An inheritable variant of the innate immune receptor MDA5 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, Rothenfusser and colleagues identified the less frequent variant of a single nucleotide polymorphism in the mda5 gene that is associated with a significantly higher rate of spontaneous recovery from hepatitis C, and 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 double-stranded RNA. This suggests that the signaling activity of MDA5 in response to cytoplasmic double-stranded RNA, 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, 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 MAVS (IPS-1) (6-8). The rapidly ensuing inflammatory response culminates in activation of the NF-κB and type I interferon 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). RIG-I and MDA5 therefore 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 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 NS3/4A protease of HCV (28, 29). Indeed
MAVS- and not the RLRs themselves- is the primary target of viral factors for
inhibiting RLR signaling (8). The study by Hoffmann, Rothenfusser and colleagues
provides the first evidence that MDA5 is directly involved in anti-HCV signaling.

Hoffmann et al. (2) analyzed fourteen known non-synonymous single-nucleotide
polymorphisms (SNPs) in the genes coding for MAVS, RIG-I, MDA5 and the RIG-I-
like co-receptor LGP2 in a patient cohort (12). 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
(LD) 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 (12, 13).
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 (Figure 1A).

SNP rs3747517 affects the codon at amino acid 843 in MDA5. Residue 843 is
an arginine in the major allele, and 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 (Figure 1B) (14-
Both residues are also near the MDA5 polymerization interface (2, 14-16). 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 (Figure 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, as 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 the 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 (12, 13). 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 (17). Interestingly rapid changes in environmental conditions can outpace genetic adaptation and lead to diseases associated with inflammation even in the absence of infection (17). 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 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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Figure legend.

Fig. 1. Single nucleotide polymorphisms (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 the 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 C-terminal domain (CTD) in complex with a 12-bp double-stranded RNA 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 grey. 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.
A

SNP rs3747517  
SNP rs1990760  

R843 (Major)  
T946 (Major)  

H843 (minor)  
T946 (Major)  

R843 (Major)  
A946 (minor)  

H843 (minor)  
A946 (minor)  

Cytokine secretion  
with dsRNA  
(IP-10, IL28B)  

\( \frac{\text{Resolved hepatitis}}{\text{Chronic hepatitis}} \)  
Patient Ratio  
0.94  
22.0  
0.75  
1.15  

B