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ORIGINAL PAPER/*PRACA ORYGINALNA*

Decreased microRNA 16 and 451a expression in hypertrophic adenoid tissue is associated with allergy

Zmniejszona ekspresja microRNA-16 i microRNA-451a w przeroście migdałka gardłowego związana jest z alergią

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ABSTRACT

Introduction: MicroRNAs (miRNAs) regulate gene expression and play a role in many biological processes. Their imbalance may result in the development of numerous diseases, including allergy. Exact mechanisms causing allergic inflammation are still unclear, but recent studies show that miRNAs are involved in its pathogenesis. Adenoid hypertrophy (AH) and allergy often coexist, although the reason for that is still being investigated.

Aim: To compare the expression of several miRNAs in adenoid tissue and nasal mucosa from children with and without allergy and to investigate whether miRNA levels correlate with the patient's allergy status.

Material and methods: Samples were taken from 37 patients and divided into two groups: allergic and non-allergic subjects. MiRNA was isolated from the adenoid tissue and nasal swabs collected during the adenoidectomy procedure, and transcribed into cDNA. MiRNA expression was measured with TaqMan MicroRNA Assays and analyzed with DataAssist software.

Results: MiR-16 and miR-451a expression was significantly decreased in the adenoid tissue of allergic children. Other miRNAs were not different between allergic and non-allergic patients. The expression of miRNA in the nasal mucosa did not differ between allergic and non-allergic patients.

Conclusions: MiRNAs are present in the adenoid tissue and have a distinct expression pattern in allergic patients compared to controls. This suggests that the molecular mechanism of AH formation in allergic patients is different and might explain why the allergy affects the prevalence of AH. Further studies are needed to better understand the role of miRNAs in the induction of allergic-type inflammation.

KEY WORDS

miRNA, allergy, adenoid hypertrophy, allergic rhinitis.

STRESZCZENIE

Wprowadzenie: MicroRNA (miRNA) regulują ekspresję genów i odgrywają rolę w wielu procesach biologicznych. Zaburzenie ich równowagi może skutkować rozwojem wielu chorób, w tym alergicznych. Dokładne mechanizmy powodujące powstawanie alergii są nadal nieznane, ale ostatnie badania pokazują, że miRNA są zaangażowane w jej patogenezę. Przerost migdałka gardłowego i alergia często współistnieją, chociaż przyczyna tego jest wciąż badana.

Cel: Porównanie ekspresji kilku miRNA w migdałku gardłowym i błonie śluzowej nosa u dzieci z alergią i bez alergii oraz zbadanie, czy poziomy miRNA korelują z diagnozą alergii u pacjenta.

Materiał i metody: Próbki pobrano od 37 pacjentów podzielonych na dwie grupy: alergików i niebędących alergikami. miRNA wyizolowano z przerośniętej tkanki migdałka gardłowego oraz nabłonka oddechowego uzyskanego z wymazów z nosa pobranych podczas adenoidektomii i przepisano na cDNA. Ekspresję miRNA mierzono za pomocą testów TaqMan MicroRNA i analizowano za pomocą oprogramowania DataAssist.

Wyniki: Ekspresja miR-16 i miR-451a była istotnie obniżona w tkance gruczołowej dzieci z alergią. Inne miRNA nie różniły się między pacjentami alergicznymi i niealergicznymi. Ekspresja miRNA w błonie śluzowej nosa nie różniła się między pacjentami alergicznymi i niealergicznymi.

Wnioski: miRNA są obecne w migdałku gardłowym i różnią się ekspresją u pacjentów z alergią i bez niej. Sugeruje to, że molekularny mechanizm powstawania przerostu migdałka gardłowego u alergików jest inny i może wyjaśniać, dlaczego alergia wpływa na częstość jego występowania. Potrzebne są dalsze badania, aby lepiej zrozumieć rolę miRNA w indukowaniu nadwrażliwości alergicznej.

SŁOWA KLUCZOWE:

miRNA, alergia, przerost migdałka gardłowego, alergiczny nieżyt nosa.

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INTRODUCTION

Adenoid hypertrophy (AH) is a common childhood pathology, which causes the obstruction of upper airways that may eventually lead to sleep apnea, craniofacial abnormal growth, or conductive hearing loss and cognitive impairment [1]. Several studies underline higher incidence of AH among children with allergy, but underlying molecular mechanisms are still not fully understood [2].

MiRNAs are small, non-coding molecules that serve as gene regulators of many biological processes, such as cell differentiation, proliferation, apoptosis or angiogenesis [3]. Their role in allergic type of inflammation has recently been described in some studies [4]. Allergic type of inflammation is based on Th cell imbalance, where the cytokines secreted in Th2 type of inflammation dominate over cytokines produced in Th1 type [5]. Recent studies showed that particular miRNAs or their clusters regulate differentiation of Th cells into Th2 type. Several murine models demonstrated that either enhancing or silencing those miRNAs might rebuild Th cell balance [6].

Our assumption that specific miRNAs might take part in adenoid enlargement pathogenesis in allergic patients comes from the concept of the united airways disease (UAD). According to the latest evidence, the upper and lower respiratory tract mucosae in allergic patients have a similar potential to trigger the Th2 immune response and secrete cytokines stimulating the allergic type of inflammation in each of its compartments. IgE-mediated allergic inflammation is based on immunoglobulin class switching (from IgM to IgE) after antigen sensitization and secretion of specific cytokines, such as interleukin 4 (IL)-4 or IL-13, which are responsible for maintenance of the allergic response. This results in vasodilatation, bronchoconstriction and increased mucus production [7]. Immunoglobulin class switching is present in respiratory mucosa of patients with AR and asthma but also has been observed in the gastrointestinal tract in patients with food allergy [8]. Nguyen *et al.* compared several levels of cytokines in adenoids, middle ear fluid and torus tubarius biopsies, and found that the eosinophils, T lymphocytes and IL-4 mRNA levels were significantly higher in the allergic group. This proved the

assumption that all three compartments share the allergic pattern of inflammation [9].

AIM

Therefore, we hypothesized that nasopharynx, with its adenoid tissue, is a part of the united airways and that the mechanism of AH formation in allergic patients might depend on a specific regulatory mechanism that is absent in non-allergic children. Taking into account the high regulatory potential of miRNAs and their involvement in allergic inflammatory processes, we sought to determine whether miRNA expression differs between patients with and without allergy, suggesting that the pathomechanism of tonsil enlargement in these two groups of patients might be distinct.

MATERIAL AND METHODS

The study was approved by the Bioethics Committee of Poznan University of Medical Sciences. Patients were recruited from inpatients at the Department of Pediatric Otolaryngology, Poznan University of Medical Sciences. The study was approved by the Poznan University of Medical Sciences Bioethics Committee. Subjects were from the Wielkopolska region of Poland, which is considered ethnically homogeneous. Written informed consent was obtained from a parent or legal guardian. The study group consisted of children diagnosed with adenoid hypertrophy.

Exclusion criteria included: craniofacial abnormalities, cleft palate, genetic syndromes, immune deficiencies, cystic fibrosis, immotile cilia syndrome, steroids, and antihistamine or leukotriene drug intake 2 weeks prior to the surgical procedure.

Full otolaryngologic examination was performed at the admission. Each child was carefully investigated for a history of allergic diseases using a detailed questionnaire. Children with positive skin prick tests or blood tests for food or inhalant allergens or with allergic rhinitis diagnosis based on ARIA [10] criteria or diagnosed allergic asthma according to GINA [11] criteria were assigned to the allergy group and compared against children without allergic history. The allergy group consisted of 16 patients whereas the non-allergic group had 21 patients.

Samples were taken during the adenoidectomy procedure, before the tonsil removal. Nasal respiratory mucosa swabs were also taken. The material was then transferred to the laboratory and frozen at –80°C for further experiments.

Total RNA was isolated with miRCURY miRNA Isolation Kit – Cell and Plant (Exiqon), according to the manufacturer's instructions and transcribed to cDNA with TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific). MiRNA expression was analyzed with TaqMan MicroRNA Assays and TaqMan Universal Master Mix II, no UNG (Thermo Fisher Scientific), according to the manufacturer's protocol. MiRNA expression datasets were analyzed with DataAssist software v.3.01 after global normalization.

Pathway enrichment analysis was performed for miRNAs that had significant changes in expression. We selected validated target genes (via reporter assay) from miRTarBase (available at http://mirtarbase.mbc.nctu.edu.tw/php/index.php). To identify KEGG pathways, the list of validated targets was analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v.6.7.

RESULTS

PATIENT CHARACTERISTICS

Samples were taken from 37 subjects (10 girls and 27 boys). Mean age was 6.22 ±2.4 years. Adenoid hypertrophy was more common in boys independently of allergy status (75% in allergic group and 71% in non-allergic group). Mean total IgE serum level was higher among allergic children (392.8 ±125.1 and 90 ±574.5 in non-allergic patients). Mean eosinophil count was also higher in the allergy group (6.2 ±2.3% in allergy group and 3.5 ±3.3% in non-allergic group). These results correspond to other studies underlining that IgE serum level as well as eosinophils is elevated in allergic patients. 69% of allergic children and 67% of non-allergic children attended kindergarten. 18.7% of allergic children and 14.2% of non-allergic children were under chronic exposure to tobacco smoke at home, which is a separate allergy factor (Table 1).

TABLE 1. Clinical description of study group according to allergy status

Parameter	Allergic	Non-allergic
Male	*n* = 12; 75%	*n* = 15; 71%
Female	*n* = 4; 25%	*n* = 6; 29%
Age, mean ± SD	6.3 ±3.3	6.1 ±3.3
Total IgE level, mean ± SD [kU/ml]	392.8 ±125.1	90 ±574.5
Eosinophil count (%), mean ± SD	6.2 ±2.3%	3.5 ±3.3%
Exposure to tobacco smoke	3; 18.7%	3; 14.2%
Attending kindergarten	11; 69%	14; 67%

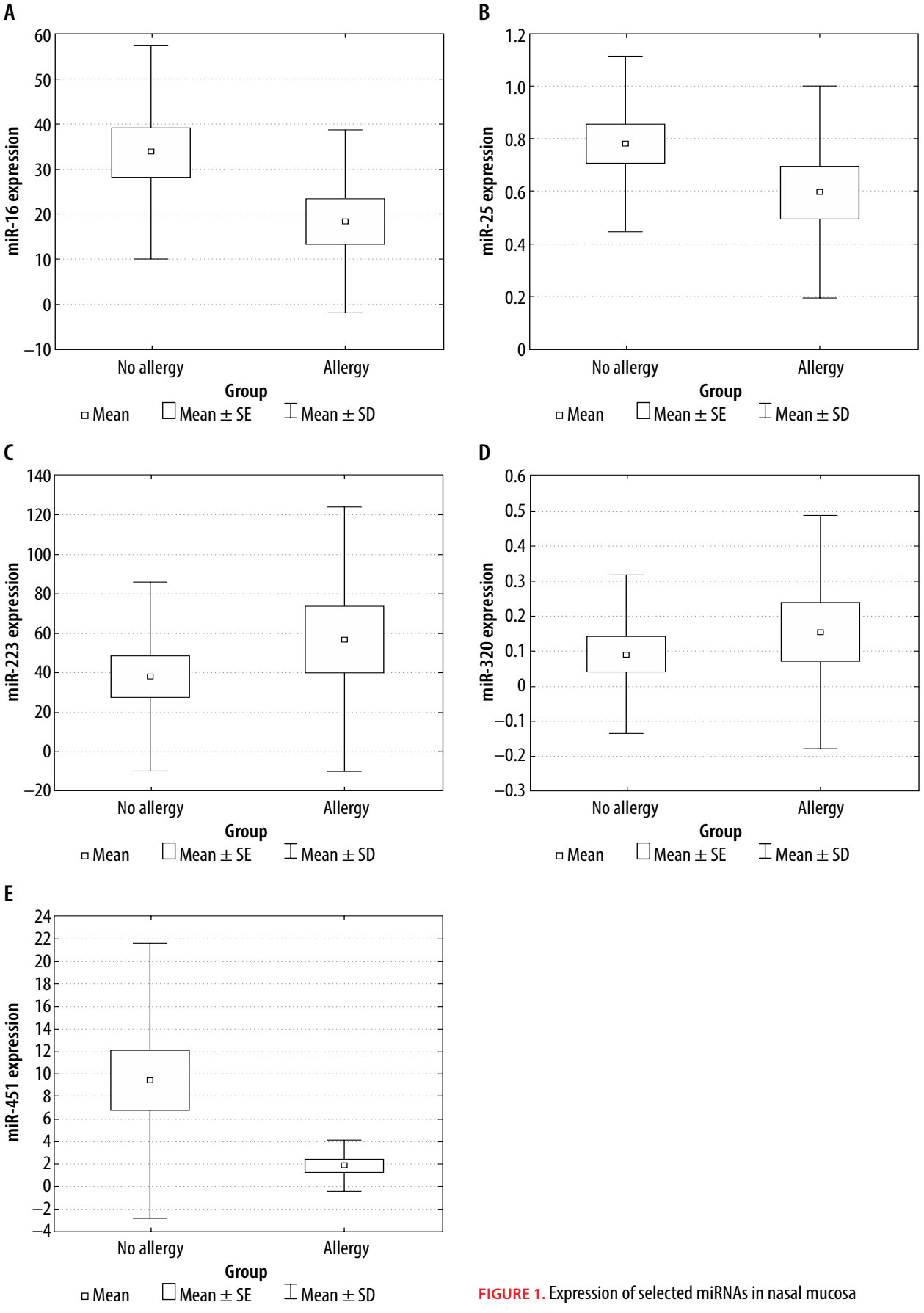

FIGURE 1. Expression of selected miRNAs in nasal mucosa

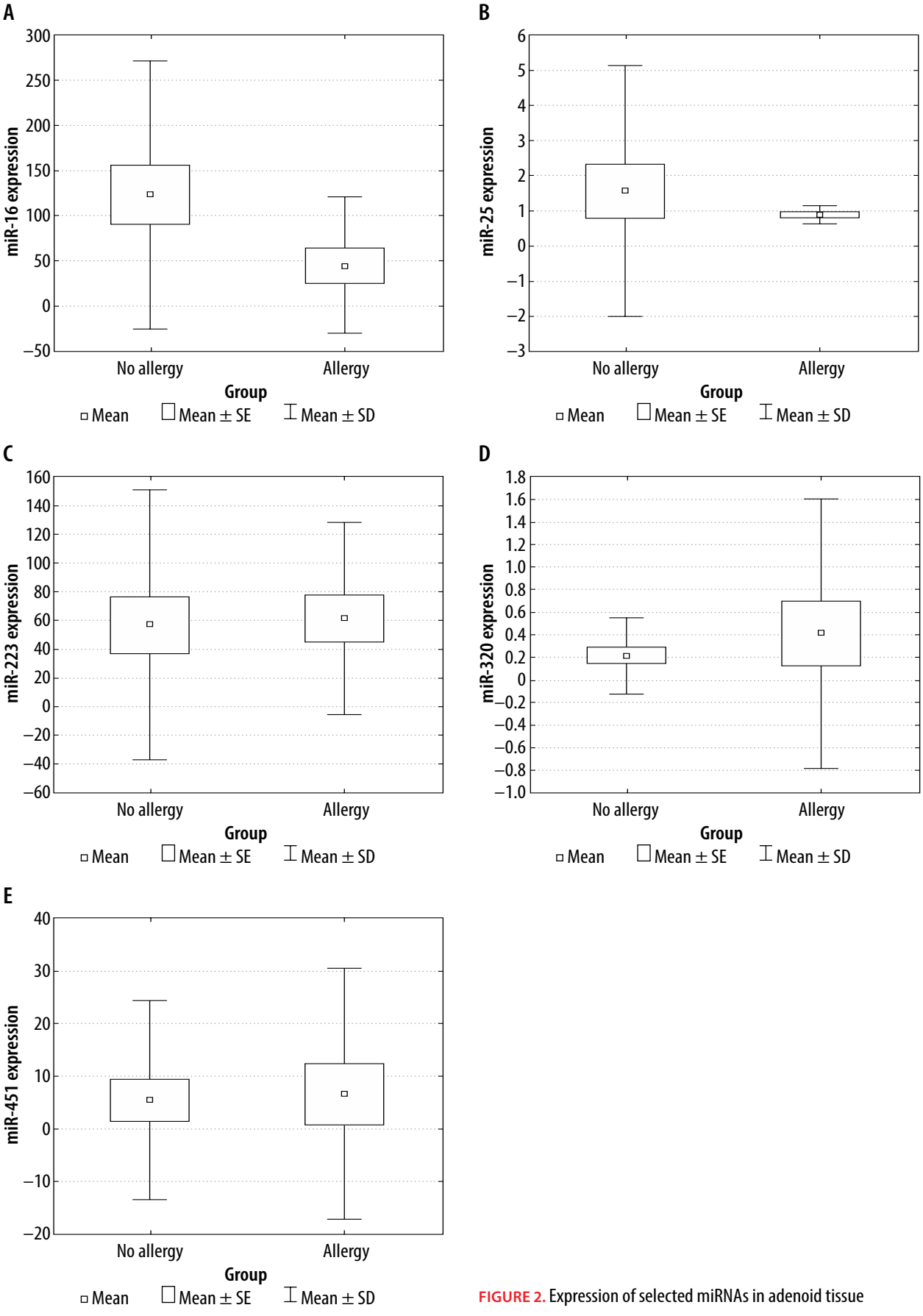

FIGURE 2. Expression of selected miRNAs in adenoid tissue

MIRNA EXPRESSION ANALYSIS

We found that expression of analyzed miRNAs in nasal mucosa did not differ significantly between allergic and non-allergic patients with AH (Figure 1). In adenoid tissue, we observed significantly decreased expression of 2 miRNAs, miR-16 ($p = 0.048$) and miR-451a ($p = 0.019$), in allergic patients as compared to non-allergic children (Figure 2).

TARGET ANALYSIS

A list of validated target genes regulated by miR-16-5p or miR-451a was analyzed with the DAVID annotation tool to identify the most enriched pathways possibly regulated by this miRNA. Several pathways were significantly enriched for both miRNAs (Tables 2 and 3).

DISCUSSION

While several studies have debated whether allergy is a separate risk factor of AH, there is still insufficient molecular evidence supporting this hypothesis [12]. Nguyen *et al.* reported that IL-4 levels and eosinophil infiltration were increased in adenoids and middle ear effusions from allergic patients compared to controls [9]. Huo *et al.* linked adenotonsillar regrowth with the allergy status. They established levels of GATA3+ cells (Th2-type cells) and found that they were increased in the adenoids from the allergic subgroup [13]. To our knowledge, our study is the first to analyze miRNA expression in adenoid tissue in the pediatric population and whether it depends on allergy status.

Expression of 5 analyzed miRNAs (miR-320e, miR-16-5p, miR-451a, miR-223-3p, miR-25-3p) was not significantly different in nasal mucosa between allergic and non-allergic children, so these miRNAs are not likely to play a role in allergic inflammation in the nose. Interestingly, we found that miR-16 and miR-451a were significantly decreased in the adenoid tissue of allergic patients, suggesting that they may favor the expression of genes participating in adenoid hypertrophy in allergic patients. MiR-16 takes part in the allergic inflammation. Pangiban et al. demonstrated that several miRNAs, including miR-16, had different expression in patients with asthma and allergic rhinitis (AR) as compared to the controls and underlined their potential as future noninvasive biomarkers of allergic diseases [14]. Another study in asthmatic patients revealed the possible role of miR-16 in asthma exacerbation by regulating Th2 cytokine expression and favoring airway inflammation [15]. Yu *et al.* also demonstrated that miR-16 has the potential to serve as an asthma biomarker. Moreover, miR-16 regulates mRNA expression of adrenoreceptor β2, which is an agonist receptor for bronchodilators; thus miR-16 may affect their efficacy [16].

Pathway analysis of predicted target genes for miR-16 and miR-451a have identified several potential regulatory mechanisms that might be involved in allergic inflammation. For example, the PI3K-Akt pathway, regulated by both the miRNAs, modulates airway inflammation and airway hyper-responsiveness [17]. MAPK signaling pathway contributes to the expression of proinflammatory genes [18]. Moreover, the neurotrophin signaling pathway modulates biological effects of infiltrated eosinophils in the allergic airways [19].

MiR-451 plays a role in pathogenesis of various cancers, but its exact role in allergic inflammation is still not fully understood. Macrophages activated by reactive oxygen species have altered miR-451 expression, suggesting its important role in macrophage maturation [20]. Chung *et al.* investigated the role of miR-451 in a mouse model of allergic asthma and found that its levels are significantly decreased, affecting macrophage activation in lungs. Macrophages isolated from mice's lungs had increased levels of CCL17 and sirtuin-2, indicating miR-451 function in regulating the allergic response [21].

Identifying the altered expression of particular miRNAs in allergic patients could help to understand the pathogenesis of AH formation, linking it to allergic inflammation. Further miRNAs studies would enable the mechanisms underlying morbidities to be explained and could possibly lead to the development of biomarkers or even therapeutic options in cases where current therapy is unfortunately insufficient.

There are two major limitations to this study that are going to be addressed in future research. The primary limitation is the small sample size. Another limitation is the lack of target gene verification addressed at altered miRNAs, which will be dealt with in a future study.

CONCLUSIONS

We have documented that miRNAs are expressed in adenoid tissue in children and that the expression of two of them, miR-16 and miR-451a, differs between allergic and non-allergic patients. These miRNAs may be involved in adenoid hypertrophy formation in allergic patients. Further studies are needed to better understand their exact role in the induction of allergic-type inflammation.

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TABLE 2. Results of pathway analysis of validated target genes ($n = 63$) for hsa-miR-16-5p in DAVID software (**bolded** p-value indicates significance after multiple testing correction with Benjamini procedure)

Category	Pathway	No. of genes	Fold enrichment	*P*-value	*P* corrected
KEGG	PI3K-Akt signaling pathway	20	7.7	< 0.001	**< 0.001**
KEGG	Proteoglycans in cancer	16	10.6	< 0.001	**< 0.001**
KEGG	MicroRNAs in cancer	17	7.9	< 0.001	**< 0.001**
KEGG	Pathways in cancer	19	6.4	< 0.001	**< 0.001**
KEGG	Prostate cancer	11	16.5	< 0.001	**< 0.001**
KEGG	Melanoma	10	18.6	< 0.001	**< 0.001**
KEGG	Hepatitis B	11	10	< 0.001	**< 0.001**
KEGG	Acute myeloid leukemia	8	18.9	< 0.001	**< 0.001**
KEGG	Colorectal cancer	8	17.1	< 0.001	**< 0.001**
KEGG	Pancreatic cancer	8	16.3	< 0.001	**< 0.001**
KEGG	Glioma	8	16.3	< 0.001	**< 0.001**
KEGG	Signaling pathways regulating pluripotency of stem cells	10	9.4	< 0.001	**< 0.001**
KEGG	Small cell lung cancer	8	12.5	< 0.001	**< 0.001**
KEGG	Measles	9	9	< 0.001	**< 0.001**
KEGG	Central carbon metabolism in cancer	7	14.5	< 0.001	**< 0.001**
KEGG	p53 signaling pathway	7	13.8	< 0.001	**< 0.001**
KEGG	Chronic myeloid leukemia	7	12.9	< 0.001	**< 0.001**
KEGG	Focal adhesion	10	6.4	< 0.001	**< 0.001**
KEGG	Rap1 signaling pathway	10	6.3	< 0.001	**< 0.001**
KEGG	Neurotrophin signaling pathway	8	8.8	< 0.001	**< 0.001**
KEGG	Ras signaling pathway	10	5.9	< 0.001	**< 0.001**
KEGG	Endometrial cancer	6	15.3	< 0.001	**< 0.001**
KEGG	Non-small cell lung cancer	6	14.2	< 0.001	**< 0.001**
KEGG	HIF-1 signaling pathway	7	9.6	< 0.001	**< 0.001**
KEGG	VEGF signaling pathway	6	13	< 0.001	**0.001**
KEGG	HTLV-I infection	10	5.2	< 0.001	**0.001**
KEGG	Bladder cancer	5	16.1	< 0.001	**0.001**
KEGG	Cell cycle	7	7.5	< 0.001	**0.002**
KEGG	MAPK signaling pathway	9	4.7	< 0.001	**0.003**
KEGG	T cell receptor signaling pathway	6	7.9	0.001	**0.004**
KEGG	mTOR signaling pathway	5	11.4	0.001	**0.004**
KEGG	Apoptosis	5	10.7	0.001	**0.005**
KEGG	Toxoplasmosis	6	7.2	0.001	**0.006**
KEGG	Renal cell carcinoma	5	10	0.001	**0.006**
KEGG	Thyroid hormone signaling pathway	6	6.9	0.002	**0.007**
KEGG	Sphingolipid signaling pathway	6	6.6	0.002	**0.008**
KEGG	Hepatitis C	6	6	0.003	**0.012**
KEGG	FoxO signaling pathway	6	5.9	0.003	**0.012**
KEGG	Viral carcinogenesis	7	4.5	0.004	**0.015**

TABLE 2. Cont.

Category	Pathway	No. of genes	Fold enrichment	*P*-value	*P* corrected
KEGG	ErbB signaling pathway	5	7.6	0.004	**0.015**
KEGG	Jak-STAT signaling pathway	6	5.5	0.004	**0.016**
KEGG	Hippo signaling pathway	6	5.3	0.005	**0.018**
KEGG	Choline metabolism in cancer	5	6.5	0.006	**0.023**
KEGG	Chagas disease (American trypanosomiasis)	5	6.4	0.007	**0.025**
KEGG	Tuberculosis	6	4.5	0.010	**0.033**
KEGG	Epstein-Barr virus infection	5	5.4	0.012	**0.041**
KEGG	AMPK signaling pathway	5	5.4	0.013	**0.041**
KEGG	Fc epsilon RI signaling pathway	4	7.8	0.014	**0.043**
KEGG	B cell receptor signaling pathway	4	7.7	0.014	**0.044**
KEGG	Adipocytokine signaling pathway	4	7.6	0.015	**0.045**
KEGG	Leishmaniasis	4	7.5	0.015	**0.046**
KEGG	Prolactin signaling pathway	4	7.5	0.015	**0.046**
KEGG	Wnt signaling pathway	5	4.8	0.019	0.054
KEGG	Insulin signaling pathway	5	4.8	0.019	0.054
KEGG	Thyroid cancer	3	13.7	0.019	0.055
KEGG	TGF-β signaling pathway	4	6.3	0.024	0.067
KEGG	African trypanosomiasis	3	12	0.025	0.067
KEGG	NF-κB signaling pathway	4	6.1	0.026	0.071
KEGG	Progesterone-mediated oocyte maturation	4	6.1	0.026	0.071
KEGG	Allograft rejection	3	10.7	0.030	0.080
KEGG	Transcriptional misregulation in cancer	5	4	0.035	0.089
KEGG	Estrogen signaling pathway	4	5.3	0.037	0.093
KEGG	Type I diabetes mellitus	3	9.4	0.038	0.095
KEGG	Influenza A	5	3.8	0.039	0.096
KEGG	Toll-like receptor signaling pathway	4	5	0.043	0.100
KEGG	TNF signaling pathway	4	4.9	0.044	0.100
KEGG	Herpes simplex infection	5	3.6	0.046	0.110
KEGG	Insulin resistance	4	4.9	0.046	0.110
KEGG	Serotonergic synapse	4	4.8	0.049	0.110
KEGG	Malaria	3	8.1	0.051	0.110
KEGG	Amyotrophic lateral sclerosis (ALS)	3	7.9	0.053	0.110
KEGG	Basal cell carcinoma	3	7.3	0.060	0.130
KEGG	Natural killer cell mediated cytotoxicity	4	4.3	0.061	0.130
KEGG	Long-term depression	3	6.6	0.073	0.150
KEGG	Osteoclast differentiation	4	4	0.073	0.150
KEGG	Inflammatory bowel disease (IBD)	3	6.2	0.081	0.160
KEGG	RIG-I-like receptor signaling pathway	3	5.7	0.095	0.190
KEGG	Oxytocin signaling pathway	4	3.5	0.099	0.190

TABLE 3. Results of pathway analysis of validated target genes for hsa-miR-451a in DAVID software (**bolded** *p*-value indicates significance after multiple testing correction with Benjamini procedure)

Category	Pathway	No. of genes	Fold enrichment	*P*-value	*P*-corrected
Kegg	mTOR signaling pathway	4	31.6	0.000	**0.012**
Kegg	PI3K-Akt signaling pathway	6	8	0.000	**0.017**
Kegg	Hepatitis B	5	15.8	0.000	**0.02**
Kegg	FoxO signaling pathway	4	13.7	0.002	0.067
Kegg	NOD-like receptor signaling pathway	3	24.6	0.006	0.13
Kegg	Acute myeloid leukemia	3	24.6	0.006	0.13
Kegg	Chronic myeloid leukemia	3	19.1	0.009	0.17
Kegg	TNF signaling pathway	3	12.9	0.019	0.18
Kegg	HTLV-I infection	4	7.2	0.013	0.19
Kegg	Toll-like receptor signaling pathway	3	13	0.019	0.19
Kegg	HIF-1 signaling pathway	3	14.3	0.016	0.2
Kegg	AMPK signaling pathway	3	11.2	0.025	0.2
Kegg	Chagas disease (American trypanosomiasis)	3	13.2	0.018	0.2
Kegg	Neurotrophin signaling pathway	3	11.5	0.024	0.21
Kegg	MAPK signaling pathway	4	7.3	0.013	0.21
Kegg	Insulin signaling pathway	3	10	0.031	0.23
Kegg	Jak-STAT signaling pathway	3	9.5	0.034	0.24
Kegg	Non-alcoholic fatty liver disease (NAFLD)	3	9.1	0.037	0.24
Kegg	Pathways in cancer	4	4.7	0.042	0.26
Kegg	Tuberculosis	3	7.8	0.049	0.27
Kegg	Influenza A	3	7.9	0.047	0.27
Kegg	Thyroid cancer	2	31.6	0.057	0.3
Kegg	Prion diseases	2	27	0.067	0.32
Kegg	Ras signaling pathway	3	6.1	0.075	0.34
Kegg	Bladder cancer	2	22.4	0.08	0.35
Kegg	Type II diabetes mellitus	2	19.1	0.093	0.39

The data that support the findings of this study are available from the corresponding author, upon request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Pereira L, Monyror J, Almeida FT, et al. Prevalence of adenoid hypertrophy: a systematic review and meta-analysis. Sleep Med Rev 2018; 38: 101-12.
2. Pagella F, De Amici M, Pusateri A, et al. Adenoids and clinical symptoms: epidemiology of a cohort of 795 pediatric patients. Int J Pediatr Otorhinolaryngol 2015; 79: 2137-41.
3. Gebert L, MacRae I. Regulation of microRNA function in animals. Nature Rev Mol Cell Biol 2018; 20: 21-37.
4. Rebane A. microRNA and allergy. Adv Exp Med Biol 2015; 888: 331-52.
5. Akdis M, Burgler S, Crameri R, et al. Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. J Allergy Clin Immunol 2011; 127: 701-21.
6. Specjalski K, Jassem E. MicroRNAs: potential biomarkers and targets of therapy in allergic diseases? Arch Immunol Ther Exp 2019; 67: 213-23.
7. Giavina-Bianchi P, Aun M, Takejima P, et al. United airway disease: current perspectives. J Asthma Allergy 2016; 9: 93-100.
8. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 2010; 125 (2 Suppl 2): S73-80.
9. Nguyen L, Manoukian J, Tewfik T, et al. Evidence of allergic inflammation in the middle ear and nasopharynx in atopic children with otitis media with effusion. J Otolaryngol 2004; 33: 345-51.

10. Brozek JL, Bousquet J, Baena-Cagnani CE, et al. Allergic rhinitis and its impact on asthma (ARIA) guidelines: 2010 revision. J Allergy Clin Immunol 2010; 126: 466-76.
11. GINA. Global Strategy for Asthma Management and Prevention, revised. www.ginaasthma.org, 2006.
12. Belhassen M, Demoly P, Bloch-Morot E, et al. Costs of perennial allergic rhinitis and allergic asthma increase with severity and poor disease control. Allergy 2017; 72: 948-58.
13. Huo Z, Shi J, Shu Y, et al. The relationship between allergic status and adenotonsillar regrowth: a retrospective research on children after adenotonsillectomy. Sci Rep 2017; 7: 46615.
14. Panganiban RP, Wang Y, Howrylak J, et al. Circulating microRNAs as biomarkers in patients with allergic rhinitis and asthma. J Allergy Clin Immunol 2016; 137: 1423-32.
15. Evcimik MF, Dogru M, Cirik AA, Nepesov MI. Adenoid hypertrophy in children with allergic disease and influential factors. Int J Pediatr Otorhinolaryngol 2015; 79: 694-7.
16. Li Q, Li Y, Zhang D, et al. Downregulation of microRNA451 improves cell migration, invasion and tube formation in hypoxiatreated HUVECs by targeting MIF. Mol Med Rep 2019; 20: 1167-77.
17. Choi Y, Jin G, Li L, Yan G. 2013. Inhibition of protein kinase C delta attenuates allergic airway inflammation through suppression of PI3K/Akt/mTOR/HIF-1 alpha/VEGF pathway. PLoS One 2013; 8: e81773.
18. Watts A, Cripps A, West N, Cox A. Modulation of allergic inflammation in the nasal mucosa of allergic rhinitis sufferers with topical pharmaceutical agents. Front Pharmacol 2019; 10: 294.
19. Nockher W, Renz H. Neurotrophins in allergic diseases: from neuronal growth factors to intercellular signaling molecules. J Allergy Clin Immunol 2006; 117: 583-9.
20. Ranjan R, Lee Y, Karpurapu M, et al. p47phox and reactive oxygen species production modulate expression of microRNA-451 in macrophages. Free Radical Res 2014; 49: 25-34.
21. Chung S, Lee Y, Karpurapu M, et al. Depletion of microRNA-451 in response to allergen exposure accentuates asthmatic inflammation by regulating sirtuin 2. Am J Physiol Lung Cell Mol Physiol 2020; 318: L921-30.