Supplementary Information

Circulating MicroRNA Signatures in Severe Alopecia Areata: Diagnostic Discrimination, Pathway Analysis, and Therapeutic Implications.

Jesús Gay-Mimbrera, BSc[1,†], Macarena Aguilar-Luque, BSc[1,†], Pedro J. Gómez-Arias, MD[1,2], Irene Rivera-Ruiz, MD[1,2], Francisco Gómez-Garcia, MD[1,2], Miguel Juan-Cencerrado, MD[1,2], Carmen Mochón-Jiménez, MD[1,2], Esmeralda Parra-Peralbo, PhD[3], Ricardo Ruiz-Villaverde, MD, PhD[4], José Liñares-Blanco, PhD[5,*], Pedro Carmona-Saez, PhD[5,†], Beatriz Isla-Tejera, PharmD, PhD[1,6,*], Juan Ruano, MD, MSc, PhD[1,2,*,†]

1 Inflammatory Immune-Mediated Chronic Skin Diseases Laboratory, IMIBIC/University of Córdoba, 14004 Córdoba, Spain

2 Department of Dermatology, Reina Sofia University Hospital, 14004 Córdoba, Spain

3 Department of Pharmacy and Nutrition, Faculty of Biomedical Science and Health, Universidad Europea, 28670 Madrid, Spain

4 Department of Dermatology, Hospital Universitario Clinico San Cecilio, Granada, Spain

5 Bioinformatics Unit, GENYO Center for Genomics and Oncological Research: Pfizer/University of Granada/Andalusian Regional Government, PTS Granada, Granada, Spain

6 Department of Pharmacy, Reina Sofia University Hospital, 14004 Córdoba, Spain

Supplementary Tables

Supplementary Table S1. Completed STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist indicating compliance with reporting guidelines for cross-sectional observational studies.

Title: Circulating MicroRNA Signatures in Severe Alopecia Areata: Diagnostic Discrimination, Pathway Analysis, and Therapeutic Implications

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Page nº	Recommendation
Title and abstract	1	1,5,6	(a) Indicate the study's design with a commonly used term in the title or the abstract
			(b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	9,10	Explain the scientific background and rationale for the investigation being reported
Objectives	3	10	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	11-16	Present key elements of study design early in the paper
Setting	5	11-16	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	11-16	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	11-16	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	11-16	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	NA	Describe any efforts to address potential sources of bias
Study size	10	NA	Explain how the study size was arrived at
Quantitative variables	11	11-16	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why

Statistical methods	12	15,16	(a) Describe all statistical methods, including those used to control for confounding
			(b) Describe any methods used to examine subgroups and interactions
			(c) Explain how missing data were addressed
			(d) If applicable, describe analytical methods taking account of sampling strategy
			(e) Describe any sensitivity analyses
Results			
Participants	13*	Table 1	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
			(b) Give reasons for non-participation at each stage
			(c) Consider use of a flow diagram
Descriptive data	14*	Table 1	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
			(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	NA	Report numbers of outcome events or summary measures
Main results	16	17-19	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
			(b) Report category boundaries when continuous variables were categorized
			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	NA	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	20,21	Summarise key results with reference to study objectives
Limitations	19	22	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	23	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	23	Discuss the generalisability (external validity) of the study results
Other information			

Funding	22	2	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Supplementary Table S2. **Sociodemographic and Clinical Characteristics of Patients with Alopecia Areata in the Discovery and Validation Phases**. The table summarizes key characteristics including age, gender, alopecia areata (AA) subtype, disease severity (based on SALT score), and study phase classification (discovery and/or technical/clinical validation) Patients with single patch, multiple patches, alopecia totalis (AT), or universalis (AU) were included.

Subject ID	Study Phase	Group	Type	Scale	Value	Severity	Gender	Age
ARE007	DIS+TV	AA	Single patch AA	SALT	2%	Mild	F	38
ARE010	DIS+TV	AA	Multiple patches AA	SALT	9%	Mild	F	43
ARE012	DIS+TV	AA	Multiple patches AA	SALT	38%	Mild	M	64
ARE013	DIS+TV	AA	Multiple patches AA	SALT	7%	Mild	M	38
ARE014	DIS+TV	AA	Multiple patches AA	SALT	55%	Severe	M	20
ARE017	DIS+TV	AA	Multiple patches AA	SALT	62%	Severe	F	38
ARE018	DIS+TV	AA	Multiple patches AA	SALT	60%	Severe	F	61
ARE020	DIS+TV	AA	Multiple patches AA	SALT	19%	Mild	F	30
ARE030	DIS+TV	AA	AT	SALT	100%	Severe	F	54
ARE031	DIS+TV	AA	AU	SALT	100%	Severe	F	49
ARE001	CV	AA	Single patch AA	SALT	2%	Mild	F	76
ARE002	CV	AA	Single patch AA	SALT	2%	Mild	F	70
ARE003	CV	AA	Single patch AA	SALT	2%	Mild	F	62
ARE005	CV	AA	Multiple patches AA	SALT	60%	Severe	F	47
ARE006	CV	AA	Single patch AA	SALT	4%	Mild	F	24
ARE008	CV	AA	Multiple patches AA	SALT	35%	Mild	F	32
ARE009	CV	AA	Multiple patches AA	SALT	60%	Severe	F	23
ARE011	CV	AA	Multiple patches AA	SALT	70%	Severe	F	41
ARE016	CV	AA	AU	SALT	100%	Severe	F	41
ARE019	CV	AA	Multiple patches AA	SALT	36%	Mild	M	58
ARE026	CV	AA	AT	SALT	100%	Severe	M	43
ARE027	CV	AA	Multiple patches AA	SALT	7%	Mild	F	18
ARE028	CV	AA	Multiple patches AA	SALT	37%	Mild	M	58
ARE029	CV	AA	Multiple patches AA	SALT	72%	Severe	F	46
ARE032	CV	AA	AT	SALT	100%	Severe	F	47
ARE033	CV	AA	Multiple patches AA	SALT	4%	Mild	M	47

Subject ID	Study Phase	Group	Type	Scale	Value	Severity	Gender	Age
ARE034	CV	AA	Multiple patches AA	SALT	31%	Mild	M	41
ARE035	CV	AA	Multiple patches AA	SALT	19%	Mild	F	50
ARE036	CV	AA	AT	SALT	100%	Severe	F	45
ARE037	CV	AA	AT	SALT	100%	Severe	F	40
ARE038	CV	AA	Single patch AA	SALT	3%	Mild	M	27
ARE039	CV	AA	Single patch AA	SALT	3%	Mild	M	38
ARE040	CV	AA	AT	SALT	100%	Severe	F	46
ARE045	CV	AA	Single patch AA	SALT	6%	Mild	F	37
ARE046	CV	AA	Single patch AA	SALT	5%	Mild	F	49
ARE047	CV	AA	AT	SALT	100%	Severe	F	49
ARE048	CV	AA	AT	SALT	100%	Severe	F	23
ARE049	CV	AA	Multiple patches AA	SALT	87%	Severe	F	27
ARE052	CV	AA	Multiple patches AA	SALT	70%	Severe	F	25
BIO021	CV	AA	Single patch AA	SALT	4%	Mild	M	34
DER026	CV	Vitiligo	Non-segmental Vitiligo	VIDA/duration	0/20ys	-	M	24
DER029	CV	Vitiligo	Non-segmental Vitiligo	VASI	12%		F	50
DER031	CV	Vitiligo	Non-segmental Vitiligo		+3/2ys		M	16
DER032	CV	Vitiligo	Non-segmental Vitiligo		+2/1ys		F	38
DER037	CV	Vitiligo	Non-segmental Vitiligo		+3/20ys		F	35
DER043	CV	Vitiligo	Non-segmental Vitiligo		+3/20ys		F	24
DER052	CV	Vitiligo	Non-segmental Vitiligo		0/3ys		F	55
DER056	CV	Vitiligo	Non-segmental Vitiligo		0/10ys		M	21
DER076	CV	Vitiligo	Non-segmental Vitiligo	VIDA/duration	0/20ys	-	M	74
BIO037	CV	Vitiligo	Non-segmental Vitiligo	VASI	0/3ys+		F	52
BIO044	CV	Vitiligo	Non-segmental Vitiligo	VASI	14%		M	51
BIO045	CV	Vitiligo	Non-segmental Vitiligo	VASI	12%	Severe	F	62
BIO046	CV	Vitiligo	Non-segmental Vitiligo	VASI	22%	Severe	M	37
BIO047	CV	Vitiligo	Non-segmental Vitiligo	VASI	17%	Severe	M	56
BIO005	CV	AD	Severe Atopic Dermatitis	IGA/EASI	3/12	Severe	M	18
BIO007	CV	AD	Severe Atopic Dermatitis	IGA/EASI	3/15	Severe	F	60
BIO012	CV	AD	Severe Atopic Dermatitis	IGA/EASI	3/17	Severe	M	63
BIO013	CV	AD	Severe Atopic Dermatitis	IGA/EASI	4/24	Severe	F	43

Subject ID	Study Phase	Group	Type	Scale	Value	Severity	Gender	Age
BIO016	CV	AD	Severe Atopic Dermatitis	IGA/EASI	4/32	Severe	M	75
BIO034	CV	AD	Severe Atopic Dermatitis	IGA/EASI	3/10	Severe	M	33
BIO039	CV	AD	Severe Atopic Dermatitis	IGA	3	Severe	F	27
DER078	CV	AD	Severe Atopic Dermatitis	IGA	4	Severe	M	47
DER080	CV	AD	Severe Atopic Dermatitis	IGA	4	Severe	F	31
DER082	CV	AD	Severe Atopic Dermatitis	IGA	4	Severe	M	36
BIO002	CV	PsO	Plaque Psoriasis	PGA	3	Severe	M	45
BIO003	CV	PsO	Plaque Psoriasis	PGA	3	Severe	M	48
DER001	CV	PsO	Plaque Psoriasis	PGA	4	Severe	M	71
DER002	CV	PsO	Plaque Psoriasis	PGA	3	Severe	M	40
DER004	CV	PsO	Plaque Psoriasis	PGA	3	Severe	M	42
DER007	CV	PsO	Plaque Psoriasis	PGA	3	Severe	M	38
DER101	CV	PsO	Plaque Psoriasis	PGA	4	Severe	F	49
DER109	CV	PsO	Plaque Psoriasis	PGA	4	Severe	F	52
BIO063	CV	PsO	Plaque Psoriasis	PGA	3	Severe	F	31
BIO064	CV	PsO	Plaque Psoriasis	PGA	4	Severe	M	62
CON187	DIS+TV	control	control	-	-	-	M	68
CON195	DIS+TV	control	control	-	-	-	M	23
CON197	DIS+TV	control	control	-	-	-	M	54
CON199	DIS+TV	control	control	-	-	-	F	28
CON202	DIS+TV	control	control	-	-	-	F	18
CON204	DIS+TV	control	control	-	-	-	M	37
CON206	DIS+TV	control	control	-	-	-	F	36
CON209	DIS+TV	control	control	-	-	-	M	21
CON211	DIS+TV	control	control	-	-	-	F	40
CON135	DIS+TV	control	control	-	-	-	F	43
CON127	CV	control	control	-	-	-	M	52
CON129	CV	control	control	-	-	-	M	50

Subject ID	Study Phase	Group	Type	Scale	Value	Severity	Gender	Age
CON136	CV	control	control	-	-	-	F	62
CON144	CV	control	control	-	-	-	M	67
CON146	CV	control	control	-	-	-	M	66
CON173	CV	control	control	-	-	-	F	38
CON175	CV	control	control	-	-	-	M	66
CON178	CV	control	control	-	-	-	F	67
CON181	CV	control	control	-	-	-	M	63
CON182	CV	control	control	-	-	-	F	76
CON183	CV	control	control	-	-	-	M	52
CON184	CV	control	control	-	-	-	M	56
CON189	CV	control	control	-	-	-	M	73
CON194	CV	control	control	-	-	-	M	51
CON196	CV	control	control	-	-	-	M	61
CON198	CV	control	control	-	-	-	M	36
CON200	CV	control	control	-	-	-	F	22
CON201	CV	control	control	-	-	-	F	22
CON203	CV	control	control	-	-	-	F	18
CON205	CV	control	control	-	-	-	M	40
CON212	CV	control	control	-	-	-	M	32
CON213	CV	control	control	-	-	-	M	27
CON214	CV	control	control	-	-	-	F	33
CON224	CV	control	control	-	-	-	F	28
CON225	CV	control	control	-	-	-	F	43
CON226	CV	control	control	-	-	-	F	33
CON227	CV	control	control	-	-	-	M	61
CON228	CV	control	control	-	-	-	F	58
CON229	CV	control	control	-	-	-	M	47
CON231	CV	control	control	-	-	-	F	44

AA: Alopecia Areata; AT: Alopecia Totalis; AU: Alopecia Universalis; AD: Atopic Dermatitis; PsO: Psoriasis; IGA: Investigator’s Global Assessment; EASI: Eczema Area and Severity Index; PGA: Physician's Global Assessment; VASI: Vitiligo Area Scoring

Index; SALT: Severity of Alopecia Tool; DIS: Discovery Phase; TV: Technical Validation; CV: Clinical Validation; F: Female; M: Male; H: Hispanic (self-reported).