A prospect for pharmacogenomics in the interferon therapy of chronic viral hepatitis

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Globally, hepatitis B or C virus persistently infects 350 million or 100 million people, respectively.¹ ² Following recurrent chronic inflammatory hepatitis, eventually ~20%–30% of chronic viral hepatitis patients develop liver cirrhosis and hepatocellular carcinoma (HCC), which usually carries a very poor prognosis.² ³ For these patients, the best strategy to prevent progression to end-stage liver diseases is to control or eradicate hepatitis B virus (HBV or hepatitis C virus (HCV)) as early as possible. In support of this, recent studies from Japan have reported an impressive reduction in the incidence of HCC following treatment of chronic hepatitis C patients with interferon (IFN).³

Current therapies for chronic viral hepatitis include two regimens, IFN or antiviral nucleoside/nucleotide analogues such as lamivudine, adefovir dipivoxil, and ribavirin.⁴ ⁵ IFN or lamivudine alone can control hepatitis B in about one-third of patients.⁶ ⁷ A combination of IFN with ribavirin is standard therapy for hepatitis C and has resulted in eradication of HCV in about 50% of patients.⁵ ⁷ Although these results are encouraging, current available therapies are still limited by a high non-response rate (more than 50% of treated patients), the frequent emergence of drug-resistant mutants (in the case of lamivudine), or unpleasant side effects. Since there is as yet no ideal therapy, it is imperative that patients are administered treatments on which they will respond the best and suffer the least. Patient characteristics that predict a response help in selecting appropriate antiviral treatment.

The outcome of viral hepatitis involves a complex interplay of viral determinants of virulence and host resistance/susceptibility factors. Important virological factors have been identified: viral load, and viral genomic variations (especially viral genotypes and certain specific genomic variants).⁸ ⁹ Usually the higher the viral load the less satisfactory the response. Recent studies have suggested that genotypes C and D of HBV are associated with more severe liver diseases and poorer IFN responses than genotypes B and A.⁸ ¹⁰ Another impressive and useful example is hepatitis C. The response to IFN and ribavirin treatment by HCV genotype 1 is much poorer than by genotypes 2 or 3 (40% versus 80%).¹¹ The mechanisms still remain to be investigated; however, they may be attributed to the effect of HBV or HCV genomic variations on the viral replication rate, or the viral ability to antagonize IFN antiviral activity. In clinical practice, viral titre and genotypes should be ascertained before treating hepatitis B or C patients.

In addition to viral factors, generally it is believed that host factors play critical roles in response to hepatitis virus infection and IFN treatment. Previous clinical studies have listed patient characteristics, such as hepatitis activity [alanine aminotransferase (ALT) levels], disease stage (cirrhosis or not), gender and age, as predictors of outcome of treatment.⁴ ⁵ The finding that viral genomic variations influence treatment response infers that the genomic composition of individual patients may also contribute to treatment response. Numerous studies have indicated that specific polymorphisms of certain host genes are probably involved. For example, an association has been demonstrated between the tumour necrosis factor-α (TNF-α) promoter polymorphism at position ~238 and the development of chronic hepatitis.¹² ¹³ In addition, major histocompatibility complex (MHC) class I and MHC class II polymorphisms,¹⁴ interleukin 10 polymorphism,¹⁵ MxA promoter single nucleotide polymorphisms (SNPs)¹⁶ and mannose-binding protein SNPs,¹⁷ have all have been purported to affect host immune and antiviral responses, and thus are associated with disease progression and treatment response. However, most studies have only focused on the analysis of a single gene, or a few genes belonging to a certain branch of host immuno-defence gene families, which cannot cover comprehensively all aspects of immune response or related signal transduction pathways. For chronic hepatitis B or C treatment, IFN remains a major regimen, and its downstream signalling regulators and related effectors are well-characterized and available for systemic study. It is thus possible that a genetic polymorphism analysis, conducted for all genes underlying the IFN signalling pathway and the subsequent induced antiviral molecules, may provide more comprehensive information on treatment response.

In the human body, humoral IFNs serve as the first lines of cellular defence in the control of virus infection. This is achieved mainly by
transmodulating the immune response, either the innate or the adaptive arm, and by initiating the cellular antiviral status through binding to the cognate receptors on the cell surface.\textsuperscript{18,19} The signals are passed into the nucleus through activation of Janus kinases–signal transducer and activator of transcription (JAK-STAT) signalling pathways.\textsuperscript{20} By binding to IFN response elements, either IFN-stimulated response element or IFN-γ-activated sequences, the transcriptional activators, either IFN-stimulated gene factor 3 or γ-activated factor, will then induce the expression of responsive genes that orchestrate specific antiviral activities.\textsuperscript{18,21} These include the following enzymes: (1) protein kinase R, which inhibits translational initiation through the phosphorylation of protein synthesis initiation factor eIF-2α; (2) the 2′-5′-oligoadenylate synthetase family and RNase L nuclease, which mediate the degradation of both viral and cellular RNAs; (3) the family of Mx protein GTPases, which appear to target viral nucleocapsids and inhibit RNA synthesis; and (4) adenosine deaminases acting on RNA, which edit double-stranded RNA by deamination of adenosine to yield inosine, increasing the number of mutated proteins in virus-infected cells. In addition, IFN induces a variety of proteins belonging to mediators of cell apoptosis, such as TNF-α-related apoptosis inducing ligand, and the promyelocytic leukaemia protein; or the modulators of immune responses, such as MHC class I and II molecules and a form of nitric oxide synthase (iNOS).\textsuperscript{2,18} These IFN-induced molecules all lead to effective control of viral expansion, either by inhibiting viral replication or by promoting infected cells to undergo apoptosis. Supposedly, a combination of these molecules in each person will determine the individual’s varying degree of response to IFN treatment. However, the genes encoding these molecules are conserved in humans with only certain genetic polymorphisms. In each individual, inheritance from parents of different alleles of genes involved in IFN pathways will constitute a unique combination of polymorphic alleles. Such combinations, especially the SNPs that affect the function of protein products, are the most plausible targets for investigating host genomic factors that influence the outcome of IFN treatment.

We tried to test the feasibility of systemic IFN pharmacogenomics, in a pilot study in 82 HBV patients receiving the standard 4–6 months of IFN-α treatment who were followed for more than 1 year.\textsuperscript{22} The patients consisted of 46 IFN responders (R) with hepatitis remission after therapy, and 36 IFN non-responders (NR). We conducted a comprehensive association analysis by comparing the SNP profiles of all the above-mentioned genes in the JAK-STAT signalling pathway and the four subsequently induced antiviral pathways between the R and NR groups. Most of the SNPs available from the public database, such as the dbSNP and JSNP, that fit in the promoter, regulatory, or exon categories of relevant genes, which may possibly affect gene expression or function, were chosen for sequencing. First, we compared individually the genotype distribution of each SNP between the R and NR groups. Two SNPs, located in two parallel antiviral pathways, were identified that may influence the IFN response. The one located at the promoter region (nt –88) of the MxA gene showed borderline significance, concurring with a previous study involving hepatitis C patients.\textsuperscript{10} The significance of this SNP in IFN treatment response was further supported in HBV-infected patients. The other SNP, located at the regulatory region of the eIF-2α gene, also showed distinct genotype distributions between R and NR. The rate of A/G heterozygotes was 22% in NR and 2% in R, with an odds ratio of 12.28 (95% CI, 1.52–107.85, \( P = 0.009 \)).\textsuperscript{22} This SNP, although present at a low frequency in the population, revealed even greater significance over conventional predictors (i.e. HBV DNA levels and serum ALT concentrations) as a marker for IFN response.

Furthermore, because the SNPs of genes located in the same antiviral pathways have been selected for analysis, it will thus be possible to conduct both haplotype analysis and combined genotype analysis. The former analysis is to evaluate the distribution of specific haplotypes, consisting of SNPs located in the same chromosome regions, between R and NR patients. It can help to derive IFN response-specific haplotypes. The latter analysis could be used to evaluate the effect of combined genotypes of two SNPs located on the same gene or pathway, which may reveal a possible interaction or synergistic effect between SNPs or genes in the same pathways. Both analyses can only be conducted in a comprehensive SNP analysis covering a great number of genes. Our results, although preliminary due to the limited number of cases, indicated a possible correlation between certain haplotypes or combined genotypes and IFN treatment response, which were not detected in the SNP association analysis for single genes.\textsuperscript{22} In addition, they might also provide valuable clues for future studies that look in detail at the interactions of related genes that affect clinical treatment response. A similar systemic SNP study for hepatitis C patients is underway.

This pilot pharmacogenomics study on hepatitis B showed that host genomic polymorphisms may correlate with response to antimicrobial immunotherapy. In a future study, to encompass the tremendous advances in elucidating IFN antiviral mechanisms, we propose to include even more SNPs to cover all of the genes involved. A whole spectrum of SNP analysis will probably be more effective in correlating with IFN treatment response. In addition, such a pharmacogenomic study should also include an analysis of genomic polymorphisms of hepatitis viruses, because the host and the pathogen always play equally key roles in infectious disease. In the light of recent great improvements in SNP detection methods and the potential for efficient mass screening, such a possibility can be tested in a large-scale analysis. By combining host and viral SNPs with conventional predictors, hopefully we can increase the precision by which patient response is predicted before treatment. In the future, this approach may aid physicians effectively to individualize the medical regimen of each patient, to achieve the best treatment response, and thus save on medical expenses.

**References**

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