlled. Although miRNA expression was validated, functional experiments linking miRNA dysregulation to specific biological outcomes were not performed, limiting causal conclusions. Lastly, while the study highlights miRNA specificity for AA, it remains to be tested whether these miRNAs are dysregulated in other autoimmune or inflammatory diseases, potentially affecting their utility as specific biomarkers for AA.

Conclusions

In conclusion, this study adds to the growing body of knowledge on systemic miRNA dysregulation in AA, highlighting the importance of both local and systemic factors in the disease's complex pathogenesis. By identifying specific dysregulated miRNAs in the blood of patients with severe AA, we reveal a broader systemic miRNA dysregulation that extends beyond the skin. These findings offer new insights into the role of miRNAs in AA and open pathways for future therapeutic development. Larger, more diverse studies are needed to confirm these results and assess their broader applicability across AA subtypes.

Author Contribution: Conceptualization: J.G-M. (equal), M.A-L. (equal), J.R. (lead); Data curation: J.G-M. (lead), I.R-R. (equal), M.A-L. (equal), A.R-M. (equal); Formal analysis: J.L-B. (lead), P.C-S. (equal), M.J-C. (supporting); Funding acquisition: B.I-T. (equal), J.R. (lead); Investigation: J.G-M. (lead), M.A-L. (equal); Methodology: J.G-M. (equal), B.I-T. (equal); Project administration: C.M-J. (lead), I.R-R. (equal), E.P-P. (lead), J.R. (equal); Resources: J.G-M. (lead), I.R-R. (equal), M.J-C. (supporting); Software: J.L-B. (lead), P.C-S. (equal); Supervision: F.J.G-G. (equal), E.P-P. (equal), J.R. (equal); Validation: J.G-M. (equal), J.L-B. (equal), M.A-L. (supporting); Visualization: J.G-M. (lead), J.L-B. (equal); Writing – original draft: J.G-M. (equal), M.A-L. (equal); Writing - review & editing: F.J.G-G. (equal), B.I-T. (equal), J.R. (equal).

Funding: This research has been exclusively supported by public foundations, with no involvement of private funds. This work received partial support from Project PI23/01590 (awarded to JR), funded by Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union. The foundation did not influence the study design, data collection and analysis, publication decision, or manuscript preparation. It is important to note that no funding was received from any pharmaceutical company.

Ethical Approval: Reviewed and approved by Provincial Research Ethics Committee of Córdoba, Spain; approval # HRP-503.

Ethics statement: The research was conducted following the standards of good clinical practice and adhering to the principles outlined in the by the Helsinki Declaration of 1975, as revised in 1983, and Belmont Report and adhered to Spanish and European laws regarding data privacy. Before participation, all patients provided informed consent after receiving and reviewing the patient information sheet.

Data availability statement: The datasets generated and/or analyzed during the current study are available in the supplementary information files accompanying this article. Additional data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: We gratefully acknowledge the support from the Plan Propio de Investigación at the Instituto Maimónides de Investigación Clínica de Córdoba (IMIBIC), with awards to JG-M and MA-L. Additionally, PG-A received funding from the International Eczema Council's 2002 Research Fellowship Program. EP-P appreciates the support from Universidad Europea de Madrid in facilitating this research. We also extend our sincere thanks to ChatGPT (AI) for its invaluable assistance in generating ideas, and providing support with R and Python for data analysis and visualization. Its contributions were instrumental throughout the planning and manuscript preparation stages of this work.

Conflicts of Interest: None declared.

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