BMJ Open

Association of IL-10 and IL-10RA single nucleotide polymorphisms with the responsiveness to HBV vaccination in Chinese infants of HBsAg(+)/HBeAg(−) mothers: a nested case–control study

Simin Wen,[1] Yanhua Wu,[1] Yuchen Pan,[1] Mengzhuo Cao,[2,3] Dan Zhao,[1] Chong Wang,[4] Chuan Wang,[5] Fei Kong,[4] Jie Li,[2] Junqi Niu,[4] Jing Jiang[1]

To cite: Wen S, Wu Y, Pan Y, *et al*. Association of IL-10 and IL-10RA single nucleotide polymorphisms with the responsiveness to HBV vaccination in Chinese infants of HBsAg(+)/HBeAg(−) mothers: a nested case–control study. *BMJ Open* 2018;**8**:e022334. doi:10.1136/bmjopen-2018-022334

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2018-022334).

SW and YW contributed equally.

Received 9 March 2018
Revised 2 July 2018
Accepted 27 September 2018

For numbered affiliations see end of article.

Correspondence to
Dr Jing Jiang;
jiangjing19702000@jlu.edu.cn

ABSTRACT

Objectives To investigate the association of interleukin (IL)-10 and IL-10 receptor A (IL-10RA) single nucleotide polymorphisms with the responsiveness to hepatitis B virus (HBV) vaccination in newborns whose mothers were hepatitis B surface antigen (HBsAg)(+)/hepatitis B e antigen (HBeAg)(−).

Design Nested case–control study.

Setting Changchun, China.

Participants 713 infants from a Han Chinese population whose mothers were HBsAg(+)/HBeAg(−) and participated in the prevention of mother-to-child transmission of HBV at the First Hospital of Jilin University from July 2012 to July 2015 were included. Infants were excluded for HBsAg-positive; unstandardised vaccination process; inadequate blood samples; not Han Chinese and failed genotyping.

Results Infants with artificial feeding pattern were correlated with low responsiveness to HBV vaccination (p=0.009). The GG genotype of IL-10 rs3021094 was correlated with a higher risk of low responsiveness to HBV vaccination (OR 2.80, 95% CI 1.35 to 5.83). No haplotype was found to be correlated with responsiveness to HBV vaccination. No gene–gene interaction was found between IL-10 and IL-10RA.

Conclusions Our study found that IL-10 gene variants were significantly associated with the immune response to the HBV vaccine. Identifying these high-risk infants who born to HBsAg(+)/HBeAg(−) mothers and low responses to hepatitis B vaccination will provide evidence for individualised prevention strategies.

Strengths and limitations of this study

- This study is the first to investigate the relationship between interleukin 10 (IL-10) and IL-10 receptor A (IL-10RA) single nucleotide polymorphisms and the responsiveness to hepatitis B virus (HBV) vaccination in infants from a Han Chinese population of hepatitis B surface antigen (HBsAg)(+)/hepatitis B e antigen (HBeAg)(−) mothers.
- We provide evidence for individualised prevention strategies to infants in high risk of low responsiveness to HBV vaccination.
- The sample size of low responders was not sufficient, because the subjects in our study were well managed and immunised regularly, infants of HBsAg-positive mothers who under the effect of long-term stimulation by maternal HBsAg will also increase their immune responsiveness.
- Analysis of long-term immune response to HBV vaccination in infants should be carried out in longer studies.
- The specific mechanism of how the IL-10/IL-10RA pathway inhibits immune responses has not been fully understood which should be further analysed.

INTRODUCTION

Hepatitis B virus (HBV) infection is still a serious global public health problem, with 2 billion people infected worldwide. There are 350 million suffering from chronic HBV infection globally, and three-quarters of them are Chinese.[1 2] It has been reported that people infected with HBV mostly contracted the virus during their perinatal period or in early childhood, mainly from mother-to-child transmission (MTCT) of HBV infection.[3 4] Approximately 90% of infants born hepatitis B surface antigen (HBsAg)-positive will become chronically infected with HBV, and finally, 25% of them will develop into hepatic cirrhosis and hepatocellular carcinoma. The most effective measure to prevent MTCT of HBV infection is by immunising all susceptible individuals with the HBV vaccination.[5–8] However, epidemiological studies have demonstrated that 5%–10% of healthy individuals who received a standard vaccination schedule with hepatitis B vaccines still failed to produce protective levels (≥10 mIU/mL) of antibodies against HBsAg (anti-HBs).[9] Several factors related

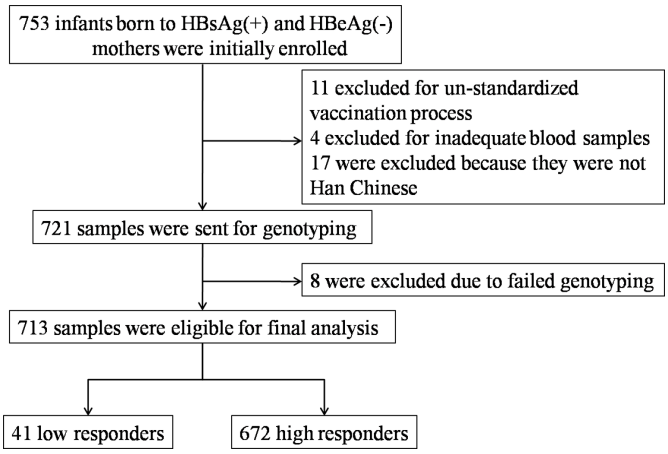

Figure 1 Study flow chart. Low responders, Ab titres <100 mIU/mL; high responders, Ab titres ≥100 mIU/mL. Ab, antibody.

to low response or non-response after vaccination, such as maternal obesity, advancing age, smoking, intramuscular vaccination and host genetic factors, have been reported.[10–12] Among the host genetic factors, many single nucleotide polymorphisms (SNPs) in human leucocyte antigen (HLA)-DP, HLA-DQ and HLA-DR have been confirmed to be strongly associated with the responsiveness to HBV vaccination through genome-wide association studies[13–15] because the HLA system plays an important role in modulating the immune response.[16 17] However, a vaccination study in twins revealed that more than half of the heritability is determined outside this complex.[18] This means that other gene variants among cytokines, such as tumour necrosis factor, interferons, and interleukin (IL)-2, IL-4 and IL-12B, are also correlated with immune responses.[19–23]

IL-10 is a cytokine recognised for its ability to inhibit the activation of antigen-presenting cells (APCs) and immune responses. It is secreted by several cells including T helper subtype 1 (Th1), Th2 and Th17 cell subsets, T Reg cells, $CD8^+$ T cells, and B cells and is also expressed by cells of the innate immune system. The immune-modulating effect of IL-10 starts with its binding to the IL-10 receptor (IL-10R). This receptor complex is composed of two subunits including IL-10RA and IL-10RB. IL-10RB contributes little to IL-10 binding affinity, thus, the effect of IL-10 is mainly due to the IL-10/IL-10RA pathway.[24–26] Recently, polymorphisms of IL-10 and IL-10RA genes have been described to correlate with the immune response to HBV vaccination in infants from black and non-Hispanic white individuals.[19 20] However, the association of the large number of infants of the Han Chinese population whose mothers were positive for HBsAg and at high risk of suffering HBV infection has not been revealed.

In this study, we detected 7 SNPs in IL-10 and IL-10RA genes in 713 infants from a Han Chinese population. The objective of our study was to elucidate the association of IL-10 and IL-10RA polymorphisms with responsiveness to HBV vaccination in newborns whose mothers were HBsAg(+)/hepatitis B e antigen (HBeAg)(-).

MATERIALS AND METHODS

Study population

A total of 753 infants whose mothers were HBsAg(+)/HBeAg(-) and participated in the prevention of MTCT of HBV at the First Hospital of Jilin University from July 2012 to July 2015 were enrolled. Infants were administered intramuscular injections of 100 IU hepatitis B immunoglobulin (HBIG) (Hualan Biological Engineering, Xinxiang, China) and 10 µg HBV vaccine (Hansenula polymorpha yeast-derived recombinant Hepatitis B vaccine; Dalian Hissen Bio-pharm, Dalian, China) within 2 hours after birth, followed by administration of 10 µg HBV vaccine at the ages of 1 month and 6 months. All infants were detected for HBsAg and anti-HBs at 7 months of age. The exclusion criteria were as follows: HBsAg-positive; unstandardised vaccination process; inadequate blood samples; not Han Chinese and failed genotyping. As a result, 40 infants were excluded, and in total, 713

Table 1 Characteristics of subject

Variants	LR (n=41)	HR (n=672)	P values
Maternal HBV DNA (IU/mL)	3.30±2.09	2.87±1.44	0.319
Maternal antiviral therapy			
No	41 (100.0%)	667 (99.3%)	>0.999*
Yes	0 (0.0%)	5 (0.7%)	
Gestational age (week)	39.00±1.38	38.94±1.14	0.231
Delivery mode			
Natural delivery	16 (40.0%)	180 (26.8%)	0.070
Caesarean delivery	24 (60.0%)	491 (73.2%)	
Sex			
Male	21 (51.2%)	356 (53.0%)	0.827
Female	20 (48.8%)	316 (47.0%)	
Birth weight (kg)	3.36±0.44	3.41±0.47	0.657
Feeding pattern			
Breast feeding	8 (20.0%)	259 (38.6%)	0.009
Mixed feeding	8 (20.0%)	167 (24.9%)	
Artificial feeding	24 (60.0%)	245 (36.5%)	
Anti-HBs (mIU/L)	1.60±0.37	3.22±0.48	<0.001

Anti-HBs and maternal HBV DNA were transformed to their logarithms.
*P value for Fisher's exact test.
HBV, hepatitis B virus; HR, high responder (Anti-HBs ≥100 mIU/mL); LR, low responder (Anti-HBs <100 mIU/mL).

Table 2 Genotype and allele distributions of SNPs in low and high responders

SNPs	Variants	LR (n=41), (%)	HR (n=672), (%)	P values	OR (95% CI)*	P values†
rs1800896	TT	35 (85.4)	544 (81.1)		1.00	
	CT	6 (14.6)	120 (17.9)	0.689	0.78 (0.32 to 1.89)	0.578
	CC	0 (0.0)	7 (1.0)		–	0.999
	T	76 (92.7)	1208 (90.0)		1.00	
	C	6 (7.3)	134 (10.0)	0.431	0.71 (0.30 to 1.67)	0.433
rs1800871	GG	18 (43.9)	270 (40.2)		1.00	
	GA	21 (51.2)	315 (46.9)	0.325	1.01 (0.52 to 1.92)	>0.999
	AA	2 (4.9)	86 (12.9)		0.35 (0.08 to 1.53)	0.163
	G	57 (69.5)	855 (63.7)		1.00	
	A	25 (30.5)	487 (36.3)	0.288	0.77 (0.48 to 1.25)	0.289
rs3021094	TT	13 (31.7)	232 (34.8)		1.00	
	GT	16 (39.0)	336 (50.4)	0.044	0.85 (0.40 to 1.80)	0.671
	GG	12 (29.3)	99 (14.8)		2.16 (0.95 to 4.91)	0.065
	T	42 (51.2)	800 (60.0)		1.00	
	G	40 (48.8)	534 (40.0)	0.117	1.43 (0.91 to 2.23)	0.119
rs3790622	GG	29 (70.7)	571 (85.1)		1.00	
	GA	12 (29.3)	96 (14.3)	0.049‡	2.46 (1.21 to 4.99)	0.012
	AA	0 (0.0)	4 (0.6)		–	0.999
	G	70 (85.4)	1238 (92.3)		1.00	
	A	12 (14.6)	104 (7.7)	0.027	2.04 (1.07 to 3.89)	0.030
rs2282494	AA	21 (51.2)	338 (52.3)		1.00	
	AG	16 (39.0)	264 (40.9)	0.771	0.98 (0.50 to 1.91)	0.942
	GG	4 (9.8)	44 (6.8)		1.46 (0.48 to 4.46)	0.503
	A	58 (70.7)	940 (72.8)		1.00	
	G	24 (29.3)	352 (27.2)	0.690	1.11 (0.68 to 1.81)	0.690
rs2508450	CC	37 (90.2)	572 (85.3)		1.00	
	CT	3 (7.4)	96 (14.3)	0.111‡	0.48 (0.15 to 1.60)	0.233
	TT	1 (2.4)	3 (0.4)		5.16 (0.52 to 50.85)	0.160
	C	77 (93.9)	1240 (92.4)		1.00	
	T	5 (6.1)	102 (7.6)	0.616	0.79 (0.31 to 2.00)	0.617
rs4252249	GG	38 (92.7)	630 (93.9)		1.00	
	AG	2 (4.9)	38 (5.7)	0.270‡	0.87 (0.20 to 3.75)	0.855
	AA	1 (2.4)	3 (0.4)		5.53 (0.56 to 54.39)	0.143
	G	78 (95.1)	1298 (96.7)		1.00	
	A	4 (4.9)	44 (3.3)	0.352‡	1.51 (0.53 to 4.32)	0.439

LR (Anti-HBs <100 mIU/mL).
HR (Anti-HBs ≥100 mIU/mL).
*ORs (95% CI) were assessed using univariate logistic regression analysis.
†P value for univariate logistic regression analysis.
‡P value for Fisher's exact test.
HR, high responder; LR, low responder; SNP, single nucleotide polymorphism.

participants were included in this study. The flow chart of our study participants is shown in figure 1.

Patient and public involvement statement

Patients and public were not involved in this work.

Data collection

Maternal parameters were collected according to the registration from enrolled mothers at the outpatient clinic. Mothers were followed up after delivery by telephone call, other parameters including gestational age,

Table 3 The association of IL-10 rs3021094 and rs3790622 with responsiveness to HBV vaccination

SNP	Genotype	Anti-HBs levels*	P values†	OR (95% CI)‡	P values§
IL-10 rs3021094	TT+GT	3.14±0.60	0.212	1.00	0.006
	GG	2.95±0.79		2.80 (1.35 to 5.83)	
IL-10 rs3790622	GG	3.11±0.61	0.244	1.00	0.019
	GA+AA	3.07±0.74		2.37 (1.15 to 4.88)	

*Hepatitis B surface antibody levels were transformed to their logarithms.
†P values were calculated using covariance analysis adjusted for delivery mode and feeding pattern.
‡ORs (95% CI) were assessed using multivariate logistic regression analysis adjusted for delivery mode and feeding pattern.
§P value for multivariate logistic regression analysis adjusted for delivery mode and feeding pattern.
HBV, hepatitis B virus; IL-10, interleukin-10; SNP, single nucleotide polymorphism.

delivery mode, feeding pattern, infant sex, infant birth weight and whether they followed the standardised vaccination process were collected. Mothers whose HBV DNA ≥2000 IU/mL and alanine transaminase (ALT) was twice as high as the upper limit of normal range received antiviral therapy.

Test of HBsAg, anti-HBs and HBV DNA

Venous blood samples were collected from infants at the age of 7 months (1 month after the last dose of the vaccine). The detection of HBsAg in infants was carried out through the chemiluminescent microparticle immunoassay (CMIA) with an Abbott ARCHITECT HBsAg Reagent Kit (Abbott Laboratories, North Chicago, Illinois, USA). An ARCHITECT anti-HBs Reagent Kit (Abbott Laboratories) from CMIA was used to determine the anti-HBs levels, with a range of 0–15000 mIU/mL after 15 dilutions. Infants with serum anti-HBs <100 mIU/mL and anti-HBs ≥100 mIU/mL at 7 months of age were classified as low responders (LRs) and high responders (HRs), respectively. Maternal HBV DNA was assessed by the Roche TaqMan HBV test (Roche Diagnostics, Grenzach, Germany).

SNP selection and genotyping

Seven tag SNPs (four SNPs including rs1800871, rs1800896, rs3021094 and rs3790622 in IL-10; three SNPs including rs2282494, rs2508450 and rs4252249 in IL-10RA) were selected using GVS: http://gvs.gs.washington.edu/GVS147/and SNPinfo: http://snpinfo.niehs.nih.gov/ with minor allele frequencies (MAFs) >0.05 in CHB and r^2>0.8. Genomic DNA was extracted using an AxyPrep Blood Genomic DNA Miniprep kit (Axygen, Union City, California, USA) according to the manufacturer's instructions. Genotyping of each SNP was conducted using the MassARRAY technology platform (Sequenom, San Diego, California, USA) and determined by BioMiao Biological Technology (Beijing, China). The call rates for SNPs in IL-10 rs1800871, rs1800896, rs3021094 and rs3790622, and IL-10RA rs2282494, rs2508450 and rs4252249 were 99.6%, 99.4%, 99.1%, 99.6%, 95.1%, 99.5% and 99.6%, respectively.

Table 4 Association between IL-10 and IL-10RA gene cluster haplotypes and risk of low responsiveness to HBV vaccination

IL-10	SNP				Frequency			Adjusted OR	
Haplotype	rs1800896	rs1800871	rs3021094	rs3790622	Total	HR	LR	(95% CI)	P values
1	T	A	G	G	0.33	0.32	0.34	1.00	–
2	T	G	T	G	0.26	0.26	0.23	0.83 (0.45 to 1.54)	0.55
3	T	A	T	G	0.23	0.21	0.21	0.83 (0.45 to 1.56)	0.57
4	C	G	T	G	0.10	0.10	0.07	0.69 (0.28 to 1.71)	0.42
5	T	A	G	A	0.08	0.08	0.15	1.82 (0.88 to 3.74)	0.10
Rare*	–	–	–	–	–	–	–	–	–
IL-10RA	**SNP**				**Frequency**			**Adjusted OR**	
Haplotype	**rs2282494**	**rs2508450**	**rs4252249**		**Total**	**HR**	**LR**	**(95% CI)**	**P values**
1	A	C	G		0.65	0.65	0.66	1.00	–
2	G	C	G		0.27	0.27	0.28	1.07 (0.65 to 1.77)	0.79
3	A	T	G		0.04	0.04	0.00	0.28 (0.04 to 2.09)	0.21
4	A	T	A		0.03	0.03	0.05	1.38 (0.52 to 3.68)	0.52
Rare*	–	–	–		–	–	–	–	–

*Rare: haplotypes with frequencies <0.01.
HBV, hepatitis B virus; HR, high responder; IL-10RA, interleukin-10 receptor A; LR, low responder; SNP, single nucleotide polymorphism.

Table 5 Gene–gene interactions between IL-10 and IL-10RA and low responsiveness to HBV vaccination

Model							Training	Testing	P values	CV consistency
rs3790622							0.579	0.519	0.828	6/10
rs2282494	rs3021094						0.612	0.486	0.828	6/10
rs1800871	rs2282494	rs3021094					0.653	0.505	0.623	7/10
rs1800871	rs2282494	rs2508450	rs3021094				0.687	0.490	0.623	4/10
rs1800871	rs1800896	rs2282494	rs2508450	rs3021094			0.722	0.543	0.172	6/10
rs1800871	rs1800896	rs2282494	rs2508450	rs3790622	rs3021094		0.747	0.528	0.377	10/10
rs1800871	rs1800896	rs2282494	rs2508450	rs3790622	rs3021094	rs4252249	0.751	0.539	0.377	10/10

CV, cross validation; HBV, hepatitis B virus; IL-10RA, interleukin-10 receptor A.

Statistical analyses

The Hardy-Weinberg equilibrium (HWE) test for assessing the SNP genotype frequency among subjects was conducted. Assessment of pairwise linkage disequilibrium (LD) was performed by the Haploview V.4.2 software. Anti-HBs and maternal HBV DNA were transformed to their logarithms. Continuous variables with normal distribution through Kolmogorov-Smirnov test were described as the mean±SD and compared by Student's t-test. Categorical data were summarised as frequencies (percentages) and compared using χ^2 test or Fisher's exact test when appropriate. Variants which p<0.20 were included in the multivariate analysis. Associations between SNPs and responsiveness to HBV vaccination were calculated using univariate and multivariate logistic regression models adjusted for delivery mode and feeding pattern. Covariance analysis was performed to assess the association of IL-10 rs3021094 and rs3790622 gene variants with anti-HBs levels adjusted for delivery mode and feeding pattern. The haplotype analysis was conducted using SNPStats (http://bioinfo.iconcologia.net/SNPStats). Gene–gene interactions were calculated with the GMDR program (V.0.9, http://sourceforge.net/projects/gmdr/). The level of statistical significance was p<0.05. The significance level was turned to p<0.007 (0.05/7=0.007) according to Bonferroni correction while analysing the relationship between SNPs and the responsiveness to HBV vaccination. All analyses were performed by the SPSS program (V.22.0).

RESULTS

Characteristics of subjects

A total of 713 subjects were included in the final analysis. As shown in table 1, 41 subjects were LRs and 672 subjects performed with high responsiveness. Infants with artificial feeding pattern were correlated with low responsiveness to HBV vaccination (p=0.009). There were no significant differences in maternal HBV DNA, maternal antiviral therapy, gestational age, delivery mode, infant sex and infant birth weight between the LR and HR groups.

Association of SNPs with responsiveness to HBV vaccination

The distributions of rs1800871, rs1800896, rs3021094, rs3790622, rs2282494, rs2508450 and rs4252249 were all in HWE (p=0.953, 0.983, 0.361, 0.955, 0.961, 0.879 and 0.089, respectively). Univariate logistic regression analysis showed that the frequency of IL-10 rs3790622 A allele was tended to higher in the LR group than that of in the HR group (p=0.030) (table 2). When associations were performed using multivariate logistic regression analysis included variants which p<0.20 (table 3), compared with the TT+GT genotype of IL-10 rs3021094, the GG genotype was correlated with a higher risk of low responsiveness to HBV vaccination adjusted for delivery mode and feeding pattern (OR 2.80, 95% CI 1.35 to 5.83), the difference was still significant after the Bonferroni correction (p=0.006). In addition, the GA+AA genotype of IL-10 rs3790622 was tended to be associated with a higher risk of low responsiveness to HBV vaccination compared with the GG genotype, but the difference was not significant after the Bonferroni correction (table 3).

Haplotype analysis and gene–gene interactions

The LD structures of four SNPs in IL-10 and three SNPs in IL-10RA are presented in online supplementary file 1, and they were not in LD with each other. We found that no haplotype was correlated with responsiveness to HBV vaccination in table 4. Gene–gene interaction analysis showed that there was no interaction between IL-10 and IL-10RA and low responsiveness to HBV vaccination (table 5).

DISCUSSION

The IL-10/IL-10RA pathway plays an important role in the immune response to HBV vaccine. The process of antibody production to HBsAg is T-cell dependent and requires Th-cell activation.[27] Velu *et al* suggested that lacking a Th1 and Th2 response may result in unresponsiveness to recombinant hepatitis B vaccines.[28] As a cross-regulator of Th1/Th2 immunity, IL-10 was demonstrated to be an important cytokine suppressing autoimmunity and inflammatory responses, according to various reports.[29–31] IL-10 can inhibit APCs by downregulating the

cell surface expression of HLA molecules,[32] which may affect the immune response. Moreover, it has also been reported that the IL-10/IL-10RA pathway may inhibit immune responses by downregulating the activation of macrophages via the janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway.

In our research, we found that IL-10 rs3021094 gene variants were correlated with the immune response to HBV vaccination in newborn children whose mothers were HBsAg(+)/HBeAg(−). The MAF of IL-10 rs3021094 in East Asian is 0.47 (Our study: 0.41), which is much higher than that of in other populations. Thus, no other related study has reported this SNP while analysing the relationship between gene variants and responses to HBV vaccination. IL-10 rs3021094 was located in transcription factor binding sites (TFBSs) according to SNPinfo. It is known that gene variants in TFBS may influence the expression of proteins by affecting the transcriptional process. As a result, we presumed that rs3021094 T to G change may enhance the binding ability of the gene to the transcription factor, then increase IL-10 gene and protein expression and downregulate the immune response to HBV vaccination. Our research did not find an association of gene variants, IL-10 rs1800896 and IL-10 rs1800871, with variable antibody responses to HBV vaccination. Related studies from Wang *et al*[21] and Macedo *et al*[23] also found no relationship between rs1800896 and rs1800871 gene variants and the responsiveness to HBV vaccination. However, Yukimasa *et al* had drawn different results.[33] They found that the IL-10 rs1800896 CT genotype was present more frequently in the low titre group than in the high titre group of HBV vaccination in a Japanese population of young adults. Reasons for the difference in immune responses between adults and infants are speculative. Further studies of larger samples in newborn children of the Han Chinese population should be carried out. We did not find the association of gene variants of IL-10RA with variable antibody responses to HBV vaccination, and the titres of anti-HBs had no significant differences between different genotypes of SNPs of IL-10RA.

There are still certain limitations to our study. First, the sample size of the outcome event was not sufficient. In our study, the rate of low responsiveness to HBV vaccination was 5.75%, which was much lower than other previous studies.[20 34 35] The well managed and regular immunisation of subjects in the MTCT cohort may contribute to a better response to HBV vaccination. Moreover, infants of HBsAg-positive mothers were under the effect of long-term stimulation by maternal HBsAg, which may also increase their immune responsiveness. Second, the testing time of anti-HBs for infants was only at 7 months (1 month after the third vaccination) in this study, at which time the levels of anti-HBs reached their peak. However, the level of anti-HBs changes over time, so long-term immune response to HBV vaccination in infants should be analysed in longer studies. In addition, the specific mechanism of how the IL-10/IL-10RA pathway inhibits immune responses is still only hypothesised and has not been fully understood. Therefore, further studies should be undertaken to investigate the relationship between IL-10 expression and the immune response to HBV vaccination as well as how IL-10 SNPs affect this.

In conclusion, our study found that IL-10 gene variants were significantly associated with the immune response to the HBV vaccine. Identifying these high-risk infants who born to HBsAg(+)/HBeAg(−) mothers and low responses to hepatitis B vaccination will provide evidence for individualised prevention strategies.

Author affiliations
[1]Department of Clinical Research, First Hospital of Jilin University, Changchun, China
[2]Department of Microbiology and Infectious Disease Center, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China
[3]Division of Education, Beijing Jishuitan Hospital, Beijing, China
[4]Department of Hepatology, First Hospital of Jilin University, Changchun, China
[5]Department of Child Healthcare, Maternal and Child Health Care and Family Planning Service Center of Chaoyang District, Beijing, China

Acknowledgements The authors would like to thank all those who participated in this study, especially Ying Song for her work on the follow-up of the subjects.

Contributors JJ, JN and JL designed the study. SW, YP, MC, DZ, ChoW, ChuW and FK collected human samples and clinical data. SW detected the HBsAg and anti-HBs. SW and YW conducted the statistical analysis of the data and wrote the paper.

Funding This study was supported by the National Major Scientific and Technological Special Project during the 13th Five-year Plan Period (2017ZX1020120100301), Health Research Project of Jilin Province (2015Z003) and China Hepatitis Prevention Foundation Project (TIAN QING Liver Disease foundation, TQGB20140137).

Competing interests None declared.

Patient consent Parental/guardian consent obtained.

Ethics approval This study was approved by the Medical Ethics Committee of the First Hospital of Jilin University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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