

RESEARCH ARTICLE

Deltex1 Polymorphisms Are Associated with Hepatitis B Vaccination Non-Response in Southwest China

Bin Xie¹✉, Penghui Zhang²✉, Menggang Liu¹, Wei Zeng¹, Juntao Yang¹, Hongming Liu¹*✉

1 Department of Hepatobiliary Surgery, Daping Hospital & Institute of Surgery Research, Third Military Medical University, Chongqing, China, **2** Department of Clinical Laboratory Medicine, Children's Hospital of Chongqing Medical University, Chongqing, China

✉ These authors contributed equally to this work.

✉ Current address: Department of Hepatobiliary Surgery, Daping Hospital & Institute of Surgery Research, Third Military Medical University, Chongqing, China.

* lhming56@163.com



Abstract

Background

Hepatitis B vaccination is the most important tool available for preventing hepatitis B virus (HBV) infection and reducing the prevalence of infection. However, epidemiological studies have demonstrated that more than 5% of patients exhibit a non- or hypo-response to the HBV vaccine. Genetic variations associated with T cell immunity contribute to the immune response to HBV vaccination. The *deltex 1* (DTX1) gene is involved in T cell anergy, which may also be associated with the immune response to the HBV vaccination.

Methods

We detected 10 single nucleotide polymorphisms (SNPs) in or around the DTX1 gene in 601 infants out of a population from Southwest China, including 299 high responders (HRs; HBsAb > 100 mIU/mL) and 302 non-responders (NRs; HBsAb < 10 mIU/mL). An additional validation study was performed, comprising 230 adult patients (135 HRs and 95 NRs) from Southwest China.

Results

This study found that the minor allele 'G' of rs2384077 (adjusted $p = 2.63E^{-04}$) and the minor allele 'C' of rs10744794 (adjusted $p = 3.69E^{-04}$) in the first intron of the DTX1 gene were remarkably associated with the immune response to HBV vaccination in both infant and adult populations. Moreover, a subsequent analysis indicated that haplotypes (A-T, G-C) of the two SNPs were significantly associated with the immune response to HBV vaccination.

Conclusions

Two SNPs (rs2384077 and rs10744794) in an intron of DTX1 and the linkage disequilibrium (LD) block are significantly associated with the immune response to HBV vaccination. The

OPEN ACCESS

Citation: Xie B, Zhang P, Liu M, Zeng W, Yang J, Liu H (2016) Deltex1 Polymorphisms Are Associated with Hepatitis B Vaccination Non-Response in Southwest China. PLoS ONE 11(2): e0149199. doi:10.1371/journal.pone.0149199

Editor: Chiaho Shih, Academia Sinica, TAIWAN

Received: October 10, 2015

Accepted: January 28, 2016

Published: February 19, 2016

Copyright: © 2016 Xie et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: Hongming Liu received funding from the Nature Science Foundation of China, grant number: 81172739 (<http://www.nsf.gov.cn>).

Competing Interests: The authors have declared that no competing interests exist.

functional element annotation of the LD block between the two SNPs contains four transcriptional regulatory elements. The results suggest that these two SNPs may be involved in the immune response to HBV vaccination.

Introduction

Hepatitis B virus (HBV) infection is a global problem that tremendously impacts affected individuals' health as well as society in general. It has been estimated that more than two billion people suffer from HBV infection, and approximately 350 million chronically infected people have a high mortality risk due to cirrhosis of the liver and liver cancer [1]. HBV vaccination is the most important tool available for preventing HBV infection and decreasing its prevalence [2, 3]; however, epidemiological studies have demonstrated that approximately 5%-10% of patients exhibit a non- or hypo-response that results in vaccine failure [4]. Therefore, uncovering the mechanism underlying this non- or hypo-response and finding solutions for vaccine failure are important medical issues.

Studies have demonstrated that HBV infection appears in family aggregations, which may be caused by both environmental and genetic factors. A non- or hypo-response to the HBV vaccine is often the result of several endogenous and exogenous factors, including psychological stress, nutrition, smoking, alcohol consumption, co-infection (along with HIV infection), gender, age, body weight, race, and the type and quantity of vaccine administered [5–7]. These factors can affect the immune response after vaccination by impacting the function of the immune system. Numerous studies have confirmed that the body's response after vaccination is closely related to the genetic background of the immune system [8, 9]. For example, the deletion of genes in human leukocyte antigen complexes and deficits in antigen-presenting cell (APC) function were previously found to be associated with a non- or hypo-response to the HBV vaccine [10, 11]. In addition, single nucleotide polymorphisms (SNPs) in human leukocyte antigen (HLA), tumor necrosis factor (TNF), interferon (IFN), interleukin (IL)-1, IL-4, IL-10, IL-15 and corresponding genes are associated with HBV vaccine failure [12–19]. Nevertheless, most of the genes evaluated in prior studies are related to T cell activation, and there has been a lack of research on the functional impact of the affected genes.

The activation and proliferation of T cells are important steps in the immune response to HBV vaccination [20–22]. Dysfunction of helper T cells and special types of B cells due to underlying disease has been demonstrated to be related to a non- or hypo-response; the HBsAg of T cells are also related to vaccine failure. The immune response is largely controlled by T cells, which can recognize the HBsAg and regulate other immunocytes to maintain the production of HBsAb. One indication of successful HBV vaccination is continued HBsAb production [23].

Deltex1 (DTX1) is a single transmembrane protein that has been identified as a key factor in T cell immunity [24]. Recently, Hsiao et al. demonstrated that DTX1 is a target of the transcription factor (TF) nuclear factor of activated T cells (NFAT). NFAT promotes T cell anergy and increased DTX1 expression, which may attenuate T cell activation [25]. We propose that DTX1 may play a role in the T cell response to HBV vaccination.

In this study, we detected 10 SNPs in or around the DTX1 gene in 601 infants from a population in Southwest China, including 299 infants with successful vaccination (HBsAb > 100 mIU/mL) and 302 with vaccine failure (HBsAb < 10 mIU/mL), using an improved multiplex ligation detection reaction (iMLDR) technique. A total of 230 adults from Southwest China were also examined in a validation study. Moreover, a haplotype analysis was performed to

assess the functional relevance of the SNPs and to detect the DNA regions highly associated with the immune response to HBV vaccination.

Materials and Methods

Study population

This study was approved by the Experimental and Ethics Committees of Daping Hospital, Third Military Medical University, and the written informed consent was obtained from each participating subject or the subject's guardian (for infants). Two independent Chinese Han populations were used in these studies. The first population was composed of infants recruited from the Chongqing Maternal and Child Care Service Centre. The following inclusion criteria were used for this population: (1) born full term; (2) body mass index (BMI) in the normal range; (3) HBsAg-negative status; and (4) an absence of underlying diseases, such as congenital heart disease, neonatal aspiration pneumonia, hypoxic-ischemic encephalopathy, or cytomegalovirus infection. Peripheral blood samples were collected, and all infants were administered intramuscular injections of 10 µg of the HBV vaccine at 0, 1, and 6 months. Infants with serum HBsAb levels >100 mIU/mL at 7 months were classified as high responders (HRs). Infants with serum HBsAb levels <10 mIU/mL received additional intramuscular injections of 10 µg of the HBV vaccine three times over a period of 3–6 months. Infants with serum HBsAb levels that remained at <10 mIU/mL were recruited as non-responders (NRs). Ultimately, 601 infants including 302 NR participants and 299 HR participants were recruited to be a part of the first study.

The second population consisted of adults recruited from Chongqing Daping Hospital. A total of 1,845 participants were initially recruited in 2012. All of these participants had received HBV vaccines within the last year and provided written informed consent for participation in the study. Demographic information, smoking history, vaccination history, chronic disease status, immunosuppressive disease status and medication lists were obtained using questionnaires. In this population, 95 participants with serum HBsAb levels <10 mIU/mL were classified as NRs, and there were 1,479 participants with serum HBsAb levels >100 mIU/mL. We chose the HR participants after taking gender, age, BMI, and smoking status into account. At first, we excluded participants whose age and BMI were significant departures from those of the NRs. We then excluded certain individuals to keep the proportions of gender and smoking status among the HRs similar to those among the NRs. Ultimately, 135 participants were classified as HRs.

SNP selection and genotyping

The DTX1 gene is located in the 12q24.13 region, spanning approximately 40.17 kb. A total of 24 SNPs (minor allele frequencies (MAFs) >5%) in the DTX1 gene have been identified in the HapMap Han Chinese population from Beijing; these SNPs are located in non-coding regions (see [S1 Fig](#)). We selected 10 tagSNPs using the HaploviewV4.2 software program (using two-marker haplotypes). Genomic DNA was extracted from frozen venous blood using the TIANamp Blood DNA Kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions.

SNP genotyping was performed using an iMLDR technique developed by Genesky Biotechnologies Inc. (Shanghai, China). We applied the iMLDR technique to genotype the 10 SNP loci in one ligation reaction. A multiplex of PCR reactions was designed to amplify the SNP loci. The first PCR reaction, performed in a 20-µL total volume, contained 1X PCR buffer (Takara, Dalian, China), 3.0 mM Mg²⁺, 0.3 mM dNTPs, 1 U of Hot-Start Taq DNA polymerase (Takara, Dalian, China), 1 µL of primer mixture 1, and ~20 ng of genomic DNA.

The second PCR reaction, also performed in a 20 μ L total volume, contained 1X GC Buffer I (Takara), 3.0 mM Mg²⁺, 0.3 mM dNTPs, 1 U of Hot-Start Taq DNA polymerase (Takara), 1 μ L of primer mixture 2, and ~20 ng of genomic DNA. The primer information is presented in [S1 Table](#).

The PCR program for both reactions was 95°C for 2 min, 11 cycles (94°C for 20 s, 65°C-0.5°C/cycle for 40 s, and 72°C for 1 min and 30 s), 24 cycles (94°C for 20 s, 59°C for 30 s, and 72°C for 1 min and 30 s), 72°C for 2 min, and hold at 4°C. The two PCR products were equally mixed and purified by digestion with 1 U of shrimp alkaline phosphatase at 37°C for 1 h and at 75°C for 15 min. The ligation reaction, performed in a 20- μ L total volume, contained 1 X ligation buffer, 80 U of Taq DNA Ligase (NEB), 1 μ L of labeling oligo mixture, 2 μ L of probe mixture, and 5 μ L of purified PCR product mixture. The oligo or probe information for these mixtures is presented in [S1 Table](#). The ligation cycling program was 95°C for 2 min, 38 cycles (94°C for 1 min and 56°C for 4 min), and hold at 4°C. A total of 0.5 μ L of ligation product was loaded into an ABI 3730xl, and the raw data were analyzed using the GeneMapper 4.1 software program. All of the primers, probes, and labeling oligos were designed by and ordered from Genesky Biotechnologies, Inc. (Shanghai, China).

Statistical analyses

SNP-specific deviations from the Hardy-Weinberg equilibrium (HWE) were tested using HWE exact tests (“genetics” package implemented in R language). Allele frequencies were compared between two groups using Pearson’s χ^2 test, and genotype frequencies were compared between two groups using Fisher’s exact test. Bonferroni correction was used to correct the *p*-values. Logistic regression analysis was used to adjust for confounding factors. The association between genotyped polymorphisms and the risk of disease was estimated using corrected *p*-values, odds ratios (ORs), and 95% confidence intervals (CIs). Certain ORs of minor alleles that were less than 1 were converted into a value greater than or equal to 1.0 by calculating the ORs of the alternative alleles. The statistical analyses were performed using the PLINK 1.07 software program. All statistical tests were two sided, and corrected *p*-values < 0.05 were considered to be statistically significant. The pairwise *D* measures of linkage disequilibrium (LD) for all SNPs as well as the haplotype distributions in the HRs and NRs were calculated using Haploview v4.2 software. A 95% CI for *D'* between 0.7 and 0.98 was considered to indicate strong LD, and a block was created if 95% of the informative comparisons were considered to indicate “strong LD”. Haplotypes occurring with a frequency < 1% were excluded. The 3 strongest LD blocks (with each block having 3 haplotypes) were then identified (see [Fig 1](#)). A haplotype association study was performed to test the set of blocks using Pearson’s χ^2 test. Logistic regression analysis was used to adjust for confounding factors (age, gender, BMI and smoking status). Correction for multiple testing was performed separately in the infants, adults and combined data set using Bonferroni correction.

Results

Basic characteristics of the two study populations

The demographic and clinical characteristics of the two study populations are presented in [Table 1](#). In the infant population, all of the infants were less than 6 years old, and no significant differences were noted in gender or BMI between the NR and the HR groups. In the adult population (1,845 healthy volunteers), 95 qualified as NRs (<10 mIU/mL), and 135 qualified as HRs (>100 mIU/mL). Moreover, no significant differences in gender or BMI were noted between the NR and the HR groups among the adults.

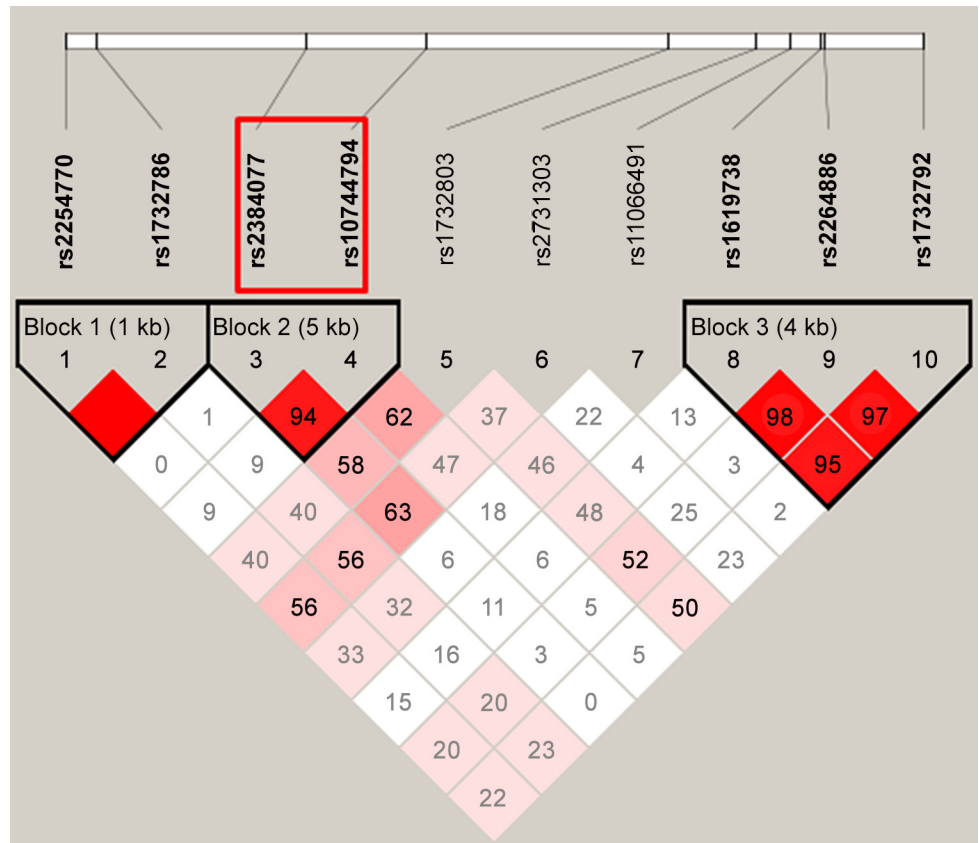


Fig 1. LD blocks of the 10 SNPs in DTX1.

doi:10.1371/journal.pone.0149199.g001

Association between SNPs and immune response to hepatitis B vaccination

Infant population. In the genotyping experiments involving the 10 SNPs in the infant population, the call rate of all 10 SNPs was greater than 99%. More specifically, only one SNP (rs11066491) had a call rate below 100% (99.64%), so the remaining nine SNPs all had a call rate of 100% (S2 Table). HWE was analyzed for the infant population using PLINK 1.07, and

Table 1. Characteristics of the infant and adult populations.

| Infants | | | | Adults | | | |
|--------------------------|------------|------------|----------|--------------------------|------------|------------|----------|
| HBsAb | NR | HR | <i>p</i> | HBsAb | NR | HR | <i>p</i> |
| | N = 302 | N = 299 | | | N = 135 | N = 95 | |
| BMI (kg/m ²) | 17.51±3.67 | 17.68±4.12 | 0.071 | BMI (kg/m ²) | 23.41±3.55 | 23.56±3.81 | 0.058 |
| Age (months) | 24.4±9.56 | 22.6±11.34 | 0.151 | Age (years) | 38.5±6.58 | 37.2±7.14 | 0.079 |
| Gender | | | | Gender | | | |
| Male | 186 | 192 | 0.505 | Male | 82 | 53 | 0.453 |
| Female | 116 | 107 | | Female | 53 | 42 | |
| | | | | Smoker | | | |
| | | | | Yes | 58 | 46 | 0.413 |
| | | | | No | 77 | 49 | |

doi:10.1371/journal.pone.0149199.t001

all SNPs conformed to HWE (corrected $p > 0.05$). All 601 samples had a call rate greater than 90%, and 600 of them had a call rate of 100%. Logistic regression analysis with an additive model was used to adjust for the confounding factors (age, gender and BMI). The allele and genotype distributions of the 10 tagSNPs in the NR and HR groups are presented in [Table 2](#). Two SNPs (rs2384077 and rs10744794) were significantly associated with the immune response to the HBV vaccination (adjusted $p < 0.05$). The SNPs rs2384077 and rs10744794 are both located in the first intron of DTX1. The frequency of the minor allele 'G' of rs2384077 was significantly reduced in the NR group relative to the HR group (adjusted $p = 2.63E^{-04}$), with an adjusted OR of 1.9948 (95% CI: 1.4518–2.5974) for the alternative allele. The distribution of the genotypes significantly differed between the NR and the HR groups (adjusted $p = 7.345E^{-05}$). Meanwhile, the frequency of the minor allele 'C' of rs10744794 was significantly reduced in the NR group compared with the HR group (adjusted $p = 3.69E^{-04}$), and the adjusted OR was 1.6883 (95% CI: 1.2799–2.2232) for the alternative allele. The distribution of the genotypes between the NR and the HR groups was also significantly different for this allele (adjusted $p = 5.23E^{-05}$).

Adult population. In the adult population, the call rates of the SNPs and samples were greater than 95%, and all SNPs conformed to HWE. Logistic regression analysis with an additive model was used to adjust for the confounding factors (age, gender, BMI and smoking status). The allele and genotype distributions of the adult population are presented in [Table 2](#). The same two SNPs (rs2384077 and rs10744794) were significantly associated with the immune response to the HBV vaccination. The frequency of the minor allele 'G' of rs2384077 was significantly reduced in the NR group compared with the HR group (adjusted $p = 2.16E^{-03}$), and the adjusted OR was 2.0268 (95% CI: 1.3016–3.87) for the alternative allele. The distribution of the genotypes between the NR and the HR groups was significantly different (adjusted $p = 4.56E^{-03}$). Meanwhile, the frequency of the minor allele 'C' of rs10744794 was significantly reduced in the NR group compared with the HR group (adjusted $p = 4.82E^{-03}$), with an adjusted OR of 1.6852 (95% CI: 1.121–2.7925) for the alternative allele. The distribution of the genotypes between the NR and the HR groups was also significantly different for this allele (adjusted $p = 4.26E^{-03}$).

Combined analyses. We also combined the two populations for a genotyping experiment. Logistic regression analysis with an additive model was used to adjust for the confounding factors (age, gender, BMI and smoking status). The same two SNPs were both highly significantly associated with the immune response to the HBV vaccination. The adjusted p -values for rs2384077 and rs10744794 were $1.17E^{-05}$ and $3.93E^{-05}$, respectively, and the adjusted ORs were 2.0412 (95% CI: 1.4892–2.586) and 1.6658 (95% CI: 1.3086–2.0433) for the respective alternative alleles. The distribution of the genotypes between the NR and the HR groups was also significantly different (adjusted $p = 3.57E^{-07}$ and $4.46E^{-07}$, respectively).

Haplotype analysis and functional element annotation for two SNPs

To assess the functional relevance of these two SNPs, we constructed haplotypes for 10 SNPs using the accelerated Expectation Maximization algorithm, implemented using HaploviewV4.2 software. As presented in [Fig 1](#), rs2384077 was in LD with rs10744794 as a block (block 2, $r^2 = 0.94$). As shown in [Table 3](#), the haplotypes of the LD block were significantly associated with the immune response to the HBV vaccination in each population and in the combined results. The significant individual haplotypes included A-T (infants: adjusted $p = 4.14E^{-05}$, adults: adjusted $p = 2.03E^{-02}$, combined: adjusted $p = 3.67E^{-06}$) and G-C (infants: adjusted $p = 6.24E^{-03}$, adults: adjusted $p = 4.10E^{-03}$, combined: adjusted $p = 5.33E^{-06}$). The other two LD blocks were not significantly associated with the vaccine-induced immune response (data not shown).

Table 2. Minor allele and genotype frequencies of the SNPs associated with the response to hepatitis B vaccination in infants and adults.

| SNP | Minor allele | | | | Genotype | | | | | | |
|-----------------|--------------|----|-----|-----|---------------------------------|-----------------|------------------------------------|----------------|----------------|--------------------------------------|-----------------|
| | A1 | A2 | NR | HR | Adjusted p^a (χ^2 test) | Corrected p^b | Adjusted OR (95% CI) ^a | NR | HR | Adjusted p^a (Fisher's exact test) | Corrected p^b |
| Infants | | | | | | | | | | | |
| rs2254770 | C | G | 214 | 212 | 0.9954 | 1 | 1.0021(0.7931–1.269) | 33/148/ 121 | 45/122/ 132 | 0.1134 | 1 |
| rs1732786 | G | A | 215 | 215 | 0.9113 | 1 | 1.0063(0.7994–1.2845) ^c | 33/149/ 120 | 45/125/ 129 | 0.1478 | 1 |
| rs2384077 | G | A | 61 | 113 | 2.63E-04 | 2.63E-03 | 1.9948(1.4518–2.5974) ^c | 4/53/245 | 5/103/ 191 | 7.35E-05 | 7.35E-04 |
| rs10744794 | C | T | 97 | 148 | 3.69E-04 | 3.69E-03 | 1.6883(1.2799–2.2232) ^c | 9/79/214 | 7/134/ 158 | 5.23E-05 | 5.23E-04 |
| rs1732803 | G | T | 158 | 170 | 0.4242 | 1 | 1.1091(0.8628–1.4312) ^c | 19/120/ 163 | 30/110/ 159 | 0.2874 | 1 |
| rs2731303 | A | C | 124 | 146 | 0.1453 | 1 | 1.2268(0.9488–1.6311) ^c | 13/98/ 191 | 13/120/ 166 | 0.18234 | 1 |
| rs11066491 | G | C | 126 | 126 | 0.934 | 1 | 1.0097(0.757–1.3021) ^c | 11/104/ 187 | 17/92/ 190 | 0.3936 | 1 |
| rs1619738 | G | C | 149 | 144 | 0.8221 | 1 | 1.011(0.7919–1.341) | 14/121/ 167 | 18/108/ 173 | 0.5234 | 1 |
| rs2264886 | G | A | 121 | 116 | 0.7943 | 1 | 1.025(0.7821–1.377) | 11/99/ 192 | 13/90/ 196 | 0.7885 | 1 |
| rs1732792 | A | G | 133 | 123 | 0.5956 | 1 | 1.071(0.8263–1.421) | 12/109/ 181 | 12/99/ 188 | 0.7793 | 1 |
| Adults | | | | | | | | | | | |
| rs2254770 | C | G | 67 | 98 | 0.8667 | 1 | 1.0121(0.7138–1.5244) ^c | 10/47/38 | 23/52/60 | 0.2034 | 1 |
| rs1732786 | G | A | 68 | 99 | 0.8942 | 1 | 1.0298(0.6789–1.5179) ^c | 10/48/37 | 23/53/59 | 0.1934 | 1 |
| rs2384077 | G | A | 21 | 61 | 2.16E-03 | 2.16E-02 | 2.0268(1.3016–3.87) ^c | 1/19/75 | 5/51/79 | 4.56E-03 | 4.56E-02 |
| rs10744794 | C | T | 33 | 78 | 4.82E-03 | 4.82E-02 | 1.6852(1.121–2.7925) ^c | 3/27/65 | 6/66/63 | 4.26E-03 | 4.26E-02 |
| rs1732803 | G | T | 50 | 86 | 0.3216 | 1 | 1.3087(0.8666–1.9759) ^c | 5/40/50 | 19/48/68 | 0.09745 | 0.9745 |
| rs2731303 | A | C | 46 | 69 | 0.7815 | 1 | 1.0678(0.6892–1.6361) ^c | 3/40/52 | 6/57/72 | 0.9683 | 1 |
| rs11066491 | G | C | 41 | 60 | 0.8734 | 1 | 1.0316(0.6575–1.6121) ^c | 4/33/58 | 9/42/84 | 0.0754 | 0.754 |
| rs1619738 | G | C | 49 | 57 | 0.2499 | 1 | 1.201(0.8367–1.989) | 6/37/52 | 8/41/86 | 0.3843 | 1 |
| rs2264886 | G | A | 38 | 45 | 0.3673 | 1 | 1.18(0.7633–1.974) | 5/28/62 | 6/33/96 | 0.6634 | 1 |
| rs1732792 | A | G | 41 | 47 | 0.2735 | 1 | 1.194(0.8061–1.995) | 5/31/59 | 5/37/93 | 0.5782 | 1 |
| Combined | | | | | | | | | | | |
| rs2254770 | C | G | 281 | 310 | 0.8992 | 1 | 1.0009(0.823–1.2309) ^c | 43/195/ 159 | 68/174/ 192 | 0.02013 | 0.2013 |
| rs1732786 | G | A | 283 | 314 | 0.8341 | 1 | 1.0118(0.834–1.2452) ^c | 43/197/ 157 | 68/178/ 188 | 0.0232 | 0.232 |
| rs2384077 | G | A | 82 | 174 | 1.17E-05 | 1.17E-04 | 2.0412(1.4892–2.586) ^c | 5/72/320 | 10/154/ 270 | 3.57E-07 | 3.57E-06 |
| rs10744794 | C | T | 130 | 226 | 3.93E-05 | 3.93E-04 | 1.6658(1.3086–2.0433) ^c | 12/106/ 279 | 13/200/ 221 | 4.46E-07 | 4.46E-06 |
| rs1732803 | G | T | 208 | 256 | 0.1547 | 1 | 1.1438(0.8993–1.4484) ^c | 24/160/ 213 | 49/158/ 227 | 0.02657 | 0.2657 |

(Continued)

Table 2. (Continued)

| SNP | Minor allele | | | | Genotype | | | | | | |
|------------|--------------|----|-----|-----|-------------------------------------|-----------------|------------------------------------|----------------|----------------|--|-----------------|
| | A1 | A2 | NR | HR | Adjusted $p^a(\chi^2 \text{ test})$ | Corrected p^b | Adjusted OR (95% CI) ^a | NR | HR | Adjusted $p^a(\text{Fisher's exact test})$ | Corrected p^b |
| rs2731303 | A | C | 170 | 215 | 0.2101 | 1 | 1.1678(0.905–1.4903) ^c | 16/138/ 243 | 19/177/ 238 | 0.1834 | 1 |
| rs11066491 | G | C | 167 | 186 | 0.9074 | 1 | 1.0147(0.8032–1.2768) ^c | 15/137/ 245 | 26/134/ 274 | 0.3721 | 1 |
| rs1619738 | G | C | 198 | 201 | 0.4526 | 1 | 1.0796(0.8806–1.388) | 20/158/ 219 | 26/149/ 259 | 0.2874 | 1 |
| rs2264886 | G | A | 159 | 161 | 0.4847 | 1 | 1.085(0.8558–1.3762) | 16/127/ 254 | 19/123/ 292 | 0.6107 | 1 |
| rs1732792 | A | G | 174 | 170 | 0.3114 | 1 | 1.113(0.8793–1.415) | 17/140/ 240 | 17/136/ 281 | 0.5035 | 1 |

a: p -values and OR (95% CI) adjusted for age, gender, BMI, and smoking status (for adults and combined) using logistic regression.

b: p -values corrected by Bonferroni correction.

c: adjusted ORs for the alternative alleles of the minor alleles (to keep the OR greater than or equal to 1).

doi:10.1371/journal.pone.0149199.t002

To further explore the functional mechanism by which the two SNPs in the 5-kb region (LD block) affect immunity following vaccination against HBV, we investigated this region by functional element annotation using the Regulatory Features of the Ensembl database (url: <http://asia.ensembl.org/info/genome/funcgen/index.html>). As presented in Fig 2, four transcriptional regulatory elements, including two enhancers (ENSR00001615188, ENSR00001615189), one TF-binding site (ENSR00000437298) and one promoter-flanking region (ENSR00000654472), were located in this region. rs10744794 is located in the promoter-flanking region (ENSR00000654472).

Table 3. Haplotype effects for DTX1 (rs2384077-rs10744794).

| Haplotype | Frequency | Case, Control Frequencies | $p(\chi^2 \text{ test})$ | Adjusted p^a | Corrected p^b |
|-----------------|-----------|---------------------------|--------------------------|-----------------|-----------------|
| Infants | | | | | |
| AT | 0.79 | 0.839, 0.740 | 2.58E-05 | 4.14E-05 | 3.73E-04 |
| GC | 0.138 | 0.101, 0.177 | 1.00E-04 | 6.24E-03 | 0.0562 |
| AC | 0.065 | 0.060, 0.071 | 0.4436 | 0.2564 | 1 |
| Adults | | | | | |
| AT | 0.747 | 0.826, 0.691 | 0.0011 | 2.30E-02 | 0.207 |
| GC | 0.166 | 0.110, 0.206 | 0.0063 | 4.10E-03 | 0.0369 |
| AC | 0.075 | 0.064, 0.083 | 0.4489 | 0.5722 | 1 |
| Combined | | | | | |
| AT | 0.778 | 0.836, 0.725 | 5.62E-08 | 3.67E-06 | 3.30E-05 |
| GC | 0.146 | 0.103, 0.186 | 1.72E-06 | 5.33E-06 | 4.80E-05 |
| AC | 0.068 | 0.061, 0.074 | 0.2697 | 0.5632 | 1 |

a: p -values adjusted for age, gender, BMI, and smoking status (adults) using logistic regression.

b: p -values corrected by Bonferroni correction.

doi:10.1371/journal.pone.0149199.t003

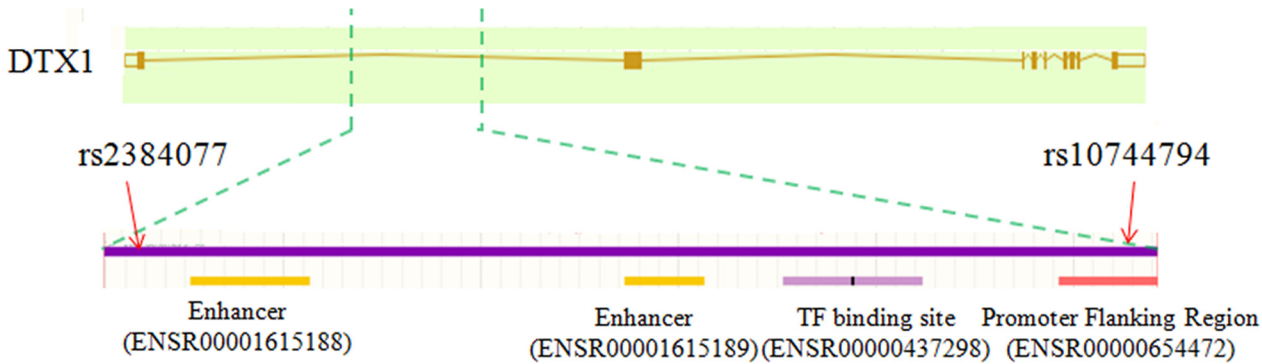


Fig 2. Regulatory elements in the LD block of rs2384077 and rs10744794.

doi:10.1371/journal.pone.0149199.g002

Discussion

Immunity following HBV vaccination is affected by several different factors; however, the molecular mechanism underlying this response is unclear. Several studies have identified numerous immune response-related genes and SNPs that were significantly associated with the degree of the immune response to HBV vaccination, including HMC, cytokine, and cytokine receptor genes [26–28]. T cell immunity plays an important role in regulating the magnitude of the immune response to HBV vaccination [29, 30] because genes related to T cell immunity are involved in the host response to the HBV vaccination; however, there have been limited reports on polymorphisms in genes involved in T cell immunity.

DTX1 is a single transmembrane protein that is involved in T cell anergy [23, 24]. Deltex (DTX) contains a proline-rich motif and a RING finger domain at its C terminus and two WWE domains at its N terminus (the WWE domains mediate its interaction with Notch) [31]. Several studies have suggested that DTX plays a critical role in the recombination signal binding protein for the immunoglobulin kappa J region (RBP-J)-independent Notch signaling pathway [32–34]. DTX1 over-expression inhibits Notch signaling, as demonstrated by enhanced B cell lymphocyte development and suppressed T cell development [35, 36]. Evaluating the relationship between DTX1 and the immune response may shed further light on the immune response to the HBV vaccination.

In the present study, we aimed to evaluate how DTX1 might be involved in the host response to the HBV vaccination in the Chinese Han population. We detected 10 SNPs in DTX1 using iMLDR. Two of these SNPs, located in the first intron of DTX1 (rs2384077 and rs10744794), were significantly associated with the degree of the immune response to HBV vaccination. Introns comprise a large portion of the eukaryotic genome, and as such, these introns contain abundant biological information [37]. Numerous studies have demonstrated that introns participate in various biological processes, such as regulating transcription initiation, mRNA modification, metabolism, transport, and protein expression [38–40]. Although various studies have found that the pathogenic risk of SNPs in intron regions is significantly reduced compared with the risk of SNPs in the gene coding and regulatory regions, the pathogenic risk of SNPs in the first intron is typically increased [41]. Therefore, to explore the functional relevance of the two identified SNPs, we constructed their haplotypes. The results demonstrated that the haplotypes were significantly associated with the immune response to HBV vaccination in both infant and adult populations.

Subsequently, to further explore the functional mechanism of the LD block (5-kb region) of the two SNPs in the immune response to vaccination against HBV, functional element annotation using the Regulatory Features of the Ensembl database was performed. We identified four

transcriptional regulatory elements, including two enhancers (ENSR00001615188, ENSR00001615189), one TF-binding site (ENSR00000437298), and one promoter-flanking region (ENSR00000654472), located in this region, with rs10744794 being located in the promoter-flanking region (ENSR00000654472). These results indicate that the SNPs in this LD block (5-kb region) may affect DTX1 expression by altering the four transcriptional regulatory elements and T cell immunity.

The induction of immunity following HBV vaccination is a complicated process affected by several factors, including environmental and host-related physical factors, such as age, smoking, and alcohol consumption. In the present study, we identified two DTX1 SNPs that are involved in the host response to the HBV vaccination using peripheral blood samples from an infant population, which allowed us to avoid interference by most exogenous factors. In a second population, we used peripheral blood samples from adults to confirm these results. In the adult population, we took age, BMI, and smoking status into account. The results from both populations demonstrated that the two identified SNPs were significantly associated with the degree of the immune response to HBV vaccination, indicating that the results of the association analysis in our study were efficient and reliable.

There are still certain limitations to our study. First, a lack of power caused by the moderate sample size in our study prevented us from assessing genetic effects in NRs to the HBV vaccine. Second, although we only chose ethnic Han for our study, there are many other ethnic populations in the Southwest than in other places in China, which might have introduced heterogeneity in our Han population. Third, we could not collect information on the doses and sources of the vaccine that was used in the adult cohort, which might have biased the outcome of the statistical analysis. Moreover, due to a lack of enough blood samples, we could not detect the expression level of DTX1 in T cells to confirm a relationship between DTX1 expression and the two identified SNPs. Therefore, we will collect more samples for further study, and greater attention will be paid to investigating the relationship between DTX1 expression and the two identified SNPs.

In summary, the results of the present study demonstrate that the minor allele 'G' of rs2384077 and the minor allele 'C' of rs10744794 in the first intron of the DTX1 gene are associated with a higher immune response to the HBV vaccination in the Chinese Han population in Southwest China. The haplotype analysis also indicated that the LD block of the two SNPs is significantly associated with the immune response to HBV vaccination. Functional element annotation of the LD block between the two SNPs found four transcriptional regulatory elements located in this 5-kb region. Considering all of these results, we speculated that the two SNPs are involved in the immunity generated by vaccination against HBV, likely by affecting DTX1 expression. Nevertheless, the relationship between DTX1 expression and the two SNPs needs further confirmatory research. Additional studies that focus on the molecular mechanism(s) underlying how DTX1 affects the immune response to vaccination against HBV might lead to a better understanding of the mechanism of action of these vaccines.

Supporting Information

S1 Fig. LD plot for the 24 SNPs (MAFs>5%).
(TIF)

S1 Table. Primer and oligo or probe information for the 10 SNPs detected by iMLDR.
(XLSX)

S2 Table. Statistical information regarding the 10 SNPs.
(XLSX)

Acknowledgments

We thank Dr. Ren Liping. and Dr. Liu Guodong for helping us to complete the revised work.

Author Contributions

Conceived and designed the experiments: BX PZ HL ML. Performed the experiments: BX PZ WZ JY ML. Analyzed the data: BX PZ JY HL ML. Contributed reagents/materials/analysis tools: PZ HL. Wrote the paper: BX HL.

References

1. World Health Organization (2008) Hepatitis B Factsheet No 204. <http://www.who.int/mediacentre/factsheets/fs204/en/>, accessed February 9, 2012.
2. Zuckerman JN, Zuckerman AJ. Current topics in hepatitis B. *J Infect.* 2000; 41:130–136. PMID: [11023756](#)
3. Hui Chee-Kin, Lau George K.K. Immune system and hepatitis B virus infection. *Journal of Clinical Virology.* 2005; 34: s44–s48. PMID: [16461223](#)
4. Desombere I, Willems A, Leroux-Roels G. Response to hepatitis B vaccine: multiple HLA genes are involved. *Tissue Antigens.* 1998; 51:593–604. PMID: [9694351](#)
5. Van LH, Van Amsterdam JG, Vandebriel RJ, Kimman TG, Rumke HC, Steerenberg PS, et al. Vaccine-induced antibody responses as parameters of the influence of endogenous and environmental factors. *Environ Health Perspect.* 2001; 109:757–764.
6. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. *N Engl J Med.* 2005; 350: 1118–1129.
7. Krawczyk A, Ludwig C, Jochum C, Fiedler M, Heinemann FM, Shouval D, et al. Induction of a robust T- and B-cell immune response in non- and low-responders to conventional vaccination against hepatitis B by using a third generation PreS/S vaccine. *Vaccine.* 2014; 32:5077–5082. doi: [10.1016/j.vaccine.2014.06.076](#) PMID: [24975813](#)
8. Tan P, Jacobson R, Poland G, Jacobsen S, Pankratz V. Twin studies of immunogenicity: determining the genetic contribution to vaccine failure. *Vaccine.* 2001; 19:2434–2439. PMID: [11257374](#)
9. Bhathena A, Spear BB. Pharmacogenetics: improving drug and dose selection. *Curr Opin Pharmacol.* 2008; 8:639–646. doi: [10.1016/j.coph.2008.07.013](#) PMID: [18762278](#)
10. Mert G, Sengul A, Gul HC, Karakas A, Eyigun CP. The role of human leukocyte antigen tissue groups in hepatitis B virus vaccination in Turkey. *J Microbiol Immunol Infect.* 2014; 47:9–14. doi: [10.1016/j.jmii.2013.01.004](#) PMID: [23523043](#)
11. Lobaina Y, Hardtke S, Wedemeyer H, Aguilar JC, Schlaphoff V. In vitro stimulation with HBV therapeutic vaccine candidate Nasvac activates B and T cells from chronic hepatitis B patients and healthy donors. *Mol Immunol.* 2015; 63: 320–327. doi: [10.1016/j.molimm.2014.08.003](#) PMID: [25193323](#)
12. Wang CB, Tang JM, Song W, Elena L, Craig MW, Richard AK. HLA and Cytokine Gene Polymorphisms Are Independently Associated With Responses to Hepatitis B Vaccination. *Hepatology.* 2004; 39: 978–988. PMID: [15057902](#)
13. Yucesoy B, Sleijffers A, Kashon M, Garssen J, de Gruijl FR, Boland GJ, et al. IL-1 beta gene polymorphisms influence hepatitis B vaccination. *Vaccine.* 2002; 20: 3193–3196. PMID: [12163271](#)
14. Vermehren J, Lötsch J, Susser S, Wicker S, Berger A, Zeuzem S, et al. A common HLA-DPA1 variant is associated with hepatitis B virus infection but fails to distinguish active from inactive Caucasian carriers. *PLoS One.* 2012; 7: e32605. doi: [10.1371/journal.pone.0032605](#) PMID: [22448225](#)
15. Pan LP, Zhang W, Zhang L, Wu XP, Zhu XL, Yan BY, et al. CD3Z genetic polymorphism in immune response to hepatitis B vaccination in two independent Chinese populations. *PLoS One.* 2012; 7: e35303. doi: [10.1371/journal.pone.0035303](#) PMID: [22536368](#)
16. Grzegorzewska AE, Wobszal PM, Sowińska A, Mostowska A, Jagodziński PP. Association of the interleukin-12 polymorphic variants with the development of antibodies to surface antigen of hepatitis B virus in hemodialysis patients in response to vaccination or infection. *Mol Biol Rep.* 2013; 40: 6899–6911. doi: [10.1007/s11033-013-2809-7](#) PMID: [24158609](#)
17. Ryckman KK, Fielding K, Hill AV, Mendy M, Rayco-Solon P, Sirugo G, et al. Host genetic factors and vaccine-induced immunity to HBV infection: haplotype analysis. *PLoS One.* 2010; 5: e12273. doi: [10.1371/journal.pone.0012273](#) PMID: [20806065](#)

18. Yucesoy B, Talzhanov Y, Johnson VJ, Wilson NW, Biagini RE, Wang W, et al. Genetic variants within the MHC region are associated with immune responsiveness to childhood vaccinations. *Vaccine*. 2013; 31: 5381–5391. doi: [10.1016/j.vaccine.2013.09.026](https://doi.org/10.1016/j.vaccine.2013.09.026) PMID: [24075919](https://pubmed.ncbi.nlm.nih.gov/24075919/)
19. Bayard F, Malmassari S, Deng Q, Lone YC, Michel ML. Hepatitis B virus (HBV)-derived DRB1*0101-restricted CD4 T-cell epitopes help in the development of HBV-specific CD8+ T cells in vivo. *Vaccine*. 2010; 28: 3818–3826. doi: [10.1016/j.vaccine.2010.03.026](https://doi.org/10.1016/j.vaccine.2010.03.026) PMID: [20362206](https://pubmed.ncbi.nlm.nih.gov/20362206/)
20. Johnson S, Berghthaler A, Graw F, Flatz L, Bonilla WV, Siegrist CA, et al. Protective efficacy of individual CD8+T cell specificities in chronic viral infection. *J Immunol*. 2015; 194: 1755–1762. doi: [10.4049/jimmunol.1401771](https://doi.org/10.4049/jimmunol.1401771) PMID: [25567678](https://pubmed.ncbi.nlm.nih.gov/25567678/)
21. Saffar H, Saffar MJ, Ajami A, Khalilian AR, Shams-Esfandabad K, Mirabi AM. Long-term T-cell-mediated immunologic memory to hepatitis B vaccine in young adults following neonatal vaccination. *Hepat Mon*. 2014; 14: e22223. doi: [10.5812/hepatmon.22223](https://doi.org/10.5812/hepatmon.22223) PMID: [25368659](https://pubmed.ncbi.nlm.nih.gov/25368659/)
22. Almeida AP, Dias MO, Vieira Cde A, Chávez-Olórtegui C, Gazzinelli RT, Rodrigues MM, et al. Long-lasting humoral and cellular immune responses elicited by immunization with recombinant chimeras of the Plasmodium vivax circumsporozoite protein. *Vaccine*. 2014; 32: 2181–2187. doi: [10.1016/j.vaccine.2014.02.053](https://doi.org/10.1016/j.vaccine.2014.02.053) PMID: [24582631](https://pubmed.ncbi.nlm.nih.gov/24582631/)
23. Wang HC, Perry SS, Sun XH. Id1 Attenuates Notch Signaling and Impairs T-Cell Commitment by Elevating Deltex1 Expression. *Mol Cell Biol*. 2009; 29: 4640–4652. doi: [10.1128/MCB.00119-09](https://doi.org/10.1128/MCB.00119-09) PMID: [19564409](https://pubmed.ncbi.nlm.nih.gov/19564409/)
24. Hsiao HW, Liu WH, Wang CJ, Lo YH, Wu YH, Jiang ST, et al. Deltex 1 is a Target of the Transcription Factor NFAT that Promotes T Cell Anergy. *Immunity*. 2009; 31: 72–83. doi: [10.1016/j.immuni.2009.04.017](https://doi.org/10.1016/j.immuni.2009.04.017) PMID: [19592273](https://pubmed.ncbi.nlm.nih.gov/19592273/)
25. Yao JY, Chao K, Li MR, Wu YQ, Zhong BH. Interleukin-21 gene polymorphisms and chronic hepatitis B infection in a Chinese population. *World J Gastroenterol*. 2015; 21: 4232–4239. doi: [10.3748/wjg.v21.i14.4232](https://doi.org/10.3748/wjg.v21.i14.4232) PMID: [25892873](https://pubmed.ncbi.nlm.nih.gov/25892873/)
26. Kimkong I, Chankaew J, Kunanopparat A, Hirankarn N, Tangkijvanich P. Gene polymorphisms of interleukin 28B and the risk to chronic hepatitis B virus infection in Thai. *Tissue Antigens*. 2015; 85: 177–181. doi: [10.1111/tan.12517](https://doi.org/10.1111/tan.12517) PMID: [25664396](https://pubmed.ncbi.nlm.nih.gov/25664396/)
27. Hou SH, Hu J, Zhang Y, Li QL, Guo JJ. Effects of interaction between genetic variants in human leukocyte antigen DQ and granulysin genes in Chinese Han subjects infected with hepatitis B virus. *Microbiol Immunol*. 2015; 59: 209–218.
28. Ye B, Liu X, Li X, Kong H, Tian L, Chen Y. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. *Cell Death Dis*. 2015; 6:e1694. doi: [10.1038/cddis.2015.42](https://doi.org/10.1038/cddis.2015.42) PMID: [25789969](https://pubmed.ncbi.nlm.nih.gov/25789969/)
29. Li X, Wang Y, Chen Y. Cellular immune response in patients with chronic hepatitis B virus infection. *Microb Pathog*. 2014; 74: 59–62. doi: [10.1016/j.micpath.2014.07.010](https://doi.org/10.1016/j.micpath.2014.07.010) PMID: [25128091](https://pubmed.ncbi.nlm.nih.gov/25128091/)
30. Zweifel ME, Leahy DJ, Barrick D. Structure and Notch receptor binding of the tandem WWE domain of Deltex. *Structure*. 2005; 13:1599–1611. PMID: [16271883](https://pubmed.ncbi.nlm.nih.gov/16271883/)
31. Romain P, Khechumian K, Seugnet L, Arbogast N, Ackermann C, Heitzler P. Novel Notch alleles reveal a Deltex-dependent pathway repressing neural fate. *Curr Biol*. 2001; 11: 1729–1738. PMID: [11719214](https://pubmed.ncbi.nlm.nih.gov/11719214/)
32. Hu QD, Ang BT, Karsak M, Hu WP, Cui XY, Duka T, et al. F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation. *Cell*. 2003; 115: 163–175. PMID: [14567914](https://pubmed.ncbi.nlm.nih.gov/14567914/)
33. Hori K, Fostier M, Ito M, Fuwa TJ, Go MJ, Okano H, et al. Drosophila deltex mediates suppressor of Hairless-independent and late-endosomal activation of Notch signaling. *Development*. 2004; 131: 5527–5537. PMID: [15496440](https://pubmed.ncbi.nlm.nih.gov/15496440/)
34. Izon DJ, Aster JC, He Y, Weng A, Karnell FG, Patriub V, et al. DTX1 redirects lymphoid progenitors to the B cell lineage by antagonizing Notch1. *Immunity*. 2002; 16: 231–243. PMID: [11869684](https://pubmed.ncbi.nlm.nih.gov/11869684/)
35. Yun TJ, Bevan MJ. Notch-regulated ankyrin-repeat protein inhibits Notch1 signaling: multiple Notch1 signaling pathways involved in T cell development. *J Immunol*. 2003; 170: 5834–5841. PMID: [12794108](https://pubmed.ncbi.nlm.nih.gov/12794108/)
36. Romano M, Buratti E, Baralle D. Role of pseudoexons and pseudointrons in human cancer. *Int J Cell Biol*. 2013; 2013:810572. doi: [10.1155/2013/810572](https://doi.org/10.1155/2013/810572) PMID: [24204383](https://pubmed.ncbi.nlm.nih.gov/24204383/)
37. Chorev M, Carmel L. The function of introns. *Front Genet*. 2012; 3:55. doi: [10.3389/fgene.2012.00055](https://doi.org/10.3389/fgene.2012.00055) PMID: [22518112](https://pubmed.ncbi.nlm.nih.gov/22518112/)
38. Yenerall P, Zhou L. Identifying the mechanisms of intron gain: progress and trends. *Biol Direct*. 2012; 7:29. doi: [10.1186/1745-6150-7-29](https://doi.org/10.1186/1745-6150-7-29) PMID: [22963364](https://pubmed.ncbi.nlm.nih.gov/22963364/)
39. Rogozin IB, Carmel L, Csuros M, Koonin EV. Origin and evolution of spliceosomal introns. *Biol Direct*. 2012; 7:11. doi: [10.1186/1745-6150-7-11](https://doi.org/10.1186/1745-6150-7-11) PMID: [22507701](https://pubmed.ncbi.nlm.nih.gov/22507701/)

40. Chen R, Davydov EV, Sirota M, Butte AJ. Non-synonymous and synonymous coding SNPs show similar likelihood and effect size of human disease association. *PLoS One*. 2010; 5: e13574. doi: [10.1371/journal.pone.0013574](https://doi.org/10.1371/journal.pone.0013574) PMID: [21042586](https://pubmed.ncbi.nlm.nih.gov/21042586/)
41. Zhang X, Sun XH, Li M, Zhou ZH, Gao YQ. A promoter polymorphism (rs3806798) of interleukin-15 gene is associated with chronic hepatitis B virus infection in the Chinese Han population. *Int J Immunogenet*. 2014; 41: 298–305. doi: [10.1111/iji.12120](https://doi.org/10.1111/iji.12120) PMID: [24762213](https://pubmed.ncbi.nlm.nih.gov/24762213/)