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An Inheritable Variant of the Innate Immune Receptor Melanoma Differentiation-Associated Gene 5 Promotes Clearance of Hepatitis C Virus

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As a leading cause of chronic liver disease and cancer, hepatitis C virus (HCV) is a serious and persistent global health threat.¹ In their article in the current issue of HEPATOLOGY, Hoffmann et al. identified the less frequent variant of a single-nucleotide polymorphism (SNP) in the melanoma differentiation-associated gene 5 (*mda5*) gene that is associated with a significantly higher rate of spontaneous recovery from hepatitis C, as well as a lower rate of chronic infection in HCV patients.² The more efficient clearance of HCV in patients harboring this minor allele of *mda5* stems from increased cytokine secretion by cells in response to double-stranded RNA (dsRNA). This suggests that the signaling activity of MDA5 in response to cytoplasmic dsRNA, which is a hallmark of HCV infection, is enhanced in the protective variant of MDA5. This new finding implicates MDA5 in the natural course of HCV infection.

Most enveloped viruses either deliver an RNA genome into the cytoplasm of the host cell or generate RNA in the cytoplasm during replication or transcription of the viral genome. Infection by these viruses is primarily detected by a pair of essential immune sensors, retinoic acid-inducible gene 1 (RIG-I) and MDA5.^{3–5} Binding of cytoplasmic viral RNA by RIG-I or MDA5 induces a conformational change in the sensor protein that directs the cooperative assembly of large oligomeric signaling platforms, leading to the recruitment and activation of the signaling adaptor, mitochondrial antiviral-signaling protein (MAVS; interferon [IFN] promoter stimulator 1 IPS-1).^{6–8} The rapidly ensuing inflammatory response culminates in activation of the nuclear factor kappa B– and type I IFN-signaling pathways. This response is one of the first and most important lines of defense against infection and is responsible for the activation of the adaptive immune system.⁹ Therefore, RIG-I and MDA5 play pivotal roles as master regulators of inflammation.

HCV is an enveloped RNA virus known to be sensed and controlled by the RIG-I-like receptor (RLR)-signaling pathway. Indeed, Huh-7.5 cells have a mutation in RIG-I that

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inactivates RIG-I signaling that renders them much more susceptible to HCV replication.^{10,11} However, MDA5 had not, until now, been directly implicated in the antiviral response against HCV. Notably, the downstream signaling adaptor of both RIG-I and MDA5, MAVS, is cleaved by the nonstructural 3/4A protease of HCV.^{12,13} Indeed, MAVS—and not the RLRs themselves—is the primary target of viral factors for inhibiting RLR signaling.⁸ The study by Hoffmann et al. provides the first evidence that MDA5 is directly involved in anti-HCV signaling.

Hoffmann et al.² analyzed 14 known nonsynonymous SNPs in the genes coding for MAVS, RIG-I, MDA5, and the RIG-I-like coreceptor, laboratory of genetics and physiology 2, in a patient cohort.¹⁴ The frequency of the minor T allele of SNP rs3747517, in the gene encoding MDA5, was identified as significantly higher in the group of patients that had spontaneously recovered from hepatitis C infection versus the group of patients that developed chronic infection. A strong linkage disequilibrium was detected between this SNP and another SNP in the *mda5* gene, rs1990760, which had been previously identified as a risk allele for type I diabetes.^{14,15} Although the SNP rs1990760 variants are not significantly associated with HCV clearance, the allele frequency for the combined “T/T” haplotype, with the minor allele of SNP rs3747517 and the major allele of SNP rs1990760 present, was significantly higher in the group of patients that spontaneously recovered from infection and conferred patients with a 16-fold higher chance of resolving HCV infection.² In other words, the minor variant of SNP rs3747517 only promotes HCV clearance when present in combination with the major variant of rs1990760 polymorphism, but neither allele alone is associated with the outcome of HCV infection (Fig. 1A).

SNP rs3747517 affects the codon at amino acid 843 in MDA5. Residue 843 is an arginine in the major allele, as well as a histidine in the protective minor allele. SNP rs1990760 affects the codon at position 946 in MDA5, which is a threonine in the protective major allele of the SNP, and an alanine in the minor allele. Notably, residues 843 and 946 are both located near the MDA5-RNA-binding interface (Fig. 1B).^{16–18} Both residues are also near the MDA5 polymerization interface.^{2,16–18} Residue 843 is in the elbow-like pincer motif of between the second Rec A-like helicase domain and the C-terminal domain (CTD) of MDA5, and residue 946 is in a surface loop within the CTD (Fig. 1B).

The study by Hoffmann et al. is significant because it broadens the known arsenal of innate immune responses to HCV to include MDA5. Moreover, the clinical and epidemiological implications are considerable, given that the T/T variant of MDA5 is present in a significant fraction of the human population, approximately 5% in Europe.² An interesting question that arises in light of this discovery is whether the potential evolutionary advantage from the increased antiviral activity conferred by the T/T variant of MDA5 may come at the cost of trade-offs. The threonine side chain at position 946 is associated with increased susceptibility to type I diabetes.^{14,15} It is conceivable that the reason this susceptibility is tolerated evolutionarily is because the advantage conferred by increased resistance to HCV and other pathogens outweighs the increased risk of diabetes in certain environments. The increased signaling activity of the MDA5 T/T variant may also impose a cost in terms of local or systemic inflammation-induced cell damage. This type of trade-off in favor of signal potency at the cost of increased cytotoxicity has been optimized over evolutionary time for

specific environmental conditions.¹⁹ Interestingly, rapid changes in environmental conditions can outpace genetic adaptation and lead to diseases associated with inflammation, even in the absence of infection.¹⁹ Further work is required to continue to dissect the interplay between viral infection, inflammation, and autoimmune diseases. For example, it would be important to establish whether the current frequency of the human T/T the MDA5 variant reflects is optimized to the current environment and hence in evolutionary equilibrium. This may not be true given the recent increase in HCV prevalence. If the spread of HCV has indeed outpaced evolutionary adaptation, an increase in the frequency of T/T MDA5, and, perhaps therapeutic strategies to increase MDA5 signaling activity, might be beneficial to global health.

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Abbreviations

CTD	C-terminal domain
dsRNA	double-stranded RNA
HCV	hepatitis C virus
IFN	interferon
MAVS	mitochondrial antiviral-signaling protein
MDA5	melanoma differentiation-associated gene 5
RIG-I	retinoic acid-inducible gene 1
RLR	RIG-I-like receptor
SNP	single-nucleotide polymorphism

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Fig. 1.

SNPs affecting residues 843 (rs3747517) and 946 (rs1990760) of MDA5 are associated with spontaneous resolution of hepatitis C and map to the MDA5-RNA-binding interface. (A) The frequencies of the four different genotypes of MDA5 resulting from SNPs rs3747517 and rs1990760 in the cohort studied by Hoffmann et al.² The ratio of patients with spontaneous resolution of hepatitis to patients with chronic hepatitis was 22 in patients with the H843/T946 genotype and approximately 1 in patients with the other three 843/946 genotypes. (B) The crystal structure of the MDA5 helicase domains and CTD in complex with a 12-base-pair dsRNA fragment.¹⁴ MDA5 is in the colors of the rainbow, from the N-terminus of the first RecA-like helicase domain (blue) to the C-terminus of the CTD (red); the RNA is in gray. Residues 843 and 946 are highlighted in magenta, as space-filling spheres (residue 946 is disordered in the MDA5 crystal structure and its approximate position is modeled based on the position of the homologous residue in RIG-I^{2,16}). Residues 843 and 946 are both close to the MDA5-RNA-binding interface and to the MDA5 polymerization interface.