Host genetic polymorphisms in interferon pathway genes are reported to be associated with response to interferon therapy. Five hundred and forty-eight interferon therapy-naive chronic hepatitis B patients were enrolled in the retrospective nested case–control study. All patients received interferon based treatment and were examined for therapy efficacy. We genotyped 115 polymorphisms from 16 interferon pathway genes using the MassArray system. We identified rs4845384 in ADAR1 gene is strongly associated with the outcome of interferon therapy allele dose-depended (P = 0.0005), with decreased odds ratios of 0.69 and 0.27 for GA and AA genotypes, respectively (95% confidence interval, 0.47–0.99 for GA; 0.11–0.64 for AA). Our study suggested that rs4845384 in ADAR1 associates with treatment-induced clearance of chronic hepatitis B.

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pathway genes are associated with IFNα therapy effect, using the tag-SNP approach.

2. Materials and methods

2.1. Subjects

The subjects enrolled in the present study were 548 Han Chinese IFNα treatment-naive CHB patients recruited from the Beijing Youan Hospital between November 2005 and May 2008. The patients were included if the following criteria were met: their serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were continuously >40 IU/L; they were HBsAg seropositive and HBeAg seropositive for 6 months; their serum HBV DNA >2000 copies/mL. Patients were excluded if: (1) there was evidence of past or current infection with other hepatitis viruses or hepatitis not caused by HBV; (2) they had cirrhosis or hepatocellular carcinoma; or (3) they were not of Han ethnicity.

All the enrolled patients received PEG-IFNα-2a (Pegasys) 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 for 12 months. Three hundred and seven patients received PEG-IFNα-2a monotherapy, and 241 patients received combined therapy with IFNα/nucleoside analogues (NA), namely, PEG-IFNα-2a plus Lamivudine 100 mg orally per day, or PEG-IFNα-2a plus Adefovir 10 mg orally per day, or PEG-IFNα-2a plus Entecavir 0.5 mg orally per day.

Patients were then followed up for 6 months to evaluate the therapeutic effects. Sustained virological response (SVR) was confirmed if the following evidences were present: 6 months after the end of therapy, the patients had normal ALT and AST levels (<40 IU/L), their serum HBV DNA levels <500 copies/mL, and achieved HBeAg seroconversion. Patients who did not satisfy all of the abovementioned criteria were categorized as non-response (NR) patients. The characteristics of participating patients are described in Table 1.

The study was carried out in accordance with the guidelines of the Helsinki Declaration after obtaining written informed consent from all the subjects and was approved by the ethics committee of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

2.2. Serological testing

Enzyme-linked immunosorbent assay was performed for the detection of serum HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc (IMX; Abbott Diagnostics, North Chicago, IL). The ALT and AST levels were measured by a continuous monitoring assay.

2.3. SNP selection and genotyping

Genomic DNA was extracted from peripheral blood by using a salting-out protocol. We selected 131 tag-SNPs for a total set of 16 candidate genes related to the IFN pathway, using the HapMap database (HapMap Data Rel 24/phase Nov 08, on NCBI B36 assembly, dbSNP b126) and pairwise tagging method. With the selection criteria of r2 greater than 0.8 and minor allele frequency (MAF) of greater than 0.05 for the Han Chinese Beijing population, tag-SNPs were selected from the entire gene region from approximately 2000 bp upstream of the transcription start site to 2000 bp downstream of the 3′ untranslated region (3′UTR) in each gene (Tsukada et al., 2009). The total number of tag-SNPs was 131. SNPs were genotyped by the MassArray system (Sequenom; Bioyong Technology Co. Ltd., Beijing, China). The detailed genotyping information is shown in Supplementary Table 1. For genotyping quality control, 5% of samples were randomly genotyped twice for duplication accuracy, which was calculated to be 100%.

2.4. Statistical analysis

We used 2 × 2 or 2 × 3 contingency tables for comparing allele and genotype frequencies. Tests for association of quantitative traits were performed using the Mann–Whitney U test for traits with abnormal distributions, or ANOVA for normally distributed traits. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 13.0 (SPSS Inc., Chicago, Illinois)). We obtained estimates of linkage disequilibrium values ( r2, D′) and the haplotype estimation using the SHEsis online software (Shi and He, 2005).

3. Results

We first searched for the association between tag-SNPs and the outcome of IFNα therapy. There were no significant differences in response rates between the subgroups of the combined therapy group, i.e. Peg IFNα-2a plus Lamivudine, Peg IFNα-2a plus Adefovir, and Peg IFNα-2a plus Entecavir (data not shown), so all the subgroups were referred as combined therapy group. Of the 131 tag-SNPs, seven were failed in designing proper primers, and nine were failed in genotyping, so we had a final dataset of genotypes consisting of 115 SNPs in 548 patients, with an average call rate of 98.4%. There were nine SNPs deviated slightly from the HWE in SVR and/ or NR groups (0.01 < P < 0.05), and one SNP rs10173099 deviated significantly from the HWE (P = 0.005) in SVR group (Supplementary Table 2). Single locus analysis indicated that four SNPs were associated with the outcome of IFNα therapy both in allele frequencies and genotype distributions, among which rs4845384 was the most significant. As shown in Table 2, the frequency of A allele was significantly lower in SVR group than in NR group (25.3% vs. 35.3%, P = 0.0007). The Cochran–Armitage trend test (assuming an additive model for A allele) revealed an allele dose-dependent association of rs4845384 with the outcome of IFNα therapy (P = 0.0005), with decreased odds ratios of 0.69 and 0.27 for GA and AA genotypes, respectively (95% CI, 0.47–0.99 for GA; 0.11–0.64 for AA). When considering unadjusted additive model for each genotype, the OR is 0.80, with 95% CI 0.45–0.80 (P = 0.0006).
Stratification analysis was then conducted. The results remained significant except in HBV genotype B patients (Supplementary Table 3). The three other associated SNPs were rs7531982, rs4636449 and rs1127313 (Table 2). To adjust the P values for multiple testing, we applied Bonferroni correction (totally 115 SNPs). In this conservative adjustment, only rs4845384 reached the borderline significance ($P = 0.08$).

The four associated SNPs were all in the ADAR1 gene. Linkage disequilibrium analysis showed that the four SNPs were in LD though not absolutely ($D > 0.911, 0.172 < r^2 < 0.896$). As there were 10 tag-SNP studied in ADAR1 gene, we conducted haplotype analysis of the 4 and 10 SNPs in ADAR1 gene, respectively. As shown in Supplementary Table 4, when analyzing haplotypes of 10 SNPs were 10 tag-SNP studied in ADAR1 gene, we conducted haplotype analysis of the 4 and 10 SNPs in ADAR1 gene, respectively. As shown in Supplementary Table 4, when analyzing haplotypes of 10 SNPs in ADAR1 gene, we conducted haplotype analysis of the 4 and 10 SNPs in ADAR1 gene, respectively. As shown in Supplementary Table 4, when analyzing haplotypes of 10 SNPs in ADAR1 gene, we conducted haplotype analysis of the 4 and 10 SNPs in ADAR1 gene, respectively. 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rs1127309 is not recorded in the HapMap database. Nevertheless, it is informative that SNP in ADAR1 gene can predict therapy effect in chronic hepatitis C patients.

To the best of our knowledge, the present work is the most systematic pharmacogenetic study with the largest sample set regarding antiviral treatment of HBV infection. Nevertheless, there are some improvements for future studies. We select tag-SNPs in the present study, but some rare variants may be not recorded in the HapMap database yet. The coding region of ADAR1 should be sequenced to identify whether there is any new SNP that results in mRNA substitution.

5. Conclusions

In conclusion, we identified in the present study that a tag-SNP rs4845384 located in the ADAR1 gene is associated with the outcome of IFNα therapy in CHB patients, and this association is still significant after stratification analysis and logistic regression. Nevertheless, the sample size involved in the present study is not large enough, and it is possible that these findings may be incidental. Therefore, it is necessary to perform further studies in other ethnic groups and to confirm the present findings in a larger sample set.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2012.03.004.

References


Glossary

ADAR1: adenosine deaminases acting on RNA

ALLT: alanine aminotransferase

AST: aspartate aminotransferase

CHB: chronic hepatitis B

GWAS: genome-wide association studies

HBV: hepatitis B virus

HCV: hepatitis C virus

HWE: Hardy–Weinberg equilibrium

IFN: interferon

JAK: janus-activated kinase

NA: nucleoside analogues

NK: non-response

PR: partial response

R: response

SC: individuals who spontaneously cleared HBV

SNP: single nucleotide polymorphism

STAT: signal transducer and activator of transcription

SIV: sustained virological response