

ADVANCES IN HEPATOLOGY

Current Developments in the Treatment of Hepatitis and Hepatobiliary Disease

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Genome-Wide Association Studies: “SNPing” Away at Liver Disease

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G&H How are genome-wide association studies improving our understanding of liver disease?

ET In the field of liver disease, 4 major findings have recently arisen from genome-wide association studies (GWAS). The first discovery occurred in 2008, when the *PNPLA3* gene was found to be associated with nonalcoholic fatty liver disease. A year later, several publications showed that single nucleotide polymorphisms (SNPs) near the *IL-28B* locus are associated with both treatment outcomes and spontaneous viral clearance in patients with hepatitis C virus (HCV) infection. In addition, SNPs in the *ITPA* gene have been found to be associated with ribavirin-induced anemia. Finally, another SNP was just recently identified that is associated with HCV-induced hepatocellular carcinoma.

G&H How does this information improve clinicians' understanding of these diseases and their treatment?

ET Of the 4 genetic associations I just mentioned, 2 of them mainly give insight into disease etiology: the *PNPLA3* gene, which is associated with fatty liver disease; and the *MICA* gene, which is associated with HCV-induced hepatocellular carcinoma. However, the other 2 genes I mentioned, *IL-28B* and *ITPA*, are also both associated with treatment outcomes. SNPs that are associated with treatment responses tend to be more

clinically useful than those associated with disease etiology, as they may be used to guide treatment decisions in the near future.

G&H How have studies of IL-28B provided insight into basic biological questions?

ET The *IL-28* gene family was discovered in 2003, but experiments performed in knockout mice initially suggested that the *IL-28* gene products were not very important for defense against viral pathogens. However, the previously mentioned GWAS then demonstrated that *IL-28* is important for human disease. In fact, *IL-28B* is interesting for 2 reasons: it has been found to be associated with response to pegylated interferon and ribavirin in HCV-infected patients, and it has been shown to be important for predicting spontaneous clearance of HCV.

Despite these insights, questions remain about *IL-28*. We know that the 3 genes in the *IL-28* gene family—*IL-28A*, *IL-28B*, and *IL-29*—are located very close to each other on chromosome 19, and they all yield gene products that are relatively similar at the protein level. However, we do not really know how these proteins are involved in HCV infection. Also, while the recently identified SNPs were found to be located very close to the *IL-28B* gene, they could also affect *IL-28A* and/or *IL-29*, which complicates our attempts to study them. Finally, there are differences between the human and mouse in terms of the *IL-28* gene locus—specifically, *IL-29* is a pseudogene in the mouse genome, whereas the human *IL-29* gene codes for a functional protein—and these differences make mouse models difficult to compare to humans.

G&H What is the mechanistic explanation for the relationship between IL-28B genotype and treatment response?

ET Unfortunately, a mechanistic insight has not been found at this time. There are numerous reasons why we think IL-28B would be important for treatment response in HCV, but the functional studies published to date have shown no clear role for this cytokine, so its role in HCV remains controversial. One hurdle in trying to develop a mechanistic explanation is that we do not have the most appropriate *in vitro* models to reflect what is happening in the human liver. When we get better models to reflect the actual processes that are occurring in patients, then we may be able to get closer to a mechanistic explanation.

G&H What is IL-28B? Does it have antiviral activity against HCV?

ET IL-28B is a cytokine classified as a type III interferon; these interferons are also known as the interferon λ s. Shortly after the *IL-28* gene locus was discovered in 2003, *in vitro* studies showed that IL-28 has antiviral activity against HCV. More interestingly, recent clinical trials have shown that recombinant type III interferon is effective against HCV when compared to pegylated interferon α -based treatment regimens.

Given these data, interferon λ s would be a realistic treatment option for HCV. One reason this treatment is particularly promising is that the interferon λ s seem to act specifically on the liver; they affect other tissues to a much lesser extent. In contrast, the interferon currently used in HCV treatment, pegylated interferon α , affects numerous tissues and organs. Indeed, clinical trials of interferon λ have demonstrated fewer side effects than interferon α , probably because of its specificity for the liver.

G&H How will the ability to detect IL-28B SNPs affect clinical management?

ET We need to be careful in using GWAS to guide treatment, because this is a very new field, and we still need more data before these studies can be used clinically. However, some of the emerging data suggest that combining *IL-28B* genotype with other markers of treatment response can yield strong prediction of nonresponse to standard therapy. I believe these combined tests are going to be developed further over time, and *IL-28* will hopefully become very useful for predicting treatment outcomes.

While more research is needed before *IL-28B* testing is ready to be routinely used in the community, an immediate application of this testing is to stratify patient cohorts by *IL-28B* genotype in clinical trials studying HCV antiviral agents used with the standard-of-care

treatment regimen. If a study has too many patients of a certain genotype in a treatment group, that imbalance could skew the study's results, so researchers will need to test patients for *IL-28B* genotype and randomize groups accordingly.

G&H If clinicians could better predict treatment outcomes, how might that affect clinical management?

ET Interferon and ribavirin treatment is very expensive, and these drugs are associated with many side effects; for these reasons, if a patient is unlikely to respond to this treatment regimen, we would want to spare him or her from incurring these costs and side effects. Instead, the recently approved direct-acting antiviral agents (eg, telaprevir [Incivek, Vertex] and boceprevir [Victrelis, Merck]) can be added to the interferon-based drug regimen, and this treatment combination should offer a higher chance of success than pegylated interferon α and ribavirin alone. When using combined treatment regimens that include the direct-acting antiviral agents, *IL-28B* genotype may not be as important.

G&H How do variants in the ITPA gene impact HCV treatment?

ET Interestingly, *ITPA* gene variants have been shown to correlate with ribavirin-induced anemia, which is the biggest side effect associated with ribavirin use in HCV-infected patients. SNPs in the *ITPA* gene determine which patients are likely to have the worst anemia due to ribavirin treatment and which patients are less likely to experience anemia.

When using *ITPA* genotype to predict anemia, a caveat clinicians need to keep in mind is that the protective allele—which is associated with resistance to ribavirin-induced anemia—is fairly rare. As a result, these SNPs are not as useful as we might have hoped they would be. However, if a patient has a low level of hemoglobin at the start of treatment and genotyping reveals that the patient does not have a protective *ITPA* allele, then the clinician can anticipate that ribavirin-induced anemia is likely to occur. In this case, the clinician could either take steps to avoid the development of anemia or follow the patient closely to ensure that significant complications related to any anemia are avoided.

G&H Overall, how does genetic information help clinicians improve treatment of liver disease?

ET It is clear that information gleaned from GWAS will eventually be of great clinical importance in the study of liver disease. Hopefully, these studies will yield mechanis-

tic insights into disease etiology; once we understand how these diseases originate and the roles that specific genes play in their development, then hopefully we can use this knowledge to generate more targeted therapies, thus improving patient care.

I should point out that we are still in the early stages of using GWAS in the clinical setting. We are starting to see signs that clinical application of GWAS will happen rapidly, but this process is just beginning. Another caveat is that different GWAS provide different types of information about a disease. For instance, *ITPA* gives information about treatment response, whereas *PNPLA3* provides information about disease etiology. Therefore, we should not expect that a single formula will explain how we can use each association; instead, how we use these studies must be specifically tailored for each genetic association and the patient cohort in which it was found. Hopefully, once all those factors are considered, GWAS can be utilized clinically, but they will not all provide the same information, which makes interpreting these studies more complicated. Also, all of these studies need to be validated with more investigations before they can be utilized clinically.

Despite these challenges, I believe that GWAS will provide specific insights into disease mechanisms and will aid in the development of new treatments. In addition, once more data are available and genetic testing is more widely available, clinicians may be able to not only test for patients' genotypes but also use this information to guide patient management. Ultimately, these studies are moving our field closer to the realization of personalized medicine, which is something that genetics has been trying to achieve for a number of years. The most critical application of these studies would be to identify high-risk patients who are likely to have the worst outcomes and then manage these patients appropriately using the insight gained from genetic studies.

G&H What further research is needed in this area?

ET We definitely need to study the mechanistic role of these genetic associations more in a laboratory setting. In addition, we are going to need validated in vitro models that accurately represent the disease pathophysiology as it occurs in patients. Once more models become available, mechanistic insights can be gained regarding the roles that certain genes play in various diseases. In

terms of *IL-28*, researchers need to identify the specific polymorphism that actually causes each of the observed clinical outcomes, and the mechanisms underlying these associations need to be determined. Once these mechanisms are understood, clinicians can then be more confident in using this information to determine a patient's treatment.

This need for further research is also true for the other SNPs that I mentioned: the *PNPLA3* gene; and the *MICA* gene, which has been associated with hepatocellular carcinoma. I should note that the *MICA* association was just recently discovered and still needs to be validated in additional patient cohorts. Of all the gene loci mentioned above, the *ITPA* gene and its role in ribavirin-induced anemia has been the best characterized.

A final point is that GWAS are beginning to reveal the untapped potential of clinical cohorts and patient samples. Clinicians who have well-defined patient cohorts—groups that have been well characterized regarding disease and treatment status—can now analyze samples from these patients in GWAS to gather more information about what genetic factors contribute to disease in these patients. If hepatologists have well-characterized patient cohorts, they may have access to an abundant amount of information that could be teased out using these molecular tools. Ultimately, my hope is that all of these studies will help the millions of patients who are suffering from liver disease by improving treatment and minimizing progression of disease.

Suggested Reading

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