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ORIGINAL ARTICLE

Retrospective Study

Impact of IL28B and *OAS* gene family polymorphisms on interferon treatment response in Caucasian children chronically infected with hepatitis B virus

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Abstract

AIM

To investigate the impact of IL28B and *OAS* gene polymorphisms on interferon treatment responses in children with chronic hepatitis B.

METHODS

We enrolled 52 children (between the ages of 4 and 18) with hepatitis B e antigen-negative chronic hepatitis B (CHB), who were treated with pegylated interferon alfa for 48 wk. Single nucleotide polymorphisms in the OAS1 (rs1131476), OAS2 (rs1293747),

OAS3 (rs2072136), OASL (rs10849829) and IL28B (rs12979860, rs12980275 and rs8099917) genes were studied to examine their associations with responses to IFN treatment in paediatric patients. We adopted two criteria for the therapeutic response, achieving an hepatitis B virus (HBV) DNA level < 2000 IU/mL and normalization of ALT activity (< 40 IU/L). To perform the analyses, we compared the patients in terms of achieving a partial response (PR) and a complete response (CR) upon measurement at the 24-wk post-treatment follow-up.

RESULTS

The PR and CR rates were 80.8% and 42.3%, respectively. Factors such as age, gender and liver histology had no impact on the type of response (partial or complete). A statistically significant relationship between higher baseline HBV DNA and ALT activity levels and lower rates of PR and CR was shown (P < 0.05). The allele association analysis revealed that only the IL-28B rs12979860 (C *vs* T) and IL28B rs12980275 (A *vs* G) markers significantly affected the achievement of PR (P = 0.021, OR = 3.3, 95%CI: 1.2-9.2 and P = 0.014, OR = 3.7, 95%CI: 1.3-10.1, respectively). However, in the genotype analysis, only IL-28B rs12980275 was significantly associated with PR (AA *vs* AG-GG, P = 0.014, OR = 10.9, 95%CI: 1.3-93.9). The association analysis for CR showed that the TT genotype of IL28B rs12979860 was present only in the no-CR group (P = 0.033) and the AA genotype of OASL rs10849829 was significantly more frequent in the no-CR group (P = 0.044, OR = 0.26, 95%CI: 0.07-0.88). The haplotype analysis revealed significant associations between PR and CR and OAS haplotype (P = 0.0002 and P = 0.001, respectively), but no association with IL28B haplotype was observed.

CONCLUSION

IL28B and OAS polymorphisms are associated with different clinical outcomes in CHB children treated with interferon.

Key words: Chronic hepatitis B; IL28B; OAS; Single-nucleotide polymorphisms; IFN therapy; Children

Core tip: The limited efficacy and side effects associated with IFN treatment limit its clinical use in paediatric patients with chronic hepatitis B (CHB). Therefore, pretreatment predictors are required to identify those patients at highest risk for treatment response failure. OAS and IL28B are well-known IFN-induced antiviral pathway players; however, the impact of host-related genetic variability in the IL28B and *OAS* genes on response rates to IFN therapy in CHB paediatric patients has not been studied. The results of our study show an association between IL28B rs12979860, OASL rs10849829 and OAS haplotypes and final IFN-treatment response in Caucasian CHB children.

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INTRODUCTION

It is estimated that at least 2 billion people worldwide have serologic evidence of contact with HBV, including approximately 350 million people who develop chronic hepatitis B (CHB)[1,2]. The largest proportion of chronic cases occurs in children infected in the first years of life; this figure reaches 90% in the case of infection in the perinatal period[1,3,4]. Despite the significant decrease in the number of new cases of CHB in children because of the introduction of compulsory vaccination in many countries, we are still struggling with the treatment of adolescent patients, especially in developing countries[5-7].

A 48-wk course of interferon therapy is recommended as a first-line treatment option for select HBeAg-negative patients, especially young patients with increased aminotransferase activity, which indicates the activation of the immune system to eliminate infected hepatocytes, and providing a therapy with antiviral and immunomodulatory activity in the form of interferon may enhance this effect[8,9]. However, PEG-IFN-based therapy is modestly effective in suppressing viral replication in comparison to nucleos(t)ide analogues, which are highly effective in suppressing HBV replication. In contrast to nucleos(t)ide analogues, PEG-IFN-based therapy has a higher HBsAg seroconversion rate[10-12]. Therefore, pretreating patients to identify those with the highest probability of success is of great clinical importance to IFN therapy.

The antiviral activity of interferons is associated with their ability to induce virus targeting proteins such as 2′-5′-oligoadenylate synthetase (2′,5′-OAS)[13]. Analytic studies in human hepatocytes have confirmed that both interferon alfa and interferon lambda, which includes interleukin 28B, have activating effects[14]. The OAS proteins are well-known IFN-induced antiviral pathway players involved in the cleavage of viral RNA molecules, resulting in the inhibition of viral replication. The human OAS family contains the OAS1, OAS2, OAS3, and OASL genes, which are located on chromosome 12 (in the 12q24.1 region)[15,16]. Single-nucleotide polymorphisms (SNPs) in the OAS family genes have been identified as a factor associated with

susceptibility to viral infection and antiviral effects during IFN-based therapy in patients infected with HCV[17-20].

The influence of host-related genetic variability on differences in response rates to IFN therapy in CHB patients is not well understood. Unlike many studies confirming that SNPs in the interleukin 28B (*IL28B*) gene play a primary role in IFN-based treatment outcomes in patients with chronic hepatitis C, the association between IL28B SNPs and the result of IFN monotherapy in CHB has been a subject of very few studies[21-23]. Additionally, there is limited information about the role of SNPs in 2',5'-oligoadenylate synthetase (OAS) family genes in the IFN response in hepatitis B patients[24,25]. Currently, there are no available results concerning the impact of IL28B or OAS SNPs on the results of IFN therapy in a group of paediatric patients with CHB.

The aim of this study was to determine the relationship between IL28B and OAS gene family SNPs and the biochemical and virological response rates to PEG-IFN alfa-2a monotherapy in a cohort of Caucasian children chronically infected with HBV.

MATERIALS AND METHODS

Patients

We retrospectively enrolled a cohort of CHB children of Caucasian ethnicity who were treated with PEG-IFN alfa-2a (Pegasys) at a dose of 180 μg per week for 48 wk at the Department of Paediatric Infectious Diseases and Hepatology.

The inclusion criteria were as follows: HBsAg positive for more than 6 mo, confirmed detection of HBV DNA > 2000 IU/mL or abnormal liver biochemistry (alanine aminotransferase (ALT) > 40 IU/L) for a period of one year prior to the start of therapy. All the included patients were treatment naïve, HBeAg-negative, and had completed a 48-wk course of PEG-IFN α-2a monotherapy with a minimum of 24 wk post-therapy follow-up.

The exclusion criteria included hepatitis C virus (HCV) coinfection, human immunodeficiency virus (HIV) coinfection, a coexisting autoimmune disease, cirrhosis, hepatocellular carcinoma (HCC) or chronic liver disease other than CHB. There were no pregnant women in the study group. Blood samples from these patients were used for IL28B and OAS genotyping. Detailed demographic characteristics and other standard clinical data, including the ALT level, HBsAg, HBeAg, and anti-HBe status, HBV DNA level, and liver histology, were obtained from the patients' clinical documentation. Pre-treatment liver histological analysis was carried out by only one pathologist using the modified Scheuer scoring system (F0–F4; A0–A4).

The study was reviewed and approved by the NCU Bioethics Committee at Collegium Medicum NCU. All procedures conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The patients' legal guardians and all patients older than 16 signed a written informed consent.

IL28B and OAS genotyping

Host genomic DNA was prepared using the QIAamp DNA Mini Kit (Qiagen) from peripheral whole-blood samples collected in 0.5 M EDTA tubes. The detection of IL28B (rs12979860 CT, rs12980275 AG and rs8099917 TG), OASL (rs10849829), OAS1 (rs1131476), OAS2 (rs1293747), and OAS3 (rs2072136) SNPs was carried out with a real-time polymerase chain reaction (real-time PCR) using the TaqMan SNP Genotyping Assays (Applied Biosystems by Life Technologies). For all SNPs, genotyping was performed on a LightCycler® 480 Instrument (Roche Diagnostics) with the following standard reaction conditions: 95 ℃ for 10 min, 92 ℃ for 15 s, followed by 35 cycles of 60 ℃ for 1 min. A population analysis for each genetic marker was performed for all patients.

Treatment responses

The main focus of this study was to determine the predictive value of IL28B and OAS SNPs in treatment outcomes. The impact of other prognostic factors on the results of therapy was also analysed. To evaluate the therapeutic effects, we assessed virological, serological and biochemical responses by analysing HBV DNA levels, HBsAg status and ALT activity before, during and 24 wk after the end of therapy. A limit of 2000 IU HBV DNA/mL, which has been used in the literature, was adopted in the virological response analysis[26]. In our study, we determined the impact of genetic markers on the efficacy of therapy by comparing patients in terms of achieving a defined therapeutic response. We adopted two criteria for the therapeutic response, achieving an HBV DNA level < 2000 IU/mL and normalization of ALT activity (< 40 IU/L). To perform the analyses, we compared the patients in terms of achieving a partial response (PR) and a complete response (CR). CR was defined as the suppression of viral replication to an HBV DNA level < 2000 IU/mL and normalization of ALT activity (< 40 IU/L) 24 wk after completing the treatment. To be classified as PR, patients were required to achieve a positive result for at least one of the two parameters. Based on the PR criterion, patients were divided into those who achieved at least one of the two considered parameters and those who did not achieve any positive response 24 wk after completing the treatment. HBV DNA level was assessed with a quantitative polymerase chain reaction assay (cobas AmpliPrep/cobas TaqMan HBV Test; Roche Diagnostics).

Statistical analysis

The summary statistics for the continuous variables are presented as the median and range. The categorical variables are presented as frequencies.

Table 1 Characteristics of hepatitis B e antigen-negative chronic hepatitis B children

Characteristic	
n	52
Age (yr)	
Median (range)	16 (4-18)
Gender	
Female/male	16 (30.8)/36 (69.2)
Staging (F)	
F0/F1/F2/F3	2 (3.9)/31 (59.6)/16 (30.7)/3 (5.8)
Grading (A)	
A0/A1/A2	2 (3.9)/ 22 (42.3)/28 (53.8)
IL28B SNPs	
rs12979860 CC/CT/TT	20 (38.5)/26 (50.0)/6 (11.5)
rs12980275 AA/AG/GG	24 (46.2)/24 (46.2)/4 (7.6)
rs8099917 TT/TG/GG	30 (57.7)/19 (36.5)/3 (5.8)
OAS SNPs	
rs10849829 AA/AG/GG	19 (36.5)/27 (52.0)/6 (11.5)
rs1131476 AA/AG/GG	26 (50.0)/17 (32.7)/9 (17.3)
rs1293747 GG/GA/AA	32 (61.5)/17 (32.7)/3 (5.8)
rs2072136 GG/GA/AA	30 (57.7)/20 (38.5)/2 (3.8)
Baseline HBV DNA (IU/mL)	
median (range), log	4.6 (3.4-8.0)
< 20000	31 (59.6)
Baseline ALT (U/L)	
median (range)	42 (12-210)
< 40	14 (26.9)
At week 24 of treatment	
HBV DNA < 100 (IU/mL)	32 (61.5)
ALT < 40 (U/L)	13 (25.0)
At the end of treatment	
HBV DNA < 2000 (IU/mL)	39 (75.0)
ALT < 40 (U/L)	28 (53.9)
24 wk post-treatment	
HBV DNA < 2000 (IU/mL)	27 (51.92)
ALT < 40 (U/L)	37 (71.15)
Partial response	
< 2000 IU/mL HBV DNA or < 40 IU/L ALT	42 (80.8)
Complete response	
< 2000 IU/mL HBV DNA with < 40 IU/L ALT	22 (42.3)

Data are presented as the number of patients (%) unless otherwise indicated.

To identify factors predicting treatment response, we evaluated statistical significance using univariate analysis. Differences between continuous variables, such as HBV viral load and ALT activity, were analysed with the Mann-Whitney *U* test (the distributions were not parametric). The Pearson χ^2 test or Fisher's exact test, where appropriate, was used for categorical variables. IL28B and OAS SNP comparisons were made using dominant and recessive models based on the minor allele frequency in the presented study group. For IL28B and OAS in the dominant model, patients carrying one or two copies of the minor allele were compared with the others, and for IL28B and OAS in the recessive model, patients carrying two copies of the minor allele were compared with the others. Multivariate analysis was performed using logistic regression models that included the variables determined to be significant in univariate analysis. The analysis of linkage disequilibrium (LD) and associations of haplotype with treatment response were performed using SHEsis online software[27]. The results were considered statistically significant when the *P* value was less than 0.05. Odds ratios (OR) and 95%CI were also calculated for the statistically significant results of compared binary clinical variables. The statistical analysis and graphing were completed with the use of IBM SPSS 20 and GraphPad Prism 6 software. The statistical methods of this study were reviewed by a biomedical statistician.

RESULTS

Patient characteristics and treatment responses

This study recruited 52 Caucasian children chronically infected with HBV, including girls (30.8%) and boys (69.2%), with a median age of 16 years. The clinical characteristics of all the patients treated for CHB are presented in Table 1. All the patients in the present study had a baseline HBV DNA level above 2000 IU/mL; 31 of them had values under 20000 IU/mL (59.6%) at baseline. The median baseline HBV DNA of the entire group was 4.6 $\log_{10}$ IU/mL. Fourteen (26.9%) patients had normal ALT activity (< 40 U/L) at baseline. The median ALT activity was 42 U/L. The pretreatment liver biopsy sample data, which was assessed in all patients, showed that most of the patients had stage F1 (59.6%) and F2 (30.7%) fibrosis and grade A1 (42.3%) and A2 (53.8%) inflammation, according to the modified Scheuer score. None of these patients had cirrhosis. The genotype distributions for the IL28B and OAS family genes are presented in Table 1.

The HBV DNA level < 2000 IU/mL and ALT normalization response rates were 75.0% and 53.9%, respectively, at the end of therapy and 51.9% and 71.2%, respectively, 24 wk post-treatment. The partial and complete response rates were 80.8% and 42.3%, respectively. Two (3.8%) of the analysed patients group achieved HBsAg seroclearance (data not shown).

Factors associated with PR

At first, we evaluated the association between clinical baseline characteristics, including the analysed polymorphisms, and PR (Table 2). In general, the univariate analysis showed no relationship between age at start of therapy, gender, liver histology and partial response. In contrast, the baseline HBV DNA level and ALT activity significantly affect the PR. Higher baseline HBV DNA levels and ALT activity were associated with decreased rates of PR.

Analysis of the IL28B and OAS gene family SNPs showed that only the IL28B SNPs had an impact on partial response. However, only the IL28B rs12980275 marker was significantly different in the PR group (AA *vs* AG-GG, *P* = 0.014, OR = 10.9, 95%CI: 1.3-93.9). The other two markers had borderline significance (0.068 and 0.075 for CC *vs* CT-TT of rs12979860 and

Table 2 Impact of clinical and genetic factors on partial response in HBeAg-negative chronic hepatitis B children

Characteristic	PR, *n* = 42	No-PR, *n* = 10	*P* value	OR (95%CI)
Age (yr)				
Median (range)	16 (4-18)	16 (5-18)	0.803	
Gender				
Female	14 (33.3)	2 (20.0)	0.412	
Staging (F)				
F0-F1	26 (61.9)	7 (70.0)	0.729	
Grading (A)				
A0-A1	20 (47.6)	5 (50.0)	0.892	
Baseline HBV DNA (IU/mL)				
Median (range), log	4.1 (3.4-8.0)	5.9 (3.6-8.0)	0.017	
< 20000	28 (66.7)	3 (30.0)	0.069	
Baseline ALT (U/L)				
Median (range)	44 (10-120)	63 (22-118)	0.003	
< 40	13 (31.0)	1 (10.0)	0.207	
IL28B rs12979860				
CC	19 (45.2)	1 (10.0)	0.068	
CT	20 (47.6)	6 (60.0)		
TT	3 (7.2)	3 (30.0)	0.077	
IL28B rs12980275				
AA	23 (54.8)	1 (10.0)	0.014	10.9 (1.3-93.9)
AG	17 (40.6)	7 (70.0)		
GG	2 (4.8)	2 (20.0)	0.163	
IL28B rs8099917				
TT	27 (64.2)	3 (30.0)	0.075	
TG	13 (31.0)	6 (60.0)		
GG	2 (4.8)	1 (10.0)	0.481	
OASL rs10849829				
AA	16 (38.1)	5 (50.0)	0.500	
AG	20 (47.6)	5 (50.0)		
GG	6 (14.3)	0 (0.0)	0.582	
OAS1 rs1131476				
AA	19 (45.2)	7 (70.0)	0.291	
AG	15 (35.7)	2 (20.0)		
GG	8 (19.1)	1 (10.0)	0.670	
OAS2 rs1293747				
GG	25 (59.5)	7 (70.0)	0.481	
GA	15 (35.7)	2 (20.0)		
AA	2 (4.8)	1 (10.0)	0.722	
OAS3 rs2072136				
GG	23 (54.8)	7 (70.0)	1.000	
GA	17 (40.6)	3 (30.0)		
AA	2 (4.8)	0 (0.0)	0.488	

Data are presented as the number of patients (%) unless otherwise indicated.

Table 3 Impact of clinical and genetic factors on partial response in HBeAg-negative chronic hepatitis B children

Characteristic	CR, *n* = 22	No-CR, *n* = 30	*P* value	OR (95%CI)
Age (yr)				
Median (range)	16 (12-18)	17 (4-18)	0.141	
Gender				
Female	8 (36.4)	8 (26.7)	0.454	
Staging (F)				
F0-F1	14 (63.6)	19 (63.3)	0.942	
Grading (A)				
A0-A1	10 (45.5)	15 (50.0)	0.746	
Baseline HBV DNA (IU/mL)				
Median (range), log	4.6 (3.4-8.0)	4.7 (3.5-8.0)	0.671	
< 20000	14 (63.6)	17 (56.7)	0.613	
Baseline ALT (U/L)				
Median (range)	43 (10-110)	48 (17-126)	0.116	
< 40	8 (36.3)	6 (20.0)	0.189	
IL28B rs12979860				
CC	8 (36.4)	12 (40.0)	0.790	
CT	14 (63.6)	12 (40.0)		
TT	0 (0.0)	6 (20.0)	0.033	NA
IL28B rs12980275				
AA	11 (50.0)	13 (43.3)	0.634	
AG	11 (50.0)	13 (43.3)		
GG	0 (0.0)	4 (13.4)	0.128	
IL28B rs8099917				
TT	15 (68.2)	15 (50.0)	0.190	
TG	7 (31.8)	12 (40.0)		
GG	0 (0.0)	3 (10.0)	0.253	
OASL rs10849829				
AA	5 (22.7)	16 (53.3)	0.026	0.26 (0.07-0.88)
AG	14 (63.6)	11 (36.7)		
GG	3 (13.6)	3 (10.0)	0.685	
OAS1 rs1131476				
AA	10 (45.5)	16 (53.3)	0.575	
AG	8 (36.4)	9 (30.0)		
GG	4 (18.1)	5 (16.7)	0.887	
OAS2 rs1293747				
GG	14 (63.6)	18 (60.0)	0.253	
GA	8 (36.4)	9 (30.0)		
AA	0 (0.0)	3 (10.0)	0.790	
OAS3 rs2072136				
GG	15 (68.2)	15 (50.0)	1.000	
GA	6 (27.3)	14 (46.7)		
AA	1 (4.5)	1 (3.3)	0.190	

Data are presented as the number of patients (%) unless otherwise indicated. NA: Not applicable.

TT *vs* TG-GG of rs8099917, respectively). However, the distributions of allele frequencies for IL28B rs12979860 (C *vs* T) were significantly different between the PR and no-PR groups (P = 0.021, OR = 3.3, 95%CI: 1.2-9.2), in contrast to rs8099917 (T *vs* G, P = 0.082) (data not shown). The factors that were significantly associated with PR in the univariate analysis were analysed by logistic regression analysis. We found significant associations for baseline HBV DNA level (P = 0.037, OR = 2.2, 95%CI: 1.1-4.7) and IL28B rs12980275 AA (P = 0.020, OR = 18.8, 95%CI: 1.6-123.2), but not for ALT activity (P = 0.70).

Factors associated with complete response

In the next step, we analysed the significance of the association between baseline factors and complete response (Table 3). The presented data revealed that overall there were no statistically significant differences in the baseline characteristics of the complete response (CR) and no-CR groups in the analysed group of children. However, ALT activity was higher in patients in the no-CR group (48 U/L) compared with patients with the CR group (43 U/L). Additionally, the percentage patients with ALT < 40 U/L was higher in the patients with a CR (36.3% *vs* 20.0%).

Analyses of the examined polymorphisms showed that only the IL28B rs12979860 and OASL rs10849829

Table 4 Association of OAS haplotypes with treatment response

Haplotype	CR	No-CR	*P* value	OR (95%CI)	PR	No-PR	*P* value	OR (95%CI)
A A A A	0.11	0.16	0.402	0.60 (0.18-1.98)	0.16	0.00	0.041	NA
A A G A	0.03	0.05	0.473	0.47 (0.06-3.78)	0.05	0.00	0.283	NA
A A G G	0.17	0.35	0.031	0.35 (0.13-0.92)	0.21	0.57	0.002	0.21 (0.07-0.61)
A G G G	0.24	0.12	0.156	2.11 (0.74-6.00)	0.17	0.13	0.543	1.55 (0.37-6.48)
G A G G	0.28	0.03	0.0003	11.96 (2.38-59.97)	0.16	0.05	0.173	3.91 (0.48-31.8)
G G G G	0.05	0.17	0.046	0.24 (0.05-1.06)	0.15	0.05	0.194	3.70 (0.45-30.25)

Data are presented as the haplotype frequency; NA: Not applicable.

markers had an impact on CR results. The recessive genotype distributions for the IL28B rs12979860 polymorphism (CC-CT *vs* TT) were significantly different between the CR and no-CR groups (P = 0.033). These results were observed because none of the 6 children with the rs12979860 TT genotype achieved CR and the proportion of patients with CC-CT genotypes was comparable between the CR and no-CR groups. Despite the lack of statistical significance such as that observed in rs12979860 for the other two IL28B polymorphisms, none of the patients carrying homozygous genotypes for the minor alleles (GG for rs8099917 and GG for rs12980275) achieved CR. In contrast to the PR results, there were no statistically marginal or significant differences in CR rates for the dominant genotype and allele frequency distributions of the IL28B markers. The genotype distributions for the OASL rs10849829 polymorphisms (AA *vs* AG-GG) were significantly different between the CR and no-CR groups. In our series, for rs10849829, the OR of being a responder for the AA genotype compared to the AG and GG genotypes was 0.26 (95%CI: 0.07-0.88). CR was achieved in 5/21 (23.8%) of patients with the AA genotype at rs10849829, 14/25 (56.0%) patients with the genotype AG, and 3/6 (50.0%) patients with the genotype GG. Multivariate analysis for factors significantly associated with CR in univariate analysis showed only borderline significance for OASL rs10849829 AA (P = 0.061) in predicting a complete response.

Impact of OAS and IL28B haplotypes on treatment responses

Because all four OAS SNPs were located in a cluster on chromosome 12, we analysed the association of 4 SNPs with treatment response. First, we determined whether the four analysed SNPs were in strong linkage disequilibrium (LD). Overall, LD analysis demonstrated that rs1131476 OAS1, rs1293747 OAS2, rs2072136 OAS3 and rs10849829 OASL were in slight linkage disequilibrium (ranges, D′ = 0.358 - 0.999, r^2 = 0.023 - 0.382). The strongest LD was noted for the OAS1 and OAS3 markers (D′ = 0.635, r^2 = 0.152) and for the OAS2 and OAS3 markers (D′ = 0.999, r^2 = 0.382). In the next step, we conducted a haplotype analysis of the 4 OAS SNPs. There were 6 common haplotypes with a frequency exceeding 5% in our study cohort (Table 4). The global tests revealed significant associations between OAS haplotype and PR and CR (P = 0.0002 and P = 0.001, respectively). Significant differences in haplotype frequencies between the CR and no-CR groups were noted for the G-A-G-G haplotype (28% *vs* 3%, P = 0.0003), the A-A-G-G haplotype (17% *vs* 34%, P = 0.031) and the G-G-G-G haplotype (5% *vs* 17%, P = 0.046). In addition, the haplotypes A-A-A-A and A-A-G-G were associated with the PR group rather than the no-PR group (16% *vs* 0%, P = 0.041 and 21% *vs* 57%, P = 0.002, respectively).

In our study cohort, the IL28B SNPs were in strong LD (D′ = 0.999 for all pairs; r^2 ranges from 0.550 to 0.712). The global IL28B haplotype analyses indicated that the response groups (PR *vs* no-PR and CR *vs* no-CR) did not contain significantly different haplotype frequencies. However, the C-A-T haplotype (major alleles of all markers) was significantly associated with achieving PR (69% in the PR group *vs* 40% in the no-PR group, P = 0.015). In addition, for the T-G-G haplotype (minor alleles of all markers) there was a borderline significant difference between the CR and no-CR groups (16% *vs* 30%, P = 0.09) and the PR and no-PR groups (21% *vs* 40%, P = 0.06).

DISCUSSION

The limited efficacy, high cost and side effects associated with PEG-IFN treatment limit its clinical use in patients with CHB; however, this therapy is currently the only option for the permanent elimination of the virus[28]. Although viral clearance is rarely achieved with IFN treatment, it is more common with treatment than the natural rate of HBsAg seroclearance, which remains at a level of 2%-3%[29-31]. In this study of a group of paediatric patients, two (3.8%) patients permanently eliminated the virus.

Since there is no method of eradicating HBV, the current realistic goal of antiviral therapy is to achieve the permanent suppression of HBV replication to a level enabling the inhibition or retardation of inflammation and fibrosis of the liver and to protect against the development of hepatocellular carcinoma[32,33]. In the case of HBeAg-negative patients treated with IFN, reducing the viral load of HBV DNA after treatment to less than 2000 IU/mL is usually considered a

therapeutic success. The other endpoint of IFN therapy is a reduction in ALT to levels considered normal and the improvement of liver histology[28]. In our study, 42.3% of the patients achieved a complete response (HBV < 2000 IU/mL with ALT normalization), which is a result similar to the results obtained in other tests carried out among adult patients negative for HBe antigen[11,34,35].

Identification of the molecular mechanisms affecting the efficacy of interferon therapies and the severity of side effects of the treatment remain one of the main objectives of the study. In the current study, we analysed the relationship between polymorphisms in the interleukin 28B gene and OAS gene family and the response to interferon therapy in children with CHB. In our previous study, which included adult patients, we demonstrated that IL28B markers are associated with response to interferon therapy in patients with HBeAg negative CHB[36]. Other previous studies tried to determine whether having favourable IL28B genotype markers is associated with a better response to interferon therapy in HBeAg-negative adult patients, but the results were inconclusive. Some reports have suggested that the presence of favourable IL28B genotypes is associated with a better response to treatment[31,37], while others have indicated that there is no association between IL28B SNPs and the response to treatment with interferon[26,38]. In the present study, we demonstrated a statistically significant relationship between the rs12979860 marker and the response to treatment with interferon in patients with CHB. However, the results indicate the importance of the unfavourable TT genotype in estimating the chances of treatment failure.

Oligoadenylate synthetase plays a critical role in innate immunity, controlling the outcome of virus production. Consistent with the important role of OAS proteins in viral infection, it was shown that polymorphisms in the OAS genes are associated with increased susceptibility to HCV infection[18] and IFN treatment outcomes[17,19,20]. However, the molecular basis for this association is not well understood. In several studies, it was shown that polymorphisms in the OAS genes result in variations in enzyme activity by influencing basal enzyme activity[39], gene expression[17] or cellular localization[40].

In our study, we tried determine whether SNPs within the OAS genes may also affect the efficacy of interferon therapy in paediatric patients with CHB. In this study, a total of four SNPs located in the OAS1 (rs1131476), OAS2 (rs1293747), OAS3 (rs2072136) and OASL (rs10849829) genes were selected. The results of our study indicate that only the OASL rs10849829 marker has an impact on final therapeutic success, which was defined as the suppression of viral replication to an HBV DNA level < 2000 IU/mL and normalization of ALT activity 24 wk after completing treatment. The connection between the OAS gene polymorphisms and the efficacy of interferon therapy has not been extensively studied in patients with CHB. A small number of available studies have been carried out only in Han Chinese treatment-naïve CHB patients, who are representative of the Asian population[24,25]. The study of Wu *et al*[24] demonstrated an association between interferon treatment and the haplotype G-T-G-A within the rs3177979G, rs1293747T, rs4767043G, and rs10849829A alleles, which occur in the OAS1, OAS2, OAS3, and OASL genes, respectively, instead of with OASL rs10849829. In this study, the authors suggested that patients with a G-T-G-A OAS haplotype were less responsive to IFN treatment. In agreement with the results of Wu *et al*[24] we demonstrated that the AA genotype of rs10849829 was associated with failure of IFN therapy; however, there were no significant differences in allele frequencies between the different response groups. Additionally, the authors showed that the allele frequencies and genotype distributions of all the examined OAS SNPs were not correlated with treatment outcomes in patients who underwent IFN therapy. In another study conducted by Ren *et al*[25], the influence of 4 SNPs - rs2285934 OAS1, rs2072138 OAS2, rs2072136 OAS3 and rs10849829 OASL - on the outcome of a 48-week IFN treatment of Han Chinese treatment-naïve CHB patients (265 HBeAg-positive, 55 HBeAg-negative, and 43 inactive HBsAg carriers) was evaluated. In this study, treatment response was defined as HBsAg seroconversion or HBeAg seroconversion for the patients who were HBeAg-positive, without HBsAg seroconversion. In contrast to our results, the allele frequencies and genotype distribution analysis of these SNPs showed that only the OAS3 SNP (rs2072136 T/C) was independently associated with IFN treatment response. However, OAS haplotype analysis demonstrated significant associations between haplotypes and response to IFN treatment. The most common haplotype C-C-T-A (rs2285934C, rs2072138C, rs2072136T and rs10849829A) was associated with non-response. Based on the results of these two studies, in our study we conducted a haplotype analysis of the 4 SNPs. Although the sample size was relatively small, we identified an association between the OAS haplotypes and PR and CR to IFN treatment.

In addition to the different endpoints of therapy, the reason for the discrepancies in the results of our work may be, as for other OAS gene polymorphisms, differences in the distribution of alleles between Caucasian and Asian populations. While the most common genotype for the rs10849829 marker is genotype AA (approx. 60%[25]) in the Asian population, in our study, the percentage of patients with the AA genotype did not exceed 40%. The differences in the distribution of alleles between populations and their role in predicting response to interferon therapy in patients infected with HCV have been documented

for IL28B polymorphisms[41]. Additionally, Lampertico *et al*[42] suggest that studies of IL28B genotype and response to peginterferon in chronic hepatitis B should be stratified by HBV genotype. Although comparing the results for the Caucasian population with the results of the Asian population is difficult due to differences in the human alleles and HBV genotype distributions, it indicates the direction required for further studies, including those in Caucasian patients.

In summary, the presented study demonstrated a relationship between the IL28B rs12979860 and rs10849829 OASL genotypes, OAS haplotypes and the final IFN treatment outcome in paediatric patients infected with CHB. We noted that the unfavourable IL28B SNP genotype was present only in the no-CR group. Unlike adult studies, paediatric studies are definitely less frequent and are carried out in relatively small populations. Even the group we studied was not large enough to produce results that can be considered final. Nevertheless, this study indicates the potential markers we should focus on during further studies involving larger groups of patients.

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COMMENTS

Background

The 48-wk course of interferon therapy is recommended as a first-line treatment option for selected HBeAg-negative patients, especially in young patients with increased aminotransferase activity. The limited efficacy and side effects associated with IFN treatment limit its clinical use in paediatric patients with chronic hepatitis B (CHB). Therefore, a pretreatment identifying children with the highest probability of success is of great clinical importance to IFN therapy.

Research frontiers

The influence of host-related genetic variability on differences in response rates to IFN therapy in CHB patients is not well understood. The association between interleukin 28B (IL28B) and 2',5'-oligoadenylate synthetase (OAS) polymorphisms and the result of IFN monotherapy in adults with CHB has been the subject of very few studies. Currently, there are no available results concerning the impact of the IL28B or OAS SNPs on the results of IFN therapy in a group of paediatric patients with CHB.

Innovations and breakthroughs

The association between OASL rs10849829 and OAS haplotypes with IFN-treatment response in Caucasian CHB children is an important finding of this study. The results of the study confirm a most significant role of IL28B rs12979860 in predicting treatment response in Caucasian patients; however, the results indicate the importance of the unfavourable TT genotype in estimating the chances of treatment failure.

Applications

The results of the study indicate potential markers that may be useful to confirm the eligibility of children with CHB for IFN treatment.

Terminology

2,5 -*oligoadenylate synthetases* are the family of interferon-induced enzymes that play a critical role in controlling the production of viral proteins. The human OAS family contains the OAS1, OAS2, OAS3, and OASL genes located on chromosome 12. Interleukin 28B is a cytokine belonging to the interferon lambda family that plays a role in immune defence against virus infection, including inducing the transcription of antiviral genes such as the *OAS* genes.

Peer-review

This study examined the association between SNPs in OAS and IL28B and the biochemical and virological response rates to PEG-IFN alfa-2a monotherapy in a cohort of Caucasian children chronically infected with HBV. The results of this study revealed that patients with the AA allele at OASL rs10849829 and the TT allele at IL28B rs12979860 have lower chances of a complete response. In addition, this study found that the OAS1 (rs1131476), OAS2 (rs1293747), OAS3 (rs2072136), and OASL (rs10849829) haplotype had an impact on estimating the treatment response.

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