Allergy Asthma Immunol Res. 2025 Mar;17(2):226-240
https://doi.org/10.4168/aair.2025.17.2.226
pISSN 2092-7355·eISSN 2092-7363

Allergy, Asthma & Immunology Research AAIR

Original Article

Check for updates

Analysis of miRNA Expression in Patients With NSAID-Exacerbated Respiratory Disease

Adrian Gajewski,[1] **Adrian Bekier**,[1] **Karolina Frachowicz-Guereirro**,[1] **Izabela Drożdż**,[2] **Rafał Ćwikliński**,[1] **Marcin Kurowski**,[1] **Marek L. Kowalski**,[1] **Ralf Baumann**,[3,4] **Carsten Schmidt-Weber**,[5,6] **Adam M. Chaker**,[5,6] **Maciej Chałubiński**,[1] **Aleksandra Wardzyńska**[1*]

[1]Department of Immunology and Allergy, Medical University of Lodz, Lodz, Poland
[2]Department of Clinical Genetics, Medical University of Lodz, Lodz, Poland
[3]Institute for Occupational, Social and Environmental Medicine, Medical Faculty, University Hospital RWTH Aachen University, Aachen, Germany
[4]Institute for Translational Medicine (ITM), Medical School Hamburg (MSH), Hamburg, Germany
[5]Center of Allergy and Environment (ZAUM), Technical University and Helmholtz Center Munich, Member of the German Center for Lung Research (DZL), Munich, Germany
[6]Department of Otorhinolaryngology and Head and Neck Surgery, TUM School of Medicine and Health, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany

OPEN ACCESS

Received: Jul 26, 2024
Revised: Oct 19, 2024
Accepted: Oct 29, 2024
Published online: Feb 5, 2025

Correspondence to
Aleksandra Wardzyńska, MD, PhD
Department of Immunology and Allergy, Medical University of Lodz, 251 Pomorska Str., 92-213 Lodz, Poland.
Tel: +48-42-675-7309
Fax: +48-42-678-2294
Email: aleksandra.wardzynska@umed.lodz.pl

ORCID iDs
Adrian Gajewski
https://orcid.org/0000-0001-7445-9752
Adrian Bekier
https://orcid.org/0000-0002-4980-1074
Karolina Frachowicz-Guereirro
https://orcid.org/0000-0003-4599-1445
Izabela Drożdż
https://orcid.org/0000-0003-1956-5063
Marcin Kurowski
https://orcid.org/0000-0002-0653-7533
Marek L. Kowalski
https://orcid.org/0000-0002-8442-2774
Ralf Baumann
https://orcid.org/0000-0002-6523-1024

ABSTRACT

Purpose: Non-steroidal anti-inflammatory drug-exacerbated respiratory disease (N-ERD) is a phenotype of bronchial asthma that is characterized by a severe course and the presence of chronic rhinosinusitis (CRS) with nasal polyps. MicroRNAs (miRNAs) belong to a family of small, non-coding RNAs whose primary function is to regulate gene transcription. The aim of this study was to determine the miRNA profile and to validate selected miRNAs in biological material from the upper respiratory tract collected with a minimally-invasive method in patients with N-ERD.
Methods: The miRNA profile was assessed in subjects with N-ERD, CRS, and allergic asthma (AA), as well as healthy controls (HCs), using microarray technique. Following this, 6 miRNAs were validated using reverse transcription polymerase chain reaction in 77 subjects.
Results: The profiling identified 23 miRNAs whose expression significantly differed between patients with N-ERD and HCs. Based on these results, 6 miRNAs were selected for further validation. It was found that patients with N-ERD had significantly different expressions of miR-34a-5p and miR-22-5p compared to those with AA. In the whole study group, significant correlations were found between miR-7d-3p/miR-34a-5p/miR-22-5p and the presence of blood eosinophilia ($r = 0.25$, $r = 0.28$ and $r = 0.26$, for all $P < 0.05$). Forced expiratory volume in 1 second/forced vital capacity was correlated with miR-149a-5p expression ($r = 0.27$, $P < 0.05$).
Conclusions: The results indicate that the miRNA profile in nasal mucosal lining fluid of patients with N-ERD differs from patients with AA, CRS, and compared to HCs. Some of the miRNAs selected on the basis of profiling may be involved in the regulation of eosinophilic inflammation in the respiratory tract. Our findings suggest that specific miRNAs may be considered as potential biomarkers of N-ERD.

Keywords: MicroRNAs; asthma; drug hypersensitivity; eosinophils; biomarker; nasal mucosa

Allergy, Asthma & Immunology Research AAIR

Carsten Schmidt-Weber
https://orcid.org/0000-0002-3203-8084
Adam M. Chaker
https://orcid.org/0000-0002-5117-4073
Maciej Chałubiński
https://orcid.org/0000-0001-8311-9530
Aleksandra Wardzyńska
https://orcid.org/0000-0001-6253-2799

Disclosure
There are no financial or other issues that might lead to conflict of interest.

INTRODUCTION

One of the phenotypes of bronchial asthma is non-steroidal anti-inflammatory drug-exacerbated respiratory disease (N-ERD), which is often characterized by a severe clinical course and intense eosinophilic inflammation in the upper and lower airways.[1] Exposure to non-steroidal anti-inflammatory drugs intensifies the underlying abnormalities, which could be manifested as acute symptoms such as nasal discharge, blockage or bronchospasm. The pathophysiology of this process is not fully understood; however, it is believed that the primary defect relates to an individual predisposition comprising overproduction of pro-inflammatory derivatives of arachidonic acid together with a simultaneous deficiency of protective metabolites such as PGE2.[1] The key factors in this process are respiratory epithelial cells and non-specific response lymphocytes producing the alarmins and chemokines responsible for the recruitment of effector cells such as mast cells, eosinophils and neutrophils into the respiratory tract.[2] The gold standard for the diagnosis of N-ERD is an aspirin challenge, most often administered orally, but there are numerous contraindications to this test, and for safety reasons, it is performed only in specialized centers. Also, no biomarkers have been identified that could form the basis for the development of *in vitro* diagnostic tests.[1]

MicroRNAs (miRNAs) belong to a family of small, non-coding RNAs whose primary function is to regulate gene transcription. They exist in both the intra- and extracellular fractions. A single miRNA can affect the transcription of many different genes and, consequently, many biochemical pathways. Several studies have shown the involvement of miRNAs in the regulation of processes related to inflammation occurring in chronic non-infectious diseases of the upper and lower respiratory tract. Besides, due to their stability and tissue specificity, they are considered potential biomarkers of inflammatory disorders.[3] miRNAs are expressed differently in asthma and allergic patients, compared to healthy subjects, and many miRNAs may be dysregulated depending on the severity or phenotype of the disease.[4] Studies on chronic rhinosinusitis (CRS) have focused on the role of miRNAs in the regulation of key proinflammatory cytokines, factors associated with T2-dependent inflammation or tissue remodeling. Similar to asthma, differential expression of several miRNAs has been demonstrated in CRS patients compared to controls.[5] One study showed that the miRNA profile varied depending on the CRS phenotype.[6] However, to the authors' knowledge, this topic has not been explored in subjects with N-ERD.

The lining fluid of the nasal mucosa consists of a variety of mediators derived from epithelial and inflammatory cells. It is a material that can be collected using minimally-invasive methods, and the mediator`s levels of the mediators may correlate with the inflammation in both the upper and lower respiratory tract.[7] We therefore hypothesized that patients with N-ERD may have a unique miRNome (disease-specific miRNA expression profile) that could be assessed by a nasal sampling. The aim of the study was to compare the miRNA profile of the fluid of the nasal mucosal lining in patients with N-ERD with that of healthy subjects and patients with CRS with nasal polyps (CRSwNP) or allergic asthma (AA). It also determined the expression of selected miRNAs in a larger group of patients to verify their potential usefulness as biomarkers.

https://doi.org/10.4168/aair.2025.17.2.226

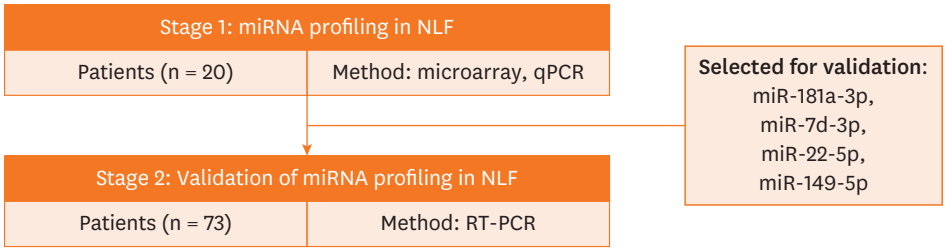

Fig. 1. A diagram describing the study design.
miRNA, microRNA; NLF, nasal lining fluid; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction.

MATERIALS AND METHODS

Study design

The study was a single-center, observational, and all procedures related to the clinical evaluation of the patient, and collection of biological material were performed during a single visit. We adopted a 2-stage approach to identify miRNAs in nasal mucosal lining fluid of potential value as biomarkers. In the first stage, we profiled miRNAs in a representative group of 20 subjects (equal numbers of patients with N-ERD, asthma, CRS, and healthy individuals) using microarrays. The second stage was aimed to validate the results obtained in the previous one. miRNAs that, according to profiling, had the ability to differentiate between N-ERD patients and other groups were determined using reverse transcription polymerase chain reaction (RT-PCR) in a larger sample. The study was approved by the local Bioethics Committee (approval number RNN/204/19/KE). All study participants gave their written and informed consent. The scheme of the study is shown in **Fig. 1**.

Patients and biological material sampling

Patients for both stages were recruited according to the same protocol. The inclusion criteria comprised age 18–75 years, and a diagnosis of one or more of the following upper or lower respiratory diseases: allergic rhinitis (AR; diagnosed according to ARIA criteria),[8] CRS with or without nasal polyps (diagnosed on the basis of EPOS),[9] bronchial asthma (based on GINA 2019),[10] N-ERD (based on EAACI guidelines).[1] The control group consisted of subjects without any of the diseases mentioned above. The exclusion criteria comprised any of the following: active atopic dermatitis, specific immunotherapy in the last 5 years, biological therapy within 6 months, active infectious diseases, exacerbation of chronic diseases, uncontrolled mental illnesses, malignant tumors in active status, taking immunosuppressive drugs, condition after organ transplantation, inability to communicate or understand the objectives of the study, or any active addictions.

Patients taking medication were asked to discontinue nasal steroids, montelukast and antihistamines 7 days before the study and oral steroids 10 days before the visit. Inhaled steroids were discontinued 24 hours before the visit.

The patients completed a questionnaire about symptoms, medications taken, and medical history. The Asthma Control Questionnaire (ACQ) was used to assess asthma control. The Sinonasal Outcome Test 22 (SNOT-22) was used to assess the severity of symptoms and quality of life in patients with CRS.

https://doi.org/10.4168/aair.2025.17.2.226

Table 1. Patient characteristics: profiling study

Variables (n = 20)	Sex	Age (year)	FEV1/FVC%	FeNO (ppb)	Atopy	Blood Eos (G/l)
N-ERD (n = 5)	Female	61	82.4	17	Y	0.14
	Male	26	83.2	17	N	0.79
	Female	67	61.0	28	Y	0.09
	Male	68	53.0	39	Y	0.55
	Female	46	71.5	13	N	NA
AA (n = 5)	Female	47	65.9	48	Y	0.12
	Female	42	83.6	25	Y	0.17
	Female	62	73.6	23	Y	0.33
	Male	45	66.2	19	Y	0.12
	Male	43	73.7	22	Y	0.16
CRS (n = 5)	Male	27	77.4	29	Y	0.07
	Female	64	82.3	17	N	0.15
	Female	49	80.7	20	N	0.08
	Male	55	76.1	71	Y	0.68
	Male	64	59.3	41	N	0.30
Controls (n = 5)	Female	52	84.1	33	N	0.04
	Male	31	82.3	21	N	0.35
	Male	34	83.5	21	N	0.34
	Female	64	72.1	16	Y	0.11
	Female	53	71.2	18	N	0.12

N-ERD, non-steroidal anti-inflammatory drug exacerbated respiratory disease; AA, allergic asthma; CRS, chronic rhinosinusitis; FEV1/FVC%, forced expiratory volume in 1 second to the forced vital capacity ratio; FeNO, fractional exhaled nitric oxide; Blood Eos, blood eosinophilia.

Table 2. Patient characteristics: validation study

Variables	N-ERD (n = 18)	AA (n = 22)	CRS (n = 23)	Controls (n = 14)	N-ERD vs. AA	N-ERD vs. CRS	N-ERD vs. Controls	AA vs. CRS	AA vs. Controls	CRS vs. Controls
Age (yr)	54.5 ± 13.7	42.7 ± 16.3	51.5 ± 14.8	32.7 ± 13.6	0.008	NS	< 0.002	0.03	0.04	0.002
Female	15/18 (83.3)	15/22 (68.2)	15/23 (65.2)	8/14 (57.1)	NS	NS	NS	NS	NS	NS
Atopy	10/18 (50.6)	22/22 (100)	10/23 (43.5)	3/14 (21.4)	NS	NS	NS	NS	NS	NS
FeNO (ppb)	33.63 ± 27.36	27.84 ± 19.68	31.6 ± 15.82	20.33 ± 7.95	NS	NS	NS	NS	NS	0.04
FEV1/FVC%	87.1 ± 6.5	86.1 ± 9.5	89.3 ± 10.4	97.9 ± 6.5	NS	NS	0.006	NS	0.002	0.03
Blood eosinophilia (G/l)	0.39 ± 0.32	0.23 ± 0.12	0.34 ± 0.19	0.14 ± 0.1	NS	NS	0.006	NS	0.01	0.02
ACQ score	1.35 ± 1.62	1.3 ± 1.22	NA	NA	NS	NS	NS	NS	NS	NS
SNOT 22 score	41.76 ± 21.48	NA	35.86 ± 18.36	NA	NS	NS	NS	NS	NS	NS

Values are presented as mean ± standard deviation or number (%).
N-ERD, non-steroidal anti-inflammatory drug exacerbated respiratory disease; AA, allergic asthma; CRS, chronic rhinosinusitis; FeNO, fractional exhaled nitric oxide; FEV1/FVC, forced expiratory volume in 1 second to the forced vital capacity ratio; ACQ, Asthma Control Questionnaire; SNOT 22, Sinonasal Outcome Test 22; NS, not significant; NA, not available.

The clinical and demographical characteristics of patients taking part in the first and second stages of the study are presented in **Tables 1** and **2**.

Fractional exhaled nitric oxide (FeNO) measurement

The patients performed single breath maneuvers online according to the American Thoracic Society/European Respiratory Society (ERS) guidelines,[11] using the HypAir FeNO (Medisoft, Sorinnes, Belgium). The mean value of at least 2 successful measurements was analyzed. FeNO measurement was performed before spirometry.

Spirometry

Spirometry was performed according to the ERS standards,[12] using a Vyntus system spirometer (Carefusion, San Diego, CA, USA).

Atopy evaluation

Atopy was evaluated using a panel of skin prick tests including the following inhalant

https://doi.org/10.4168/aair.2025.17.2.226

allergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Acarus siro*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, *cat* dander, *Alternaria tenuis*, birch pollen, mix of grass pollen, mugwort pollen, nettle pollen and ash pollen. A positive result was defined as a wheal of 3 mm in diameter. Atopy was diagnosed in the presence of at least one positive skin test.

Blood samples

Venous blood samples were obtained from study subjects (stages 1 and 2). These were collected into an anticoagulant tube (Sarstedt, Nümbrecht, Germany) to obtain serum and into an EDTA tube (Sarstedt) to determine differential blood count.

Nasal sampling

Nasal mucosal lining fluid was collected using Nasosorption devices (Hunt Developments Ltd., Midhurst, United Kingdom). After short anterior rhinoscopy, the fluid was sampled by gently inserting the Nasosorption into both nostrils for 60 seconds, while gently pressing on the wing of the nose. Then procedure was repeated twice, after a 60 minutes and then a 70 minutes interval in each nostril.

Evaluation of blood eosinophilia and nasal sample processing

Blood eosinophilia

Cell count and differentiation were determined with an XN-1000 hematology analyzer (Sysmex, Kobe, Japan).

Nasal sample processing

The nasal samples were eluted in 300 µl assay buffer (Ab-33k; Merck Millipore Life Science, Burlington, MA, USA), centrifuged at 16,000 × *g* at 4°C for 20 minutes and then pooled: first Nasosorption samples from both nostrils were pooled, and then the second and third from both nostrils were pooled. Samples were stored in −80°C. miRNA profiling was performed on pooled samples from the 2nd and 3rd collections, while the validation study was performed on the first collection.

miRNA isolation, profiling and validation with RT-PCR

miRNA isolation and complementary DNA (cDNA) synthesis

miRNA was isolated using miRNeasy Serum/Plasma Advanced Kit (Qiagen, Copenhagen, Denmark) from 200 µL of nasal samples according to the manufacturer's instructions. miRNA elution was performed in 20 µL nuclease-free water (Qiagen). Then the reverse transcription of RNA was performed using the miRCURY LNA RT Kit (Qiagen) and ratio 1:45 miRNA to nuclease-free water, was used.

Verification of the presence of miRNA in nasal lining fluid (NLF)

Due to the fact that miRNAs have never been determined before in NLF collected with Nasosorption, we conducted a preliminary study in a group of volunteers prior the profiling. Its aim was to verify whether miRNAs could be found in NLF obtained by this method. For this purpose, nasal samples were collected according to the protocol described above in a group of eleven volunteers aged 28–40 with asthma (n = 2) AR (n = 2), AR with asthma (n = 3) and healthy controls (n = 4).

miRNA isolation and cDNA synthesis were performed as described above. The RNA isolation efficiency and quality of the isolated miRNA was determined using miRCURY miRNA QC polymerase chain reaction (PCR) Panels (Qiagen). Quantitative PCR (qPCR) reaction

https://doi.org/10.4168/aair.2025.17.2.226

was performed on a LightCycler 480 II (Roche, Hvidovre, Denmark) thermal cycler with LightCycler 480 software (Roche). All samples demonstrated miR-103a-3p, miR-191-5p, miR-45a, miR-23a-3p and miR-30c-5p expression (relatively stable miRNAs in different cells, tissues and body fluids). These results indicated that the material collected using Nasosorption is a suitable source of miRNAs.

Stage 1: miRNA profiling

The initial steps (miRNA isolation and cDNA synthesis) were the same for stage 1 and 2 and were performed according to the method described above. Synthesized cDNA combined with the miRCURY LNA SYBR Green master mix (Qiagen) was added into the 384-well PCR plate containing the pre-aliquoted miRCURY LNA miRNA Human miRNome I & II Panels (Qiagen). qPCR reaction was performed on a LightCycler 480 II (Roche) thermal cycler with LightCycler 480 software (Roche). Cycle threshold (CT) values were exported to an Excel file and then uploaded on to the data analysis web portal at http://www.qiagen.com/geneglobe. Samples were assigned to a control group and test groups (N-ERD, CRS, AA). CT values were normalized based on the global CT mean of expressed miRNAs. The fold change in miRNA levels was calculated by the 2−ΔΔCt method.

Stage 2: validation study: qPCR

The selection of miRNAs for this part of the study was based on the profiling results. Any miRNAs that were differently expressed in N-ERD patients compared to both the controls and patients with AA or CRS were selected for validation. Primers specific for miR-181a-3p, -7d-3p, -22-5p, -149-5p, -34a-5p and -497-5p were purchased from Qiagen. Real-time PCRs were performed using the LightCycler 96 (Roche) and analyzed in LightCycler 96 software. Results were normalized to miR-103a-3p, whose expression showed the least variance in the profiling samples. miRNA expression is presented as a relative ratio. which is a mathematical delta–delta method for comparing relative expression results between treatments in real-time PCR.[13]

Statistical analysis

Differences in miRNA expressions in profiling study were calculated in Gene Globe application with a Student's *t*-test. Differences in miRNA expression showing more than a 2-fold up- or down-regulation ($P < 0.05$) were considered significant. In validation study as the samples were small and consisted of non-normal data, nonparametric tests were used. Comparisons between the groups were made with the Mann-Whitney *U* test. Spearman's rank correlation was used to evaluate the correlation between the variables. Analysis was not corrected for the multiplicity given the exploratory nature of the study. The statistical analysis was performed using Statistica 13.1 (TIBCO Software Inc., Palo Alto, CA, USA). A 2-tailed *P* value < 0.05 was considered as significant.

Molecular pathway and gene ontology (GO) analyses

GO functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using DIANA TOOLS MirPath version 3. Analysis was performed separately for 17 miRNAs that differentiated N-ERD patients from healthy people (**Supplementary Table S1**) and for those miRNAs which were selected for validation study (**Supplementary Table S2**).

https://doi.org/10.4168/aair.2025.17.2.226

RESULTS

Results of miRNA profiling

For miRNA profiling, a representative group of 20 participants (equal numbers of patients with N-ERD, CRS, AA and controls) was selected from the study population. The group was matched for sex (all groups) and age (patients with asthma, CRS and N-ERD) (**Table 1**). Out of the 767 miRNAs on the microarray, only 12 were not expressed in any of the samples. It was found that 639 miRNAs were expressed in at least 50% of the samples and 397 in all samples. Compared to the control group, 23 miRNAs were differently expressed in N-ERD patients, 8 in allergic asthmatics and 12 in CRS patients (**Fig. 2**, **Supplementary Table S3**). Some of these miRNAs were the same in the 2 groups, as shown in **Fig. 2**. Compared to the N-ERD patients, 4 miRNAs were differently expressed in CRS, 20 in allergic asthmatics (**Fig. 3**, **Supplementary Table S4**). Any miRNAs which were differently expressed in N-ERD patients compared to both controls and CRS or asthma patients and were not shared by 2 groups (**Fig. 3**, **Supplementary Table S4**) were chosen for further validation.

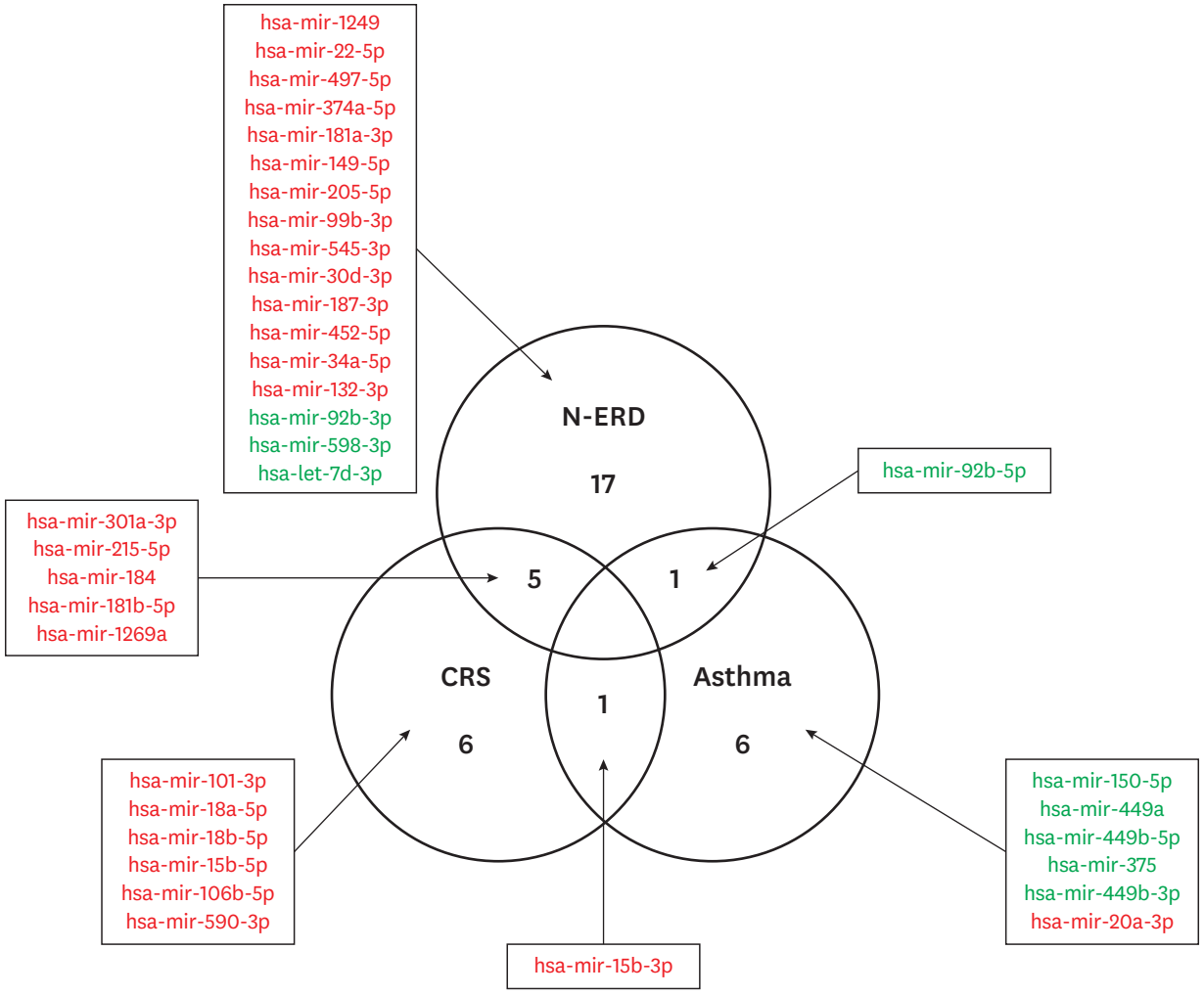

Fig. 2. Venn diagram with miRNAs unique and common for N-ERD (n = 5), AA (n = 5) and CRS (n = 5) compared to controls (n = 5). miRNAs showing more than a 2-fold up-regulation ($P < 0.05$) are marked with green; miRNAs showing more than 2-fold down-regulation ($P < 0.05$) are marked with red.
N-ERD, non-steroidal anti-inflammatory drug exacerbated respiratory disease; AA, allergic asthma; CRS, chronic rhinosinusitis; miRNA, microRNA.

https://doi.org/10.4168/aair.2025.17.2.226

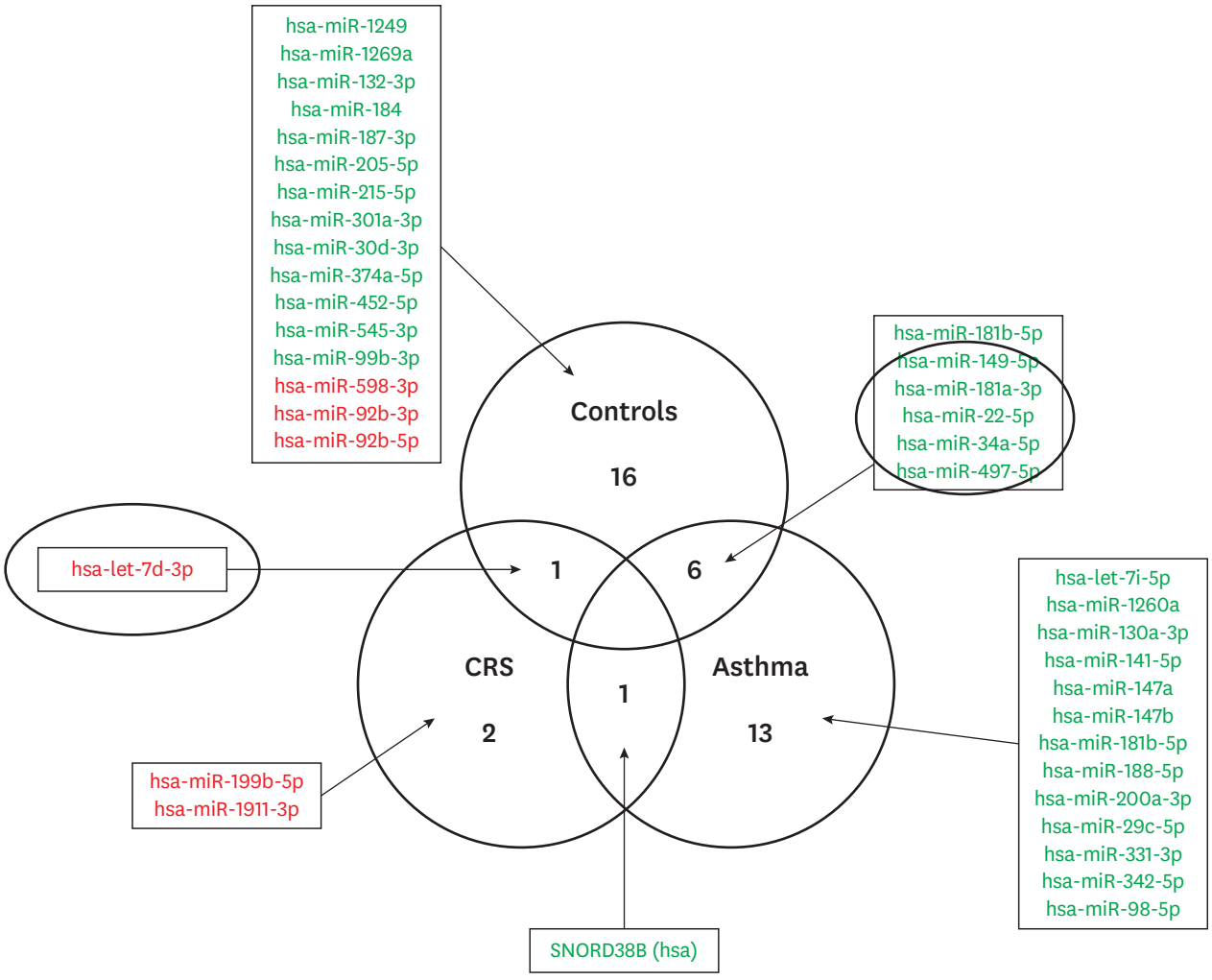

Fig. 3. Venn diagram with miRNAs unique and common for AA (n = 5), CRS (n = 5) and controls (n = 5) compared to N-ERD (n = 5). miRNAs showing more than a 2-fold up-regulation ($P < 0.05$) are marked with green; miRNAs showing more than 2-fold down-regulation ($P < 0.05$) are marked with red. miRNAs selected for validation are circled.
AA, allergic asthma; CRS, chronic rhinosinusitis; N-ERD, non-steroidal anti-inflammatory drug exacerbated respiratory disease; miRNA, microRNA.

Results of the miRNA validation

The patients with N-ERD were older than those with AA and controls, had a lower FEV1/FVC% and higher blood eosinophilia than controls. There was no difference in ACQ between N-ERD and AA patients. Similarly, the quality of life and the severity of upper respiratory tract symptoms assessed using the SNOT-22 questionnaire were comparable in patients with N-ERD and CRS. Patients with AA were younger than those with CRS, and in comparison to controls, a lower FEV1/FVC and higher blood eosinophilia were observed. Higher FeNO level and blood eosinophilia, but a lower FEV1/FVC was found in CRS patients compared to controls (**Table 2**).

The patients with N-ERD demonstrated significantly different expressions of miR-34a-5p and -22-5p than those with AA (**Fig. 4**). miR-22-5p, miR-149-5p and miR-497-5p were differently expressed in CRS patients in comparison to those with AA. miR-149a-5p was differently expressed in patients with AA compared to controls. In the whole study group, weak-moderate correlations were found between miR-7d-3p/miR-34a-5p/miR-22-5p and blood eosinophilia (**Fig. 5**). FEV1/FVC was moderately correlated with miR-149a-5p expression ($r = 0.27$; $P = 0.04$).

https://doi.org/10.4168/aair.2025.17.2.226

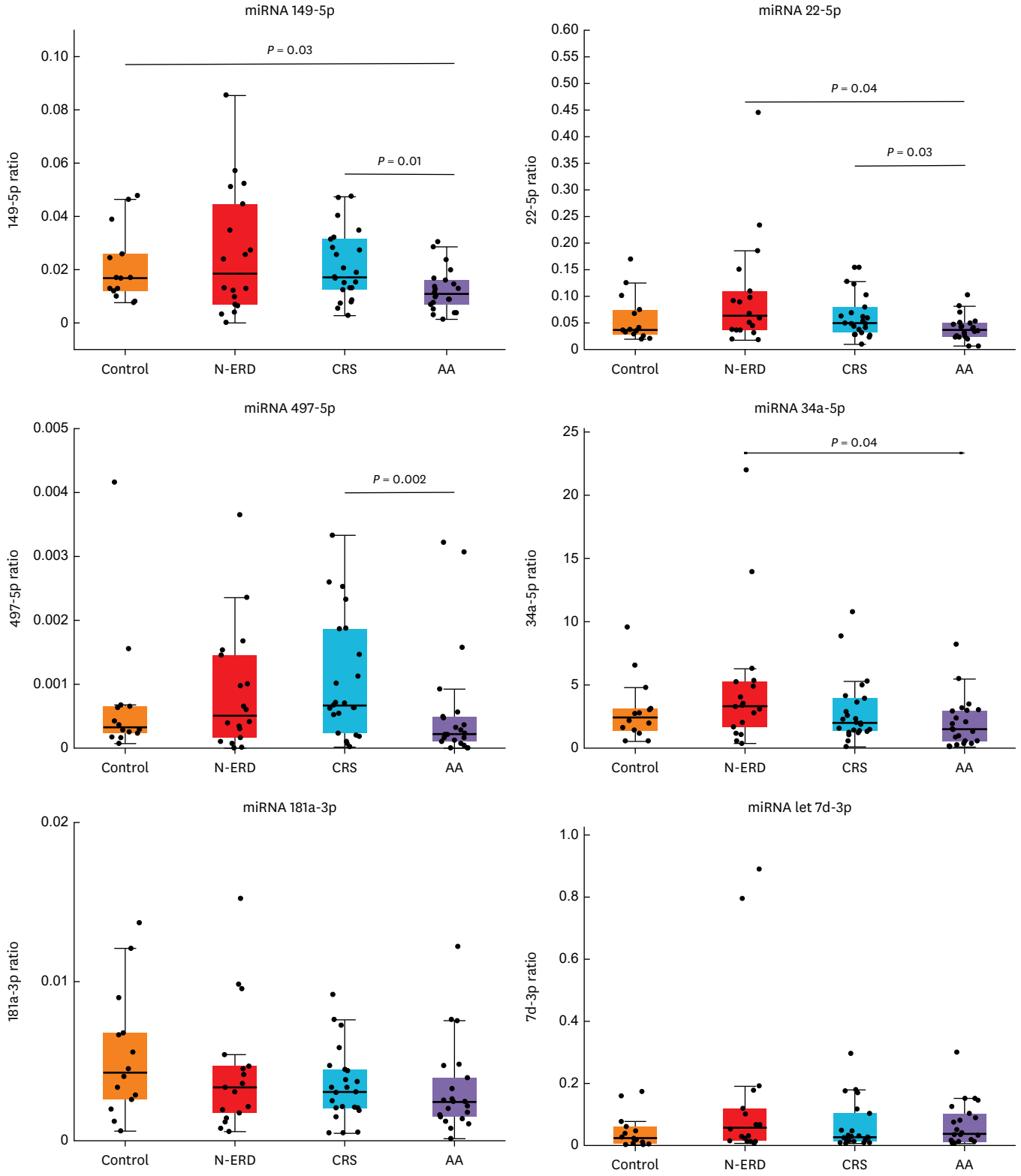

Fig. 4. Differences in expression (relative ratio) of miRNAs between patients with N-ERD/CRS/AA and controls. Comparison between groups was made with the Mann-Whitney test. Boxes: 25%–75% percentile; bars: median value; whiskers: non-outliers range.
miRNA, microRNA; N-ERD, non-steroidal anti-inflammatory drug exacerbated respiratory disease; CRS, chronic rhinosinusitis; AA, allergic asthma.

https://doi.org/10.4168/aair.2025.17.2.226

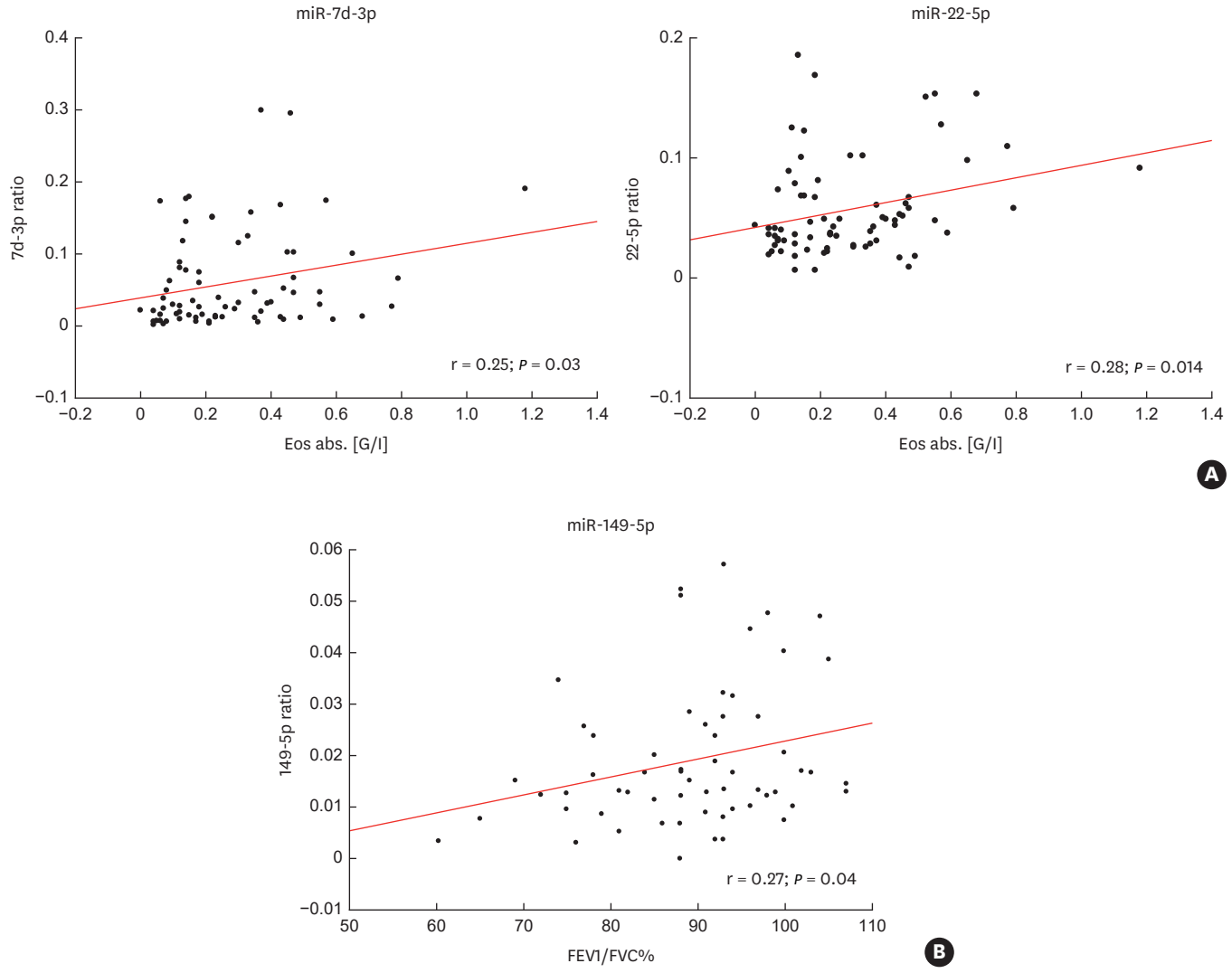

Fig. 5. Correlations between miRNA expression and clinical parameters in the study patients. (A) Correlation between miRNA expressions and blood eosinophilia. (B) Correlation between miRNA expression and forced expiratory volume in the first one second to the forced vital capacity. Correlation was performed with the use of Spearman's rank correlation test.
FEV1/FVC%, forced expiratory volume in 1 second to the forced vital capacity ratio; Eos abs., eosinophilia absolute; miRNA, microRNA.
Include exact *P* value in the **Fig. 5A**.

DISCUSSION

The pathogenesis of N-ERD is not fully understood and is associated with dysregulation of the production of arachidonic acid metabolites and the presence of chronic eosinophilic inflammation. The role of epigenetic regulation in this context is poorly researched; as such, a key observation from our study is the fact that the miRNome of N-ERD patients is not only different from healthy controls, but also from patients with AA or CRS. Our findings indicate 17 miRNAs whose expression differed uniquely in patients with N-ERD compared to controls. In addition, only 5 miRNAs differentiating between patients and healthy subjects were common to both the N-ERD and CRS subjects, and only one was shared between N-ERD and AA. Other studies also indicate that some miRNAs can not only differentiate patients with a disease of interest, such as asthma or AR, from healthy controls, but also can indicate the phenotype of the condition, suggesting that the miRNAs may have potential utility as biomarkers.[4]

https://doi.org/10.4168/aair.2025.17.2.226

This study was part of a European multicenter ADAPT project, whose primary aim was to identify, and validate a set of biomarkers associated with AR, CRS or different phenotypes of asthma measured in NLF collected using a minimally-invasive method. NLF contains cytokines and other mediators originating from epithelial and inflammatory cells. Our findings in this exploratory sub-analysis indicate that it is also a rich source of circulating miRNAs, probably secreted by these cells. In the case of bronchial asthma, inflammation covers the entire airways, and it has even been suggested that collecting material from the upper respiratory tract may better illustrate the inflammation in the lower airways than serum or plasma.[14] miRNAs have previously been determined in materials from the upper respiratory tract in eluates from nasal swabs[15] or in nasal lavage fluid.[16] However, it is worth mentioning that the miRNA profile and expression will probably differ in the material from the upper respiratory tract and, for example, in serum. One study in patients with coronavirus disease 2019 showed that the miRNA profile differed between plasma and nasal swab eluates, and only 2 miRNAs were shared between the 2 materials.[17]

Among the list of miRNAs we identified in the profiling, a few have been studied previously as biomarkers in studies on asthma, CRS and AR. Some authors additionally noted an association of miRNAs expression with clinical features, lung, the presence of eosinophilic inflammation in the airways, or the phenotype or severity of the disease.

Huo *et al.*[18] report decreased epithelial and plasma miR-181b-5p expression in asthmatics and note that the level correlated with those of sputum and bronchial eosinophilia. Also, miR-181b-5p regulated interleukin (IL)-13 -induced IL-1β and CCL11 expression by targeting SPP1 in human bronchial epithelial cells *in vitro*. In our group, both N-ERD and CRS patients demonstrated decreased miR-181b-5p expression compared to controls. miR-34a was another one miRNA that differentiated asthmatic patients from controls.[19] In our group, it was downregulated in N-ERD patients compared to controls.

Another miRNAs of interest, miR-18a-5p and miR-374 were included in the set of miRNAs used to differentiate asthma in the pediatric population.[20] Our profiling also indicated that miR-375 was upregulated in asthmatics and miR-18a-5p was downregulated in CRS patients.

Hirai *et al.*[21] identified 5 miRNAs that distinguished between asthma and chronic obstructive pulmonary disease, with miR-15b-5p showing the highest predictive value. In another study, miR-15a-5p, miR-15b-5p and miR-374a-5p were correlated with changes in ventilation parameters and FeNO in patients with AA challenged with house dust mites. Based on the results, the authors suggested the role of these miRNAs as predictors of the delayed reaction occurring during allergen challenge.[22] Interestingly, miR-15b-5p was down-regulated in our patients with asthma and CRS, while miRNA-374a-5p was downregulated in N-ERD patients. In a study on asthma phenotypes, miRNA-215-5p was among 5 miRNAs identified as discriminating between mild and moderate/severe asthmatics.[23] In our study, this miRNA was downregulated in patients with N-ERD and CRS compared to controls. Another study found a total of 26 miRNAs, including miR-101-3p, to be differentially expressed in asthmatics as compared to healthy controls after rhinovirus challenge[24]; in contrast, this miRNA was downregulated in patients with CRS in this study.

Based on microarray and qPCR analyses, in a study of Silveira *et al.*[25], 6 miRNAs found to be differently expressed in CRSwNP patients as compared to controls including miR-205-5p. Moreover, miR-205-5p correlated with IL-5 levels, eosinophil counts at the tissue and SNOT-

https://doi.org/10.4168/aair.2025.17.2.226

22 score. The present study demonstrated decreased expression of miR-205-5p in the NLF of N-ERD patients.

In a study of Yu *et al.*[26] downregulation of 192 miRNA was demonstrated in epithelial cells collected form patients with CRSwNP. Validation of the profiling result in these patients using RT-PCR showed that, among others, miR-132-3p expression was significantly downregulated in those with CRSwNP in comparison to healthy controls. Similarly, in our patients with N-ERD, the expression of this particular miRNA was also lower than in controls.

In contrast to our present results, a previous study found miR-150-5p expression to be decreased in AR patients compared to controls. In addition, miR-150-5p was also found to be responsible for downregulating intercellular adhesion molecule 1 expression and suppressing innate lymphoid cells function in a study based on a mouse model of AR.[27] MiR-150-5p was also observed to be up-regulated in dendritic cells in peripheral bloods from CRS patients.[28]

On the other hand, *in vitro* studies have shown potential mechanisms that may be influenced by the identified miRNAs. Most of them focused on the role in the regulation of cytokine secretion through influencing transcription factors or participating in remodeling-related processes.

In a mouse model of asthma, CircZNF652 activated miR-452-5p/JAK/STAT signaling, resulting in goblet cell metaplasia, airway hypersecretion, and hyperresponsiveness.[29]

Overexpression of miR-34a-5p suppressed the proliferation and migration of airway smooth muscle cells isolated from bronchoalveolar lavage specimens stimulated by platelet-derived growth factor-BB (PDGF-BB).[30] miR-34a-5p was also found to be an important factor influencing senescence in-human and mouse lung epithelial cells[31] and in normal human lung fibroblasts.[32] In animal model of asthma miR-301a-3p suppressed the PDGF-BB-stimulated proliferation and migration of airway smooth muscle cells, enhanced apoptosis, as well as decreased inflammation via targeting STAT3.[33] In addition, miR-497-5p inhibited lung fibroblast activation *in vitro*,[34] and miR-106b-5p inhibited transforming growth factor-β1-induced fibrosis in BEAS-2B cells.[35] In another study, miR-375 was found to be decreased in the epithelia of nasal mucosa of mice with AR, and the authors suggest that miR-375 may prevent cells from apoptosis and ameliorates AR via inhibiting JAK2/STAT3 pathway.[36] In a study by Song *et al.*,[37] a role for miR-181b-5p in regulating neutrophil-dependent asthma was demonstrated in a mouse model of asthma through inhibition of NETs release, DEK/p-GSK-3βSer9/β-catenin/MMP-9 pathway, DEK/Wnt/DRP1/MMP-9 and mitochondria damage. Experimental validation of miRNA profiling with another method only partially confirmed the initial results. Among the 6 selected miRNAs included in the second part of the study, only miR-34a-5p and -22-5p expression significantly differed between patients with N-ERD and those with AA. In addition, allergic asthmatics had significantly lower expression of miR-149 than controls. Three of the miRNAs selected for validation, miR-7d-3p, miR-34a-5p and miR-22-5p, were also weakly associated with blood eosinophilia, which may suggest their potential action mechanisms.

The study has the following limitations: first, the selected group for profiling may not be representative of the general population. In addition, the patients were not selected randomly, and the study group consisted of subjects who were treated in our clinic and voluntarily underwent the study. Hence, the selected miRNAs require further confirmation in an independent population. Secondly, only a limited number of miRNAs were included in the

validation part, omitting those which might potentially be more representative for the study group. It is also possible that the patients could have overlapping respiratory diseases, *e.g.* 50% of patients with N-ERD had atopy which could also be associated with the occurrence of AR. Thirdly, although study subjects were asked to discontinue inhaled steroids, this may also be a factor potentially influencing miRNA expression. Finally, in order to complete the study, it would also be appropriate to perform functional studies of the mechanism of action of the selected miRNAs, *e.g.* on cell lines or animal models.

Nevertheless, our results indicate that the miRNome of patients with N-ERD in NLF differed from those with AA/CRS or controls. The validation of miRNA profiling showed that miR-34a-5p and miR-22 were differently expressed in patients with N-ERD compared to allergic asthmatics. The selected miRNAs were found to correlate with clinical features: blood eosinophilia and spirometric parameters.

ACKNOWLEDGMENTS

This study was supported by EIT Health (ADAPT - Airway Diseases, Analysis and Prevention; GRANT NR 19065), a body of the European Union. The authors would like to thank the study nurse involved in the study, Mrs Dorota Żaromińska, for her assistance.

SUPPLEMENTARY MATERIALS

Supplementary Table S1

KEGG pathway enrichment analysis and GO functional enrichment analysis of miRNAs significantly different between N-ERD patients and healthy controls

Supplementary Table S2

KEGG pathway enrichment analysis and GO functional enrichment analysis of miRNAs selected for the validation study

Supplementary Table S3

Results of miRNA profiling (stage 1)

Supplementary Table S4

Results of miRNA profiling (stage 1)

REFERENCES

1. Kowalski ML, Agache I, Bavbek S, Bakirtas A, Blanca M, Bochenek G, et al. Diagnosis and management of NSAID-exacerbated respiratory disease (N-ERD)-a EAACI position paper. Allergy 2019;74:28-39. PUBMED | CROSSREF
2. Kohanski MA, Cohen NA, Barrett NA. Epithelial dysregulation in chronic rhinosinusitis with nasal polyposis (CRSwNP) and aspirin-exacerbated respiratory disease (AERD). J Allergy Clin Immunol 2021;148:1161-4. PUBMED | CROSSREF
3. Gil-Martínez M, Lorente-Sorolla C, Naharro S, Rodrigo-Muñoz JM, Del Pozo V. Advances and highlights of miRNAs in asthma: biomarkers for diagnosis and treatment. Int J Mol Sci 2023;24:1628. PUBMED | CROSSREF

4. Weidner J, Bartel S, Kılıç A, Zissler UM, Renz H, Schwarze J, et al. Spotlight on microRNAs in allergy and asthma. Allergy 2021;76:1661-78. PUBMED | CROSSREF
5. Brar T, Marks L, Lal D. Insights into the epigenetics of chronic rhinosinusitis with and without nasal polyps: a systematic review. Front Allergy 2023;4:1165271. PUBMED | CROSSREF
6. Zhang XH, Zhang YN, Li HB, Hu CY, Wang N, Cao PP, et al. Overexpression of miR-125b, a novel regulator of innate immunity, in eosinophilic chronic rhinosinusitis with nasal polyps. Am J Respir Crit Care Med 2012;185:140-51. PUBMED | CROSSREF
7. Hansel TT, Tunstall T, Trujillo-Torralbo MB, Shamji B, Del-Rosario A, Dhariwal J, et al. A comprehensive evaluation of nasal and bronchial cytokines and chemokines following experimental rhinovirus infection in allergic asthma: increased interferons (IFN-γ and IFN-λ) and type 2 inflammation (IL-5 and IL-13). EBioMedicine 2017;19:128-38. PUBMED | CROSSREF
8. Bousquet J, Schünemann HJ, Togias A, Bachert C, Erhola M, Hellings PW, et al. Next-generation allergic rhinitis and its impact on asthma (ARIA) guidelines for allergic rhinitis based on grading of recommendations assessment, development and evaluation (GRADE) and real-world evidence. J Allergy Clin Immunol 2020;145:70-80.e3. PUBMED | CROSSREF
9. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European position paper on rhinosinusitis and nasal polyps 2020. Rhinology 2020;58:1-464. PUBMED | CROSSREF
10. Global Initiative for Asthma. Global strategy for asthma management and prevention, 2019 [Internet]. Fontana (WI): Global Initiative for Asthma; 2019 [cited 2024 Jul 1]. Available from: www.ginasthma.org.
11. American Thoracic SocietyEuropean Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171:912-30. PUBMED | CROSSREF
12. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005;26:319-38. PUBMED | CROSSREF
13. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:e45. PUBMED | CROSSREF
14. Poulsen NN, Bjerregaard A, Khoo SK, Laing IA, Le Souëf P, Backer V, et al. Airway interleukin-33 and type 2 cytokines in adult patients with acute asthma. Respir Med 2018;140:50-6. PUBMED | CROSSREF
15. Trifunovic A, Dombkowski A, Cukovic D, Mahajan P. The potential of microRNAs as noninvasive biomarkers in acute pediatric asthma. J Allergy Clin Immunol 2020;145:1706-1708.e5. PUBMED | CROSSREF
16. Cha S, Seo EH, Lee SH, Kim KS, Oh CS, Moon JS, et al. MicroRNA expression in extracellular vesicles from nasal lavage fluid in chronic rhinosinusitis. Biomedicines 2021;9:471. PUBMED | CROSSREF
17. Farr RJ, Rootes CL, Stenos J, Foo CH, Cowled C, Stewart CR. Detection of SARS-CoV-2 infection by microRNA profiling of the upper respiratory tract. PLoS One 2022;17:e0265670. PUBMED | CROSSREF
18. Huo X, Zhang K, Yi L, Mo Y, Liang Y, Zhao J, et al. Decreased epithelial and plasma miR-181b-5p expression associates with airway eosinophilic inflammation in asthma. Clin Exp Allergy 2016;46:1281-90. PUBMED | CROSSREF
19. Mirra D, Cione E, Spaziano G, Esposito R, Sorgenti M, Granato E, et al. Circulating microRNAs expression profile in lung inflammation: a preliminary study. J Clin Med 2022;11:5446. PUBMED | CROSSREF
20. He L, Liu J, Wang X, Wang Y, Zhu J, Kang X. Identifying a novel serum microRNA biomarker panel for the diagnosis of childhood asthma. Exp Biol Med (Maywood) 2022;247:1732-40. PUBMED | CROSSREF
21. Hirai K, Shirai T, Shimoshikiryo T, Ueda M, Gon Y, Maruoka S, et al. Circulating microRNA-15b-5p as a biomarker for asthma-COPD overlap. Allergy 2021;76:766-74. PUBMED | CROSSREF
22. Duecker RP, Alemdar O, Wimmers A, Gronau L, Chiocchetti AG, Valesky EM, et al. MicroRNA profiling of the inflammatory response after early and late asthmatic reaction. Int J Mol Sci 2024;25:1356. PUBMED | CROSSREF
23. Vázquez-Mera S, Martelo-Vidal L, Miguéns-Suárez P, Saavedra-Nieves P, Arias P, González-Fernández C, et al. Serum exosome inflamma-miRs are surrogate biomarkers for asthma phenotype and severity. Allergy 2023;78:141-55. PUBMED | CROSSREF
24. Wang W, Sinha A, Lutter R, Yang J, Ascoli C, Sterk PJ, et al. Analysis of exosomal microRNA dynamics in response to rhinovirus challenge in a longitudinal case-control study of asthma. Viruses 2022;14:2444. PUBMED | CROSSREF
25. Silveira MLC, Tamashiro E, Santos ARD, Martins RB, Faria FM, Silva LECM, et al. miRNA-205-5p can be related to T2-polarity in chronic rhinosinusitis with nasal polyps. Rhinology 2021;59:567-76. PUBMED | CROSSREF

26. Yu J, Kang X, Xiong Y, Luo Q, Dai D, Ye J. Gene expression profiles of circular RNAs and microRNAs in chronic rhinosinusitis with nasal polyps. Front Mol Biosci 2021;8:643504. PUBMED | CROSSREF
27. Zhang L, Meng W, Chen X, Ning Y, Sun M, Wang R. MiR-150-5p regulates the functions of type 2 innate lymphoid cells via the ICAM-1/p38 MAPK axis in allergic rhinitis. Mol Cell Biochem 2022;477:1009-22. PUBMED | CROSSREF
28. Ma Z, Shen Y, Zeng Q, Liu J, Yang L, Fu R, et al. MiR-150-5p regulates EGR2 to promote the development of chronic rhinosinusitis via the DC-Th axis. Int Immunopharmacol 2018;54:188-97. PUBMED | CROSSREF
29. Wang X, Xu C, Cai Y, Zou X, Chao Y, Yan Z, et al. CircZNF652 promotes the goblet cell metaplasia by targeting the miR-452-5p/JAK2 signaling pathway in allergic airway epithelia. J Allergy Clin Immunol 2022;150:192-203. PUBMED | CROSSREF
30. Ding L, Liu GL, Lu L, Ge L, Wang JY. circ_CSNK1E modulates airway smooth muscle cells proliferation and migration via miR-34a-5p/VAMP2 axis in asthma. Cell Signal 2022;95:110340. PUBMED | CROSSREF
31. Maeda H, Yao H, Go H, Huntington KE, De Paepe ME, Dennery PA. Involvement of miRNA-34a regulated Krüppel-like factor 4 expression in hyperoxia-induced senescence in lung epithelial cells. Respir Res 2022;23:340. PUBMED | CROSSREF
32. Markopoulos GS, Roupakia E, Tokamani M, Vartholomatos G, Tzavaras T, Hatziapostolou M, et al. Senescence-associated microRNAs target cell cycle regulatory genes in normal human lung fibroblasts. Exp Gerontol 2017;96:110-22. PUBMED | CROSSREF
33. Feng CY, Bai SY, Li ML, Zhao JY, Sun JM, Bao HJ, et al. Adipose-derived mesenchymal stem cell-derived exosomal miR-301a-3p regulates airway smooth muscle cells during asthma by targeting STAT3. J Asthma Allergy 2022;15:99-110. PUBMED | CROSSREF
34. Li Y, Sun W, Pan H, Yuan J, Xu Q, Xu T, et al. LncRNA-PVT1 activates lung fibroblasts via miR-497-5p and is facilitated by FOXM1. Ecotoxicol Environ Saf 2021;213:112030. PUBMED | CROSSREF
35. Liu S, Chen X, Zhang S, Wang X, Du X, Chen J, et al. miR-106b-5p targeting SIX1 inhibits TGF-β1-induced pulmonary fibrosis and epithelial-mesenchymal transition in asthma through regulation of E2F1. Int J Mol Med 2021;47:04855. PUBMED | CROSSREF
36. Wang T, Chen D, Wang P, Xu Z, Li Y. miR-375 prevents nasal mucosa cells from apoptosis and ameliorates allergic rhinitis via inhibiting JAK2/STAT3 pathway. Biomed Pharmacother 2018;103:621-7. PUBMED | CROSSREF
37. Song Y, Wang Z, Jiang J, Piao Y, Bai Q, Piao Q, et al. miR-181-5p attenuates neutrophilic inflammation in asthma by targeting DEK. Int Immunopharmacol 2022;112:109243. PUBMED | CROSSREF