Research Highlights

Highlights from the latest articles in pharmacogenomics

Pharmacogenomics of chronic hepatitis C

Evaluation of: Younossi ZM, Baranova A, Afendy A *et al.*: Early gene expression profiles of patients with chronic hepatitis C treated with pegylated interferon- α and ribavirin. *Hepatology* 49, 763–774 (2009).

Tsukada H, Ochi H, Maekawa T *et al.*: A polymorphism in *MAPKAPK3* affects response to interferon therapy for chronic hepatitis C. *Gastroenterology* 136(5), 1796–805 (2009).

Wada M, Marusawa H, Yamada R *et al.*: Association of genetic polymorphisms with interferon-induced haematologic adverse effects in chronic hepatitis C patients. *J. Viral Hepat.* 16(6), 388–396 (2009).

Hayashi K, Katano Y, Honda T *et al.*: Mutations in the interferon sensitivity-determining region of hepatitis C virus genotype 2a correlate with response to pegylated-interferon- α 2a monotherapy. *J. Med. Virol.* 81(3), 459–466 (2009).

Healthcare will soon be tailored to a patient's genetic make up. One of the best examples comes from the experience of treating patients with chronic hepatitis C virus (HCV) infection [1]. Currently, pegylated interferon (peg-IFN) and ribavirin (RBV) combination therapy is the standard therapy for the treatment of HCV patients. Sustained virologic response (SVR) rates of up to approximately 60–70% have been achieved [1–4]. However, the treatment regimen takes 6–12 months and causes significant adverse effects in many patients, leading to the refusal of the treatment or premature cessation of therapy in 10–20%

of patients. In addition, the cost is high. Therefore, it will be useful to predict which patients will respond to treatment and which will develop treatment-related serious adverse effects prior to the start of antiviral therapy. This personalized strategy will maximize the treatment efficacy, limit the unnecessary adverse effects and reduce the expense for patients who are unlikely to achieve the SVR or are likely to develop serious adverse effects.

At present, several viral and host factors have been demonstrated to influence the treatment outcomes of patients with chronic hepatitis C [1–4]. Recently, the virologic response of patients receiving treatment has become an important parameter to monitor [5–7]. Reduction in serum HCV load after 4 weeks (rapid virologic response [RVR]) and 12 weeks (early virologic response [EVR]) of therapy predicts the likelihood of SVR [6–8].

Although these viral factors in patients receiving treatment markedly sharpen the prediction of the responses to anti-HCV therapy, the proportion of patients achieving RVR and EVR varies among different ethnicities. Furthermore, patients should be exposed to 4–12 weeks of therapy before the decision is made to continue or stop treatment. Thus, we still need more accessible predictors that are obtained either at baseline or shortly after the start of therapy. Besides, the mechanisms underlying differences in treatment outcomes are still unknown. To address this issue, exploration of host and virus genomic make-up will be an optimistic approach.

HCV-related hepatitis activity is essentially an interaction between viral genomes (RNA) and infected host genomes (DNA) and this partially dictates the eventual outcome. Therefore, a study of the genomic factors determining the individual's response to medical treatments

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is reasonable and important [9,10]. Some relevant findings have recently been published [11–14].

These papers provide interesting data regarding the predictive value of host (DNA) and viral (RNA) genomic profiles, gene expression and gene polymorphisms. However, there are some common limitations. First, the design of these studies was retrospective. Large prospective studies are needed to validate these findings. Second, all these studies basically focused on the candidate host or viral gene approach and were not genome wide. Thus, certain critical host or viral genomic backgrounds are likely to have been missed. Third, and most importantly, as aforementioned, RVR and EVR have been found to be reliable predictors for the response to anti-HCV therapy. The value of the identified DNA or RNA markers in these studies should be compared with those of the ‘gold standard’ RVR and EVR in future studies.

Early gene-expression profiles of chronic hepatitis C patients receiving peg-IFN/RBV

The aim of the study was to assess if SVR was associated with early gene expression in chronic hepatitis C patients receiving peg-IFN plus RBV standard combination therapy [11]. To address this issue, peripheral blood mononuclear cells were collected prior to treatment, as well as 1, 7, 28 and 56 days after treatment from 68 patients, and in a one-step reverse transcription PCR in order to profile 154 mRNAs that were previously implicated in the host response to various viral infections.

It was found that the SVR could be predicted by the gene expression of signal transducer and activator of transcription-6 (STAT-6) and suppressor of cytokine signaling-1 in the pretreatment samples. After 24 h, SVR was predicted by the expression of interferon (IFN)-dependent genes and this dependence continued to be prominent throughout the treatment.

This study clearly demonstrated that very early gene-expression profiles can predict response to anti-HCV therapy. Since peripheral blood mononuclear cells can be collected easily from each patient, any biomarker derived from peripheral blood mononuclear cells could potentially be applied in future clinical medicine.

Furthermore, the gene-expression profiles identified in this study may help elucidate important molecular pathways that may influence the probability of achieving virologic responses in patients with different viral genotypes and treatment-naïve versus -experienced patients. These data may also help to develop more effective anti-HCV therapy in the future.

Of course, several limitations of this study should be addressed further, particularly, the fact that the case number was small. External validation of the predictive value of the identified mRNAs was only carried out in a few cases. Furthermore, whether profiles of mRNA expression in the circulation could reflect those occurring in the liver compartment awaits further investigation.

MAPKAPK3 & IFN therapy for HCV infection

The study by Tsukada *et al.* aimed to identify host SNPs that are associated with the efficacy of IFN monotherapy in patients with chronic HCV infection [12]. This study population was quite large and included 468 sustained responders and 587 nonresponders, 61% being infected with HCV genotype 1b. First, they examined whether 116 tagging SNPs from 13 genes that are involved in the type I IFN signaling pathway are associated with the outcome of IFN therapy. Two SNPs (rs3792323 [A/T] and rs616589 [G/A]), located in intron 2 of mitogen-activated protein kinase-activated protein kinase 3 (MAPKAPK3), were identified in patients infected with HCV genotype 1b. Using various modeling, statistical analyses, including population stratification analysis and haplotype analysis, and internal validation, the authors confirmed that rs3792323 is an independent factor associated with IFN efficacy. The identified SNP was located in an intronic part of the gene and its biological significance is poorly understood. Using an allele-specific transcript-quantification assay in liver biopsy, they tried to demonstrate that allele-specific expression of *MAPKAPK3* mRNA, corresponding to the allele for risk of nonresponse, was significantly higher than that of the other allele. Using a luciferase reporter assay, the authors revealed that overexpression of *MAPKAPK3* may

inhibit IFN- α -induced gene transcription via the IFN-stimulated response element and IFN- γ activated site.

This is an example of a classical candidate gene study focusing on genes possibly involved in IFN-signaling pathways. First, the SNPs relevant to IFN-therapy outcome were successfully identified. Then, the role of the identified SNPs in the expression of the *MAPAPK3* gene was further investigated. Unfortunately, understanding the exact biological function of MAPKAPK3 in IFN-related gene expression is still in its infancy. Besides, whether current findings could be repeated in chronic hepatitis C patients receiving standard-of-care peg-IFN and RBV combination therapy also awaits confirmation from further studies.

SNPs associated with IFN-induced cytopenia

Hematologic toxicities, such as neutropenia, thrombocytopenia and anemia, frequently cause poor treatment tolerance and diminish therapeutic efficacy. Thus, the aim of the study by Wada *et al.* was to identify host genetic polymorphisms associated with the efficacy or hematologic toxicity of IFN-based combination therapy in chronic hepatitis C patients [13]. First, the authors performed comprehensive SNP detection in all exonic regions of the 12 genes involved in the IFN signaling pathway in 32 healthy Japanese volunteers. Of 167 identified polymorphisms, 35 were genotyped and tested for possible association with efficacy or toxicity of IFN plus RBV therapy in 240 chronic hepatitis C patients. Multiple logistic regression analysis revealed that none of the genetic polymorphisms were associated with the SVR. Interestingly, two polymorphisms (*IFNAR1* 10848-A/G and *STAT2* 4757-G/T) were found to be significantly associated with IFN-induced neutropenia; thrombocytopenia was associated with the IRF7 789-G/A polymorphisms.

This was the first study demonstrating that genetic polymorphisms in IFN signaling pathway-related genes correlate with IFN-induced neutropenia and thrombocytopenia in chronic hepatitis C patients. Indeed, data obtained from pharmacogenomics uncovers both the

positive side of predicting efficacy, demonstrated in many other studies, as well as the negative side, that is, predicting unfavorable adverse events as is demonstrated in this study. Nevertheless, the biological plausibility of these findings remains unclarified. Besides, SNPs of the genes possibly involved in the turnover of blood cells were not investigated. Furthermore, since multiple testing was carried out, to adjust the p-values with each individual SNP as an independent variable, Bonferroni correction should be considered.

IFN sensitivity-determining region of HCV genotype 2a and Peg-IFN monotherapy

The IFN sensitivity-determining region (ISDR) of HCV genotype 2a is thought to be inhibited by the double stranded RNA-dependent protein kinase. Several studies from Japan have reported a relationship between the ISDR and IFN responsiveness in patients with chronic HCV infection. However, this relationship is controversial and relevant information regarding HCV genotype 2a is still limited. The aim of this study was to investigate whether genomic heterogeneity of the ISDR among patients with HCV genotype 2a affects the response to peg-IFN- α 2a monotherapy [14]. A total of 80 patients infected with HCV genotype 2a were evaluated. The ISDR (amino acids 2193–2228) was examined by direct sequencing. A total of 31 patients received subcutaneous injections of peg-IFN- α 2a (180 mg) once weekly for 24 weeks, and 35 patients received injections for 48 weeks. A total of 14 patients withdrew from treatment. Of the remaining 66 patients, 51 (77.3%) exhibited a SVR. Factors related to the SVR on multivariate analysis only related to RVR and the number of mutations in the ISDR. These findings provided accumulative data to this area of research. However, this still did not resolve why similar observations could not be obtained in the studies from other countries. The data regarding SVR in patients with genotype 2a being treated with peg-IFN- α and RBV combination therapy, current standard-of-care, was also not elucidated [15].

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