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ORIGINAL ARTICLE

MicroRNA expression in the affected skin of adult patients with IgA vasculitis

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Abstract

IgA vasculitis (IgAV) represents a common systemic vasculitis in pediatric and adult population. Our current knowledge of disease pathogenesis is still very limited, without information on miRNAs in IgAV. The aim of our study was to determine the expression of five pre-selected miRNAs (miRNA-146a-5p, miRNA-148-3p, miRNA-155-5p, miRNA-223-3p, and let-7b) in the affected skin of adult IgAV patients. The study included 65 skin samples from consecutive, untreated IgAV patients (61.5% male, median age 67.6 years, range 29–91), diagnosed between October 2014 and September 2016, and 20 samples of normal skin from healthy volunteers. Total RNA was isolated from tissue sections of formalin-fixed, paraffin-embedded samples. Expression of miRNAs was measured using qRT-PCR. To present relative miRNA expression, the ΔΔCT method was used. Skin miRNA expression was correlated to clinical characteristics of adult IgAV patients. We found significantly higher levels of miRNA-155-5p, miRNA-223-3p, and let-7b in the affected skin compared to controls (18.6-fold, 6.4-fold, and 7.9-fold higher respectively). Contrary, the miRNA 148-3p expression was significantly lower (2.2-fold). The expression of the miRNA-146-5p showed near normal levels. Patients with necrotic skin lesions had significantly higher miRNA-223 tissue expression than those with non-necrotic purpura ($p = 0.029$). Gastrointestinal tract involvement inversely correlated with the expression of miRNA-155-5p and/or miRNA-146a-5p in affected skin. Altered expression of miRNA-148b-3p, miRNA-155-5p, miRNA-223-3p, and let-7b was found in vasculitic skin lesions in IgAV. Additionally, we found a positive association between the severity of purpura and skin miRNA-223-3p expression. Aberrantly expressed miRNAs could represent a biomarker in adult IgAV.

Keywords Henoch Schönlein purpura · IgA vasculitis · MicroRNA expression · Vasculitic skin lesions

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Rheumatology in Slovenia: Clinical practice and translational research

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Introduction

IgA vasculitis (IgAV; formerly the Henoch-Schönlein purpura) is a small vessel leukocytoclastic vasculitis, clinically characterized by palpable purpura, arthralgia or arthritis, gastrointestinal, and renal involvement. Histologically, the disease is characterized by the predominantly immunoglobulin (Ig) A deposits in the vascular wall [1]. It is a common vasculitis in the pediatric, but also in the adult population [2, 3]. Our current knowledge of disease etiopathogenesis is still very limited. In patients with kidney involvement, similarities to IgA nephropathy were frequently drawn [4]. Yet, the latest observations point additionally to endothelial cell and neutrophil activation through IgA binding on IgA Fc receptor I leading to inflammation and vascular damage [5].

In the recent decade, microRNAs (miRNAs) have emerged as potential (diagnostic, prognostic) biomarkers and/or therapeutic targets in different diseases. miRNAs function as posttranscriptional regulators of gene expression, regulating cellular functions, such as proliferation, migration, signal transduction, and others. The dysregulation of miRNAs has been associated with several diseases, including autoimmune diseases [6, 7]. In IgA nephropathy, several miRNAs have been investigated. Bao H et al. showed that miRNA-223 downregulation promoted glomerular endothelial cell activation by the upregulation of importins α4 and α5, and suggested that monitoring miRNA-223 could provide a non-invasive method for evaluating the severity of IgA nephropathy [8]. Serino G et al. found an association between abnormal miRNA-148b and let-7b expression in blood mononuclear cells and aberrant IgA glycosylation, and proposed a predictive model for diagnosing IgA nephropathy based on the serum levels of miRNA-148b and let-7b [9]. Furthermore, Wang G. et al. reported that an increased expression of renal and urinary miRNA-146a and miRNA-155, showed a positive correlation to one another, as well as to the level of proteinuria and an inverse correlation with the glomerular filtration rate [10].

Since information is lacking with regard to miRNA expression in IgAV, we aimed to study, for the first time, selected miRNA expression in vasculitic skin lesions of adult patients with IgAV.

Patients, methods, and materials

Setting

This observational study was conducted at the Department of Rheumatology, University Medical Centre (UMC) Ljubljana, with the collaboration of the Institute of Pathology, Faculty of Medicine, University of Ljubljana.

Patients

Adult patients (aged ≥ 18 years) with IgAV diagnosed for the first time between October 1, 2014 and September 30, 2016 participated in the study. The patient's data were collected in a prospective manner. All patients underwent an extensive clinical evaluation and laboratory workup. In the study, we used the definitions of generalized purpura, gastrointestinal (GI), and renal involvement, as previously reported in detail [11].

Skin biopsies, performed in treatment-naive patients, were evaluated using bright-field microscopy and direct immunofluorescence.

The diagnosis of IgAV was established according to the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides [1]. In order for the IgAV patient to be included in the study, the patient had to fulfill the 2010 EULAR/PRINTO/PRES classification criteria and in addition, had to have a histologically proven disease [12].

Control group

Samples of normal skin taken from the lower leg from 20 healthy volunteers (age- and sex- matched to the IgAV cohort) served as a control group.

miRNA analysis

RNA isolation

For miRNA expression analysis, IgAV and control skin tissue samples were cut at 10 μm from formalin-fixed paraffin-embedded (FFPE) tissue blocks. In the RNA isolation procedure, two to six 10-μm sections were used. The total RNA was isolated from the FFPE samples by using the AllPrep® DNA/RNA FFPE Kit (80234, Qiagen, Germany), according to the manufacturer's instructions. The total RNA was eluted in 30 μl of nuclease-free water, supplied with the kit. The RNA yield and integrity was measured spectrophotometrically with NanoDrop™ 1000 (Thermo Fisher Scientific, USA). The isolated RNA was stored at − 80 °C until further used.

Reverse transcription and quantitative real-time PCR analysis

A reverse transcription of the total RNA from the skin tissue samples was performed by using the miScript II RT Kit (218161, Qiagen, Germany), according to the manufacturer's instructions. Briefly, a reverse transcription was performed in 20 μl reaction volumes, containing 100 ng (5 ng/μl) total RNA per reaction, 4 μl 5× miScript HiFlex Buffer, 2 μl miScript Reverse Transcriptase Mix and 2 μl 10× miScript Nucleics Mix. cDNA synthesis was performed by incubating the prepared reaction mixes for 60 min at 37 °C, which was

followed by a 5-min incubation at 95 °C to inactivate the miScript Reverse Transcriptase Mix. The synthesized cDNA was stored at − 20 °C until the qRT-PCR analysis was performed.

The qRT-PCR experiments were performed according to the MIQE guidelines (minimum information for the publication of quantitative real-time PCR experiments) [13]. All qRT-PCR assays were performed using the SYBR Green-based technology on the Rotor-Gene Q real-time PCR cycler (Qiagen, Germany). The quantitative RT-PCR assays were performed in 10 μl reaction mixtures, containing a 3 μl 250-fold diluted cDNA sample (0.06 ng), 5 μl 2× QuantiTect SYBR Green PCR Master Mix, 1 μl 10× miScript Universal Primer, and 1 μl 10× miScript Primer Assay. The MiScript Primer Assays used in the analysis are presented in Table 1. All qRT-PCR reactions were performed in duplicates, following the recommended manufacturer's program (95 °C/15 min, 94 °C/15 s, 55 °C/30 s, 70 °C/30s for 40 cycles). The signal was collected at the endpoint of every cycle. Following amplification, a melting curve analysis of the PCR products was performed to verify their specificity. Melting curves were acquired on the SYBR channel, using a ramping rate of 0.7 °C/s for 60–95 °C.

Prior to the qRT-PCR analysis, two separate cDNA pools were created (Pool IgAV and Pool Ctrl), which included the cDNA obtained from the IgAV and corresponding control tissues, respectively. To determine the qRT-PCR primer amplification efficiency (E) for each primer, standard curves were generated from an eight-step dilution series of each cDNA pool. Primer E were calculated from the slopes of the standard curves by using $E = 10^{(-1/\text{slope of the standard curve})}$ and used in subsequent calculations [14]. All qRT-PCR E reactions were performed in triplicates.

Computational and statistical analysis

The relative miRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method, where ΔΔCt represents the difference between the quantity of the target transcript in the IgAV samples, relative to the normal condition (normal control skin tissue samples), after being normalized to the expression of the reference genes (ΔCt) [15]. The target miRNA expression was normalized to the reference RNU6B and SNORD72 miRNA genes, whose Ct values were geometrically averaged, to enhance the accuracy of normalization [16]. RNU6B and SNORD72 were used based on our preliminary testing results. Before performing the calculations, we used the determined primer E of each miRNA for each cDNA pool to correct the values of the exported Ct data, as previously described [17]. The efficiency-corrected Ct values were then used for calculating the miRNA expression by the $2^{-\Delta\Delta Ct}$ method.

The statistical analysis was performed using the Mann-Whitney U test. The ΔCt values were used to analyze the statistical significance of miRNA expression differences between IgAV and the normal skin sample groups and the ΔΔCt values for testing the association of miRNA expression with ordinal variables (e.g., sex, age group, smoking, necrosis, kidney failure, and IgA levels). For all statistical tests, the IBM SPSS Statistics 24.0 software (IBM Corporation, USA) was used, with a cutoff point at $p < 0.05$.

Ethics committee approval

All patients and controls signed a written consent form. The study was approved by the ethical approval # 99/04/15 obtained from the Slovenian National Medical Ethics Committee.

Results

Patients

During the 24-month observation period, 65 new, histologically proven, treatment-naive IgAV cases were identified (61.5% male, 50.8% ever-smokers, the median age at presentation was 67.6 years (range 29–91, the interquartile range

Table 1 MiScript Primer Assays (Qiagen, Germany) used for a qRT-PCR analysis of miRNA expression in IgAV and normal skin tissues

miRNA	Symbol	Primer sequence	Catalogue number[a]
miR-146a-5p	Hs_miR-146a_1	5′-UGAGAACUGAAUUCCAUGGGUU-3′	MS00003535
miR-148b-3p	Hs_miR-148b_2	5′-UCAGUGCAUCACAGAACUUUGU-3′	MS00031458
miR-155-5p	Hs_miR-155_1	5′-CUCCUACAUAUUAGCAUUAACA-3′	MS00008778
miR-223-3p	Hs_miR-223_1	5′-UGUCAGUUUGUCAAAUACCCCA-3′	MS00003871
let-7b	Hs_let-7b_1	5′-UGAGGUAGUAGGUUGUGUGGUU-3′	MS00003122
RNU6B	Hs_RNU6-2_11	NA[b]	MS00033740
SNORD72	Hs_SNORD72_11	NA	MS00033719

[a] Qiagen (Germany) catalogue number

[b] Not available

(IQR) 51.8–78.2), the median (IQR) symptom duration time 8 (5–14) days). Purpura was present in all patients (necrotic in 32 (49.2%) and generalized above the waist in 37 (56.9%) cases). Twenty-eight patients (43.1%) had renal involvement, which was severe with acute kidney injury or nephrotic syndrome in nine cases. Sixteen patients (24.6%) had GI tract involvement, five of them severe with bloody diarrhea or in need of surgical intervention. Joint involvement developed in 28 (43.1%) cases. The serum IgA level was elevated in 30 cases (46.2%).

miRNA analysis

Characteristics of the standard curves used for the relative quantification of miRNA expression in IgAV and control tissue samples are presented in Table 2. The standard curves were generated for two cDNA pools, which included cDNA obtained from IgAV (Pool IgAV) and corresponding control tissues (Pool Ctrl). Primer *E* was used for Ct efficiency corrections and subsequent fold change calculations, as described in the "Patients, Methods, and Materials" section.

Relative changes in the expression of miRNA-146a-5p, miRNA-148b-3p, miRNA-155-5p, miRNA-223-3p, and let-7b are shown in Fig. 1. The tissue expression of miRNA-155-5p, miR-223-3p, and let-7b was significantly upregulated in IgAV skin compared to normal skin (6.4-fold, 18.6-fold, and 7.9-fold, respectively, all $p < 0.001$). The expression of miRNA-148b-3p was significantly downregulated (2.2-fold, $p < 0.001$) and of miRNA-146a-5p had near normal levels (1.1-fold decrease, $p = 0.901$).

The tissue expression of individual miRNA depending on the clinical characteristics of IgAV are presented in Table 3.

Table 2 Characteristics of standard curves used for a relative quantification of miRNA expression in IgAV and normal skin tissues

	Pool IgAV[a]			Pool Ctrl[b]		
miRNA	Slope[c]	E[d]	R^{2e}	Slope	E	R^2
miR-146a-5p	− 3.39	1.97	0.99	− 3.37	1.98	0.99
miR-148b-3p	− 3.32	2.00	0.98	− 3.35	1.99	0.99
miR-155-5p	− 3.32	2.00	0.98	− 3.48	1.94	0.98
miR-223-3p	− 3.32	2.00	0.98	− 3.38	1.98	0.99
let-7b	− 3.53	1.92	0.99	− 3.39	1.97	0.99
RNU6B	− 3.42	1.96	0.98	− 3.40	1.97	0.99
SNORD72	− 3.32	2.00	0.98	− 3.34	1.99	0.99

[a] Pooled cDNA obtained from IgAV skin tissues ($n = 65$)

[b] Pooled cDNA obtained from control skin tissues ($n = 20$)

[c] Slope of the standard curve

[d] PCR primer amplification efficiency

[e] Coefficient of determination

Except for the lower expression of miRNA-146a-5p in the skin samples of ever-smokers vs. non-smokers ($p = 0.003$), we found no further significant differences in miRNA expression with regard to the baseline demographic data (age, gender, smoking history, history of prior infection).

Considering the IgAV clinical manifestations, patients with necrotic skin lesions had significantly higher miRNA-223-3p tissue expression than those with non-necrotic purpura ($p = 0.029$). There was a trend toward inverse correlation between the presence of GI tract involvement and its severity, and miRNA-223-3p expression ($p = 0.054$ and 0.069, respectively). We found two additional negative correlations between GI tract involvement and the analyzed miRNAs—with miR-146a-5p ($p = 0.041$) and miRNA-155-5p ($p = 0.004$). No significant association between renal involvement and/or its severity, and the skin expression of the investigated miRNAs was detected. Patients with elevated serum IgA had a borderline upregulated skin expression of miRNA-223-3p ($p = 0.053$).

Discussion

MicroRNAs play a central role in the development and homeostasis of the immune system. Their aberrant expression is associated with immune system dysfunction and with (autoimmune) disease. Nevertheless, the role of miRNAs has been poorly investigated in IgAV. Indeed, to our knowledge, this study is the first to evaluate the expression of miRNAs in the skin lesions of patients with adult IgAV. More information about miRNA expression is known from IgA nephropathy, a disease that is similar to IgAV glomerulonephritis [8–10, 18, 19]. Data derived from miRNA studies in IgA nephropathy served as a baseline for the selection of five miRNAs tested in our pilot miRNA study. As IgAV is characterized by skin involvement (the latter is a mandatory criterion according to EULAR/PRINTO/PRES IgAV classification criteria), we decided to evaluate the miRNAs' expression in the affected skin of patients with this small vessel systemic vasculitis and compared it to the normal skin of healthy volunteers. Our data showed significant aberrant expression in 4 out of 5 examined miRNAs (an upregulation of miRNA-155a-5p, miRNA-223-3p, and let-7b and a downregulation of miRNA-148-3p). The expression of miRNA-223-3p in vasculitic skin was the most severely deregulated. miRNA-223 functions as a regulator of granulocyte differentiation and maturation and our finding could be interpreted as an indirect support of the recently hypothesized central role of neutrophils in IgAV pathogenesis. Furthermore, we found a positive correlation between the severity of IgAV skin involvement and skin miRNA-223-3p expression. IgAV patients with necrotic skin lesions exhibited significantly higher tissue expression of miRNA-223-3p than those with non-necrotic purpura. Interestingly, the extent of skin involvement (i.e., generalized

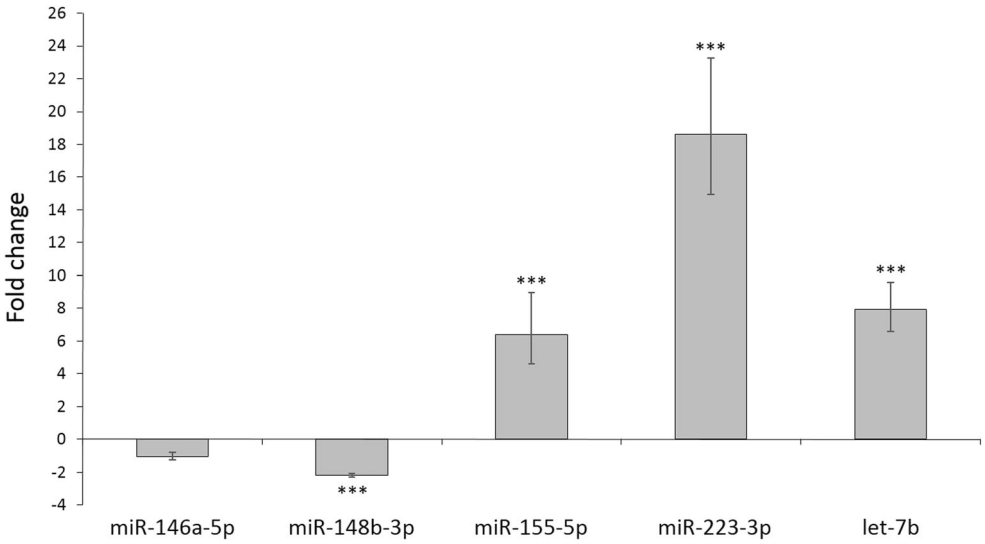

Fig. 1 Expression of miRNAs miR-146a-5p, miR-148b-3p, miR-155-5p, miR-223-3p, and let-7b in skin samples of IgAV patients. miRNA fold change values were generated by the $2^{-\Delta\Delta Ct}$ method. The bars represent the means ± SD (n = 65). The expression of each miRNA was compared to the control (skin samples of healthy individuals) and statistically evaluated by using the Mann-Whitney U test. A p value of < 0.05 was considered statistically significant (***$p < 0.001$)

vs. limited purpura) did not correlate with the intensity of skin miRNA-223-3p expression. Our group previously demonstrated that generalized purpura represents, along with smoking and abdominal pain, one of the three clinical predictors of severe GI and renal involvement in adult IgAV [20]. miR-233 could indeed represent a therapeutic target in IgAV. The potential of miR-233 as a therapeutic target has been evaluated in patients with inflammatory bowel disease (IBD), where the upregulated miR-223 positively mediated the pathogenesis of IBD by negatively regulating FOXO3a, the direct target of miRNAs, and enhanced the expression of inflammatory cytokines [21].

Regarding GI tract involvement, our results uncovered a borderline downregulation of skin miRNA-223-3p expression in the case of bowel involvement, as well as severe GI tract involvement. Next, we found two additional negative correlations between GI tract involvement and the skin expression of miRNA-146a-5p and miRNA-155-5p. MicroRNA 155 exerts several functions in an adaptive immune system: it promotes the differentiation of regulatory T cells, promotes Th1 and Th17 differentiation and immunoglobulin class switching in B cells. MicroRNA-146a plays an important role in the maintenance of self-tolerance. It is significantly downregulated in the plasma of patients with systemic lupus erythematosus, where decreased miRNA-146a levels are also associated with increased clinical disease activity [7]. One might speculate that decreased skin miRNA-146a-5p expression points to a more aggressive acute IgAV disease with GI tract involvement.

In IgAV patients with renal involvement, no significant differences in either skin miRNA-146a-5p expression or other tested miRNAs were found. However, despite these negative results, any further conclusions are premature, since we did not evaluate miRNA expression in the kidney biopsy or urine samples.

In contrast to the expression of miRNA-146a-5p, miRNA-155-5p, and miRNA-223-3p in IgAV skin tissue, the upregulated let-7b expression showed no significant correlation with the evaluated IgAV clinical characteristics. The same was also determined for downregulated miRNA-148b-3p expression. Previous studies have associated the upregulation of miRNA-148b and let-7b with an aberrant regulation of *O*-linked glycosylation of IgA1 in the peripheral blood mononuclear cells (PBMCs) of patients with IgA nephropathy [18, 19]. Furthermore, both miRNAs were proposed as a robust non-invasive combined biomarker for diagnosing IgA nephropathy from a patient's serum samples [9].

We are aware of the limitations of our study. As a pilot study, we analyzed only a pre-selected and limited set of miRNAs and these solely in the skin. Evaluating a larger number of miRNAs and getting data on the miRNA profiles, also from other tissues (e.g., renal tissue) and easily collected body fluids (like peripheral blood and/or urine), would have further improved our understanding of IgAV. A larger number of patients and controls included would also add to the quality of the study.

The major strengths of our study are its prospective design and the quality of the collected clinical data in a well-defined IgAV cohort. All patients had a histologically verified disease and were not treated with steroids at the time when a skin biopsy was performed. Next, the skin biopsies in patients and controls were primarily taken from a single body region (i.e., shin), and the controls included were age- and sex-matched to the patient cohort. The meticulous control selection minimized the potential influence of gender, age, and location of the skin biopsy on the miRNA expression profile.

In conclusion, this is the first study to date on miRNA expression in IgAV which showed that several miRNAs might play an important role in the vasculitic process of IgAV. In addition, the study revealed potential associations between the

Table 3 Clinical characteristic of IgAV cohort and miRNA expression

IgAV characteristics		ΔΔCT miR-146a-5p			*p*	ΔΔCT miR-148b-3p			*p*	ΔΔCT miR-155-5p			*p*	ΔΔCT miR-223-3p			*p*	ΔΔCT let-7b			*p*
		Median	IQR1	IQR2		Median	IQR1	IQR2		Median	IQR1	IQR2		Median	IQR1	IQR2		Median	IQR1	IQR2	
Male	Yes	−0.10	−1.06	0.65	0.726	−0.99	−1.41	−0.60	0.239	2.43	1.99	3.18	0.353	4.31	3.13	6.16	0.225	3.03	2.49	3.60	0.212
	No	0.13	−0.65	0.87		−1.26	−2.03	−0.68		2.92	2.19	3.42		3.45	2.74	5.54		2.65	2.27	3.38	
Prior infection	Yes	0.21	−0.48	0.66	0.749	−0.99	−1.25	−0.39	0.179	2.50	2.06	3.18	0.402	3.88	2.72	5.90	0.957	3.28	2.71	3.64	0.058
	No	−0.14	−1.11	0.77		−1.18	−1.85	−0.69		2.72	2.09	3.47		4.45	2.89	5.61		2.89	2.30	3.31	
Ever smoker	Yes	−0.35	−1.50	0.41	0.003	−1.21	−1.84	−0.84	0.217	2.48	1.99	3.16	0.301	4.58	3.09	6.21	0.125	2.90	2.29	3.24	0.105
	No	0.38	−0.33	0.96		−0.94	−1.60	−0.37		2.74	2.19	3.52		3.45	2.71	5.39		3.20	2.48	3.66	
General symptoms	Yes	0.18	−1.32	0.38	0.643	−1.43	−1.95	−0.95	0.174	2.49	2.29	3.41	0.739	4.11	2.92	5.81	0.928	2.98	2.32	3.35	0.573
	No	0.04	−0.67	0.70		−0.99	−1.69	−0.58		2.64	2.00	3.20		3.97	2.86	5.67		2.99	2.42	3.53	
Skin necroses	Yes	0.08	−0.85	0.70	0.768	−1.13	−1.67	−0.78	0.424	2.96	2.18	3.48	0.131	5.06	3.20	6.09	0.029	2.92	2.31	3.50	0.447
	No	0.13	−0.83	0.69		−0.99	−1.83	−0.28		2.41	1.95	2.96		3.45	2.64	5.02		3.06	2.53	3.63	
Generalized purpura	Yes	0.12	−1.15	0.60	0.782	−1.06	−1.73	−0.71	0.574	2.41	2.02	2.99	0.121	4.13	2.85	6.12	0.521	2.99	2.32	3.54	0.172
	No	0.13	−0.83	0.69		−0.89	−1.81	−0.40		3.07	2.22	3.56		3.89	2.87	5.53		3.03	2.48	3.45	
Articular involvement	Yes	0.21	−1.29	0.68	0.858	−0.99	−1.73	−0.77	0.903	2.44	2.08	3.12	0.383	4.29	2.62	5.74	0.974	2.90	2.42	3.31	0.470
	No	−0.14	−0.64	0.70		−1.00	−1.81	−0.54		2.82	2.09	3.43		3.95	2.87	5.72		3.14	2.32	3.63	
GI involvement	Yes	−0.75	−1.59	0.38	0.041	−1.11	−2.03	−0.90	0.198	2.22	1.94	2.35	0.004	3.21	2.41	4.36	0.054	3.02	2.15	3.44	0.599
	No	0.13	−0.52	0.84		−0.95	−1.68	−0.54		2.92	2.25	3.48		4.54	2.94	5.87		2.96	2.45	3.54	
Severe GI involvement	Yes	0.24	−1.45	0.61	0.694	−0.99	−2.02	−0.85	0.557	2.27	2.02	2.59	0.311	2.92	2.13	3.48	0.069	2.99	2.11	3.83	0.941
	No	0.08	−0.71	0.72		−1.01	−1.75	0.60		2.70	2.06	3.25		4.47	2.89	5.82		2.98	2.39	3.52	
Renal involvement	Yes	0.15	−0.89	0.61	0.884	−1.21	−1.78	−0.78	0.209	2.75	2.17	3.35	0.532	4.54	2.94	5.97	0.311	2.94	2.31	3.57	0.791
	No	0.04	−0.81	0.71		−0.94	−1.78	−0.34		2.48	2.01	3.22		3.95	2.67	5.67		3.03	2.39	3.50	
Severe renal involvement	Yes	−0.21	−1.94	0.43	0.247	−1.39	−2.31	−0.91	0.284	2.88	2.12	3.23	0.969	5.02	2.90	5.80	0.805	2.38	2.00	3.30	0.217
	No	0.13	−0.65	0.73		−0.99	−1.75	−0.58		2.57	2.07	3.25		4.02	2.82	5.76		3.02	2.46	3.55	
↑ IgA	Yes	−0.10	−0.55	0.58	0.572	−0.99	−1.62	−0.64	0.752	2.88	2.02	3.05	0.701	5.06	2.96	6.18	0.053	2.98	2.31	3.62	0.767
	No	0.18	−1.16	0.97		−1.17	−1.92	−0.43		2.48	2.19	3.17		3.82	2.41	5.06		2.99	2.42	3.39	

GI gastrointestinal

selected miRNA expression patterns and specific disease manifestations. Our results should be confirmed in a larger study.

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Compliance with ethical standards

All patients and controls signed a written consent form. The study was approved by the ethical approval # 99/04/15 obtained from the Slovenian National Medical Ethics Committee.

Disclosures None.

References

1. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, Flores-Suarez LF, Gross WL, Guillevin L, Hagen EC, Hoffman GS, Jayne DR, Kallenberg CG, Lamprecht P, Langford CA, Luqmani RA, Mahr AD, Matteson EL, Merkel PA, Ozen S, Pusey CD, Rasmussen N, Rees AJ, Scott DG, Specks U, Stone JH, Takahashi K, Watts RA (2013) 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 65:1–11. https://doi.org/10.1002/art.37715
2. Piram M, Mahr A (2013) Epidemiology of immunoglobulin A vasculitis (Henoch-Schönlein): current state of knowledge. Curr Opin Rheumatol 25:171–178. https://doi.org/10.1097/BOR.0b013e32835d8e2a
3. Hočevar A, Rotar Z, Ostrovršnik J, Jurčić V, Vizjak A, Dolenc Voljč M, Lindič J, Tomšič M (2014) Incidence of IgA vasculitis in the adult Slovenian population. Br J Dermatol 171:524–527. https://doi.org/10.1111/bjd.12946
4. Kiryluk K, Moldoveanu Z, Sanders JT, Eison TM, Suzuki H, Julian BA, Novak J, Gharavi AG, Wyatt RJ (2011) Aberrant glycosylation of IgA1 is inherited in both pediatric IgA nephropathy and Henoch-Schönlein purpura nephritis. Kidney Int 80:79–87. https://doi.org/10.1038/ki.2011.16
5. Heineke MH, Ballering AV, Jamin A, Ben Mkaddem S, Monteiro RC, van Egmond M (2017) New insights in the pathogenesis of immunoglobulin A vasculitis (Henoch-Schönlein purpura). Autoimmun Rev 16:1246–1253. https://doi.org/10.1016/j.autrev.2017.10.009
6. Ceribelli A, Yao B, Dominguez-Gutierrez PR, Nahid MA, Satoh M, Chan EK (2011) MicroRNAs in systemic rheumatic diseases. Arthritis Res Ther 13:229. https://doi.org/10.1186/ar3377
7. Garo LP, Murugaiyan G (2016) Contribution of MicroRNAs to autoimmune diseases. Cell Mol Life Sci 73:2041–2051. https://doi.org/10.1007/s00018-016-2167-4
8. Bao H, Chen H, Zhu X, Zhang M, Yao G, Yu Y, Qin W, Zeng C, Zen K, Liu Z (2014) MiR-223 downregulation promotes glomerular endothelial cell activation by upregulating importin α4 and α5 in IgA nephropathy. Kidney Int 85:624–635. https://doi.org/10.1038/ki.2013.469
9. Serino G, Pesce F, Sallustio F, de Palma G, Cox SN, Curci C, Zaza G, Lai KN, Leung JC, Tang SC, Papagianni A, Stangou M, Goumenos D, Gerolymos M, Takahashi K, Yuzawa Y, Maruyama S, Imai E, Schena FP (2016) In a retrospective international study, circulating miR-148b and let-7b were found to be serum markers for detecting primary IgA nephropathy. Kidney Int 89:683–692. https://doi.org/10.1038/ki.2015.333
10. Wang G, Kwan BC, Lai FM, Chow KM, Li PK, Szeto CC (2011) Elevated levels of miR-146a and miR-155 in kidney biopsy and urine from patients with IgA nephropathy. Dis Markers 30:171–179. https://doi.org/10.3233/DMA-2011-0766
11. Hočevar A, Rotar Ž, Jurčić V, Čučnik S, Tomšič M (2015) Patient age, gender and extent of purpura may suggest short-term outcomes in adults with IgA vasculitis. Rheumatology (Oxford) 54:1330–1332. https://doi.org/10.1093/rheumatology/kev122
12. Ozen S, Pistorio A, Iusan SM, Bakkaloglu A, Herlin T, Brik R, Buoncompagni A, Lazar C, Bilge I, Uziel Y, Rigante D, Cantarini L, Hilario MO, Silva CA, Alegria M, Norambuena X, Belot A, Berkun Y, Estrella AI, Olivieri AN, Alpigiani MG, Rumba I, Sztajnbok F, Tambic-Bukovac L, Breda L, Al-Mayouf S, Mihaylova D, Chasnyk V, Sengler C, Klein-Gitelman M, Djeddi D, Nuno L, Pruunsild C, Brunner J, Kondi A, Pagava K, Pederzoli S, Martini A, Ruperto N, for the Paediatric Rheumatology International Trials Organisation (PRINTO) (2010) EULAR/PRINTO/PRES criteria for Henoch-Schonlein purpura, childhood polyarteritis nodosa, childhood Wegener granulomatosis and childhood Takayasu arteritis: Ankara 2008. Part II: final classification criteria. Ann Rheum Dis 69:798–806. https://doi.org/10.1136/ard.2009.116657
13. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55:611–622. https://doi.org/10.1373/clinchem.2008.112797
14. Rasmussen R (2001) Quantification on the LightCycler. In: Meuer S, Wittwer C, Nakagawara K (eds) Rapid cycle real-time PCR, Methods and Applications. Springer Press, Heidelberg, pp 21–34
15. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262
16. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3:RESEARCH0034
17. Kubista M, Sindelka R, Tichopad A et al (2007) The prime technique. Real-time PCR data analysis. G.I.T. Lab J 9–10:33–35
18. Serino G, Sallustio F, Cox SN, Pesce F, Schena FP (2012) Abnormal miR-148b expression promotes aberrant glycosylation of IgA1 in IgA nephropathy. J Am Soc Nephrol 23:814–824. https://doi.org/10.1681/ASN.2011060567
19. Serino G, Sallustio F, Curci C, Cox SN, Pesce F, de Palma G, Schena FP (2015) Role of let-7b in the regulation of N-acetylgalactosaminyltransferase 2 in IgA nephropathy. Nephrol Dial Transplant 30:1132–1139. https://doi.org/10.1093/ndt/gfv032
20. Hočevar A, Rotar Z, Kejžar N, Tomšič M (2017) A model predicting short term severity of IgA vasculitis in adults. J Clin Exp Dermatol Res 8:397. https://doi.org/10.4172/2155-9554.1000397
21. Wang H, Zhang S, Yu Q, Yang G, Guo J, Li M, Zeng Z, He Y, Chen B, Chen M (2016) Circulating MicroRNA223 is a new biomarker for inflammatory bowel disease. Medicine (Baltimore) 95:e2703. https://doi.org/10.1097/MD.0000000000002703