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Polymorphisms of interferon-inducible genes OAS associated with interferon-α treatment response in chronic HBV infection

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ABSTRACT

To evaluate the role of host single nucleotide polymorphisms (SNPs) of 2′,5′-oligoadenylate synthetase (OAS) in predicting IFN response in patients with HBV infection, OAS gene and four SNPs were examined in 363 patients with chronic HBV infection (including 41 patients with HBsAg seroconversion) and 57 healthy controls. One SNP and three haplotypes were identified after adjustment for age, sex, HBV DNA. The frequency of OAS3T/C heterozygotes is 52.2% in responders (R) and 38.2% in non-responders (NR), with an odds ratio (OR) of 1.511 ($P = 0.018$). For complete responders (CR) and NR, the OR reached 2.323($P = 0.023$). Haplotype analyses revealed significant association between three OAS haplotypes and response to IFN-α treatment. Genotype combination and interaction between gene–gene analyses disclosed that there was a positive interaction between OAS2/OAS3 and OAS3/OASL, and the rate of OR was 2.46 (likelihood test, $P = 0.004$) and 4.46 (likelihood test, $P = 0.004$), respectively. Our results suggest that OAS gene variations may play an important role in response to IFN-α and provide a novel strategy for the resolution of HBV infection.

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1. Introduction

Interferon alpha (IFN-α) is one of the mainstay for the treatment of chronic hepatitis B (CHB) due to its antiviral and immunomodulatory activities. However, the response to INF-α may differ from individual to individual. Except for the reported factors such as alanine aminotransferase (ALT) levels, female gender and HBV genotype B, mutations in the coding region of interferon-inducible genes may influence the response to IFN-α therapy (King et al., 2002; Ahmadi et al., 2005). In respect of host genetic background, the role of single nucleotide polymorphisms (SNPs) in relation to treatment response has become increasingly important in a variety of illnesses (Suzuki et al., 2004; Yakub et al., 2005; Ovsyannikova et al., 2009). Fortunately, advances in elucidating the IFN pathway have provided significant clues to possible key genes for SNP selection (King et al., 2002; Kong et al., 2007). As a critical component of the innate immune response to viruses, the antiviral enzyme 2′,5′-oligoadenylate synthetase (OAS) encoded by OAS gene uses adenosine triphosphate in 2′-specific nucleotidyl transfer reactions to synthesize 2′,5′-oligoadenylates (2′,5′AS), which activate latent ribonuclease, resulting in degradation of viral RNA and inhibition of virus replication (Hovanessian and Justesen, 2007). Thus, any genetic variants that affect 2′,5′AS activity could be important determinants of susceptibility/resistance to viral infection and to virus-related diseases. In the present study, we therefore sought to determine whether IFN-α treatment response is genetically controlled and, if so, whether the relevant variants reside in the OAS gene cluster.

2. Materials and methods

2.1. Subjects

In accordance with the principle of informed consent, this open-label randomized study enrolled three hundred and sixty-three Han Chinese treatment-naïve CHB patients (265 HBeAg-positive, 55 HBeAg-negative, and 43 inactive HBsAg carriers) in Beijing You'an Hospital from September 2007 to March 2010. The patients had a history of HBV or HBsAg positive for more than 6

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Table 1
Profile of SNP genotyping.

Loci	Primer sequences (5′–3′)	T_m(°C)[a]	PCR product (bp)	Restriction enzyme	Genotype
rs2285934	F:AAATAGTCCTCTCTCTGTGA R:CTCTTCTGTGTTTTTCATCC	58	413	ECORI	AA: 413bp AC:413 + 219 + 194bp CC:219 + 194bp
rs2072138	F:CCCCAGAATCTAGTGTCTCA R:ACAGAACTTCAGCTTATCCC	58	191	KpnI	GG:191bp GC:191 + 127 + 64bp CC:127 + 64bp
rs2072136	F:TCAGCTTGACAGGGCGAATG R:TGGTGAATGGATGGGTTGGT	60	1061	XhoI	TT:1061bp TC:1060 + 611 + 450 CC:611 + 450bp
rs10849829	F:ACCTTCTCCAAAGAGCAACT R:TGGATGTGAGGATACGCAGT	60	403	HaeIII	AA:403bp AG:403 + 262 + 141bp GG:262 + 141bp

[a] Tm, annealing temperature.

months and remained HBsAg-and/or HBV DNA-positive at baseline. Indications for treatment included: whether HBeAg-positive or -negative, CHB patients who present HBV replication and abnormal liver biochemistry; alanine aminotransferase (ALT) > 60 IU/L, but <400 IU/L; pathological grade G2 and/or S2 for some patients whose ALT < 40 IU/L. According to the histological examination of biopsy specimens, G and S were referred to the grades and stages of inflammation and fibrosis. Inflammation grade (G) was divided from G0 to G4, while fibrosis stage(S) was from S0 to S4. Patients should be considered for treatment when liver biopsy shows moderate to severe active necroinflammation and/or fibrosis using a standardised scoring system (EASL, 2009. Clinical Practice Guidelines: management of chronic hepatitis B). Patients were excluded if (1) there was evidence of past or current infection with hepatitis viruses except HBV; (2) they suffered from cirrhosis or hepatocellular carcinoma; or (3) they were not of Han ethnicity. As a control group, fifty-seven HBV self-limited infections were analyzed.

All enrolled patients accepted PEG-IFNα-2a-based antiviral therapy, with the dose of 180 μg for body weight ≥70 kg or 135 μg for body weight <70 kg, subcutaneously once a week at least for 12 months. Therapeutic effects were evaluated on the following criteria. Complete response (CR) was defined as HBsAg seroconversion (anti-HBs > 100 IU/ml); Partial response (PR) was defined as HBeAg seroconversion for the patients which were HBeAg-positive, without HBsAg seroconversion; Patients who did not satisfy any of the above mentioned criteria were categorized as non-responders (NR).

2.2. Serological testing

HBsAg and HBeAg were measured by the use of ARCHITECT I2000 test (Abbott). If HBsAg > 250 IU/ml, ARCHITECT I2000 test (Abbott) was used for the detection and quantification of hepatitis B surface antigen (HBsAg), linear correlation curve in range from 0.05 to 250 IU/ml. Samples with HBsAg levels >250 IU/ml at 1:100 dilution were retested in a fully automated chemiluminescent microparticle immunoassay (Architect HBsAg QT). Serum HBV DNA was measured by Real-time PCR with the use of Biosystems 5700. The ALT and AST levels were measured by a continuous monitoring assay with the normal range of 0–40 IU/L.

2.3. SNP selection and genotyping

Genomic DNA was extracted from peripheral blood using a salting-out protocol. The four SNPs (rs2285934 in OAS1; rs2072138 in OAS2; rs2072136 in OAS3; rs10849829 in OASL) selected for the present study are recorded in the public dbSNP database. The SNP ID numbers and detailed sequence information are available at http://www.ncbi.nlm.nih.gov/SNP/. The four SNPs were genotyped using protocol of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The details of the PCR-RFLP genotyping experiments are summarized in Table 1.

2.4. Statistical analysis

Whether the studied SNPs were in the Hardy–Weinberg equilibrium (HWE) was analyzed by χ^2 test. Genotype frequencies of each SNP among CR, R and NR were analyzed using χ^2 test or Fisher exact test. Multiple logistic regressions were performed to evaluate whether there were differences in treatment response between each SNP after adjustment for age, sex, HBV DNA. Linkage disequilibrium (LD) values (D'), r^2 values, and haplotypes were estimated using the online software SHEsis (Shi and He, 2005). Multiple logistic regressions were also performed to evaluate the combined genotypes of four SNPs in OAS and gene–gene interaction. All statistical tests were 2-tailed. *P*-values less than 0.05 were considered statistically significant. The analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 11.5.

3. Results

3.1. Demographic and clinical characteristics

A total of 363 patients were analyzed, among which 208 patients were infected with HBV C genotype, 112 with HBV B genotype. Baseline HBV DNA levels were 6.4 ± 1.5 log copies/ml, ALT levels were 120.2 ± 126.7 IU/L. Efficacy of IFN-α was evaluated in the 363 patients based on the criteria described in Section 2. Since the CR rate was low, CR and PR groups were combined into one group (response group, R) for analysis. The rate of R was 43.8% (159/363), including CR 11.29% (41/363) and PR 32.51% (118/363), and the rate of NR was 56.20% (204/363). There were no significant differences in distribution of age and gender among the three groups of patients.

3.2. Genotype distribution

Genotype distribution analysis showed that the studied SNPs were in HWE. No significant SNP-specific deviation ($P > 0.05$) was observed. This result came from a final sample size of 420 subjects (363 chronic HBV infections and 57 controls) and four SNPs in the candidate antiviral genes.

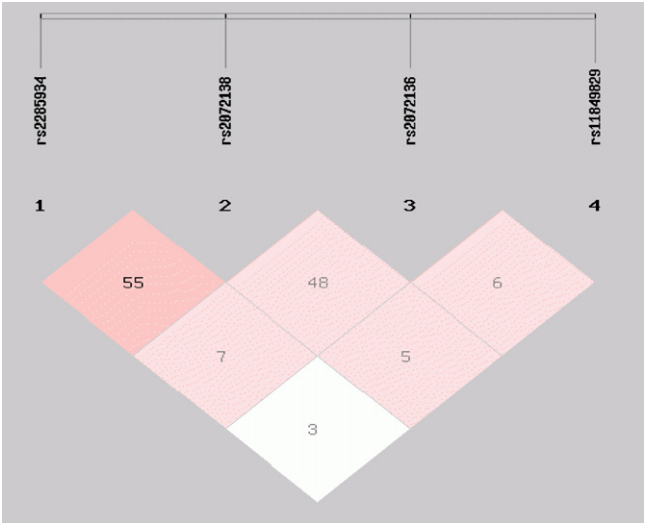

Fig. 1. Haplotype block structure of the OAS gene region in the study cohort. The LD block structure was analyzed using SHEsis software, online. SNPs in the OAS region clustered into two blocks of linkage disequilibrium.

3.2.1. Associations between OAS genotype distributions and IFN therapy response

The allele frequencies and genotype distributions of SNPs were compared between R and NR groups (see Table 2). There was a significant difference in OAS3 (rs2072136 T/C) polymorphism ($\chi^2 = 7.201$, $P = 0.027$). The frequency of T/T homozygote in the non-responders was significantly higher than that in the responders (52.5% vs 39.6%). However, the frequency of T/C heterozygote was 38.2% in NR and 52.2% in R.

Although the sample size was relatively small, the frequency of T/T homozygote was 52.5% (107/204) in NR group and 34.1%(14/41) in complete responders ($P = 0.054$). To determine if OAS3 SNP (rs2072136) was independently associated with IFN treatment response, we analyzed the SNPs along with other covariates such as age, sex, HBV DNA, which was previously reported to be significantly associated with the response in a multivariable regression model. There were statistically significant associations after adjustment for other covariates in HBV carriers of OAS3 rs2072136 T/C genotype between CR + PR and NR groups (OR = 1.511; $P = 0.018$), and CR and NR groups (OR = 2.323; $P = 0.023$).

3.2.2. Associations between OAS haplotypes and IFN therapy response

In order to understand whether the four associated OAS SNPs were in strong linkage disequilibrium (LD) with each other or whether each contributed to the association with treatment response independently, we performed LD analysis. As shown in Fig. 1, the four SNPs were slightly associated with each other (D ranges from 0.033 to 0.554; r^2 ranges from 0.02 to 0.066). There were no evidences of apparent LD. Thus, it was likely that some of these SNPs may independently contribute to the association.

Because all of the four SNPs associations were within the OAS gene region, we focused on haplotype analysis, including all genotyped SNPs in the specific gene region of interest. Seven OAS haplotypes were identified with frequencies exceeding 5% in our study cohort. The global tests of OAS haplotype analyses demonstrated significant associations between haplotypes and response to IFN treatment (see Table 3). The most common haplotype C-C-T-A (major alleles of all genetic variants) was associated with NR, rather than CR ($P = 0.005$). So was the haplotype A-C-T-A ($P = 0.046$). In addition, haplotype C-C-C-A was associated with complete response (CR) to IFN treatment, compared with NR group ($P = 0.042$).

3.2.3. Genotype combination and interaction between genes of OAS family

There were no significant differences in allele frequencies and genotype distributions of the four SNPs except OAS3 between the response and non-response groups. There were three haplotypes were associated with response to IFN treatment. Genotype combination analysis was performed to investigate whether OAS gene family contributed to IFN response in case–control study, and whether there were gene–gene interactions contributing to the response by case–control analysis and, if so, whether a dose-dependent effect existed.

We regarded the patients who responsed to IFN as case group, the non-responsers as control group, and the patients carrying no

Table 2
Genotype distribution and allele frequencies of SNPs in OAS genes in chronic HBV infections treated with IFN.

SNPs	CR + PR ($n = 118 + 41$)	NR ($n = 204$)	χ^2	P-value	OR (95%CI)
OAS1	rs2285934		0.291	0.865	
AA	21(13.2)	24(11.8)			
AC	64(40.3)	87(42.6)			
CC	74(46.5)	93(45.6)			
A	53(33.3)	67(33.0)	0.01	0.922	0.83(0.925–0.958)
C	106(66.7)	137(67.0)			
OAS2	rs2072138		1.841	0.392[a]	
GG	4(2.5)	4(2.0)			
GC	33(20.8)	32(15.7)			
CC	122(76.7)	168(82.3)			
G	20.5(12.9)	20(9.8)	1.033	0.309	0.78(0.303–0.369)
C	137.5(87.1)	184(90.2)			
OAS3	rs2072136		7.201	0.027	
TT	63(39.6)	107(52.5)			
TC	83(52.2)	78(38.2)			
CC	13(8.2)	19(9.3)			
T	104.5(65.7)	121(65.7)	1.786	0.181	0.84(0.299–0.365)
C	54.5(34.3)	46(34.3)			
OASL	rs10849829		0.063	0.969	
AA	93(58.5)	120(58.8)			
AG	54(34.0)	70(34.3)			
GG	12(9.75)	14(6.8)			
A	120(75.5)	155(76.0)	0.013	0.911	0.94(0.918–0.963)
G	39(24.5)	49(24.0)			

[a] Fisher exact test.

Table 3
Comparison of haplotype frequencies among different groups.

Haplotpye	NR (freq)	CR (freq)	R (freq)	χ^2	Fisher's *P*	OR (95%CI)
C C T A	145.13(0.36)	18.34(0.22)		7.80	0.005	2.21(1.255–3.900)
C C C A	37.77(0.09)	14.57(0.18)		4.12	0.042	0.51(0.262–0.986)
A C T A	73.20(0.18)		40.57(0.13)	3.99	0.046	1.53(1.006–2.335)
A C C A	24.33(0.06)	4.54(0.06)		0.09	0.764	1.17(0.416–3.297)
A C T G	21.91(0.05)	6.25(0.08)		0.40	0.527	0.74(0.294–1.874)
C C T G	36.20(0.09)	9.46(0.12)		0.30	0.583	0.81(0.377–1.733)
C G C A	12.56(0.03)	6.23(0.08)		3.18	0.075	0.42(0.154–1.122)

protective genotypes as reference group (see Table 4). Genotypes of OAS2 CG/GG and OAS3 CC/TT were compared with other genotypes as nonprotective reference to IFN response. For OAS2 CG/GG and OAS3 CT (protective genotype) genotype combination, and OAS2 CC (protective genotype) and OAS3 CC/TT genotype combination, the OR of response to IFN was 0.96 (95%CI: 0.52–2.03, $P = 0.94$) and 1.04 (95%CI: 0.43–2.34, $P = 0.87$), respectively. Interestingly, the OR was 1.7 with OAS2 CC and OAS3 CT genotype combination. The difference of OR between R and NR group was remarkable (95%CI: 1.19–3.11, $P = 0.01$). The results indicated that there was a dose-dependent effect between genotypes and clinical IFN treatment response. In addition, we found that the two SNPs presented a positive interaction, and the rate of OR was 2.46 (likelihood test, $P = 0.004$).

Likewise, OAS3 CT and OASL AA also presented a positive interaction (likelihood test, $P = 0.004$), if the two genotypes co-exist, their benefit to treatment response was increased (OR = 1.87, $P = 0.05$). However, the difference of OR between OAS1 and OAS3 genotypes was not remarkable.

4. Discussion

In this study, we established a CHB cohort of 363 patients treated with INF-α. Based on the characteristics of IFN efficacy, HBsAg seroconversion was used as a parameter of complete response to evaluate the treatment effects. Of the 363 patients, 118 underwent HBeAg seroconversion, 41 achieved HBsAg seroconversion. With respect to HBsAg seroconversion as the indicator of complete response to INF therapy, we considered the following. First, it is hard for cccDNA to be cleared in CHB, which is the major reason for HBV relapse. There are no drugs to act directly on cccDNA, and reliable alternative indicators to evaluate the change of cccDNA. It was reported (Akman et al., 2007) that the level of HBsAg in liver tissue reflected the level of HBV cccDNA, which was a good non-invasive indicator of antiviral effects, even outcome and prognosis of HBV infection. Second, there are two types of drugs approved to treat hepatitis B: nucleos (t) ide analogues and interferons. The nucleos(t)ide analogues are potent inhibitors of HBV polymerase/reverse transcriptase activity and are highly effective in the suppression of HBV replication, but the rate of HBsAg seroconversion is very low, similar to natural seroconversion rate. IFNs have antiviral and immunomodulatory effects through initiating an innate antiviral response via induction of numerous interferon-stimulated genes, which encode the critical effector enzymes such as myxovirus resistance 1 (MxA), eukaryotic translation initiation factor 2-alpha kinase 2 (PKR), OAS, adenosine deaminase (ADAR). These antiviral proteins degrade viral as well as cellular mRNA (mRNA encoding HBsAg) and inhibit viral protein synthesis. Thus, in contrast to nucleos(t)ide analogues, IFNs have a higher HBsAg clearance rate (García-Sastre and Biron, 2006; Randall and Goodbourn, 2008; Sadler and Williams, 2008). It has been reported (Deguchi et al., 2004; Ozaras et al., 2008; Rodella et al., 2006) that HBsAg titer decreased inconsistently with the decrease of the level of viral replication in patients treated with pegylated interferon alone or in combination with lamivudine, but there was no association between the two parameters in the lamivudine alone group. The main reason could be that the nucleos(t)ide analogues can only inhibit reverse transcription of HBV DNA, rather than production of mRNA. However, mRNA is just the target of IFNs. Thus, it is inaccurate to take negative HBV DNA as the indicator of effect of IFN therapy (Wu et al., 2009), negative HBV DNA is more like the effect of the nucleos(t)ide analogues. The HBeAg and HBsAg seroconversions (especially the latter) are a reflection of interferon efficacy. That we take HBsAg seroconversion as the indicator of complete response to INF therapy is just based on the mechanism of IFNs.

Table 4
Genotype combination and gene-gene interaction analysis of OAS family contributing to IFN response in case–control study.

Gene		R	NR	OR (95%CI)	*P*	Likelihood test
OAS2	OAS3					ORint = 2.46[a]
CG/GG	CC/TT	13	13	1		
CG/GG	CT	24	23	0.96(0.52–2.03)	0.94	
CC	CC/TT	63	113	1.04(0.43–2.34)	0.87	
CC	CT	59	55	1.70(1.19–3.11)	0.01	
						0.004
OAS3	OASL					ORint = 4.46
CC/TT	AG/GG	26	50	1		
CC/TT	AA	50	76	1.27(0.726–2.19)	0.16	
CT	AG/GG	40	34	2.26(0.64–4.22)	0.56	
CT	AA	43	44	1.87(0.28–1.88)	0.05	
						0.004
OAS1	OAS3					ORint = 1.54
AA/AC	CC/TT	36	69	1		
AA/AC	CT	49	42	1.23(0.66–2.30)	0.51	
CC	CC/TT	40	57	0.72(0.40–1.38)	0.35	
CC	CT	34	36	1.81(1.11–2.10)	0.06	
						1.00

[a] ORint: odds ratios. Logistic regression analysis was used to determine the overall gene effect, an likelihood ratio test was then applied to assess whether there was a positive interaction between OAS2 gene and OAS3 according to analysis of multiplicative model.

The rate of HBsAg seroconversion is relatively low under the treatment of IFN-α. It has been reported that natural rate seroclearance of HBsAg is only 2.26% in Taiwan patients (Liu et al., 2010). Therefore, it is difficult to use the similar standard to analyze the relationship with treatment efficacy (Chan et al., 2006; Kong et al., 2007). Forty-one patients in our cohort achieved HBsAg seroconversion under the INF-based combination therapy and extended treatment duration (Cao et al., 2010a,b), with the rate of seroconversion up to 11.29%. This enables us to analyze the correlation effect according to the serologic criteria based on the mechanism of IFNs. Moreover, all of the 41 patients meet not only the satisfactory end-point in 2009 EASL guideline (EASL Clinical Practice Guidelines, 2009), but also our strict definition of HBsAb over 100 IU/ml. The reason is that in our previous study (Cao et al., 2010a,b), we found that if HBsAg disappeared alone or HBsAb was at a low level, it was hard to maintain the status of HBsAg seroconversion and easy to relapse after treatment stopped. In HBeAg-positive patients, durable HBeAg seroconversion is a satisfactory end-point due to its association with improved prognosis (EASL Clinical Practice Guidelines, 2009).

Multiple viral and host factors may be related to IFN treatment response. For the host factors, such as age, gender, fibrotic stage of the liver, and the presence of steatosis are associated with the treatment outcome. Besides, the present study highlights the important role of host genetic factors, and variations in the OAS gene cluster (including functional polymorphisms in the OAS gene) and OAS/RNase L pathway in the modulation of IFN-induced immune response (Knapp et al., 2003; Su et al., 2008). Our data showed that the rate of OAS3 (rs2072136) T/T homozygous in nonresponders was significantly higher than in responders, while the rate T/C heterozygotes in response individuals was higher than in the NR group, suggesting a possible association of OAS3 SNP with increased responses to IFN. OAS3 rs2072136 is located in exon8 region. C to T mutation will make arginine 567 linked with Opal mutation (UGA stop codon also utilized for tryptophan) resulting in lower expression of OAS3. Previous report identified highly significant association between TT homozygous and "low enzyme activities" (Bonnevie-Nielsen et al., 2005). The frequency of the common T allele could vary significantly across 2,5, AS cluster, resulting in differences in their response to IFN. However, this observation is not completely in agreement with previous reports. In 2002, King et al. carried out the first and most systematic pharmacogenetic study, and identified that the T/C henotype of rs2072136 in the OAS3 gene was more frequently in NR group than in response group,but there was no significant difference ($P = 0.234$). The reasons for the differences from our results may be as follows. First, as discussed above, since the sample size about IFN response in patients with HBV infection in the previous study was limited ($n = 87$), their findings may be incidental. Second and most importantly, there were 41 patients in our cohort, who achieved HBsAg seroconversion and anti-HBs >100 IU/ml, which may really present the response to IFN treatment.

We conducted haplotype and combined genotype analysis in the study and identified haplotypes, namely C-C-C-A, resulting from SNPs in the OAS1, OAS2, OAS3, and OASL genes, which were associated with complete response to IFN therapy, while C-C-T-A was associated with non-response. Notably, only the OAS3 rs2072136T/C allele was different in the two haplotypes. More interestingly, it had been demonstrated that the SNP of rs2072136 T/C genotype was remarkably susceptible to high immune responses to IFN treatment. The human OAS family contains the OAS1, OAS2, OAS3, and OASL genes (Bonnevie-Nielsen et al., 2005), which are all located on chromosome 12q24. The findings therefore indicate that the OAS family combined contribute to genetic susceptibility to viral infection treatment. Haplotype analyses provide additional evidence that OAS gene polymorphisms might be involved in the mechanisms underlying immune response heterogeneity to IFN treatment. Finally, potentially interesting findings in our study include genotype combination, interaction between gene–gene and dose-dependent effects in the promoter and regulatory regions of OAS gene, associated with response to IFN treatment. We found that the rate of OR to IFN response was increasing in patients carrying OAS3C/T or OAS2C/C genotype. Moreover, together with OAS2C/C and OAS3C/T genotype combination, the rate of OR of response increased strikingly. Such strend could be seen in OAS3C/T and OASL A/A genotype combination analyses. Because complex trait associations are more likely dependent on several/multiple genetic variants, it is reasonable to suggest that the observed effects in our study may be a result of several functional genetic variants (Ovsyannikova et al., 2009). The results indicated that there was a dose-dependent effect between genotypes and clinical IFN treatment response. This may be due to the cumulative effect of each allele on different genes in the OAS gene family, which when combined, showed significantly differences.

In summary, we showed that the polymorphisms in OAS3 gene and its related haplotypes were associated with response to IFN treatment in chronic HBV infection. Other SNPs of IFN-induced antiviral genes and immunological factors need to be studied. So far, no functional phenotypes were identified. Further investigations on more SNPs and related genetic factors will probably provide important findings about pathophysiological and therapeutic aspects of HBV infection.

Competing interests

The authors declare that they have no competing interests.

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