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Original Scientific Paper

ASSOCIATION BETWEEN HIGH MOBILITY GROUP BOX 1 PROTEIN GENE (rs41369348) POLYMORPHISM AND IMMUNOGLOBULIN A VASCULITIS IN CHILDREN

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SUMMARY – Immunoglobulin A vasculitis (IgAV) or Henoch-Schönlein purpura is the most prevalent systemic small vessel vasculitis in childhood. High mobility group box 1 protein (HMBG1) is a pleiotropic cytokine that functions as a pro-inflammatory signal, important for the activation of antigen-presenting cells and propagation of inflammation. HMGB1 is implicated in the pathophysiology of a variety of inflammatory diseases. The aim of this study was to investigate the role of single nucleotide polymorphism rs41369348 for HMGB1 gene in the susceptibility and clinical features of patients meeting the classification criteria for IgAV. DNA was extracted from blood cells of 76 children with IgAV and 150 age-matched healthy controls. Clinical data and laboratory parameters were collected for all IgAV patients. Although there was a higher frequency of heterozygous A/delA genotype of this gene polymorphism in IgAV group as compared with control group, no genotype difference was observed between these two groups. No statistically significant genotype differences were disclosed when patients with different IgAV clinical features were compared. In conclusion, in this study, polymorphism rs41369348 for HMGB1 was not associated with increased susceptibility to childhood IgAV, its severity or different clinical manifestations.

Key words: *Henoch-Schönlein purpura; HMGB1 protein; Single nucleotide polymorphism*

Introduction

Immunoglobulin A vasculitis (IgAV) or Henoch-Schönlein purpura is the most prevalent systemic small vessel vasculitis in childhood, with the incidence of 3-26.7 *per* 100 000[1]. In Croatia, the mean annual incidence is 6.79 *per* 100 000 children[2]. More

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than 90% of patients with IgAV are younger than 10 years of age. Childhood onset has a slight male predominance (1.5:1 male:female ratio) and a decreasing incidence with increasing age[3]. Its clinical features are well recorded and include non-thrombocytopenic palpable purpura, arthritis, and involvement of renal and gastrointestinal system. IgAV is usually self-limiting, with an average duration of 4 weeks and most cases require no treatment. Despite that, various acute and chronic complications are possible[4,5]. Nephritis (IgAVN) is the most important chronic complication of IgAV and thus the main prognostic factor[6-8]. The prevalence of IgAVN in Croatia is 19.6%[2], which is consistent with the previously reported rates[3,9]. Although it is acknowledged to be a systemic immune-complex mediated disease, detailed pathogenic mechanisms of IgAV have not been fully elucidated[10]. Key components of the genetic network implicated in the pathogenesis of IgAV are cytokines signaling pathway genes[11].

High mobility group box 1 protein (HMGB1) is a ubiquitously expressed, highly conserved 25-kDaDNA binding protein that has been identified as an endogenous damage-associated molecular pattern (DAMP)[12]. It exerts extracellularly its biological functions and plays an important role in the pathogenesis of various inflammatory and autoimmune diseases[13-18]. A group of authors have demonstrated that inflammation triggered by HMGB1 is important in the pathogenesis of juvenile idiopathic arthritis and childhood-onset systemic lupus erythematosus, suggesting HMGB1 as a biomarker and a potential target of biological therapy in these patients[17].

Several HMGB1 polymorphisms have been described, some of which have pro-inflammatory effects: certain single nucleotide polymorphisms (SNPs) are associated with more severe inflammatory response or increased risk of mortality in septic patients or in patients with the systemic inflammatory response syndrome (SIRS) (rs41369348, rs2249825, rs1045411, rs1060348), while others are related with susceptibility to colorectal cancer and development of hypertension[19-23].

In this study, we examined the influence of HMGB1 polymorphism rs41369348 (delT; chr13:30467220-30467227(GRCh38.p12)) in the susceptibility and clinical features of patients with IgAV.

Patients and Methods

Patient selection and study design

This case-control study was conducted in two Croatian university centers for pediatric rheumatology and included 226 subjects. The experimental group consisted of 76 children with IgAV and control group included 150 healthy individuals without any history of autoimmune disease. Diagnosis of IgAV was based on the criteria defined by the European League Against Rheumatism (EULAR), Pediatric Rheumatology International Trials Organization (PRINTO), and Pediatric Rheumatology European Society (PRES)[24]. This study was conducted according to the Declaration of Helsinki and its amendments, and approved by the Ethics Committee of the Josip Juraj Strossmayer University of Osijek Faculty of Medicine and Ethics Committee of the University of Zagreb School of Medicine. A written informed consent was obtained from parents of all individuals enrolled into the study.

Data collection

Clinical data and laboratory parameters were collected from medical records of all IgAV patients. Clinical data included sex; age at disease onset; place of residence; season of disease onset; time of follow up; prodromal infections before the appearance of purpura; first symptom at disease onset; number of relapses; joint, renal, scrotum and gastrointestinal system involvement; and distribution of skin changes. Big city as a place of residence was defined as a city with more than 100 000 inhabitants.

Laboratory findings included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), hemoglobin, hematocrit, leukocytes, platelets, blood urea nitrogen, serum creatinine, fibrinogen, prothrombin time (PT), D-dimer, total serum protein, serum albumin, immunoglobulins (IgA, IgG, and IgM), serum complement C3, complement C4 and quantification of proteinuria in 24-h urine collection. Samples of venous blood and urine were obtained in all patients at the disease diagnosis.

HMGB1 gene (rs41369348) polymorphism analysis

Genomic DNA was extracted from the whole peripheral blood using the spin column method and according to the manufacturer's protocol (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany). Genotyping was carried out by the real-time polymerase chain reaction (rt-PCR) method performed on 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using TaqMan SNP genotyping assays. The PCR reaction mix of 25 μL final volume consisted of 2 μL of genomic DNA, 12.5 μL of

TaqMan Genotyping PCR Master Mix 2X, 0.625 μL SNP Assay Mix 40X, and 9.875 μL H2O. The protocol for PCR amplification was as follows: initial denaturation step at 95 ˚C for 10 minutes, then 40 cycles of denaturation at 92 ˚C for 15 seconds, followed by 60 ˚C for 1 minute, and a final extension step at 60 ˚C for 1 minute. The allelic discrimination analysis was performed using SDS 7500 Software Version 2.3 (Applied Biosystems, Foster City, CA, USA).

Statistics

Statistical analysis was performed using Statistica for Windows v. 13.4.0.14. All genotype data were checked for deviation from Hardy-Weinberg equilibrium (HWE) by the χ^2-test. HMGB1 rs41369348 genotype frequencies were compared by the χ^2-test where $p<0.05$ was considered significant. The strength of association between different groups and allele or genotype of HMGB1 was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either χ^2 or Fisher exact analysis. The mean and standard deviation (SD) were calculated for normally distributed continuous variables, and percentages were calculated for categorical variables. Categorical data were analyzed using Pearson's χ^2-test. Furthermore, continuous variables were analyzed using Student's t-test. Univariate logistic regression analyses were conducted to identify the independent predictors of relapses and IgAVN.

Results

Out of 226 children, 76 of them were diagnosed with IgAV. There was no statistically significant difference between gender proportions considering the age at diagnosis in the group of patients with IgAV (p=0.87). Their main demographic and clinical characteristics are shown in Table 1.

Out of 76 patients with IgAV, 19 (25%) of them had IgAVN. In five patients (26%), there were signs of renal involvement detected at the time of diagnosis. The mean age of patients with IgAVN was not statistically significantly higher as compared with patients without renal involvement (6.44±2.86 *vs*. 7.7±3.35 years). The mean number of relapses was significantly higher in the group of patients with IgAVN as compared to patients without renal involvement (1.27±1.42 *vs*. 0.24±0.73, p<0.05). Of all laboratory parameters listed in Table 2, only the values of proteinuria in 24-h urine collection (0.29±0.38 *vs*. 0.09±0.04 g/day), serum creatinine (45.48±14.98 *vs*. 36.72±10.50 μmol/L) and blood urea nitrogen (5.50±1.86 *vs*. 4.63±1.37 mmol/L) were statistically significantly higher in the group of patients with IgAVN as compared to the group without renal involvement (p<0.05). The whole comparison of clinical and laboratory parameters between patients with and without renal involvement is illustrated in Table 2.

Twenty-one (27%) IgAV patients had gastrointestinal (GI) involvement. In seven (33%) of them, this was the first symptom of IgAV. The mean number of

Table 1. Demographic and clinical features of IgAV patients

Demographic and clinical features	Patients (N=76)
Age at disease onset (years), mean ± SD	6.78±3.03
Time from symptom onset to diagnosis (days), mean ± SD	3.19±5.22
Gender (female/male), n	34/42
Big city as the place of residence, n (%)	33 (43)
Onset of disease in cold season, n (%)	47 (62)
Infection preceded the appearance of IgAV, n (%)	56 (74)
- respiratory, n (%)	52 (93)
- gastrointestinal, n (%)	4 (7)
Follow up (months), mean ± SD	32.13±19.24
Purpura as initial symptom, n (%)	39 (51)
Abdominal symptoms as initial symptom, n (%)	6 (8)
Arthritis/arthralgia as initial symptom, n (%)	30 (40)
Acute scrotum as initial symptom, n (%)	1 (1)
Generalized rash, n (%)	32 (42)
Arthralgia and/or arthritis, n (%)	53 (70)
Gastrointestinal manifestations, n (%)	21 (27)
Renal involvement, n (%)	19 (25)
Erythrocyturia/proteinuria at onset of disease, n (%)	5 (7)
Steroid therapy, n (%)	39 (51)
At least one relapse, n (%)	20 (26)

IgAV = IgA vasculitis; SD = standard deviation

Table 2. Comparison of clinical and laboratory parameters between IgAV patients with and without renal involvement

Parameter	Renal involvement (mean ± SD)	No renal involvement (mean ± SD)	p value
Age	7.70±3.35	6.44±2.86	0.35
Number of relapses	1.27±1.42	0.24±0.73	<0.05
ESR (mm/1st hour)	19.86±11.16	22.16±13.98	0.50
CRP (mg/L)	16.5±29.74	10.91±11.38	0.23
Leukocytes (*10^9/L)	10.95±3.68	10.95±3.86	0.99
Hemoglobin (g/L)	124.10±14.04	125.28±9.31	0.67
Hematocrit (L/l)	0.36±0.04	0.37±0.03	0.56
Platelets (*10^9/L)	358.19±84.09	334.96±94.23	0.32
Proteinuria in 24-h urine collection (g/day)	0.29±0.38	0.09±0.04	<0.05
Serum creatinine (μmol/L)	45.48±14.98	36.72±10.50	<0.05
Blood urea nitrogen (mmol/L)	5.50±.86	4.63±1.37	<0.05
Prothrombin time (s)	0.91±0.21	1.00±0.14	<0.05
D-dimer (mg/L)	4.15±5.96	3.39±3.74	0.51
Total serum protein (g/L)	67.63±5.79	68.94±5.53	0.36
Serum albumin (g/L)	38.05±4.19	38.69±4.52	0.58
IgA (g/L)	2.29± 0.99	1.86±0.74	0.05
IgG (g/L)	10.18±2.79	10.14±2.4	0.94
IgM (g/L)	0.95±0.30	1.13±1.12	0.47
Fibrinogen (g/L)	3.21±1.17	3.53±0.84	0.20
Complement C3 (g/L)	1.09±0.21	1.34±0.25	0.05
Complement C4 (g/L)	0.22±0.08	0.28±0.20	0.31

ESR = erythrocyte sedimentation rate; CRP = C-reactive protein, MPV = mean platelet volume; MPR = mean platelet volume/platelet count ratio; RDW = red cell distribution width; IgG = immunoglobulin G; IgA = immunoglobulin A; IgM = immunoglobulin M; SD = standard deviation

relapses was significantly higher in the group of patients with GI involvement as compared to patients without it (0.91±1.38 *vs*. 0.22±0.59, p<0.05). The values of CRP (18.71±29.53 *vs*. 9.39±9.59 mg/L), leukocytes (16.65±5.34 *vs*. 12.65±3.93*10^9/L), proteinuria in 24-h urine collection (0.22±0.34 *vs*. 0.1±0.07 g/day) and blood urea nitrogen (0.22±0.34 *vs*. 0.1±0.07 mmol/L) were statistically significantly higher in the group of patients with GI involvement as compared to the group without it (p<0.05).

Thirty-two (42%) IgAV patients had generalized rash. The mean number of relapses was significantly higher in the group of patients with generalized rash than in the group with typically distributed rash in IgAV (0.91±1.35 *vs*. 0.35±0.90, p<0.05). The values of hemoglobin (134±12.61 *vs*. 128.52±8.9 g/L), hematocrit (0.39±0.04 *vs*. 0.38±0.03), proteinuria in 24-h urine collection (0.22±0.32 *vs*. 0.09±0.04 g/day) and serum creatinine (45.77±16.1 *vs*. 39.18±11.35 μmol/L) were statistically significantly higher in the group of patients with generalized rash as compared to the group with typically distributed rash (p<0.05). There was no statistically significant gender difference in the number of relapses (p=0.86). Age at onset of the disease showed a positive correlation to the number of relapses (p<0.05) (Fig. 1).

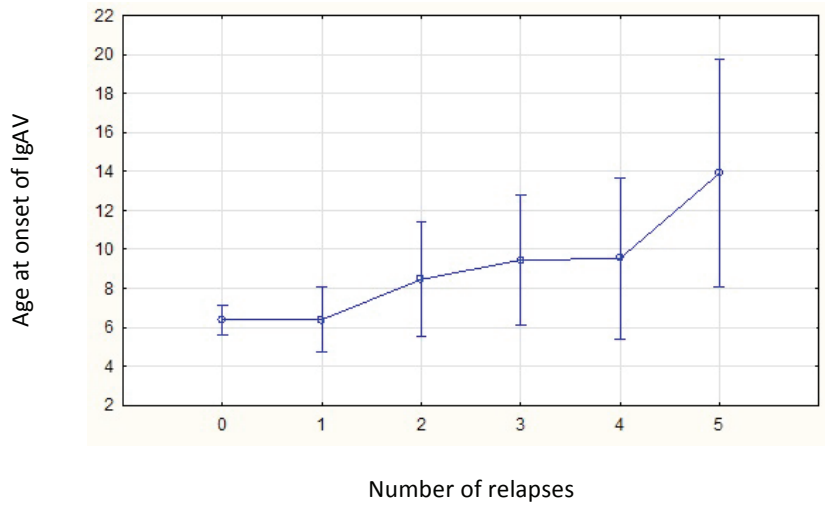

Fig. 1. Positive correlation between age at onset of immunoglobulin A vasculitis (IgAV) and number of relapses.

Table 3. Association between various factors and renal involvement in IgAV patients by univariate analysis

Factor	OR	95% CI	p
Gender (male)	0.55	0.22-1.37	0.20
Age ≥5 years	1.92	0.56-0.56	0.29
Big city as the place of residence	0.21	0.07-0.64	<0.05
Purpura as initial symptom	1.78	0.66 - 4.81	0.26
GI manifestation as initial symptom	1.71	0.37 - 7.83	0.49
Joint manifestation as initial symptom	0.44	0.15-1.29	0.14
Prodromal infections before the appearance of IgAV	0.37	0.13-1.06	0.06
Generalized rash	5.47	1.84-16.22	<0.05
Number of relapses	11.16	3.55-35.04	<0.05
ESR ≥20 mm/1st hour	0.97	0.35-2.67	0.95
CRP ≥5 mg/L	0.90	0.32-2.48	0.84
Leukocytes ≥15*10^{9}/L	1.21	0.28-5.20	0.79
Hemoglobin <115 g/L	1.68	0.43-6.46	0.44
Hematocrit <0.35 L/L	1.40	0.47-4.13	0.54
Platelets 150-300*10^{9}/L	0.49	0.12-1.96	0.31
Fibrinogen >4 g/L	0.80	0.22-2.85	0.73
Prothrombin time >0.7	0.11	0.01-1.13	0.06
IgG >15.7 g/L	2.85	0.17-47.73	0.47
IgA >2.36 g/L	2.13	0.72-6.24	0.17
IgM >2.4 g/L	0.88	0.03-22.34	0.93
Proteinuria in 24-h urine collection >0.15 g/day	9.75	2.57-37.03	<0.05
Corticosteroid therapy	1.81	0.66-4.96	0.24

IgAV = IgA vasculitis; OR = odds ratio; CI = confidence interval; GI = gastrointestinal; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; IgG = immunoglobulin G; IgA = immunoglobulin A; IgM = immunoglobulin M

Of laboratory parameters, positive correlation was only recorded between ESR at the onset of the disease, proteinuria in 24-h urine collection and number of relapses ($p<0.01$).

We evaluated the association between various factors and renal involvement in IgAV patients using univariate analysis, as shown in Table 3.

The number of relapses, generalized rash in clinical presentation, and increased proteinuria in 24-h urine collection were associated with an increased risk of IgAVN development (OR: 11.16; 95% CI: 3.55-35.04; $p<0.05$; OR: 5,47; 95% CI: 1.84-16.22; $p<0.05$; and OR: 9.75; 95% CI 2.57-37.03; $p<0.05$, respectively). Living in a big city was associated with a decreased risk of IgAVN development (OR: 0.21; 95% CI: 0.07-0.64; $p<0.05$).

In controls and patients fulfilling IgAV classification criteria, no evidence of departure from Hardy-Weinberg equilibrium was observed. Normal AA genotype was found in 83% of the IgAV patients and 91% of the control group. Heterozygous A/delA genotype was detected in 11% and 8% of the patient group and healthy controls, respectively. Homozygous mutant delA genotype was found in only one IgAV patient. The genotype and allele frequencies of both groups and p-values are shown in Table 4. When genotype frequencies of HMGB1 gene polymorphism of IgAV patients were compared to genotype frequencies in the control group, no significant differences were found (Table 4). No statistically significant differences in genotype or allele were disclosed when patients with different IgAV clinical manifestations were compared ($p>0.05$).

Discussion

Immunoglobulin A vasculitis is a leukocytoclastic vasculitis characterized by IgA dominant immune deposits involving mainly the skin but other tissues as well. The aim of this study was to investigate one part of the genetic component of this disease.

When comparing basic demographic features of our patients, they were found to fit well within the range reported in the literature. The mean age of patients in our research was similar to the data published in the study by Gardner-Medwin *et al.*[3]. We did not observe age difference between patients who developed IgAVN and those without nephritis, and age was not a risk factor for the incidence of IgAVN, which corresponds to the results reported by Feng *et al.*[25] and Peru *et al.*[26]. However, several meta-analyses have shown that older age at disease onset is a risk factor for the occurrence of nephritis[27,28], especially older than ten years[29].

In our study, distribution of IgAV patients by gender was in favor of males, which is consistent with most studies[3,25,30-32]. This result is expected if we take into account that IgAV belongs to the group of a few autoimmune diseases that occur more frequently in males, without clear explanation in the available literature, except for the assumption that it is at least partially conditioned by the male-related hormones[27].

Regarding the prevalence and timeframe of IgAVN development, nephritis was detected in 22 (27%) children, and among them, in seven (32%) it was present at the onset of IgAV, whereas in most of them (73%) it was diagnosed during the first 4 weeks of illness. A large number of previous studies confirmed our observations[7,9,33,34].

The occurrence of generalized rash as a form of severe skin involvement within the clinical course significantly increased the likelihood of relapse and nephritis, and the occurrence of at least one relapse of IgAV increased the risk of IgAVN. Other authors report similar results[7,27,35,36].

Patients with IgAV had higher values of inflammatory markers in comparison with controls. Elevated values of ESR and CRP are a reflection of systemic inflammation and are present in almost all patients with IgAV, so these parameters cannot be used to distinguish patients with nephritis from those without nephritis. In only about a quarter of our patients with IgAV, elevated serum IgA values were observed, which is in contrast to the study by Pillebout *et al.*[37] and Oni and Sampath[10].

Previous studies have clearly suggested that common genetic components may underlie different immune-related diseases[38,39]. The etiopathogenesis of IgAV is complex and not fully understood, and may involve a number of interactions between various environmental and genetic factors[10,11,40]. So far, not much is known about the genes that may contribute to the development of the disease. Although genome-wide association studies point to the significance of the HLA class II genes[41-45], specifically HLA-DRB1, various studies have also shown that variants in non-HLA genes associated with immune and inflammatory response, and in particular SNPs, may also have significance in the etiopathogenesis. Precisely,

Table 4. Genotypic (a) and allelic (b) frequencies of HMGB1 gene polymorphism rs41369348 in IgAV and control groups.

Genotype/ Allele	Controls, n (%)	IgAV (all patients), n (%)	IgAV with nephritis, n (%)	IgAV without nephritis, n (%)	IgAV with GI symptoms, n (%)	IgAV without GI symptoms, n (%)	IgAV with generalized rash, n (%)	IgAV without generalized rash, n (%)	IgAV with arthritis/ arthralgia, n (%)	IgAV without arthritis/ arthralgia, n (%)	OR (95% CI)	p value
(a)												
AA	138 (92)	68 (90)									1.716 (0.706-4.174)	0.29
A/delA	12 (8)	7 (9)									0.657 (0.264-1.635)	0.29
delA/delA	0	1 (1)									5.980 (0.241-148.565)	0.28
AA			16 (84)	52 (91)							8.889 (1.344-58.798)	0.27
A/delA			3 (16)	4 (7)							2.484 (0.503-12.28)	0.26
delA/delA			0	1 (2)							0.966 (0.038-24.705)	0.98
AA					19 (90)	49 (89)					1.163 (0.216-6.277)	0.86
A/delA					2 (10)	5 (9)					1.053 (0.188-5.895)	0.95
delA/delA					0	1 (2)					0.845 (0.033-21.561)	0.92
AA							31 (97)	37 (84)			0.144 (0.016-1.258)	0.08
A/delA							1 (3)	6 (14)			0.199 (0.023-1.742)	0.15
delA/delA							0	1 (2)			0.446 (0.018-11.31)	0.63
AA									46 (87)	22 (96)	0.144 (0.016-1.258)	0.08
A/delA									6 (11)	1 (4)	0.199 (0.023-1.742)	0.15
delA/delA									1 (2)	0	0.149 (0.006-3.8)	0.25
(b)												
delA	12	9									1.511 (0.622-3.668)	0.36
A	288	143									0.662 (0.273-1.608)	0.36
delA			3	6							1.543 (0.367-6.495)	0.55
A			35	108							0.648 (0.154-2.729)	0.55
delA					2	7					0.736 (0.147-3.693)	0.71
A					40	103					1.359 (0.271-6.823)	0.71
delA							1	8			0.155 (0.019-1.270)	0.08
A							63	78			6.461 (0.787-53.043)	0.08
delA									8	1	3.673 (0.446-30.259)	0.23
A									98	45	0.272 (0.029-2.026)	0.23

IgAV = immunoglobulin A vasculitis; OR = odds ratio; CI = confidence interval

different gene polymorphisms located outside the HLA region, including those related to aberrant glycosylation of IgA1, nitric oxide production, coding cytokines, adhesion molecules, and chemokines, may be implicated not only in the predisposition to IgAV but also in its severity[11,46,47].

In this research, we focused on HMGB1, which acts as a proinflammatory mediator with an important role in the pathogenesis of various inflammatory and autoimmune diseases such as systemic lupus erythematosus, Behçet disease, rheumatoid arthritis (RA), and antineutrophil cytoplasmic antibody (ANCA)-vasculitis[13-17]. Several studies have demonstrated elevated serum levels of HMGB1 in patients with IgAV[48-50]. Furthermore, it was found that HMGB1 serum level was significantly higher in IgAVN patients as compared with healthy controls[51]. Kornblit *et al*. found that HMGB1 gene polymorphism rs41369348 (delT; chr13:30467220-30467227(GRCh38.p12)) was significantly associated with an increased risk of delayed mortality in patients with the SIRS[19].

Taking these observations into consideration, we evaluated the potential implication of HMGB1 gene polymorphism rs41369348 in the pathogenesis of IgAV. Although there was a higher frequency of heterozygous A/delA genotype of this gene polymorphism in IgAV group compared to control group, the difference did not reach statistical significance. Since previous studies disclosed that some gene polymorphisms were associated with different IgAV phenotypes[46,52], we also aimed to determine whether this selected polymorphism might account for the increased risk of renal and GI implications or generalized rash, especially because in this research we found an increased number of relapses in all these manifestations. Therefore, we decided to verify whether there was a genetic background for this observation in the investigated HMGB1 gene polymorphism. Indeed, a higher frequency of heterozygous A/delA genotype was observed in the group of IgAV patients with IgAVN and in the group with joint involvement as compared to IgAV patients without these clinical manifestations, however, statistical significance was not reached. Our results may indicate that HMGB1 gene polymorphism rs41369348 was not associated with an increased susceptibility to childhood IgAV, its severity or clinical manifestation.

Considering the impact of HMGB1 on joint manifestations in IgAV, the lack of association is not unexpected. Specifically, in the joints of children with IgA vasculitis there is no evidence for synovitis or intra-articular inflammation[33], unlike patients with rheumatoid arthritis in whom HMGB1 is released within the inflamed joint[53].

Nonetheless, the lack of association between IgAV, IgAVN and rs41369348 polymorphism in the HMGB1 gene in the present study was unexpected. The absence of interdependence cannot exclude any possible association in another population. It may also depend on the functionality of the SNP, or an alternative mechanism for HMGB1 activation in IgAV. The pathogenic role of HMGB1 in IgAV may be regulated by other nucleotide polymorphisms, but this needs further study.

Up to now, this is the first study to investigate the association between genetic variation in the HMGB1 and IgAV, as well as its clinical manifestations. However, there were some limitations to this study that should be considered. Firstly, more SNPs of the HMGB1 gene should be studied in the future and interaction analysis should be conducted for the gene-environment interaction with some factors. Although the number of study participants met the requirement for analysis, the sample size of our study was relatively small, so we suggest that larger sample studies be conducted in the future to verify this association.

In conclusion, this study did not prove the influence of HMGB1 gene (rs41369348) polymorphism on the susceptibility and clinical features of patients with IgAV. Given the marked multifactorial nature of this disease, as a result of the interaction of environmental and genetic factors, and the insufficient statistically significant association of this polymorphism for HMGB1 gene with IgAV susceptibility, we do not recommend special follow up in children with heterozygous A/delA genotype or with homozygous mutant delA genotype of this polymorphism. Until finding reliable predictors of IgAVN in the future, all children with IgAV should be carefully followed up for at least 6 months, and optimally up to one year, as this is the time period within which nephritis most often develops.

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Sažetak

POVEZANOST POLIMORFIZMA GENA ZA PROTEIN VISOKE POKRETLJIVOSTI IZ SKUPINE 1 (rs41369348) I IMUNOGLOBULIN A VASKULITISA KOD DJECE

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Immunoglobulin A vaskulitis (IgAV) ili Henoch-Schönleinova purpura najčešći je sistemski vaskulitis malih krvnih žila u dječjoj dobi. Protein visoke pokretljivosti iz skupine 1 (*high mobility group box-1 protein*, HMGB1) pleiotropni je citokin koji djeluje kao proupalni signal, važan za aktiviranje antigen prezentirajućih stanica i širenje upale. HMGB1 ima ulogu u patofiziologiji različitih upalnih bolesti. Cilj ovog rada bio je istražiti povezanost polimorfizma gena (SNP)-rs41369348 za HMGB1 s predispozicijom za IgAV i kliničkom slikom bolesnika koji ispunjavaju kriterije za IgAV. DNA je ektstrahirana iz krvnih stanica 76 djece s IgAV-om i 150 zdrave kontrolne djece koja se po dobi nisu razlikovala. Klinički podaci i laboratorijski parametri prikupljeni su za sve bolesnike s IgAV-om. Iako postoji veća učestalost heterozigotnog genotipa A/delA ovog genskog polimorfizma u skupini s IgAV-om u odnosu na kontrolnu skupinu, nije uočena genotipska razlika između navedenih skupina. Nije nađena statistički značajna genotipska razlika između bolesnika s različitom kliničkom slikom IgAV-a. Zaključno, u ovom istraživanju nije nađena povezanost polimorfizma rs41369348 za HMGB1 s predispozicijom za nastanak IgAV-a u djece, kao niti s težinom bolesti ili njezinim različitim kliničkim manifestacijama.

Ključne riječi: *Henoch-Schönleinova purpura; Protein HMGB1; Polimorfizam nukleotida*