The relationship between genetic variation of genes in RLR-MAVS and cGAS-STING signaling pathway and other genes with HBV intrauterine transmission

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Research Article

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Abstract

Background

Hepatitis B virus is a major public health problem worldwide. The genetic susceptibility of HBV intrauterine transmission has also attracted extensive attention from researchers. We explored the relationship between genetic variation of genes in RLR-MAVS and cGAS-STING signaling pathway and other genes with intrauterine transmission.

Methods

A case-control study was performed on HBsAg-positive pregnant women who delivered from January 2013 to August 2017 at Wuhan Medical Care Center for Women and Children, Wuhan, China. In total, 1356 infants born from those women were included, and there were 107 infants with HBV intrauterine infection. RLR-MAVS and cGAS-STING signaling pathway-related genes and IL10, IL12A, IL12B, TLR3, TLR9, TNFSF14 were selected as candidates. MassARRAY gene detection system was used for genotyping. Multivariate unconditional logistic regression, cumulative effect analysis, and multifactor dimensionality reduction were used for statistical analysis.

Result

The risk of HBV intrauterine transmission in infants with rs28413168 CC/CG genotype in INTS10 gene increased by 100% compared with those with GG genotype, and GG/AG genotype in MB21D1 gene was significantly lower than that in infants with AA genotype. Compared with the rs1800872 CC genotype in IL10 gene, the risk of HBV intrauterine transmission in infants with AA genotype was significantly increased. Compared with the rs3775291 CT/CC genotype in TLR3 gene, infants with TT genotype had significantly increased risk of HBV intrauterine transmission. The risk of HBV intrauterine transmission in infants with 5–8 risk alleles was 4.34 times higher than that in those with 0–2 risk alleles after adjustment for the age of delivery, parity, HBeAg, HBV DNA.

Conclusions

The genetic polymorphisms of INTS10 rs28413168, MB21D1 rs311678, IL10 rs1800872, TLR3 rs3775291 are closely related to the susceptibility of HBV intrauterine transmission. The more risk alleles an infant carries, the higher the risk of HBV intrauterine transmission.

Introduction

About 350 million people worldwide are chronically infected with the hepatitis B virus (HBV)[1]. Controlling the further spread of HBV has become a major public health problem in the world. In highly

endemic areas, about half of HBV infections originate from mother-to-child transmission (MTCT)[2–4]. Therefore, preventing MTCT of HBV has become an important way to eliminate HBV infection. In recent years, the development of next-generation sequencing technology has provided an important guarantee for a comprehensive and systematic study of the genetic susceptibility of diseases. Therefore, the genetic susceptibility of HBV intrauterine transmission has also attracted extensive attention from researchers.

In clinical practice, it is often found that HBV pregnant women with similar general conditions have different risks of intrauterine transmission. The reasons behind this phenomenon have always been the focus of attention of researchers. In recent years, studies have reported the correlation between host gene polymorphism and the risk of HBV intrauterine transmission. Gao[5] reported that TLR3 (c.1377C/T) allele T and TLR9 (G2848A) allele A are protective factors for HBV intrauterine transmission. Another study[6] showed that the frequency of the G allele at the TNF-α-308 locus was significantly higher than that of the intrauterine uninfected group and the normal control group. Our previous research also found that genetic mutations in PDCD1 and LTA genes are associated with HBV intrauterine infection [7]. Wan[8] reported that CXCL13 rs355687 genotype CT has a lower risk of HBV intrauterine infection than genotype TT. This study also found that the neonatal HLA-C gene locus rs3130542 polymorphism is closely related to the risk of HBV intrauterine infection. But these studies are from the perspective of a single gene or a single site to analyze its relationship with HBV intrauterine transmission. At present, there is a lack of systematic and multi-level research on the genetic susceptibility of HBV intrauterine transmission.

Retinoic acid-inducible gene I(RIG-I) is a pattern recognition receptor(PRR) discovered in recent years. HBV nucleic acid is recognized by RIG-I-like receptors to trigger signal transduction, induce the production of type I interferon(IFN-I) and pro-inflammatory cytokines, and then exert antiviral effects[9]. A study[10] found that RIG-I has a dual anti-HBV effect. On the one hand, RIG-I can recognize HBV pregenomic RNA (pgRNA), and further, induce the production of type III interferon to exert its anti-HBV effect. On the other hand, RIG-I can interfere with the interaction between HBV pgRNA and HBV polymerase to inhibit HBV replication. At present, there are limited studies on the association between the MAVS, IRF3, and IFNL1 gene polymorphisms of the key genes in the RIG-I signaling pathway and the intrauterine transmission of HBV.

Cyclic GMP-AMP synthase(cGAS) is a nucleic acid transferase, which functions as a DNA sensor in mammals and recognizes DNA in the cytoplasm and produces cyclic GMP-AMP, activates stimulator of interferon genes(STING), regulates the secretion of downstream IFN-I and other cytokines, and initiates immune responses[11, 12]. A German study[13] found that the silent expression of cGAS, STING, and TBK1 can lead to a large increase in HBV infection in HepG2-NTCP cells. After CRISPR/Cas9 gene knockout, HBV infection and replication increase. The study also found that overexpression of cGAS will lead to a reduction in HBV infection. He[14] reported that when the cGAS/STING pathway is activated by dsDNA or cGAMP, cultured cells and HBV replication in mice are significantly inhibited. In addition, knocking out cGAS in human peripheral blood mononuclear cells will lead to an increase in the level of intracellular HBV DNA. Therefore, the cGAS/STING pathway has played a role in the monitoring of HBV infection and may be used to develop new anti-HBV strategies. However, whether genetic polymorphisms

in this pathway will affect the expression and function of the corresponding genes, which in turn affect the risk of intrauterine transmission in infants, remains unknown.

Toll-like receptors (TLRs) are pattern recognition receptors that play an important role as a bridge between natural immunity and acquired immunity[15, 16]. TLR3 and TLR9 are both important family members of TLRs. TLR3 can recognize double-stranded RNA viruses and dsRNA produced during virus replication. TLR9 can recognize viral DNA, induce specific immunity, and promote the body's elimination of viruses[17–20]. A study showed that TLR3 (c.1377C/T) allele T and TLR9 (G2848A) allele A are protective factors for HBV intrauterine transmission[5]. Research by Jiang found that compared with normal people, IL10 levels in HBV patients increased and IL12 levels decreased, indicating that IL10 and IL12 are closely related to hepatitis B infection[21]. Wang reported that in hemodialysis patients, IL12B 3'UTR gene polymorphism may be related to HBV susceptibility, and IL-10 gene polymorphism may be related to persistent HBV infection in China[22]. TNFSF14 is a gene encoding CD258, a member of the Tumor Necrosis Factor (TNF) superfamily, and its encoding protein CD258 is a ligand for the lymphotoxin β (LTβ) receptor. After T cells are activated, CD258 and LTB2LTA heterotrimers are expressed on the cell membrane, which binds and activates the LTβ receptor on liver cells[23]. The activated LTβ receptor can further increase the expression level of apolipoprotein B messenger RNA-editing enzyme catalytic subunit 3B gene in the nucleus, and then degrade HBV ccDNA in the nucleus, thereby exerting an anti-HBV effect [24]. To a certain extent, the above-mentioned genes are related to HBV clearance or persistent infection, but there is currently a lack of research on the association between these gene polymorphisms and HBV intrauterine transmission.

Based on the genetic susceptibility of infants, this study selected key genes in the RLR-MAVS and cGAS-STING signaling pathways, including MAVS, IRF3, IFNL1, INTS10 in the RLR-MAVS pathway, MB21D1, TMEM173, IRF7, IFNB1 in the cGAS-STING pathway to explore the relationship between these candidate gene mutations and the risk of HBV intrauterine transmission. In addition, the influence of some candidate genes IL10, IL12A, IL12B, TLR3, TLR9, TNFSF14 gene polymorphisms on the intrauterine transmission of HBV was also discussed, and the susceptible genes and sites of HBV intrauterine transmission were identified.

Materials And Methods

Study subjects

This study adopts a case-control study design. HBsAg-positive pregnant women and their infants were collected in Wuhan Maternity and Child Health Hospital from January 2013 to August 2017. A total of 1356 HBsAg-positive pregnant women and their infants were included.

Methods

Data and samples

A self-designed questionnaire was used to collect the basic information of the research objects, including general demographic characteristics of pregnant women, basic conditions during pregnancy, and relevant information about infants. The samples in this study came from infants delivered by HBsAg-positive pregnant women. After obtaining the informed consent of the guardian, blood samples were collected.

Screening of susceptibility sites for candidate genes

This study screened out 4 key genes—MAVS, IRF3, IFNL1, INTS10 in the RLR-MAVS pathway, and 4 key genes—MB21D1, TMEM173, IRF7, IFNB1 in the cGAS-STING pathway, and 6 scattered genes—IL10, IL12A, IL12B, TLR3, TLR9, TNFSF14. Using bioinformatics analysis methods to select potential functional SNPs on candidate genes, the specific screening methods[25, 26] are as follows: (1) Use the F-SNP database (http://compbio.cs.queensu.ca/F-SNP) to screen candidate gene loci with potential functional effects in protein-coding, splicing regulation, transcription regulation or post-translational processes,(2) Filter the above loci, and the filter condition is that the minimum allele frequency (MAF) of the Chinese Han population (CHB) in Beijing, China reported in the genetic database of the Thousand Genome Project is greater than 5%, (3) On the SNAP Pairwise LD website (http://www.broadinstitute.org/mpg/snap/ldsearchpw.php), use 1000 Genomes Pilot1, CHBJPT, r2=0.8, distance limit=500kb as the benchmark to test whether there is a strong linkage disequilibrium between the above-mentioned sites. If it exists, choose one of the best. After screening, the final 22 SNP loci were eligible to be included for subsequent genotyping.2.3 Genomic DNA extraction and genotyping of infants.

Genomic DNA was extracted from the blood samples using a TIANamp Blood DNA Kit DP318 (TIANGEN BIOTECH (BEIJING) CO., LTD, China) under the instruction of the manufacturer. DNA concentration and optical density were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

The DNA samples that passed the concentration and purity testing were sent to Beijing Bomiao Biological Company, and the company was entrusted to use the MassARRAY platform to perform genotyping testing on 23 SNP loci among the 15 candidates genes of the batch of samples.

Estimation of power

The power and sample size calculation v3.1.2 software was used to estimate the test power of this study. For genetic variants with MAF of 0.09, 0.28, and 0.47 at candidate loci, at the test level of α=0.05, with the current sample size, the confidence in detecting an association with an OR of 1.6 is 37.7%, 61.5%, and 64.2%, respectively.

Statistical analysis

Statistical analyses were performed by SPSS18. All *P* values were two-tailed with a statistically significant level set at 0.05. Comparison between groups was performed by two-tailed t test for continuous data, and Pearson Chi-Square ($\chi 2$) test in cross-tabulations for categorical data. Hardy-Weinberg balance test was used to estimate the reliability of group survey data. Multivariate

unconditional logistic regression was used to analyze the association between each gene locus of infants and the risk of HBV intrauterine transmission under four different genetic models (codominant model, dominant model, recessive model, additive model). MDR software was used to analyze the interaction between genes and genes, and a model with good test sample accuracy, high cross-validation consistency, and significant replacement test results was selected as the best model for interaction. The cumulative effect of the positive sites found in the correlation analysis between SNP at a single site and intrauterine infection of HBV was performed. Multivariate unconditional Logistic regression analysis was used to calculate the risk of HBV intrauterine infection in infants with different numbers of alleles, expressed by OR value and its 95% CI. The linear trend test was used to analyze whether the risk of HBV intrauterine infection increased with the increase in the number of risk alleles.

Results

Comparison of basic characteristics

Among the 1356 infants delivered by HBsAg-positive pregnant women, there were 107 infants with the intrauterine transmission. The age of delivery of pregnant women in the HBV intrauterine transmission group (case group) was significantly higher than that of the control group (P = 0.006). Among pregnant women in the case group, the proportion of first delivery was significantly higher than that in the control group (P = 0.002). The positive rate of HBeAg during pregnancy and the positive rate of HBV DNA during the third trimester in the case group were significantly higher than the control group (P <0.001). The proportion of cesarean section in the control group was significantly higher than that case group (P <0.001). (Table 1)

Table1 Comparison of general characteristics of pregnant women

	case	control	χ^2/t	*P*
Maternal Characteristics	N=107	N=1249		
Age (years)	28.00 ± 3.98	29.14 ± 4.08	-2.774	**0.006**
Pre-pregnancy BMI(kg/m^2)	26.83 ± 3.09	27.19 ± 3.19	-1.106	0.269
Primiparous			9.147	**0.002**
Yes	91 (85.8)	899 (72.3)		
No	15 (14.2)	344 (27.7)		
HBeAg			60.980	**<0.001**
positive	49 (46.7)	193 (15.9)		
negative	56 (53.3)	1020 (84.1)		
HBV DNA			31.643	**<0.001**
positive	59 (61.5)	364 (32.9)		
negative	37 (38.5)	743 (67.1)		
Infant characteristics	N=107	N=1249		
Gender			0.031	0.861
Male	55 (51.4)	653 (52.3)		
Famale	52 (48.6)	596 (47.7)		
Pre-term birth(<37)			0.261	0.610
Yes	5 (4.7)	73 (5.9)		
No	102 (95.3)	1170 (94.1)		
Delivery			23.365	**<0.001**
Vaginal	63 (58.49)	440 (35.3)		
Cesarean	44 (41.1)	805 (64.7)		

SNPs selection

22 SNPs on 14 candidate genes were included in this study, as shown in Supplementary Table S1. The success rate of rs8126207 on the MAVS gene and rs311674 on the MB21D1 gene was less than 95%, and they were not included in the study. In addition, the Hardy-Weinberg balance test found that the distribution frequency of the genotypes of MAVS rs3746661, MB21D1 rs9359033, and TMEM173 rs1131769 in the control group did not meet the Hardy-Weinberg genetic balance (P > 0.05) and was also eliminated. A total of 17 gene loci were finally included in the subsequent analysis.

The association between single gene locus polymorphism and intrauterine transmission

After adjusting for maternal delivery age, parity, HBeAg, HBV DNA, and delivery, the results of multivariate logistic regression analysis are shown in Table 2. Only statistically significant data are listed here, and basic information about these SNPs is shown in Supplementary Table S2.

In the RIG-I/MAVS signaling pathway, compared with the INTS10 rs28413168 GG genotype, the risk of intrauterine transmission of HBV in infants carrying the CC/CG genotype at this site increased by 100% (Dominant model: OR = 2.00, 95% CI: 1.26-3.19, P = 0.004), additive model showed that for each additional C allele, the risk of intrauterine transmission of HBV in infants increased by 57% (OR = 1.57, 95% CI: 1.09-2.27, P = 0.016). In the cGAS-STING pathway, there was a significant difference in the genotype distribution of MB21D1 rs311678 between the two groups of infants (P = 0.041), under the dominant model, the risk of intrauterine transmission of HBV in infants with GG/AG genotype was significantly lower than that of infants with AA genotype (OR=0.48, 95% CI: 0.30-0.78, P = 0.003), additive model showed that for every additional G allele in an infant, the risk of intrauterine transmission of HBV decreased by 45% (OR = 0.55, 95% CI: 0.36-0.82, P = 0.003).

Compared with the IL10 rs1800872 CC genotype, infants with AA genotype had a significantly higher risk of intrauterine transmission of HBV (OR = 3.01, 95% CI: 1.02-9.84, P = 0.047). The SNP at rs3775291 locus of TLR3 in infants was also closely related to the intrauterine transmission of HBV. Compared with the TT genotype, infants with CC genotype had a significantly lower risk of intrauterine transmission of HBV (OR = 0.44, 95% CI: 0.21-0.91, P = 0.027), under the recessive model, infants with TT genotype have a significantly higher risk of HBV intrauterine transmission than those with CT/CC genotype (OR = 2.09, 95% CI: 1.07-4.12 , P = 0.032).

Table 2 Association analysis of neonatal candidate gene locus polymorphisms and intrauterine transmission of HBV

Gene	Genotype	control	case	P^a	OR (95% CI)#	P^b
INTS10	rs28413168	N=1231	N=103	0.160*		
	CC	55 (4.5)	5 (4.9)		Ref	
	CG	402 (32.7)	43 (41.7)		0.78 (0.25-2.39)	0.659
	GG	774 (62.9)	55 (53.4)		1.64 (0.53-5.11)	0.390
	Dominant model				**2.00 (1.26-3.19)**	**0.004**
	Implicit model				0.96 (0.32-2.89)	0.945
	Additive model				**1.57 (1.09-2.27)**	**0.016**
MB21D1	rs311678	N=1218	N=106	**0.041**		
	GG	100 (8.2)	4 (3.8)		Ref	
	AG	492(40.4)	35 (33.0)		2.67 (0.90-7.91)	0.077
	AA	626 (51.4)	67 (63.2)		1.33 (0.43-4.11)	0.616
	Dominant model				**0.48 (0.30-0.78)**	**0.003**
	Implicit model				0.49 (0.17-1.43)	0.192
	Additive model				**0.55 (0.36-0.82)**	**0.003**
IL10	rs1800872	N=1218	N=104	0.733		
	CC	109 (8.9)	7 (6.7)		Ref	
	CA	525 (43.1)	45 (43.3)		2.44 (0.82-7.32)	0.111
	AA	584 (47.9)	52 (50.0)		3.01 (1.02-9.84)	**0.047**
	Dominant model				0.72 (0.45-1.13)	0.152
	Implicit model				0.37 (0.13-1.07)	0.064
	Additive model				0.69 (0.48-1.00)	0.051
TLR3	rs3775291	N=1215	N=98	0.007		
	TT	116 (9.5)	19 (19.4)		Ref	
	CT	549 (45.2)	36 (36.7)		0.52 (0.25-1.05)	0.069
	CC	550 (45.3)	43 (43.9)		**0.44 (0.21-0.91)**	**0.027**
	Dominant model				1.02 (0.64-1.63)	0.940
	Implicit model				**2.09 (1.07-4.12)**	**0.032**
	Additive model				1.21 (0.85-1.72)	0.303

* Fisher's exact test.

a P-value obtained from chi-square test

† OR and 95% CI obtained from the logistic regression model, adjusted for maternal age, parity, HBeAg, HBV DNA, and delivery

b P-value obtained from the logistic regression model

Multigene cumulative effect analysis

The cumulative effect of the four positive sites found in the correlation analysis between a single SNP and HBV intrauterine transmission was analyzed. According to the results of single-site association analysis, INTS10 rs28413168 C allele, MB21D1 rs311678 A allele, IL10 rs1800872 A allele, and TLR3 rs3775291 T allele were considered as risk genes. The number of risk alleles carried by each infant was calculated, and this variable was included in the logistic regression model. The age of delivery, parity, HBeAg, HBV DNA, and after delivery found that the risk of intrauterine transmission of HBV in infants carrying 5-8 risk alleles is 4.34 times that of carrying 0-2 risk alleles (95%CI: 1.44-13.09, P = 0.009). The linear trend test results showed that the risk of HBV intrauterine transmission increased as the number of risk alleles carried by infants increased (P = 0.030). (Table 3).

Table 3 Analysis of the cumulative effect of neonatal positive sites

Number of risk genotypes	case	control	OR (95%CI)	*P*
0-2	10 (9.7)	190 (15.4)	Ref	
3-4	55 (53.4)	653 (56.4)	2.29 (0.78-6.76)	0.134
5-8	38 (36.9)	348 (28.2)	4.34 (1.44-13.09)	**0.009**
linear trend test				**0.030**

*Adjusted for maternal age, parity, HBeAg, HBV DNA, and delivery

Gene interaction analysis

Table 4 lists 3 different combinations of models. Taking the 1-site model MD21D1 rs311678 as an example, the cross-validation consistency is the largest (CVC value is 9/10), and the prediction error value is the smallest 45.1%. But after the 1000 permutation test, the model is not statistically significant. In addition to the two-site and three-site models, the P-value of the replacement test is also greater than 0.05, indicating that the 3 models are not statistically significant.

Table 4 Analysis of 17 SNPs Interaction

Number of SNPs	Best model	Training accuracy	Test accuracy	CVC
1	rs311678	0.568	0.549	9/10
2	rs3212227, rs7447927	0.612	0.509	5/10
3	rs3212227,rs352140, rs7447927	0.659	0.438	4/10

Discussion

Polymorphism of key genes in RIG-I/MAVS signaling pathway

In the RIG-I/MAVS signaling pathway, the INTS10 rs28413168 polymorphism was statistically associated with HBV intrauterine transmission. There is no research report on the association between the INTS10 rs28413168 gene polymorphism and intrauterine transmission of HBV or other diseases, but there have been related reports on the association study between the INTS10 gene polymorphisms at other loci and diseases. A South African study found that three polymorphisms (rs55830938, rs73599609, rs73667448) located between the INTS10/LPL gene are associated with systolic blood pressure[27]. The results of another American study suggest that the INTS10/LPL rs149352150 gene polymorphism is associated with male BMI[28].

INTS10 is a member of the integration factor complex family, which can affect the splicing of RNA precursors, which in turn affects the synthesis and expression of mRNA in the cell. A study found that INST10 has an effect of inhibiting virus infection that has never been discovered before[29]. The study found that the INTS10 rs7000921 locus polymorphism is closely related to the chronicity of HBV infection through the genome-wide association study. Subsequently, the team also found in vitro cell culture experiments that INTS10 was significantly related to HBV replication. In the RIG-I/MAVS pathway, overexpression of INST10 can increase the phosphorylation of IRF3, which in turn activates the activity of downstream IFN-stimulated response element (ISRE), increases the expression level of IFN-λ, and ultimately inhibits HBV replication. Since no functional study on INST10 rs28413168 has been found so far, we found that this site is located in the 5'non-transcribed region through the Ensembl database, suggesting that this site may be involved in the translation, transcription, and stability maintenance of INST10 mRNA. Through the website of rSNP and Fast SNP gene function prediction, our study found that INST10 rs28413168 gene polymorphism may directly participate in gene transcription regulation, and then affect the anti-HBV function of INST10 in the body.

Polymorphism of key genes in cGAS-STING signaling pathway

In the cGAS-STING pathway, there were significant differences in the genotype distribution of MB21D1 rs311678 infants between the case group and the control group. MB21D1, also known as cGAS, can be used as a cytoplasmic DNA sensor to participate in the recognition of different viruses. The combination of cGAS and DNAs can activate the interferon-stimulating gene STING, which in turn stimulates the production of IFN-I and antiviral ISGs. The lack of cGAS will lead to the reduction of the early antiviral inherent response to the body's viral infection[30]. Dansako[31] reported that cGAS and its adaptor protein

(STING) recognize and bind dsDNA derived from the HBV genome to generate an innate immune response. In addition, the study also proved that HBV infection can induce the production of interferon-inducible gene 56 (ISG56) through the cGAS-STING signaling pathway, thereby inhibiting the assembly of HBV virus particles. A Japanese study found that daunorubicin can trigger an endogenous cGAS-dependent innate immune response to inhibit the production of HBV[32]. Therefore, the antiviral effect induced by the cGAS-STING signaling pathway plays an important role in identifying and controlling HBV infection.

rs311678 is located in the 5th exon coding region of the cGAS gene. The mutation at this site is synonymous and will not cause any changes in the amino acids it encodes. However, Xiao[33] reported that the cGAS rs311678 locus polymorphism is associated with cervical precancerous lesions, and the cGAS rs311678 G allele reduces the risk of cervical precancerous lesions. Our research used the SNP function prediction website rSNP for function prediction, and the results suggested that this site is involved in post-transcriptional regulation and can bind to a variety of RNA-binding proteins, thereby affecting the expression level and function of cGAS.

Polymorphism of IL-10 genes

Previous studies have shown that IL-10 can regulate the immune response and inflammatory response by inhibiting the activity of antigen-presenting cells[34]. In addition, IL-10 can inhibit the maturation of various immune cells and the production of cytokines, reducing the immune response level[35]. The IL-10 promoter is highly polymorphic, but currently, only the rs1800871 (-819) and rs1800872 (-592) polymorphisms in the promoter region have been extensively studied. The main reason is that these two sites are respectively related to the MsII and RsaI restriction enzyme (re) sites, which may change the activity of the IL-10 gene[36]. It is reported that IL-10 promoter region polymorphism can affect IL-10 gene transcription and translation, leading to abnormal cell proliferation and cancer development[37]. Wu[38] reported that IL-10 rs1800872 (-592a/c) gene polymorphism is associated with spontaneous HBsAg seroconversion and good outcome of hepatitis B. Compared with hepatitis B carriers of CA and CC genotypes, patients with IL-10 rs1800872 AA genotype have higher serum IL-10 levels. In addition, Li[39] found that IL-10 levels in patients with chronic hepatitis B were significantly higher than those in the general population. These results were confirmed by another research[40]. However, a Japanese study showed that the IL-10rs1800872 genotype is not significantly associated with the risk of HBV infection[41]. Our study found that compared with infants with CC genotype, infants with AA genotype at this locus have a significantly higher risk of intrauterine transmission of HBV. We speculate that infants with the AA genotype may have higher levels of IL-10, which inhibits the maturation of various immune cells and the synthesis of cytokines. This will reduce the body's immune response level, leading to a significant increase in the risk of HBV intrauterine infection.

Polymorphism of TLR3 genes

TLR3 can recognize type I IFN, double-stranded RNA (dsRNA) viruses and dsRNA produced during virus replication[42], activate IRF3 and NF-κB, and also promote the expression of IFN-β and other pro-

inflammatory factors[43]. TLR3 is mainly expressed in hepatocytes, macrophages, NK cells, and biliary epithelial cells, located in the plasma membrane or acidic endosome[44]. And macrophages and NK cells play a vital role in the process of clearing HBV[45, 46]. In addition, during HBV infection, TLR3 can activate hepatic non-parenchymal cells, produce interferon β, and inhibit virus replication[47].

This study found that the SNP at rs3775291 of neonatal TLR3 is closely related to the intrauterine transmission of HBV, which is similar to the results of previous studies. Fischer[48] found that compared with HBeAg-positive patients, the CC genotype of TLR3 rs3775291 appeared more frequently in patients with HBeAg seroclearance, while the TT genotype was more common in HBeAg-positive patients. This further supports that the rs3775291 mutation is related to the innate immune response during HBV infection. In addition, Rong's[49] research also showed that compared with the CC genotype, subjects carrying CT genotype and TT genotype at this locus had an increased risk of chronic HBV infection by 1.42 folds and 2.31 folds, respectively. The specific mechanism of this phenomenon is not yet clear. It may be that mutations at this site cause changes in protein or gene expression, affecting the function and efficiency of signal transduction and thereby altering the immune response.

SNPs rs3775291 is a non-synonymous mutant gene locus located in the fourth exon region of TLR3. The mutation at this locus reduces the location of the soluble outer domain and the dimerization of TLR3 on the membrane, resulting in a decrease in its ability to bind to dsRNA and the signal transduction efficiency. Svensson A[50] detected the expression level of TLR3 mRNA in peripheral blood mononuclear cells (PBMC) of multiple patients infected with human herpesvirus type 2 (HSV-2). It was found that the appearance of the T allele increased the expression of TLR3 mRNA in PBMC relative to the allele C. However, a study showed that the polymorphism of TLR3 rs3775291 locus does not affect the expression of TLR3 in the cell and the localization in the cell, but it will affect the activity of TLR3 and the expression of the receptor on the cell surface[51].

Multiple genes and HBV intrauterine transmission

According to the theory of common diseases-common mutations, common complex diseases are affected by multiple common mutations, and the effect of each common mutation is weak. As a complex disease, the MTCT of HBV is often affected by multiple genes or multiple sites during its occurrence and development. These genes or loci may interact with each other. This study explored the cumulative effect of multiple positive gene loci on the risk of HBV intrauterine transmission. It was found that infants carrying the INTS10 rs28413168 C allele, MB21D1 rs311678 A allele, IL10 rs1800872 A allele, and TLR3 rs3775291 T allele at the same time significantly increased the risk of intrauterine transmission of HBV. This suggested that these four sites may have an additive effect on the risk of HBV intrauterine transmission, but the specific biological mechanism depends on the elucidation of the function of each gene and in-depth functional experimental research.

Conclusion

Infants carrying the INTS10 rs28413168 C allele, MB21D1 rs311678 A allele, IL10 rs1800872 A allele, and TLR3 rs3775291 T allele will significantly increase the risk of HBV intrauterine infection. The above four sites may have an additive effect on the risk of HBV intrauterine transmission, but no gene-gene interaction between these genes has been found.

Declarations

Ethics approval and consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Tongji Hospital, Tongji Medical College, HUST. Written informed consent was obtained from individual or guardian participants.

Consent for publication

This article was published with the consent of all the authors.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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