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The relationship between *TLR4* rs4986790 and rs4986791 gene polymorphisms and *Helicobacter pylori* infection in children with gastritis

Lorena Elena Meliţ[a,1], Cristina Oana Mărginean[a,*], Claudia Bănescu[b,1], Alina Bogliş[b], Simona Mocan[c], Mihaela Iancu[d]

[a] Department of Pediatrics, University of Medicine, Pharmacy, Sciences and Technology Târgu Mureş, Gheorghe Marinescu street no 38, Târgu Mureş, 540136, Romania
[b] Genetics Laboratory, Center for Advanced Medical and Pharmaceutical Research, University of Medicine, Pharmacy, Sciences and Technology Târgu Mureş, Gheorghe Marinescu street no 38, Târgu Mureş, 540136, Romania
[c] Department of Pathology, County Hospital Târgu Mureş, Gheorghe Marinescu street no 50, Târgu Mureş, 540136, Romania
[d] Department of Medical Informatics and Biostatistics, University of Medicine and Pharmacy Cluj Napoca, Victor Babes street no 8, Cluj Napoca, Romania

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ABSTRACT

Background: *TLR4* is involved in *H. pylori* lipopolysaccharide recognition and its SNPs might be related to increased risk of developing premalignant conditions and gastric cancer. The objectives of the study were to evaluate the associations between both *TLR4* rs4986790 and rs4986791 gene polymorphisms and *H. pylori* infection in children with gastritis.
Methods: We performed a cross-sectional study on 150 children admitted in a Tertiary Centre from Romania, between March 2016 and July 2018 in order to evaluate them regarding demographic, endoscopic, histopathological and *TLR4* gene polymorphisms.
Results: Our final sample consisted of 50 children with *H.pylori* associated gastritis (group 1-Ghp group) and 97 children with gastritis without *H.pylori* infection (group 2). Poor socioeconomic status was a significant risk factor for *H.pylori* infection. We found no significant differences regarding the clinical symptoms and laboratory parameters between the two groups. Concordant results were found between the histopathological exam and rapid urease test. Variant genotypes of *TLR4*rs4986790 *and TLR4*rs4986791 gene polymorphisms acted as protective factors against *H. pylori* infection, without statistical significance.
Conclusions: The variant genotype of the *TLR4* gene polymorphisms might be protective factors for *H.pylori* infection, while socioeconomic status is an risk factor for *H. pylori* infection. Urease test is a usefull diagnostic tool for *H. pylori* infection.

1. Introduction

Helicobacter pylori (*H. pylori*) is a microaerophilic, Gram-negative bacterium that infects the human gastric mucous gel layer during childhood leading to long-life inflammation at this level [1]. Up to one half of the people worldwide are infected with this bacteria resulting in a wide spectrum of pathologies, such as: chronic gastritis, peptic ulcer, or different gastric cancers [2]. Thus, the World Health Organization stated that *H. pylori* might definitely be considered as a class I carcinogen being reported as a major leading cause of gastric cancer-associated deaths worldwide [3]. The diagnosis of *H. pylori*-related gastritis must be based on clinical, endoscopic, histologic and immunological examinations [4], ruling out other conditions that may determine a similar clinical picture [5,6]. Molecular genetic testing for the detection of high risk individuals might be an option in individuals with a family history of gastric cancer [7].

Innate and adaptive immunity as part of the immune system, own a major role in controlling infections [8]. Gastric epithelial cells represent

Abbreviations: *GHp*, *H. pylori* gastritis group; *H. pylori*, *Helicobacter pylori*; *CI*, *confidence interval*; *OR*, *odds ratio, p-values obtained from univariate and multivariate logistic regression,* [(a] *adjusted odds ratio for age group (with age category of 1-6 years as the reference group) and sex (with females as the reference group)*; *SNPs*, *single nucleotide polymorphisms*; *TLR*, toll-like receptor; *TLR 4*, *toll-like receptor 4, TLR4* rs4986790 SNP; *AA*, *homozygous for A allele*; *GA*, *heterozygous*; *GG*, *homozygous for G allele*, *TLR4* rs4986791SNP; *CC*, *homozygous for C allele*; *CT*, *heterozygous*; *TT*, *homozygous for T allele*

* Corresponding author at: Department of Pediatrics, University of Medicine, Pharmacy, Sciences and Technology Târgu Mureş, 38 Gh. Marinescu Street, 540139, Târgu Mureş, Romania.
E-mail address: marginean.oana@gmail.com (C.O. Mărginean).
[1] These authors contributed equally to this paper.

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the first line of innate immunity against the acquirement of *H. pylori* infection [9]. Innate immunity owns an important role in the stimulation of adaptive immunity resulting in an essential partnership between the two components of the human immune system in the successful eradication of different microorganisms. The pathogen recognition receptors are well-documented to exert a major role in the previously mentioned process [10]. Mammalian toll-like receptors (TLRs) belong to the family of the pathogen recognition receptors, and their activation result in the induction of chemokines, inflammatory cytokines, costimulatory and antigen-presenting molecules [11]. TLRs express the structure of type I transmembrane proteins involving the following components: a leucine-rich repeat-containing ectodomain, a transmembrane region, and an intracellular portion resulting in the activation of downstream signaling pathways [10]. Among all TLRs identified in the humans, TLR1, TLR2, TLR4, TLR5 and TLR6 were proved to recognize different membrane constituents, such as lipoproteins, lipids, proteins; while TLR3, TLR7, TLR8 and TLR9 were shown to recognize microbial nucleic acids [10].

TLR4 was the first of all *TLRs* discovered in the human being, and it was proved to exert a role in recognition of bacterial lipopolysaccharide, a major component of the Gram-negative bacteria outer membrane [12]. Moreover, this TLR, along with TLR2, is also involved in recognition of *H. pylori* heat shock protein 60 [13]. The proper functioning of TLR4 required its internalization into the cytoplasmic compartment. Individuals that carry hyporeactive polymorphisms of TLR4 resulting it its hypofunction were proven to express a reduced inflammatory response associated with a persistent infection [14]. *TLR4* SNPs was related to an increased risk of developing premalignant conditions and gastric cancer [15], even though the reported data remain contradictory [16]. Most of the studies performed with the aim of identifying the role of *TLR4* SNPs in the development of *H. pylori* induced gastropathies, and their association with gastric cancer involved adult populations. Thus, we consider of great importance for future research to focus on studying these polymorphisms on children in order to identify high-risk groups for the development of gastric cancer during adulthood.

The objectives of this study was to identify the associations between studied *TLR4* gene polymorphisms (rs4986790 and rs4986791) and *H. pylori* infection in children with gastritis.

2. Material and methods

2.1. Study sample

We performed a cross-sectional study on 150 children admitted in the Pediatrics Clinic 1 Târgu Mureş, Romania, prospectively included, between March 2016 and July 2018. The inclusion criteria in our study comprised gastrointestinal symptoms (nausea, heartburn, abdominal pain, vomiting, etc.) suggestive for acute gastritis, the age between 1 and 18 years, the lack of any signs suggesting an infectious disease, chronic disorders. The exclusion criteria consisted in the age below 2 years due to the characteristics of the video endoscope, clinical signs and symptoms suggesting an infectious disease (fever, diarrhea), incomplete clinical or laboratory data, the parents'/caregivers' refusal to performed the upper digestive endoscopy, parents'/caregivers' refusal to sign the informed consent for their children to participate in the study. Based on the exclusion criteria, 3 of the 150 children were excluded from the study because 2 of them had incomplete laboratory data, and in 1 case, the parents refused to sign the informed consent.

Initially, all children were clinically assessed and the parents/caregivers provided a thorough anamnesis. The laboratory data comprised the following parameters: complete cellular blood count, C-reactive protein, transaminases, serum iron. After the laboratory assessment, the children underwent upper digestive endoscopy with three biopsies from the gastric antrum. The first biopsy was used for the rapid urease test in order to an early detection of *H. pylori* infection. The second one was sent to the Pathology department for histological exam in order to confirm the presence or absence of *H. pylori*, and the third one was collected in an Eppendorf tube filled with a stabilization solution in order to be properly transported to the Genetics Laboratory and to eliminate the need for immediate DNA isolation and purification. All upper digestive endoscopies were performed by a single trained experienced gastroenterologist. The histopathological exam used Giemsa staining for the detection of *H. pylori* and all these exams were also performed by a single person. The histopathological exam established not only the presence or absence of *H. pylori* within the gastric mucosa, but also the acute or chronic pattern of the inflammation based on the histopathological changes at this level, such as the type of inflammatory cells, the presence of architectural changes or the presence of metaplasia.

2.2. Ethics

All the parents/caregivers signed the informed consent for their children inclusion in the study, which was performed according to the Helsinki Declaration. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy Târgu Mureş, No 27/ March 17th 2016.

Informed consent was obtained from all individual participants included in the study (the mothers signed informed consent for them and their newborns)

2.3. Genotyping analysis

The PureLink Genomic DNA Mini Kit (from ThermoFischer Scientific, USA) was used for extraction of genomic DNA (gDNA) from fresh gastric tissue samples obtained by upper digestive endoscopies. gDNA obtained according to the manufacturer's protocol was quantified by using an Eppendorf BioSpectrometer (from Eppendorf Austria GmbH). For genotyping of *TLR4* SNPs we used TaqMan technology on a 7500Fast Dx Real-*Time PCR* System and predesigned assays namely, C_11722238_20 for rs4986790and C_11722237_20 for rs4986791.

2.4. Statistical analysis

The statistical analysis was performed with R software environment for statistical computing (version R 3.5.2).

The descriptive measures as mean and sample standard deviation or median and interquartile range (IQR) were used to summarize quantitative data. The choice of a set of indicators was based on testing the existence of a Gaussian distribution for the outcome of interest. The assessment of the differences regarding quantitative medical characteristics on the two groups was achieved using the Student-t test on independent groups with equal variances or the nonparametric Mann-Whitney test.

The qualitative data were described for the two groups using the absolute and relative frequency (defined as the ratio between the absolute frequency and the total number of the studied group). The assessment of the bivariate associations between the qualitative characteristics and *H. pylori* infection were performed using Chi-square tests (asymptotic Chi-square or Exact Fisher's tests). In case of associations between the nominal characteristics and the presence of *H. pylori* infection, the bivariate analysis was followed by a post-hoc analysis (Pairwise comparisons using Fisher test with p-values adjusted using method of Benjamini and Hochberg to control the false discovery rate) in order to identify the objective of identifying the source of associations.

Regarding paired qualitative data, we used McNemar test and Cohen's Kappa as measures of concordance results.

In order to quantify the relationship between the studied *TLR4* SNPs and *H. pylori* infection in children with gastritis, we used the multiple logistic regression and adjusted odds ratio with a 95% confidence

Table 1
Association between socio-demographic characteristics, anamnestic findings, endoscopic aspect, and *H. pylori*-associated gastritis.

Variables	Gastritis group (n_1 = 97)	GHp group (n_2 = 50)	p-value*
Age(years), mean ± SD	11.94 ± 4.55	12.84 ± 3.76	0.231
Age group (years), F_a(%)			
1-6	16 (16.5)	3 (6.0)	0.153
7-14	43 (44.3)	28 (56.0)	
15-18	38 (39.2)	19 (38.0)	
Gender, male:female, F_a(%)	35(36.1):62 (63.9)	19 (38.0):31 (62.0)	0.819
Residence F_a(%)			
urban	42 (43.3)	16 (32.0)	0.184
rural	55 (56.7)	34 (68.0)	
Socioeconomic status, F_a(%)			
appropriate	86 (88.7)	33 (66.0)	**0.002**$^{\#}$
low	11 (11.3)	17 (34.0)	
Epigastric pain			
no	71 (73.2)	30(60.0)	0.102
yes	26 (26.8)	20 (40.0)	
Abdominal pain			
no	36 (37.1)	25(50.0)	0.133
yes	61 (62.9)	25 (50.0)	
Heartburn			
no	91 (93.8)	45(90.0)	0.510
yes	6 (6.2)	5 (10.0)	
Nausea/loss of appetite/ vomiting			
no	72.2 (70)	33(66.0)	0.439
yes	27 (27.8)	17 (34.0)	
Gastroesophageal reflux			
no	80 (82.5)	41 (82)	> 0.05
yes	17 (17.5)	9 (18)	
Biliary reflux			
no	64 (66)	38 (76)	> 0.05
yes	33 (34)	12 (24)	
Cobblestone aspect			
no	85 (87.6)	17 (34)	< **0.001**
yes	12 (12.4)	33 (66)	
Granular, congestive, edematous aspect			
no	52 (53.6)	33 (66)	> 0.05
yes	45 (46.4)	17 (34)	
Normal aspect			
no	57 (58.8)	50 (100)	> 0.05
yes	40 (41.2)	0 (0)	

Legend: F_a = absolute frequencies; * estimated significance level obtained from Chi-square, Fisher's Exact tests or Student-t test for independent samples with equal variances; $^{\#}$statistical significance ($p < 0.05$), SD = sample standard deviation.

interval. The evaluation of Hardy-Weinberg equilibrium and Linkage disequilibrium was done in "genetics" R package.

For all bilateral statistical tests, the chosen significance level was 0.05.

3. Results

3.1. Characteristics of studied groups

A sample containing 147 patients was divided into two groups: group 1 comprising 50 cases with gastritis and *H. pylori* infection (GHp group), and group 2 including 97 patients with gastritis without *H. pylori* infection based on histopathology results. Patients' characteristics for both studied groups were summarized in Table 1. The mean age of GHp patients was 12.84 ± 3.76 years, whereas for gastritis group it was 11.94 ± 4.55 years, without a significant difference regarding the age distribution for the two groups (Student-t test, p = 0.231). The distribution of gender (sex ratio, M/F: 19/31 for GHp group and 35/62 for gastritis group) was similar in both groups (p = 0.819). There was a significant association between *H. pylori* infection and the socio-economic status of children with gastritis (p = 0.002), the risk of *H. pylori* infection was approximately 4 folds higher in children with poor socioeconomic status (OR = 4.03, 95% CI: [1.71; 9.50]). The most frequent symptoms encountered in children with H. pylori infection were: epigastric pain, heartburn, nausea, vomiting or loss of appetite, but without significant association ($p > 0.05$).

3.2. Associations between laboratory and endoscopic parameters and H. pylori-associated infection

Most of the hematological and biochemical parameters presented higher values in case of Ghp patients compared to gastritis group, but without statistical significance (Table 2). Despite the lack of statistical significance, the values of leukocytes, neutrophils and ESR were higher in children with *H. pylori*-positive gastritis. Regarding the CRP, we found higher values in patients with gastritis without *H. pylori* infection, but without statistical significance regarding the CRP distribution on the two groups. Additionally, we noticed a number of 6 (6.2%) patients with values above the normal limit (> 5 mg/L) in gastritis group, while all patients included in GHp group were found to have normal values of CRP.

3.3. Associations between TLR-4 gene polymorphisms and H. pylori infection in children with gastritis

Frequencies of genotypes of *TLR4* rs4986791 SNP (χ^2 (1) = 3.63, simulated p-value based on 10,000 replicates = 0.156) and *TLR4* rs4986790 SNP (χ^2 (1) = 0.11, simulated p-value based on 10,000 replicates = 1.00) were observed to be in Hardy-Weinberg equilibrium on the studied sample. The *TLR4* rs4986790 and rs4986791 were in Linkage disequilibrium, the two SNPs being highly correlated (D = 0.02, D' = 0.99, r = 0.85, $\chi 2 = 224.67$, df = 1, $p < 0.0001$).

Even though we noticed that the variant genotype of both polymorphisms was a protective factor for the risk of *H. pylori* infection on our sample (*TLR4* rs4986790 SNP: adjusted OR = 0.81 and for *TLR4* rs4986791 SNP: adjusted OR = 0.54), the association between the

Table 2
Laboratory parameters values in patients with *H. pylori*-associated gastritis and gastritis group.

Variables	Gastritis group (n_1 = 97)	GHp group (n_2 = 50)	p-value*
Hemoglobin, g/dL, median[IQR]	13.3 [12.8; 14.0]	13.3 [12.7; 14.5]	0.768
Leucocytes, 10^3/μL, median[IQR]	7.0 [6.1; 8.6]	7.6 [6.3; 9.3]	0.506
Lymphocytes, 10^3/μL, median[IQR]	2.5 [2.0; 3.2]	2.5 [2.1; 2.9]	0.644
Neutrophils, 10^3/μL, median[IQR]	3.6 [2.6; 5.0]	3.9 [2.9; 5.6]	0.374
Eosinophils, 10^3/μL, median[IQR]	0.14 [0.08; 0.30]	0.12 [0.08; 0.30]	0.342
CRP mg/L, median[IQR]†	0.46 [0.20; 2.42]	0.21 [0.20; 0.41]	0.079
ESR, mm/h, median[IQR]	7.0 [5.0; 12.00]	9.0 [5.0; 11.0]	0.567

Legend: AST = aspartate transaminase, ALT = alanine transaminase, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, Fe = iron, GGT = gamma-glutamyl transpeptidase, IQR = interquartile range [25th percentile, 75th percentile]; † = 95 patients benefited by quantitative CRP determination; *estimated significance value obtained from non-parametric Mann-Whitney test; $^{\#}$statistical significance ($p < 0.05$).

Table 3
Associations between TLR4 gene polymorphisms and *H. pylori* infection in children with gastritis.

TLR4 SNPs	Genotype	Gastritis group (n_1 = 97)	GHp group (n_2 = 50)	p-value	OR [95% CI]	p-value	OR[a] [95% CI]
rs4986790	AA	92 (94.8)	48 (96.0)	0.756	Reference	0.805	Reference
	AG	5 (5.2)	2 (4.0)		0.77 [0.14; 4.10]		0.81 [0.15; 4.43]
rs4986791	CC	90 (92.8)	48 (96.0)	0.447	Reference	0.451	Reference
	CT + TT	7 (7.2)	2 (4.0)		0.54 [0.11; 2.68]		0.54 [0.11; 2.73]

Legend: CI – confidence interval, OR – odds ratio, p-values obtained from univariate and multivariate logistic regression; [a] adjusted odds ratio for age group (with age category of 1–6 years as the reference group) and sex (with females as the reference group); SNPs = single nucleotide polymorphisms, TLR 4 = toll-like receptor 4, TLR4 rs4986790 SNP: AA – homozygous for *A allele*; GA – heterozygous; GG - homozygous for *G allele*; TLR4 rs4986791SNP: CC – homozygous for *C allele*; CT – heterozygous; TT - homozygous for *T allele*.

variant genotype of the investigated polymorphisms and *H. pylori* infection in children with gastritis was not statistically significant for our pediatric population (Table 3).

Regarding *TLR4* rs4986791 gene polymorphism, we identified the homozygote CC genotype in 90 patients of those without *H. pylori* infection (92.8%), while the variant one was detected in 7 patients from the same group (1 patient with TT genotype and 6 with CT genotype). In case of GHp group, the CC normal genotype of the same polymorphism was found in 48 children (96%), and the remaining 2 children (4%) carried the CT variant genotype (p = 0.812). In terms of *TLR4* rs4986790, we noticed the AA normal homozygote genotype in 92 children (94.8%) of *H. pylori*-negative gastritis group, and the variant AG one in 5 patients (5.2%) of the same group. On the other hand, 48 children (96%) from GHp group carried the AA genotype of the same polymorphism, whereas the other 2 (4%) of this group were detected with AG heterozygote polymorphism (p = 0.756).

4. Discussions

H. pylori infection is one of the most frequent infections worldwide, up to 50% of the world population being infected with this bacteria [2]. Since this infection is usually acquired during childhood, it can result in chronic inflammation of the gastric mucosa and subsequent increased risk for gastric cancer [1]. Low socioeconomic status was found as one of the main predisposing factors associated with an increased risk for *H. pylori* infection [17]. Moreover, practices within the household, such as bed-sharing between children and adults lead to increased transmission of this bacterium from infected to uninfected subjects [18]. Therefore, a proper anamnesis and adequate pediatrician's communication skills are essential in the management of this pathology [19]. Similarly, our study also proved a significant association between *H. pylori* infection and low socioeconomic status (p = 0.002), the low socioeconomic status being a risk factor for *H. pylori* infection. Nevertheless, we found no associations between *H. pylori* infection and patients living in rural or urban areas meaning that the rural area is not mandatory to be associated with a low socioeconomic status in our country. The symptoms of *H. pylori* infection in children were reported to be either unspecific or absent [20]. Nevertheless, recurrent abdominal pain was associated with *H. pylori* infection [21]. The most common symptoms encountered in our study were: epigastric pain, abdominal pain, heartburn, nausea, vomiting or loss of appetite. Despite the increased frequency of these symptoms in children with *H. pylori* gastritis, we found no association between them and the presence of this bacterium. Moreover, abdominal pain was the only one that did not express a higher frequency in the case of *H. pylori*-related gastritis patients. Different laboratory parameters, such as leukocytes, neutrophils, CRP and ESR were found to be increased in patients with *H. pylori* associated gastritis [22–24]. Our findings support the previously mentioned studies in terms of leukocytes, neutrophils and ESR, which were higher among children with *H. pylori* infection, except for CRP values that were encountered to be increased in patients with gastritis but without *H. pylori* infection. This fact might suggest that CRP is rather related to the inflammatory process within the gastric mucosa, and not the presence of *H. pylori* infection.

Most of the studies performed until now that aimed to assess the association between *H. pylori* infection and different *TLRs* gene polymorphisms compared patients with *H. pylori* gastritis with healthy controls. In our study, we intended to assess the role of *H. pylori* infection in children with gastritis. Multiple TLRs were found to be associated with *H. pylori* infection and the development of gastric cancer [25]. The relationship between *H. pylori* infection and chronic inflammation is obvious. Moreover, chronic inflammation and gastric carcinogenesis are strongly related, and it represents the fourth most common type of neoplasia and the second cancer-related leading cause of death worldwide [26,27]. *H. pylori*, an imminent carcinogen, binds the gastric epithelial cells and activates the host innate immune system through TLRs resulting in increased secretion of different pro- and anti-inflammatory cytokines that will lead consequently to hypochlorhydria, gastric atrophy and increased risk for gastric carcinogenesis [28]. Different SNPs of receptor genes of the immune system were proved to influence the host's response to *H. pylori* and to act as a potential contributor to variations in the risk of gastric carcinogenesis [29]. The most studied *TLR4* gene polymorphisms are *rs4986790* or Asp299Gly and *rs4986791* or Thr399Ile, and they are associated with an increased risk of premalignant gastric lesions and even gastric cancer [30]. These SNPs are located in the fourth exon and they impair the extracellular domain of *TLR4* causing adenine-guanine (A–G) and cytosine-thymine (C–T) transitions, respectively. These transitions are translated by amino acid substitutions and subsequent alteration in the appropriate functioning of ligand-binding receptor site [31,32]. A study performed on adult population involving 195 patients with *H. pylori* infection and 241 patients with non-ulcer dyspepsia without *H. pylori* infection, found no association between *TLR4* rs4986790 variant genotype and *H. pylori* infection patients suggesting that Asp299Gly *TLR4* polymorphism in not related to *H. pylori* infection susceptibility [33]. In agreement to these findings, our study also proved no association between the variant genotype of *TLR4* rs4986790 and *H. pylori* infection in children with gastritis. The same *TLR4* SNP was proved to alter cytokine expression in *H. pylori*-infected adult patients with gastritis [34]. On the other hand, Achyut et al. proved on adult patients that *TLR4* rs4986791 variant allele carriers presented a higher risk of gastric atrophy and intestinal metaplasia [35]. Contrariwise, our study failed in proving any significant association between the variant genotype of this polymorphism and *H. pylori* infection. Moreover, the same study of Achyut el al. also failed in finding an association between *TLR4* rs4986790 polymorphism and precancerous gastric lesions [35] sustaining the previous findings of Kato et al. [36]. In exchange, in our sample, we noticed that the variant genotypes of both *TLR4* rs4986790 and rs4986791 polymorphisms seems to be a protective factor for *H. pylori* infection, though without statistical significance in our study population. These controversies might be explained mainly by the fact that all previously mentioned studies were performed on adult subjects while our study involved pediatric patients. Regarding the involvement of these two *TLR4* SNPs in the development of *H. pylori*-related gastropathies, the results of the studies reported in the literature remain contradictory. Thus, certain studies proved an association between *TLR4* rs4986790

and rs4986791 polymorphisms and various types of gastric cancers [37], while others failed in proving this association [37]. Moreover, the study of Santini et al. performed on adult subjects also failed in identifying a correlation between these *TLR4* SNPs and the presence of *H. pylori* [37]. The controversial results mentioned above, and the fact that in our study, performed on children, these two gene polymorphisms seem to have a protective role against *H. pylori* infection, might be explained by the possibility that in time, this infection, if left untreated, might alter the function of these receptors resulting in their hypofunction or hyporeactivity. Thus, further longitudinal studies would be of great importance in order to assess this possibility.

The *limitations of our study* consist in the relatively small number of patients from a single area of Romania, the lack of assessment of *H. pylori* pathogenicity features, the fact that we did not repeated the upper digestive endoscopy and histopathological exam after the eradication of *H. pylori* infection. The main *strength* of our study is the fact that it involved pediatric patients. Most of the previous studies that assessed the association between *TLR4* SNPs and *H. pylori* infection were performed on the adult population. Thus, this is among the few studies, if not the first to the best of our knowledge that involved children and it is certainly of great importance due to the fact that establishing the role of these polymorphisms in small ages might provide important data regarding the prevention of gastric cancer during adulthood. Moreover, this is certainly the first study from Romania that assessed the role of two major *TLR4* gene polymorphisms in the development of *H. pylori* infection in children. Among other strengths, we should also recall the fact that all upper digestive endoscopies were performed by a single experienced person, as well as all the interpretations of the histopathological exams.

5. Conclusions

H. pylori infection is one of the most frequent infections worldwide, being usually acquired during childhood. Our study proved that poor socioeconomic status is a significant risk factor for *H. pylori* infection. Although our findings were not significantly statistic, the variant genotype of the *TLR4* rs4986790 and *TLR4* rs4986791 gene polymorphisms might be protective factors against *H. pylori* infection on our sample of children with gastritis. Nevertheless, further studies are needed, on larger samples to assess longitudinally the role of the studied SNPs in the development and persistence of *H. pylori* infection taking into account also other factors such as environmental ones, bacterial pathogenicity characteristics, bacterial resistance or even the pattern of gastric microbiota.

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Contributors

Lorena Elena Meliț, Cristina Oana Mărginean, Claudia Bănescu and Mihaela Iancu conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript.

Dr. Lorena Elena Meliț, Dr Cristina Oana Mărginean designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.

Dr Simona Mocan performed the histopathological exams.

Dr. Alina Bogliș performed molecular genetic analyses, drafted and reviewed original manuscript

and Prof Claudia Bănescu performed molecular genetic analyses.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Data availability

The data used to support the findings of the current study are available from the corresponding authors upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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