American Journal of Medicine and Medical Sciences 2024, 14(11): 2900-2904
DOI: 10.5923/j.ajmms.20241411.48

Association of the CCR2 Gene rs1799864 with Progression of Chronic HBV Infection in Children

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Abstract **The aim** of the study was to evaluate the effect of the CCR2 chemokine receptor rs1799864 gene polymorphism on the course of chronic HBV infection in children and its association with disease progression. **Background.** Chronic HBV infection remains one of the world's most serious public health problems, with complications such as decompensation, cirrhosis and liver cancer as the primary endpoint. Investigation of molecular mechanisms based on genetic regulation of the immune system mediated by the chemokine receptor gene CCR2. The CCL2 family of cytokines realize various immune effects up to the progression and development of adverse disease outcomes from its level of regulatory capacity. **Material and methods.** 120 children with chronic HBV infection living in the Republic of Uzbekistan were studied to achieve the set goal. As coordination and control we used a population sample of practically healthy children aged 4-18(n=65) years, residents of Tashkent city and Tashkent region. **Results.** When evaluating the results of genotyping of CCR2 gene rs1799864 polymorphism at three polymorphic loci V/V, V/I and I/I in all studied Uzbek children, it was revealed that the actual distribution of genotypes in both groups theoretically agreed with the Hardy-Weinberg equation, which indicated the preservation of the law of genetic equilibrium in the population ($p>0.05$). **Discussion.** There are studies supporting an association between the presence of a rare mutant I allele and mild disease course in other pathologies. The analysis of clinical and biochemical syndromes of chronic HBV-infection in children depending on the polymorphism of CCR2 gene showed a diverse nature of manifestations.

Keywords Polymorphism, Chronic HBV-infection, Interferon

1. Introduction

Investigation of molecular mechanisms based on genetic regulation of the immune system mediated by the chemokine receptor gene CCR2. The CCL2 family of cytokines realize various immune effects up to the progression and development of adverse disease outcomes from its level of regulatory capacity [1-6]. Its gene modification polymorphism rs1799864 (64V>I) is associated with a different degree of CCR2-mediated effects on the induction of inflammatory response and production of type I interferons with high antiviral activity, as well as with an increased risk of chronicity of infection [7-9]. Interferon effects are mediated through intracellular signaling mechanisms, the central components of which are NF-kB and interferon-regulatory factor - IRF3 [5,10-13]. Chronic HBV infection remains one of the world's most serious public health problems, with complications such as decompensation, cirrhosis and liver cancer as the primary endpoint. which has a high prevalence and variability (up to 47%) depending on geographic areas of the world [14-16], which has a high prevalence and variability (up to 47%) depending on geographic areas of the world [14,17]. Currently, there is no doubt that it is the chronic and latent variants of HBV that determine most of the epidemiologic process and prognosis of this infection. Of particular concern is the high risk of infection of children, which, according to the analysis of epidemiologists, about 75% of cases of chronic HBV infection are due to mother-to-child transmission, as in 95% of cases chronic HBV infection is developed in children under 5 years old infected by their mother [18-20]. At the same time, hemocontact familial transmission among children in endemic territorial zones also has a certain place from the epidemiological point of view, and a number of researchers have indicated that in children, especially older children, the formation of chronic HBV-infection is often noted in the outcome of jaundice-free and subclinical forms of the infection [18-19,3,21]. In this regard, all the above facts led us to study this area from the perspective of analyzing the influence of immunogenetic factors on the formation of progression of chronic HBV infection in children.

The aim of the study was to evaluate the effect of the CCR2 chemokine receptor rs1799864 gene polymorphism on the course of chronic HBV infection in children and its association with disease progression.

Received: Oct. 23, 2024; Accepted: Nov. 19, 2024; Published: Nov. 21, 2024
Published online at http://journal.sapub.org/ajmms

2. Material and Methods

One hundred and twenty (n=120) children with chronic HBV infection living in the Republic of Uzbekistan were studied to achieve the set goal. All children examined were from the Uzbek population. There was no history of consanguinity. The mean age of patients was 9 [3,18] years. The mean duration of the disease corresponded to 7.5±0.8 years. There were 91 (75,8%) boys and 29(24,1%) girls. All patients underwent inpatient examination in the Department of Hepatology of the Republican Specialized Scientific and Practical Medical Center of Pediatrics of the Ministry of Health of the Republic of Uzbekistan from 2021 to 2023. As coordination and control we used a population sample of practically healthy children aged 4-18 (n=65) years, residents of Tashkent city and Tashkent region. The laboratory part was performed in the experimental and laboratory department, molecular genetic research laboratory of the Center of Advanced Technology and "Kani-med Helthcare" Ltd. The diagnosis was made on the basis of anamnesis, data of clinical, laboratory and instrumental examinations with the use of diagnostic criteria of the degree of the pathological process activity in the liver in children. Virological verification was based on the detection of HBV markers (HBsAg, HBsAb, HBeAg, HBeAb, HBsAb total, HCVAb, HDVAb) by ELISA on MULTISCAN FC using HUMAN kits. HBV-DNA detection was performed by Real Time PCR on the amplifier "BIO-RAD iQ5" (USA) using "AmpliSenseR HBV-FL" kits (Russia) with hybridization-fluorescence detection in real time mode on the amplifier "BIO-RAD iQ5" (USA) using "AmpliSenseR HBV-FL" kits (Russia), Eppendorf thermocycler "Master Cycler 5415" (Germany). Synthetic deoxynucleotides for PCR were synthesized on Synostat device of "Biotronic" company (USA) and after removal of protecting groups were used for PCR.

Venous blood samples of 5.0 ml were collected for genetic analysis. DNA extraction was performed using a standard method with phenol-chloroform extraction. Genotyping of the rs1799864 (64V>I) polymorphism of the CCR2 gene was performed by PCR using restrictionase primers based on the NCBI Primers Tool and Nebcutter online programs. The PCR Core kit (Isogen, Russia) was used for PCR amplification. Weight of PCR product = 380 bp. Then, the PCR product was subjected to restriction analysis using BstF5I enzyme at 650 C for 16 hours. Weight of restricted products was as follows: GG (V/V)- 380 bp, GA (V/I) –380 bp/255 bp/ 155 bp, AA (I/I) –255 bp/ 155 bp. The results of RFLP analysis were visualized in a 3% agarose gel.

To confirm the diagnosis of chronic hepatitis, an ultrasound examination of the liver, spleen and biliary tract with dopplerography of the vessels of the portal system was performed on a Philips "ClearVue 650" device (USA), as well as elastometry of the liver tissue with calculation of the total density in kPa and subsequent evaluation according to the Metavir scale (F0-F4).

Statistical processing of the material was carried out using EXELL (2019) application program package and variation statistics. Differences in the distribution of CCR2 allele and genotype frequencies between groups were assessed by applying the t-criterion and the $\chi 2$ criterion. The level of reliable significance was determined at a critical value of $\chi 2>3,84$ (Df=1) and $\chi 2>5,99$ (Df=2) etc. according to Pearson's table. The odds ratio (OR) to assess the association between specific genotypes (factors) and the risk of disease (outcome) was calculated using the standard formula OR=a/bxd/c, where ***a*** and ***b*** are the number of patients with/ without mutant genotype and, ***d*** and ***c*** are the number of children in the control group with/ without mutant genotype. OR is given with 95% confidence interval with upper and lower bounds (CI). A statistically significant difference was considered to be $p<0.05$.

3. Results

When evaluating the results of genotyping of CCR2 gene rs1799864 polymorphism at three polymorphic loci V/V, V/I and I/I in all studied Uzbek children, it was revealed that the actual distribution of genotypes in both groups theoretically agreed with the Hardy-Weinberg equation, which indicated the preservation of the law of genetic equilibrium in the population ($p>0.05$). Thus, the results of comparative analysis in the study of CCR2 gene polymorphism genotypes among patients with chronic HBV infection and control group revealed the reliable significance of mutant homozygous genotype 64I/I, the carriers of which were only sick children (10.8% vs. 0.0%, $p<0.001$). The majority of children in both groups were almost equally identified as carriers of the homozygous genotype with the wild-type V allele, 72.3% and 55.8%, respectively. Heterozygous 64V/I was identified in second place with 27.7% and 33.3%, respectively, which was 1.2 times more frequent in sick children, but without statistical difference, $p>0.05$ (Table 1).

Consequently, according to the law of H. Weinberg, the studied population has ideal conditions for genetic cleavage and stability of the gene pool, which indicates the preservation of free crossing, absence of new mutations and selections. However, there is an association between the risk of disease chronicization and, to some extent, susceptibility to HBV infection in carriers of the rare I allele, where its frequency proportion of different variations in homo- and heterozygotes SNP of the CCR2 gene was 1.2 times higher than in the control population. This fact was also confirmed in the analysis of allele frequencies of the 64V>I polymorphism of the CCR2 gene. Meanwhile, the allele ratio in both groups also showed a significant importance of both alleles, especially of the rare 64I mutant allele in the sick children (27.5% vs. 13.8% of control group (OR=0.5) [95%CI 0.27-0.92], $p<0.001$) and mainly due to the compound heterozygote ratio. In the main group, carriers of the V allele of the CCR2 gene were 1.18 times less frequent than in the control group (72.5% vs. 86.1%), with an odds ratio of OR=0.47 [95%CI 0.28-0.70], $p<0.001$

and a degree of freedom of Df=1. Further, intragroup analysis of the distribution of genotypes and alleles of the 64V>I polymorphism of the CCR2 gene depending on the activity of the pathologic process in the liver and the severity of the disease, in general, revealed slightly different associative relationships. Considering the comparable distribution of genotypes and allele frequencies of the CCR2 polymorphic gene in the multifield pairing table using the Pearson method, a direct associative relationship with the activity and severity of the course of chronic HBV infection in children was revealed. These findings are consistent with the findings of many researchers regarding other various chronic diseases [5,7].

4. Discussion

Thus, considering the dynamics of allele frequencies in the comparative distribution between different degrees of activity of chronic HBV-infection in children, a tendency to increase the number of cases of V allele carriers towards children with a pronounced pathological process in the liver with reliable significance ($\chi 2$ - 138.6; P<0.001) was revealed, where its frequency in pronounced activity amounted to 45.6% of cases (OR=10.25) (95%CI 5.98-17.59) against children of the control group - 18.1%. Among patients with moderate disease activity, V allele carriers were detected in one third (36.3%) of children, which was 1.3 times more frequent relative to patients with minimal activity (OR=0.15) (95%CI 0.10-0.22) and less frequent relative to patients with severe disease (OR=1.05) (95%CI 0.73-1.50). The distribution of I-allele carriage showed an opposite pattern, with a predominance of the frequency of the rare allele among sick children with minimal activity (63.9% vs. 27.8% and 8.3% of children with moderate and severe activity, respectively (p<0.001) (Figure 1).

Therefore, we can state the association of the V-allele in potentiating the progression of the natural course of the disease, in contrast to the I-allele in protecting the sluggish course of chronic HBV-infection in children. This probability was partially confirmed by the following analysis of the distribution of homo- and heterozygous CCR2 genotypes in sick children depending on the activity of chronic HBV infection (Table 2).

Table 1. Analysis of genotype and allele frequencies of the CCR2 64V>I gene polymorphism in children and their correlation with chronic HBV infection

Genotypes and alleles CCR2 64V>I	Control group (n=65)		Main group (n=120)		Genotype type	OR (95%CI)	P_2-value
	n	%±m	n	%±m			
64-V/V	47	72.3±5.5	67	55.8±4.5	Homozygote	1.29(0.80-2.1)	0.29
64-V/I	18	27.7±5.5	40	33.3±4.3	Heterozygote	0.83(0.44-1.56)	0.33
64-I/I	0	0.0	13	10.8±2.8	Homozygote with a rare allele	-	**0.009**
V-allele	112	86.1±3.2	174	72.5±3.4	Wild	1.18 (0.8-1.7)	0.37
I-allele	18	13.8±8.1	66	27.5±5.5	Minor	**0.5(0.27-0.92)**	**0.02**
$\chi 2$	1.67		3.23				
P_1-value	**0.19**		**0.07**				

Note: $\chi 2$ – Pearson's criterion value; P_1 – intra-group significance level;
P_2-value – significance level between control and main groups;
OR – odds ratio with 95% confidence interval (CI).

Table 2. Frequency of CCR2 64V>I genotypes depending on the activity of chronic HBV-infection in children

Genotypes	Activity of chronic HBV infection								
	Minimal		$\chi 2_{(1)}$	Moderate		$\chi 2_{(2)}$	Pronounced		$\chi 2_{(3)}$
	Abs.	%		Abs.	%		Abs.	%	
64-V/V	10	14.9±4.4		22	32.8±5.7		35	52.2±6.1	
OR (95%CI)	0.467 (0.19-1.11)			0.693 (0.35-1.38)			**0.323 (0.14-0.73)**		
64-V/I	18	45.0±7.9	8.75 **P=0.01**	14	35.0±7.5	5.99 **P=0.02**	8	20.0±6.3	27.5 **P<0.001**
OR (95%CI)	1.320 (0.57-3.0)			1.924 (0.73-5.1)			2.546 (0.99-6.51)		
64-I/I	10	76.9±11.7		3	23.1±11.7		0	0.0	
OR (95%CI)	3.421 (0.87-13.4)			---			---		

Note: $\chi 2_{(1)}$ *– significance criterion between minimal and moderate activity,* $\chi 2_{(2)}$ *– significance criterion between moderate and pronounced activity,* $\chi 2_{(3)}$ *– significance criterion between minimal and pronounced activity of chronic HBV-infection;* ***P*** *– significance criterion and confidence level;* ***OR*** *– odds ratio with 95% confidence interval (CI).*

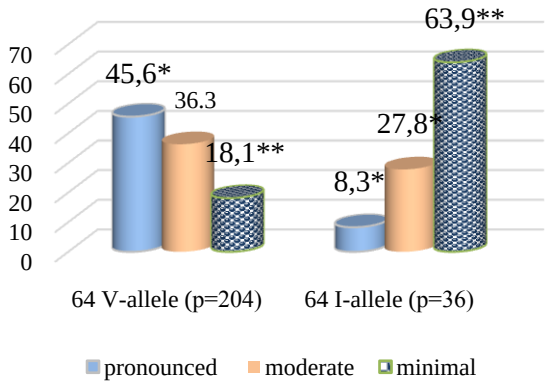

Figure 1. Frequency of CCR2 64V>I allelic positions depending on the activity of chronic HBV infection in children (***Note:*** *t-criterion * – reliability of values between activity groups within allele groups; ** – reliability of values between allele groups within activity; χ2 – significance criterion between activity groups (P<0.05-0.001); p – total number of alleles.)*

Thus, a comparative analysis of the distribution of homo- and heterozygous CCR2 genotypes in sick children depending on the activity of chronic HBV infection partially confirmed the statistically significant relationship between V-allele genotypes and severe course of the disease. Children with minimal disease activity were significantly less likely to be identified as carriers of the homozygous 64-V/V genotype compared to children with moderate and severe disease activity (14.9% vs. 32.8% and 52.2%, respectively, p<0.05-0.001) with OR=0.323 [95%CI 0.14-0.73]. While children with moderate and severe disease activity were not represented in this group with the homozygous I/I genotype, this genotype was found exclusively in children with minimal activity (16.7%, p<0.001). There are studies supporting an association between the presence of a rare mutant I allele and mild disease course in other pathologies [17]. Based on these data, it can be concluded that there is an association between genotype frequencies and disease course activity, and CCR2 gene polymorphism can be considered as one of the factors predisposing to different severity of chronic HBV infection in children.

The analysis of clinical and biochemical syndromes of chronic HBV-infection in children depending on the polymorphism of the CCR2 gene showed a diverse character of manifestations during the dynamic study. At the same time, the presence of correlations in the frequency of progressive forms of the disease depending on the carrier of the V-allele homozygous position of the CCR2 gene drew attention. Thus, in the vast majority (73.1%) of sick children with homozygous V/V-genotype, the course of the disease was progressive (p<0.05-0.01 to other groups). In children carrying the heterozygous V/I-genotype, disease progression was registered only in 45.0%. No progression was observed among children with I/I genotype (p<0.05-0.01 to other groups) (Figure 2).

Analysis of expressed forms of chronic HBV-infection occurrence in children revealed a predisposition to a severe course of the disease in patients carrying the V/V-genotype, where the frequency of expressed disease activity corresponded to 52.2% (p<0.001 between the studied groups). Overall, it can be argued that the high frequency of the 64V allele in various comparative analyses between groups indicates its potential role in accelerating the natural course of the disease in children. At the same time, the 64I allele may have a protective effect in the sluggish course of chronic HBV-infection.

5. Conclusions

The conducted studies allowed to conclude that the genetic polymorphism rs1799864 of CCR2 gene in amino acid position 64V>I in children with chronic HBV-infection has a statistically confirmed association with the peculiarities of the disease course.

In children, the presence of the mutant allele, especially in the homozygous form, has a protective effect, resulting in a more prolonged course of the disease.

Consequently, carriage of different variants of rs1799864 polymorphism of the CCR2 chemokine receptor gene in children can be considered as a factor in differentiating the severity and predicting the course of chronic HBV infection in patients.

A – children with the genotype 64-I/I CCR2
B – children with the genotype 64-V/I CCR2
C – children with the genotype 64-V/V CCR2

Figure 2. Frequency of severe and progressive forms of chronic HBV infection in children depending on CCR2 gene polymorphism (***Note:*** *reliability of differences between the studied groups – [a]-A/B; [b]-B/C; [c]-A/C (p<0.05 up to p<0.001).*)

Conflict of Interests' Statement

The authors declare no conflict of interest.

This study does not include the involvement of any budgetary, grant or other funds.

The article is published for the first time and is part of a scientific work.

ACKNOWLEDGMENTS

The authors express their gratitude to the management of the multidisciplinary clinic of Republican Specialized Scientific and Practical Medical Center of Pediatrics for the material provided for our study.

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