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Review Article

Hepatocellular carcinoma: Towards personalized medicine

Daiki Miki,[1,2] Hidenori Ochi,[1,2] C. Nelson Hayes,[1,2] Hiroshi Aikata[2] and Kazuaki Chayama[1,2,3]

[1]Laboratory for Digestive Diseases, RIKEN Center for Genomic Medicine, Hiroshima; [2]Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

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Over the past several years, the success of genome-wide association studies (GWAS) and pharmacogenomics has gradually begun to enable personalized medicine in some fields. In the field of liver diseases, host genetic factors are now very useful in clinical practice for predicting treatment outcome and adverse reactions for pegylated interferon plus ribavirin combination therapy against chronic hepatitis C virus (HCV) infection. Recently, three virus-related hepatocellular carcinoma (HCC) GWAS were reported from Asia. One study examined hepatitis B virus-related HCC in China, where hepatitis B is very prevalent, and the other two examined HCV-related HCC in Japan. We identified a common variant in the *DEPDC5* locus associated with HCV-related HCC, and another group identified an association involving the *MICA* locus. In this review, we compare the results of these GWAS and earlier candidate gene studies. Further research is needed to determine the role of these single nucleotide polymorphisms on HCC risk, but identification of these markers could make it possible to assess the magnitude of the risk of cancer based on each patient's genetic background. Consideration of the genetic background of the patients will likely play a role in personalized medicine for HCC, and understanding the mechanism underlying the association could suggest novel promising therapeutic targets in the future. (*Cancer Sci* 2012; 103: 846–850)

Over the last several years, the success of GWAS and the International HapMap Project, a large-scale database of SNPs, has identified genetic risk factors for more than 150 diseases, as well as genetic differences in drug response.[1–4] The success of these studies as well as pharmacogenomics has gradually begun to enable personalized medicine in some fields.[5–8] The goal of personalized medicine is to optimize the medical care and outcomes for each patient based on clinical, genetic, and environmental information.[9] In the field of liver diseases, host genetic factors are now very useful in clinical practice for predicting treatment outcome and adverse reactions of PEG-IFN-α plus ribavirin combination therapy against chronic HCV infection,[10–17] which causes chronic hepatitis and HCC.

Epidemiology and Risk Factors of HCC

Hepatocellular carcinoma is the third leading cancer-related cause of death and the seventh most common form of cancer worldwide.[18] There are 750 000 new cases of HCC and nearly 700 000 deaths each year, making it a lethal form of cancer.[18] A variety of risk factors for HCC have been reported, including hepatitis viruses, vinyl chloride, tobacco, aflatoxin B1, alcohol consumption, non-alcoholic fatty liver disease, diabetes mellitus, obesity, diet, coffee, oral contraceptives, and hemochromatosis.[19] Incidence of HCC varies around the world, largely reflecting the distribution of HBV and HCV. As HBV infection is highly prevalent in many Asian countries and in Africa, HBV is the most common etiology of HCC in these regions, whereas in many developed countries, including Japan, HCV infection is the most common risk factor for HCC.[18–21] Chronic hepatitis caused by HCV often leads to fibrosis and cirrhosis (stage F4 fibrosis), which markedly increase the risk of developing HCC.[22] However, the incidence and progression of HCC varies by region, and only a fraction of HCV-infected patients develop HCC. To date, many studies have examined patients with HCV and identified several predictive factors for HCC, including liver fibrosis, age, male gender, alcohol consumption, diabetes mellitus, obesity, ethnicity, and co-infection with HBV.[18,23–25] In contrast to chronic HBV carriers, the influence of viral load and viral genotype on HCC is still controversial in chronic HCV carriers.[26] In addition to these factors, multiple host genetic factors are thought to contribute to HCV-related HCC development. Single nucleotide polymorphisms are the most common form of genomic variation, involving change at a single nucleotide in either coding or non-coding DNA. The contribution of SNPs in the development of HCC has been investigated by various means. For decades, numerous studies have been undertaken using a candidate gene approach, in which candidate genes are selected prior to analysis on the basis of known functions thought to be relevant to disease risk, for example, inflammatory genes and oncogenes, and the corresponding genomic region is intensively screened for disease-associated SNPs. For example, the association between HCV-related HCC and SNPs in the region of the *IL-1beta, MDM2*, and *UGT1A7* genes have been reported from Japan and other countries.[27–32] It has been reported that these gene polymorphisms are also associated with HBV-related HCC.[33–36] In addition, the influence of *HFE* and *MnSOD* gene polymorphisms on HCV-related HCC has been reported from many countries, although not from East Asian countries.[37–39] Gene polymorphisms associated with activity of hepatitis and liver fibrosis progression, which contribute to the development of HCC, have also been reported in HCV patients.[40,41] In spite of this effort, most studies had insufficient sample sizes, and the associations with HCV-related HCC were not robust. Therefore, better predictive genetic markers are still needed.

Genome-Wide Association Studies of HCV Treatment Response

Recently, methods for searching SNPs associated with diseases or drug responses have been changing dramatically. In contrast

[3]To whom correspondence should be addressed.
E-mail: chayama@hiroshima-u.ac.jp

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to the older candidate gene approach, the GWAS approach investigates not only the region around candidate genes with a known or predicted role in disease but across the entire genome using an SNP array, which simultaneously genotypes hundreds of thousands to millions of marker SNPs (also called tag SNPs). An SNP is often in strong linkage disequilibrium with multiple other SNPs in the same region, making it possible for tagging SNPs to serve as proxy markers for nearby SNPs that are not genotyped, and marker SNPs on genotyping platforms are selected to provide maximum coverage of the genome.[42] Over the past few years, this new high-throughput genotyping technology has revealed thousands of SNPs that are significantly associated with disease and drug responses, and this approach has been particularly promising in the field of liver diseases.

Anti-HCV therapy is prescribed in many countries to prevent the progression of liver fibrosis and development of HCC.[22,43,44] The current standard of care is PEG-IFN plus ribavirin combination therapy, but this costly and poorly tolerated treatment leads to SVR in only 50% of patients with HCV genotype 1, which is the most prevalent genotype in many developed countries such as the USA, UK, France, Italy, Spain and Japan.[45] To attempt to improve treatment efficacy, several viral and host factors responsible for SVR have been identified and studied extensively. Both HCV genotype and viral load are strong predictors of SVR.[46] In HCV genotype 1b, amino acid substitutions at positions 70 and 91 of the HCV core protein and the presence of multiple substitutions in the interferon sensitivity determining region of the NS5A protein were also reported to affect treatment outcome, especially among Japanese patients.[17,47,48] Host factors responsible for SVR include age, gender,[15] degree of hepatic fibrosis,[49] obesity, hepatic steatosis,[50] low-density lipoprotein cholesterol, gamma-glutamyl transpeptidase,[48] and insulin resistance.[51] In addition, although the individual effects of genetic polymorphisms are typically small and of limited use for prediction, we recently identified an SNP in *MAPKAPK3* that affects response to interferon therapy using a candidate gene approach.[52] Using the GWAS approach, a series of studies independently revealed that a common polymorphism within the non-coding region of the *IL28* locus is strongly associated with both outcome of PEG-IFN plus ribavirin therapy for chronic HCV infection[10–12] as well as spontaneous clearance of the virus.[53] Similarly, a polymorphism within the *ITPA* locus was found to strongly predict incidence of ribavirin-induced anemia during therapy.[13,14] It is likely that future treatment regimens will involve screening for these and other SNPs in an effort to select the most promising treatment candidates, as well as to identify patients at risk for serious side-effects. Direct-acting antiviral agents, such as the protease inhibitors telaprevir and boceprevir, have recently become available, and in the near future triple therapy consisting of PEG-IFN, ribavirin, and a protease inhibitor will likely become the standard of care.[54,55] In a recent clinical trial, we found that both *IL28* and *ITPA* polymorphisms are also useful predictive factors for outcome and occurrence of side-effects in triple therapy.[56,57]

Genome-Wide Association Studies of HCV-Related HCC

The GWAS approach has also been used to identify HCV patients at greatest risk for developing HCC. The primary goal of antiviral therapy is to prevent development of HCC and advanced liver disease and improve prognosis of patients. Particularly among HCV and HBV patients who are unable to clear the virus, screening of additional SNPs associated with susceptibility to HCC may help improve prognosis and better target surveillance to high-risk patients. As for HBV, which is the major cause of HCC in many Asian countries other than Japan, we identified variants in the *HLA-DP* locus associated with persistent HBV infection in Japanese and Thai study groups using a GWAS approach,[58] and this result was also confirmed in a Han Chinese patient group.[59] Subsequently, in the first GWAS for HCC, Zhang *et al.*[60] recently identified an SNP within the *KIF1B* locus associated with progression to HCC among chronic HBV carriers. However, it is known that the epidemiology is quite different between HBV-related and HCV-related HCC, and different virological effects of HBV and HCV have been reported.[61–63] Hepatitis B infection alters pro-apoptotic and DNA repair pathways, whereas HCV infection primarily affects anti-apoptotic and inflammatory pathways.[63] Two GWAS studies were reported very recently from Japan identifying genetic factors specific to HCV-related HCC.[64,65] Kumar *et al.* identified the *MICA* locus associated with HCV-related HCC, and we identified the *DEPDC5* locus (Table 1).

Study design. A flowchart of our study is shown in Figure 1. To identify genetic markers associated with the risk of HCV-related HCC development in the Japanese population, we carried out a two-phase case–control study consisting of a GWAS and a replication study using a total of 3312 Japanese patients over the age of 55 with chronic HCV infection. An important point is that the controls used in this study were not healthy controls, but chronic HCV carriers who have the potential of developing HCC in the future. This choice of control helps to avoid confounding risk factors for developing HCV-related

Table 1. Recently reported genome-wide association studies of hepatocellular carcinoma (HCC)

Etiology	Ethnicity	Characteristics	SNP	Chr. (locus)	Sample size		RAF		OR	95% CI	*P*-value	References
		Case/control			Case	Control	Case	Control				
HBV	Chinese	Chronic HBV carriers with HCC/without HCC	rs17401966	1 (*KIF1B*)	348	359	0.833	0.731	0.53	0.41–0.70	5.8×10^{-6}	(60)
HCV	Japanese	Chronic HCV carriers with HCC/non-HCC controls	rs2596542	6 (*MICA*)	721	2890	0.388	0.331	1.34	1.16–1.53	4.5×10^{-6}	(64)
HCV	Japanese	Chronic HCV carriers (age $\geq$ 55 years) with HCC/without HCC	rs1012068	22 (*DEPDC5*)	212	765	0.189	0.095	2.20	1.64–2.97	8.0×10^{-8}	(65)

Chr., chromosome; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; OR, odds ratio; RAF, risk allele frequency; SNP, single nucleotide polymorphism.

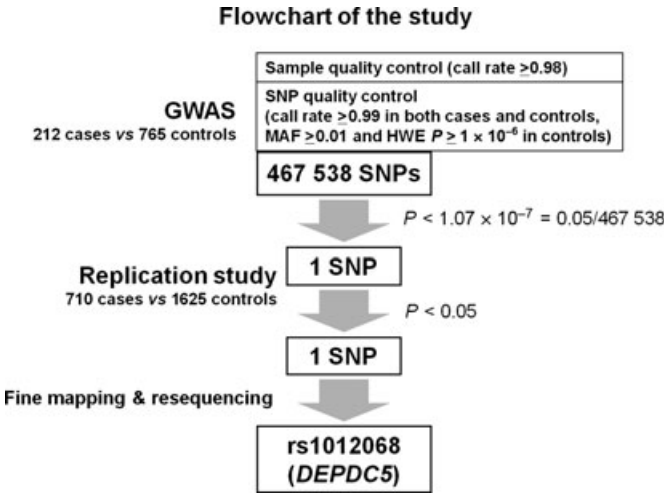

Fig. 1. Flowchart of our two-phase case–control study. For the genome-wide association study (GWAS) stage, we used the Illumina HumanHap610-Quad BeadChip. After we excluded two samples with call rate < 0.98, 467 538 single nucleotide polymorphisms (SNPs) passed the SNP quality control filters (call rate $\geq$ 0.99 in cases and controls, minor allele frequency [MAF] $\geq$0.01 and Hardy–Weinberg equilibrium [HWE] *P*-value $\geq$ 1.0×10^{-6} in controls). Only one SNP, rs1012068, within the *DEPDC5* gene reached statistical significance. We used multiplex-PCR-based Invader assays for the replication study and fine mapping. Finally, SNP rs1012068 had the strongest independent association with hepatitis C virus-related hepatocellular carcinoma.[65]

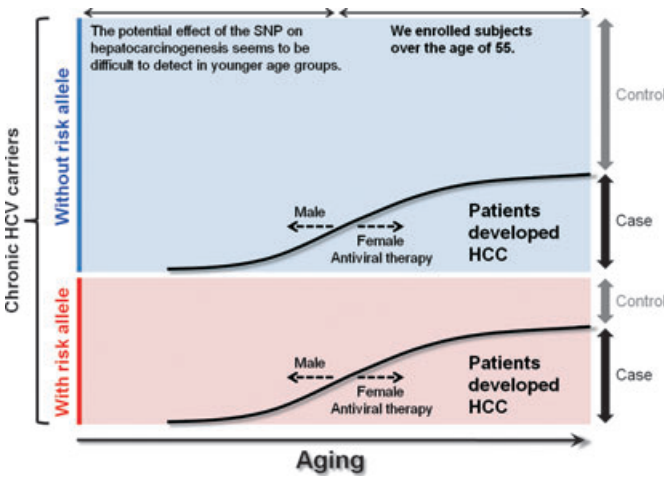

Fig. 2. Scheme of our study design considering the age range for developing hepatocellular carcinoma (HCC). All subjects were Japanese patients with chronic hepatitis C virus (HCV) infection, therefore, the controls used in this study were not healthy controls but chronic HCV carriers.[65] We enrolled subjects over the age of 55 years because most HCC patients are diagnosed at age 55 or older.[23,66–68] The potential effect of the SNP on hepatocarcinogenesis seems to be more difficult to detect in younger age groups, although males generally develop HCC at a younger age than females,[23,66,67] and antiviral therapy may prevent development of HCC.[22,43,44] SNP, single nucleotide polymorphism.

HCC with risk factors for chronic HCV. Another important point is that we enrolled subjects over the age of 55 years (Fig. 2) because age at initial diagnosis of HCV-related HCC has been increasing in Japan since the identification of HCV in 1989, and most patients are diagnosed at age 55 or older.[23,66–68] These two points represent major differences between the two Japanese GWAS studies of HCV-related HCC, and we speculate that these differences partially explain their inconsistent results, even though both studies focus on Japanese patients (Table 1).

Results. We initially carried out a GWAS using the Illumina HumanHap610-Quad BeadChip (Illumina, San Diego, CA, USA). After applying strict quality control filters, 467 538 autosomal SNPs remained and were analyzed using an additive model for genotype–phenotype association in 212 chronic HCV carriers with HCC (cases) and 765 chronic HCV carriers without HCC (controls). Principal component analysis revealed no population substructure in our study group, and the Cochran–Armitage trend test indicated a low probability of false-positive associations resulting from population stratification. Only one intronic SNP, rs1012068, within the *DEPDC5* locus on chromosome 22, showed a statistically significant association with HCC ($P = 8.05 \times 10^{-8}$) after Bonferroni correction for multiple testing (calculated as $P < 0.05/467\ 538 = 1.07 \times 10^{-7}$) with OR 2.20. To validate these results, we carried out a replication study using 710 cases and 1625 controls and confirmed the association between the SNP and HCC ($P = 2.41 \times 10^{-8}$, OR = 1.63). After adjusting for age, gender, and platelet count, which is known to correlate with the stage of liver fibrosis in HCV patients,[22] the significance level of rs1012068 increased. However, there are many confounding factors in the analysis of HCC, so we cannot rule out the possibility that other confounding factors influenced the results. To investigate causative SNPs, we carried out fine mapping of the *DEPDC5* locus including neighboring genes, and resequenced all 42 exons of the *DEPDC5* gene, but found no SNP with a stronger association than rs1012068. In contrast to *MICA*, which has previously been proposed to have a functional association with HCC,[69] *DEPDC5* has not been reported in association with HCC, and its function remains unknown.[70] Further functional analysis is needed to clarify which SNP is the true causative variant and to define the role of *DEPDC5* on the susceptibility of HCV-related HCC.

Limitations and future plans. An important limitation of our GWAS is the relatively small number of cases and the consequent lack of statistical power to detect other associations that are less robust, including rare variants and SNPs with weak effects. It remains to be determined whether other SNPs influence susceptibility to HCV-related HCC in the Japanese population. For a process as complex as HCV-related hepatocarcinogenesis, interactions among two or more SNPs as well as interactions with environmental factors should also be studied. In addition to SNPs, other types of genetic association, such as copy number variation, should be examined in the future. The question also remains whether the susceptibility loci within *MICA* and *DEPDC5* are associated with HCV-related HCC in other ethnic groups. Additional studies on other ethnic populations as well as stratification based on viral subgenotypes will provide more comprehensive information on the genetic etiology and heterogeneity of HCV-related HCC.

Towards Personalized Medicine

In current clinical practice in Japan, patients with chronic hepatitis C are recommended for surveillance for progression of liver fibrosis and early detection of cancer.[71,72] The susceptibility SNPs are relatively weak markers, but in combination with other clinical predictors, SNP genotyping could constitute a useful addition to assess the magnitude of the risk of HCC (Fig. 3). Intervention using PEG-IFN, ribavirin, and novel agents such as telaprevir[54–57] for reducing the risk for HCC[22,43,44] is planned in the future, and some SNPs

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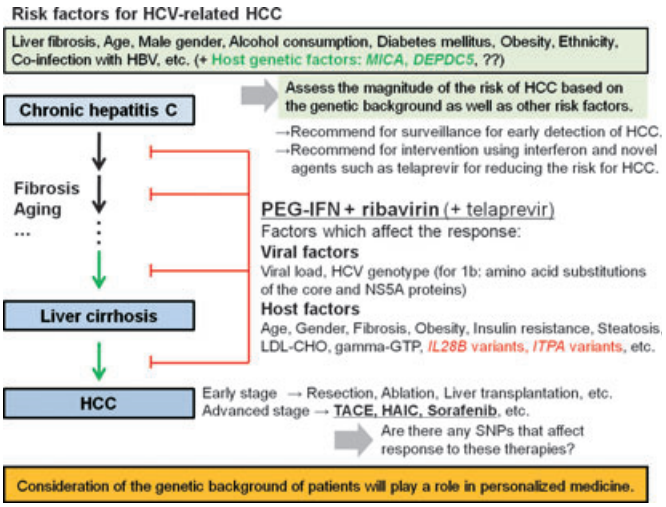

Fig. 3. Suggested outline of management of hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) incorporating genetic markers. Consideration of the genetic background of HCV patients will likely play a role in personalized medicine for HCV-related HCC. HAIC, hepatic artery infusion chemotherapy; HBV, hepatitis B virus; gamma-GTP, gamma-glutamyl transpeptidase; LDL-CHO, low-density lipoprotein cholesterol; PEG-IFN, pegylated interferon; SNP, single nucleotide polymorphism; TACE, transarterial chemoembolization.

might provide information useful in deciding whether or not intervention should be carried out. Once HCC has developed, the most promising treatment is determined based on clinical practice guidelines that are mainly based on tumor stage as well as liver function.[71–74] For treating advanced HCC, various anticancer agents and new molecular-targeted agents such as sorafenib have been advanced, but treatment outcome is still insufficient, and severe adverse drug reactions have occurred in some cases.[75–77] Host genetic factors affecting drug responses have not yet been thoroughly studied, and recent research on HCC genomes have identified several previously uncharacterized mutation patterns.[78,79] Host as well as cancer genomes should be studied further, and both may bring about benefits to HCC treatment in the future.

Conclusion

In conclusion, consideration of the genetic background of HCV patients will likely play a role in personalized medicine for HCV-related HCC, and understanding the mechanism underlying the association may suggest novel therapeutic targets.

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Disclosure Statement

The authors have no conflicts of interest.

Abbreviations

GWAS	genome-wide association study
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
OR	odds ratio
PEG-IFN	pegylated interferon
SNP	single nucleotide polymorphism
SVR	sustained virological response

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