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Inborn Errors of Type I Interferon Immunity in Patients with Symptomatic Acute Hepatitis E

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CONFLICTS OF INTEREST

The authors have no conflicts to report.

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Graphical Abstract

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ABSTRACT

Background and Aims: The clinical spectrum of human infection by hepatitis E virus (HEV) ranges from asymptomatic to severe acute hepatitis. Furthermore, HEV can cause diverse neurological manifestations, especially Parsonage-Turner syndrome (PTS). Here, we used a large-scale human genomic approach to search for genetic determinants of severe clinical presentations of HEV infection.

Approach and Results: We performed whole genome sequencing in three groups of study participants with PCR-proven acute HEV infection: 1) 24 patients with symptomatic acute hepatitis E; 2) 12 patients with HEV-associated PTS; and 3) 16 asymptomatic blood donors (controls). For variant calling and annotation, we used GATK4 best practices followed by VEP and Annovar. For variant classification, we implemented the ACMG/AMP Bayesian classification framework in R. Variants with a probability of pathogenicity > 0.9 were considered damaging. We used all genes with at least one damaging variant as input for pathway enrichment analyses.

We observed a significant enrichment of type I interferon (IFN) response pathways in the symptomatic hepatitis group: 10 out of 24 patients carried a damaging variant in one of 9 genes encoding either intracellular viral sensors (*IFIH1*, *DDX58*, *TLR3*, *POLR3B*, *POLR3C*) or other molecules involved in type I IFN response (*IRF7*, *MYD88*, *OAS3*, *GAPDH*). We did not find any enriched pathway in the PTS group nor in the controls.

Conclusion: Our results highlight the essential role of type I IFN to prevent symptomatic acute hepatitis E.

INTRODUCTION

Hepatitis E virus (HEV) is one of the most common causes of acute hepatitis and jaundice in the world [1-2]. HEV is a positive-strand RNA virus belonging to the *Hepeviridae* family [3]. HEV genotypes 1-4 (HEV-1 to HEV-4) are responsible of most infections in humans. HEV-1 and 2 only infect humans and are responsible for primarily waterborne hepatitis outbreaks in resource-limited settings with poor sanitation, while HEV-3 and 4 have emerged as porcine zoonoses in middle- and high-income countries, including Switzerland [4]. Their transmission occurs primarily through the consumption of raw or undercooked pork or game meat [1-2].

The clinical spectrum of disease caused by HEV-3 is highly variable. While the majority of infections remain asymptomatic [1-2], the most frequent clinical presentation in symptomatic cases is acute, self-limiting hepatitis. In some instances, however, the infection may be associated with severe hepatitis, acute or acute-on-chronic liver failure and death in patients with preexisting cirrhosis [4-5]. In addition, HEV-3 may cause diverse neurological manifestations, especially neuralgic amyotrophy, also referred to as Parsonage-Turner syndrome (PTS), a painful and debilitating condition resulting in potentially irreversible handicap [6].

The precise reasons for this wide clinical spectrum are unclear. Both host and viral factors are believed to contribute. Known host risk factors for severe hepatitis include preexisting liver disease, male sex, age above 50, diabetes and immunosuppressive medication [7-8]. However, even in the absence of any of these risk factors, some individuals develop a severe clinical phenotype, which might rarely progress to acute liver failure necessitating urgent liver transplantation [9].

The potential contribution of intrinsic viral factors such as viral load or virus genetics to disease severity is still debated. Zoonotic HEV-3 has been divided into three major clades harboring subtypes abchijklm (HEV-3.1), efg (HEV-3.2) and ra (HEV-3.3) [10]. Clade HEV-3.2 has recently been associated with more severe disease [11-12].

Recent investigations showed that the viral isolates identified in Switzerland are genetically very close, belonging to a specific cluster within genotype 3h, designated here as 3h_s [4,13-14]. This homogeneity reduces the possible impact of viral diversity on clinical phenotype.

A potential influence of human genetic variation on HEV-3 infection outcome has been hypothesized. However, so far only a small number of candidate gene studies concentrating on susceptibility to HEV infection have been reported, with conflicting results [15-16]. Here, we used a whole genome sequencing approach to search for human genetic determinants of outcomes of HEV infection, including symptomatic acute hepatitis in patients without any preexisting liver disease and HEV-associated PTS.

EXPERIMENTAL PROCEDURES

Study participants

Three distinct groups of participants were included in this study: 1) Patients with symptomatic acute hepatitis, with an elevation of alanine aminotransferase (ALT) > 5 times the upper limit of the norm in analogy to grade ≥ 3 Common Terminology Criteria for Adverse Events [17] and/or jaundice (peak total bilirubin > 40 µmol/L); 2) Patients with PTS as a typical neurological complication of HEV infection; and 3) Asymptomatic blood donors with HEV infection documented on routine blood donation screening serving as controls. All study participants were > 18 years old and HEV infection was documented in all cases by a positive PCR.

Patients with evidence of preexisting liver disease were excluded from group 1 based on detailed review of clinical, laboratory and imaging data as well as liver histology when available. Patients under immunosuppressive treatment or who had travelled to a region endemic for HEV-1 and -2 infections were excluded.

Patients with symptomatic acute hepatitis and patients with HEV-associated PTS were included both retrospectively [4] and prospectively between February 1, 2020 and October 31, 2022. Blood donors with asymptomatic HEV infection discovered by PCR-based routine blood donation screening between January 1, 2021 and October 30, 2022, were also included both retrospectively and prospectively by the participating Swiss Blood Donation Centers (Lausanne, Bern, Zurich). Within this period (from January to May 2021), the Swiss Federal Office of Public Health recorded an unusual wave of acute HEV infections predominantly caused by genotype 3h_s [18].

This study was approved by the Ethical Committee of the Canton de Vaud (protocol 2020-00197). All study participants provided written informed consent.

Data and sample collection

The following data was collected from each patient with symptomatic acute hepatitis or PTS (groups 1 and 2): 1) demographical data including sex, age, ethnicity, place of residence and travel history; 2) clinical data including presence of alcohol use disorder, diabetes, chronic underlying liver disease, immunosuppressive treatment, neurological symptoms at presentation, disease evolution (complete recovery, partial recovery, death) and any history of other severe infection (Supplementary Methods, http://links.lww.com/HEP/I129); 3) laboratory data including peak ALT, peak total bilirubin and INR (albumin and creatinine were retrieved when available); 4) virological data including HEV RNA quantitation, HEV genotype and, when technically feasible, subtype.

A standardized questionnaire was completed by each blood donor to ensure the absence of symptoms at the time of blood donation (Supplementary Methods, http://links.lww.com/HEP/I129). The following data were collected for each asymptomatic blood donor (group 3): demographical data including sex, age, ethnicity, place of residence and travel history, contact with animals; clinical data including chronic diseases, diabetes, ongoing treatments, and presence of any symptoms in the last 6 weeks (myalgia, shoulder and/or upper limb pain, loss of sensitivity and/or strength, jaundice, abdominal pain, fever); and quantitative HEV RNA.

A 10-mL EDTA blood sample was collected from each study participant. DNA was extracted using the DNeasy Blood and Tissue Kit from Qiagen (Hilden, Germany) or on the Maxwell RSC Instrument using the Maxwell RSC DNA Blood Kit (Promega, Madison, WI). DNA concentration was measured on Qubit (Thermo Fischer Scientific, Waltham, MA), the purity and DNA integrity number were checked on Nanodrop (Thermo Fischer Scientific; absorption at 260/280 nm) and Agilent 2200 TapeStation Systems (Santa Clara, CA), respectively. Samples were stored at -80°C at the Institutional Biobank of Lausanne University Hospital.

Human genome sequencing and short-read alignment

Whole genome sequencing was performed at the Health2030 Genome Center in Geneva. Library preparation and sequencing were performed using Illumina (San Diego, CA) reagents and the Novaseq 6000 sequencer. The reads were subsequently aligned against the human reference genome (hg38) using the maximum exact matches algorithm in Burrows-Wheeler Aligner (BWA v0.7.17) [19].

Variant calling

The Genome Analysis Software Kit (GATK v4.2.2.0) best-practice pipeline was used to call variants in the multi-sample mode [20]. In brief, PCR duplicates were removed and base quality scores were recalibrated to correct for sequencing artifacts. We called individual-level variants with GATK HaplotypeCaller before combining single-sample callsets for joint genotyping. To exclude low quality variants, we applied variant quality score recalibration and manual filtering (depth ≥ 20, genotype quality ≥ 20, and 0.2 ≤ heterozygous allele balance ≤ 0.8).

Variant annotation

To predict the potential impact of each variant, we used Variant Effect Predictor (VEP v104) [21]. To identify loss-of-function variants, we used Loss-of-Function Transcript Effect Estimator (LOFTEE v1.0.3) as a VEP plugin [22].

To classify the variant into putative pathogenicity groups, we implemented the ACMG/AMP guidelines [23] in R (https://www.r-project.org) (see full description in the Supplementary Methods, http://links.lww.com/HEP/I129). A probability of pathogenicity (PoP) was assigned to each variant according to the ACMG/AMP Bayesian classification framework [23]. Variants in patients that were also found in healthy donors received a lower PoP, as suggested by ACMG/AMP guidelines (Supplementary Methods, http://links.lww.com/HEP/I129, rule *BS2* and *BS2_moderate*). Variants with PoP ≥ 0.9 were considered as damaging.

Single variant and gene-based analyses

Three types of analyses were performed to identify variants and genes potentially involved in severe HEV infection. The analyses were run separately in the symptomatic acute hepatitis group and in the PTS group: 1) Identification of damaging homozygous, compound heterozygous, and X-linked variants; 2) Gene ranking based on the number of damaging variants identified in each gene; 3) Identification of damaging heterozygous variants in genes known to be associated with immune deficiency, i.e. in one of the 485 inborn errors of immunity genes from the updated list of the International Union of Immunological Societies Expert Committee [24].

Pathway enrichment analysis

Pathway enrichment analysis, also known as over-representation analysis, was used to determine whether pre-defined gene sets were enriched in the genes harboring pathogenic variants found in our study [25]. We included all genes that carried at least one damaging

variant and calculated enrichment using the clusterProfiler package in R [25] and the pathways listed in the "biological process" category from Gene Ontology as reference pathways [26]. Pathways with a false-discovery-rate < 5% were considered as significantly enriched.

RESULTS

Study participants

We enrolled 24 study participants with symptomatic acute hepatitis (group 1), 12 participants with HEV-associated PTS (group 2), and 16 asymptomatic blood donors with acute HEV infection (group 3) (Figure 1). Table 1 summarizes their demographic, clinical, laboratory and virological data.

Of the 24 patients with symptomatic acute hepatitis, 22 (91.7%) were male and the median age was 54 years (range, 25-80 years). The majority of these patients were hospitalized (17/24, 70.8%). Median viral load was 5.7 $\log_{10}$ IU/mL (range, 2.8-7.0 $\log_{10}$ IU/mL). Viral genotyping was possible in 14/24 (58.3%) patients, revealing subtype 3h_s in 81.8% of samples that could be subtyped (Table 1); genotyping was not possible in 3/24 patients due to low viral load and in 7/24 patients due to lack of material. Median peak ALT was 2232 U/L (range, 745-5600 U/L). The majority of patients included in this group had jaundice; median peak total bilirubin was 77 µmol/L (range, 28-469 µmol/L). None of the patients developed coagulopathy, hepatic encephalopathy or ascites. A liver biopsy was available in 7/24 (29.2%) patients, confirming in all the absence of significant liver fibrosis. All 24 patients recovered completely.

All 12 patients with HEV-associated PTS were male and the median age was 53 years (range, 39-69 years). Damage outside of the brachial plexus was noted in 4/12 (33.3%) patients. Median viral load was 4.2 $\log_{10}$ IU/mL (range, 2.6-6.1 $\log_{10}$ IU/mL). Viral genotyping was possible in 9/12 (75.0%) patients, revealing genotype 3 in all and subtype 3h_s in 6, i.e. in 85.7% of the samples that could be subtyped (Table 1); genotyping was not possible in 3/12 patients due to low viral load. Median peak ALT was 365 U/L (range, 99-3582 U/L), median peak total bilirubin was 14 µmol/L (range,10-83 µmol/L).

Of the 16 asymptomatic blood donors with acute HEV infection, 12 (75.0%) were male and the median age was 56 years (range, 24-74 years). Median viral load was low (3.1 log10 IU/mL; range, 1.1-5.3 log10 IU/mL) and did not allow for HEV genotyping. Liver function tests were not performed at time of blood donation.

Variant classification

In total, 13,768,097 variants in symptomatic patients passed the quality control criteria and were annotated. 44,006 variants were mapped to coding regions (including nonsense, frameshift, splicing, missense, start lost, stop lost, in-frame insertion, and in-frame deletion variants) and were scored with the ACMG/AMP Bayesian classification framework. 41,146 variants had a PoP ≤ 0.1 and were considered benign. 2,279 variants had an intermediate PoP (between 0.1 and 0.9), resulting in their classification as variants of unknown significance (VUS). 566 variants exceeded the pathogenicity threshold (≥ 0.9) and were considered as damaging. Among them, 345 occurred only in patients with symptomatic acute hepatitis, 204 were observed only in PTS participants, and 17 were found in both groups. For quality assessment, the average number of variants per individual were calculated for the three groups (symptomatic acute hepatitis, PTS, and asymptomatic controls) separately (Supplementary Table 1, http://links.lww.com/HEP/I129). We observed comparable distributions of variants and variant types across the three groups (Supplementary Table 1, http://links.lww.com/HEP/I129).

Identification of variants and genes of interest

Three groups of potentially interesting variants were extracted from the 566 damaging variants for the two symptomatic cohorts (severe hepatitis group and PTS group) separately (Supplementary Tables 2, http://links.lww.com/HEP/I129, 3A and 3B, http://links.lww.com/HEP/I129 as well as 4A and 4B, http://links.lww.com/HEP/I129). 1) Homozygous, compound heterozygous, and X-linked variants: one homozygous variant in *OCM* and one X-linked variant in *TSPAN7* were observed in two symptomatic acute hepatitis subjects. One X-linked variant in *CXorf58* was found in one PTS patient. No compound heterozygous variant was observed. 2) Gene enrichment: 9 genes with at least two damaging variants were detected in the symptomatic acute hepatitis group and 3 in the PTS group. 3) Heterozygous variants in genes associated with immune deficiency: 18 damaging variants were observed in the symptomatic acute hepatitis group and 4 in the PTS group.

Pathway-based analysis

We performed separate pathway enrichment analyses for the two groups, using all genes with at least one damaging variant as input, i.e., a total of 348 genes for the symptomatic acute hepatitis group and of 218 genes for the PTS group. In the symptomatic acute hepatitis group, we observed a significant enrichment of pathways linked to type I interferon (IFN) response

(Figure 2, left panel; Supplementary Table 5, http://links.lww.com/HEP/I129): positive regulation of type I IFN production (GO:0032481), positive regulation of IFN-β production (GO:0032728), IFN-β production (GO:0032608), regulation of IFN-β production (GO:0032648), regulation of type I IFN production (GO:0032479), and type I IFN production (GO:0032606). The similarity of enriched pathways is due to the hierarchical structure of the Gene Ontology database; for example, IFN-β production is a sub-pathway of type I IFN production. No pathway was significantly enriched in the PTS group (Figure 2, right panel). We also performed an enrichment analysis for the asymptomatic blood donors, who carried a total of 286 pathogenic variants in 269 genes: no pathway passed the significance threshold.

Deleterious variants in genes of the type I IFN pathway

We then focused on the damaging variants found in genes encoding protein of the enriched type I IFN pathway. In total, 10 study participants, who all belonged to the symptomatic acute hepatitis group, carried a damaging variant in one of the 9 genes identified as from type I IFN response (Table 2, Figure 3). These genes can be classified into two groups: 1) Genes encoding intracellular viral sensors: *IFIH1*, *DDX58, TLR3*, *POLR3B*, and *POLR3C* that recognize virus infection and trigger an antiviral innate immune response. 2) Genes encoding regulators or effectors of the IFN response: *IRF7*, *MYD88*, *OAS3*, and *GAPDH*.

We identified two known loss-of-function variants in *IFIH1*: rs35337543 (carried by 2 study participants) and rs35744605. rs35337543 is a splicing variant that causes skipping of exon 8 followed by a premature stop codon; rs35744605 is a stop-gained variant in exon 10, which removes 399 amino acids (aa) from the C-terminal domain of IFIH1. The minor allele frequency (MAF) of rs35337543 and rs35744605 in genome Aggregation Database (gnomAD) is 0.7% and 0.4%, respectively [26].

One study participant carried the *DDX58* splicing variant rs61756274 (gnomAD MAF = 0.001%). Using SpliceAI [27], we predicted with high confidence that the variant leads to loss of the normal splice donor site at the junction of exon 12 and intron 12 (probability = 1) and to the creation of a novel donor site on the very next base in the 5' direction (probability = 0.65). Consequently, the reading frame is shifted by one base pair, introducing a new stop codon in exon 13. This results in the truncation of 329 aa from the RIG-I protein, including the RIG-I-like receptor C-terminal regulatory domain and the helicase C-terminal domain.

The Toll-like receptor 3 (*TLR3*) rs121434431 missense variant (gnomAD MAF = 0.05%), observed in another participant, results in the replacement of a proline by a serine at aa

position 554 (TLR3 P554S), thereby destabilizing leucine-rich repeat 20, which is an essential region for dsRNA binding to TLR3.

We observed two variants in genes encoding RNA polymerase III subunits: the stop-gained variant c.2341C>T in *POLR3B* (gnomAD MAF = 0.0007%), resulting in a premature stop codon in exon 21 and the loss of 353 out of 1133 aa from the C-terminal end of the protein; and a 1-base pair deletion (c.1533del) in *POLR3C* (gnomAD MAF = 0.1%), resulting in a frameshift and the creation of a new stop codon in the last exon.

A novel splicing variant was observed in the interferon regulatory factor 7 (*IRF7*) gene, c.679+1G>C, resulting in the loss of a splice donor site (SpliceAI probability = 0.88), skipping of exon 6 and causing a premature stop in exon 7. Therefore, 339 (out of 504) aa are truncated from the resulting protein.

In *GAPDH*, we identified a deletion of 14 base pairs at the junction of exon 3 and intron 3 (gnomAD MAF = 0.004%), which removes the splice donor site. However, due to a repeat sequence, the reading frame and the canonical splice site can be restored, and the resulting protein is predicted to be functional.

Finally, a study participant carried two rare IFN-related variants predicted to be damaging: the c.615del frameshift variant (gnomAD MAF = 0.0007%) in the myeloid differentiation primary response 88 (*MYD88*) gene, which leads to a frameshift and the introduction of a premature stop codon in exon 5, thereby destroying the TIR domain, a critical region for the interaction of MyD88 with toll-like receptors; and the c.2530C>T variant (gnomAD MAF = 1%) in the 2'-5'-oligoadenylate synthetase 3 (*OAS3*) gene, which creates a premature stop codon in exon 12, removing the last 245 aa of the full-length OAS3 protein.

DISCUSSION

Human genetic diversity explains part of the interindividual differences observed in response to diverse infections [28]. Previous host genetic studies of HEV infection were limited by very small sample size or a focus on a few candidate genes [15].

Recent investigations revealed that the viral isolates circulating in Switzerland are genetically very close, constituting a distinct cluster within genotype 3h, designated here as 3h_s and belonging to clade HEV-3.1 [13]. This viral genetic homogeneity in Switzerland offered a unique opportunity to study the influence of host genetics in disease manifestation. To test the hypothesis that human genetic factors can increase susceptibility to severe outcomes, we recruited 24 well-characterized patients with HEV-induced symptomatic acute hepatitis in the

absence of preexisting liver disease and 12 patients with PTS in the context of acute HEV infection, sequenced their whole genome and performed an in-depth bioinformatic analysis of variants, genes, and pathways. Blood donors with asymptomatic HEV infection documented on routine blood donation screening-served as control group.

Our variant prioritization strategy, based on careful quality control and annotation followed by assessment of pathogenicity based on the ACMG/AMP criteria, allowed the identification of 566 variants predicted to be damaging in a total of 540 genes. In the first step, we investigated the variants and genes of interest, identifying 1) one homozygous and one X-linked variant in symptomatic acute hepatitis subjects and one homozygous variant in one PTS patient; 2) 26 genes with more than one damaging variant in severe hepatitis patients and 10 in the PTS cohort; and 3) 18 heterozygous variants in genes linked to immune-deficiency in the symptomatic acute hepatitis participants and 4 in the PTS group. These variant- and gene-based analyses did not provide statistical support to explain the underlying mechanism of severe HEV infection.

We then performed a pathway enrichment analysis using as input all genes containing at least one damaging variant. The most enriched pathways - and the only ones still significant after correction for multiple testing - were the those linked to type I IFN response. Importantly, this enrichment was only observed among patients with symptomatic acute hepatitis and not in the PTS group, suggesting that different pathogenic mechanisms are responsible for neurological complications of acute HEV infection. However, the small number of PTS patients included in our analyses precludes any definitive conclusion.

In our study, impaired type I IFN response might explain the severe clinical presentation of HEV infection in up to 10 (out of 24) participants. Of note, the impaired type I IFN response was not associated, among our study subjects, with a history of severe COVID-19 or other viral infections (Supplementary Table 6, http://links.lww.com/HEP/I129). On the one hand, this could be explained by the fact most study participants who harbored a loss-of-function variant in the IFN pathway had been vaccinated against SARS-CoV-2, possibly contributing to the benign course of Covid-19. On the other hand, and most importantly, when discussing genetic susceptibility to severe viral infections, it is crucial to consider the incomplete penetrance of a given variant. Indeed, large redundancy in innate immunity results in a narrow pattern of susceptibility to infection. In other words, vulnerability to severe viral infection might arise from exposure to a specific pathogen in a particular tissue at a specific age [29].

A subset of the variants identified in our study has been previously tested functionally and shown to alter immunity against specific human viruses, while others map to genes with known association with impaired antiviral immunity.

Three of the variants were found in *IFIH1* and *DDX58*, the genes encoding the RIG-I-like receptors MDA5 and RIG-I, respectively. These cytoplasmic viral RNA sensors recognize single- or double-stranded RNA (dsRNA) to launch a type I IFN response, with the purpose to limit the replication of RNA viruses [30]. We have previously shown that the two *IFIH1* variants identified here (rs35337543 and rs35744605) lead to an impaired induction of IFN-β, decreased stability of the MDA5 protein and lower ATPase activity [31]. Importantly, they also have a dominant negative effect when heterozygously represented, as it is the case for the two study participants with symptomatic acute hepatitis. Patients carrying either variant developed severe forms of respiratory syncytial virus and human rhinovirus infection [31]. The *DDX58* splicing variant rs61756274, leading to +1 frameshift, impacts the C-terminal RNA recognition domain and is predicted to result in a dysfunctional RIG-I isoform; however, it has not been validated *in vitro*. Of note, defective *DDX58* was previously associated with severe influenza A virus (IAV) infection [32]. While *in vitro* cell culture models for HEV infection were limited until recently, recent studies allowed to uncover that both RIG-I and MDA5 may play a role in the sensing of HEV RNA upon infection [33, 34].

The rs121434431 variant in TLR3, resulting in substitution of proline by serine in position 554 (P554S), carried by one study participant, is known to increase susceptibility to severe viral infections. TLR3 is a Toll-like receptor localized in the endosome, which detects dsRNA intermediates and promotes type I IFN expression [35]. The P554S substitution is known to have a dominant negative effect on TLR3 signaling [36]. As a result, heterozygous carriers have been shown to be highly susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [37], IAV [38] and herpes simplex virus 1 infection [36]. The identification of this *TLR3* variant in a HEV-infected individual presenting with severe hepatitis meets the conclusion of *in vitro* data suggesting a role for this pattern recognition receptor (PRR) in HEV RNA sensing [39]. Moreover, although TLR3 is not signaling through MyD88 but TRIF, we identified a damaging variant in the gene coding for this downstream adaptor which may indicate that other TLRs can be involved in HEV sensing, including TLR7, -2 and -4, as suggested by *in vitro* studies [40].

Downstream of the PRRs, the signal is conveyed by phosphorylation of interferon regulatory factors (IRF) such as IRF3 and -7 which leads to the transcriptional regulation of IFN-α/β

[41]. We identified here, in an individual presenting with severe hepatitis, a novel splicing variant in *IRF7* (c.679+1G>C), which may impair signal transduction toward IFN production. Interestingly, multiple other loss-of-function variants have been identified earlier to result in *IRF7*-dependent inborn errors of type I IFN immunity and increased susceptibility to SARS-CoV-2 [37] or IAV [42].

Among the hundreds of interferon-stimulated gene products, 2'-5'-oligoadenylate synthetases (OAS) are known as enzymes which, upon recognition of cytosolic double-strand RNA, activate host RNase L to degrade viral RNA. In our study, we identified the c.2530C>T variant in the *OAS3* gene which creates a premature stop codon in exon 12 and thereby a 245-aa deletion in the C-terminal end of the protein. A recent report revealed that inborn errors of the OAS-RNase L pathway are responsible of multisystem inflammatory syndrome associated with SARS-CoV-2 infection in children [43]. Hence, a defective OAS-RNase L response pathway might also be involved in poor control HEV infection and severe liver inflammation.

The variants observed in RNA polymerase III subunit genes (*POLR3B* and *POLR3C*) are less likely to be causal. Indeed, RNA polymerase III is supposed to induce type I IFN upon recognition of AT-rich DNA [44] and not RNA. It has been shown that patients with inborn errors of immunity due to *POLR3A* and *POLR3C* pathogenic variants have a normal IFN response against RNA viruses [45]. Whether HEV can directly or indirectly trigger RNA polymerase III remains to be tested.

Activation of the type I IFN pathway is an essential step in the host immunity response to control viral infection. Earlier studies provided evidence of the importance of this pathway in host-HEV interactions, including the strong IFN response observed by transcriptomics in human hepatocytes following HEV infection [46], the sensitivity of the virus to IFN treatment [47], but also the interference of the different HEV proteins with the type I IFN pathway [39, 48, 49]. The importance of the host IFN response in the control of HEV infection has been recently addressed using downstream inhibitors of the cascade, namely Janus kinase (Jak) inhibitors [50]. The study demonstrates that impaired IFN response facilitates HEV production in primary human hepatocytes and may contribute to increase viral pathogenicity, especially in patients treated with Jak inhibitors. We here provide direct evidence, based on unbiased genomic approach that an impaired IFN response can lead to severe liver disease during acute HEV infection.

We acknowledge the relatively limited number of study participants. However, the patients and asymptomatic blood donors included in this study all had PCR-proven acute HEV infection and were very well characterized, especially to rule out preexisting chronic liver disease as a known risk factor for autochthonous symptomatic acute hepatitis E. The lack of availability of an ALT test for blood donors is another potential point of criticism. Despite this limitation, HEV-infected asymptomatic blood donors represent a suitable control group. Indeed, only individuals who do not show any symptom suggestive of an infection are accepted as blood donors. This was further documented in the standardized questionnaire submitted to each blood donor within the framework of this study (Supplementary Methods, http://links.lww.com/HEP/I129). More importantly, the high prevalence of pathogenic variants in genes involved in type I IFN immunity in patients with symptomatic acute hepatitis due to HEV infection is a strong observation, as it is in strong contrast to patients with HEV-associated PTS or blood donors with asymptomatic HEV infection. Finally, several of the identified damaging variants have already been functionally characterized and associated with severe courses of other viral infections, including SARS-CoV2, IAV and RSV.

In conclusion, our study demonstrates an important modulating role of host genetics in hepatitis E clinical presentation. Deleterious variants in type I IFN response genes were observed at an unusually high prevalence among patients who presented a severe hepatitis in the absence of known risk factors. Interestingly, we did not find any such variants among patients with PTS, alike in asymptomatic HEV-infected individuals, suggesting that etiology of HEV-associated neurological complications might be due to different pathogenic mechanisms. Altogether, our findings contribute to a better understanding of HEV pathogenesis and might pave the way toward individualized preventive and therapeutic approaches to severe infection.

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Figure 1. Study population. A total of 52 participants with acute hepatitis E virus (HEV) infection documented by PCR were included in this study, including 1) 24 patients with severe acute hepatitis E, 2) 12 patients with HEV-associated Parsonage-Turner syndrome, and 3) 16 asymptomatic blood donors. Please refer to the Study Population and Methods section for inclusion and exclusion criteria.

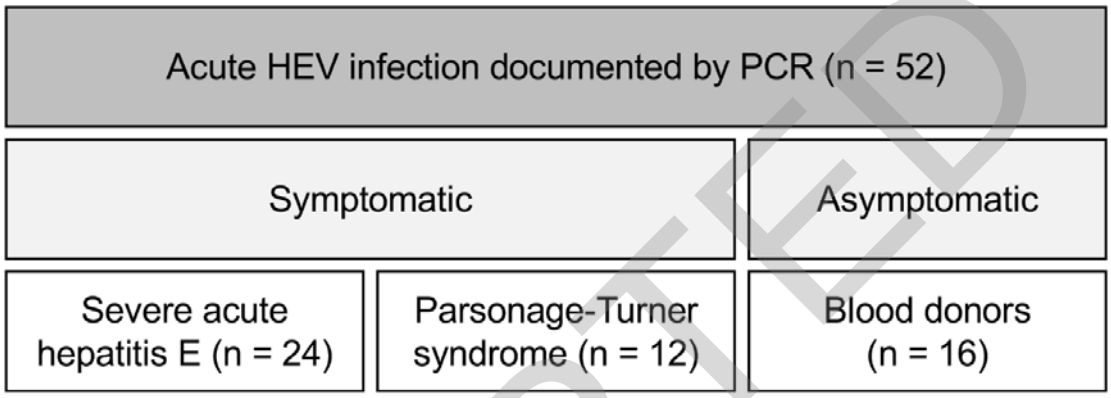

Figure 2. Results of pathway enrichment analyses. Left: Deleterious variants in the interferon I pathway are significantly enriched in the group of patients with severe acute hepatitis E. Pathway enrichment was analyzed using 348 genes in the severe hepatitis group. Each dot represents a pathway in Gene Ontology. **Right:** Pathogenic variants in the group of patients with Parsonage-Turner syndrome (PTS) are not enriched in any pathway. Pathway enrichment was analyzed using 218 genes in the PTS group. Each dot represents a pathway in Gene Ontology. The dashed line denotes the significance cutoff ($\alpha = 0.05$). The y-axis represents the log scale of adjusted p-values, and the x-axis is an arbitrary order of pathways.

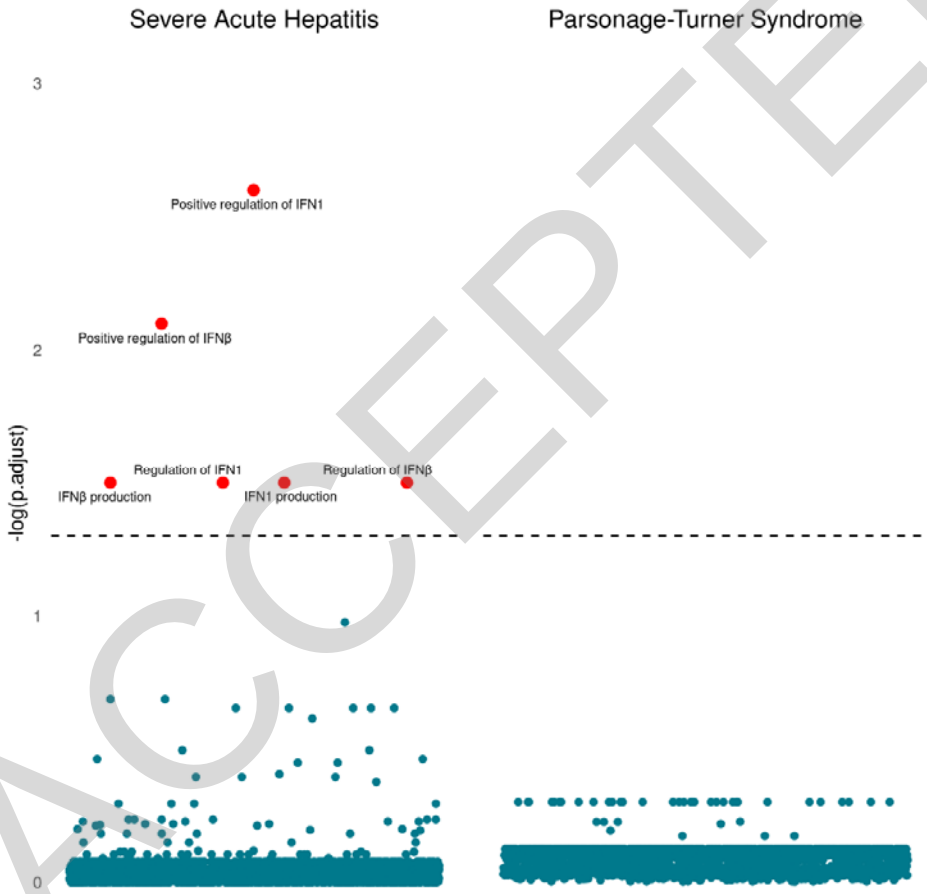

Figure 3. Inborn errors of type I interferon response underlie HEV-induced symptomatic acute hepatitis. Molecules in red represent key elements in the antiviral immunity against hepatitis E virus (HEV). Our findings reveal that deleterious variants in these pivotal proteins predisposed to symptomatic acute hepatitis after HEV infection in patients without any pre-existing liver disease. Figure created with BioRender.com.

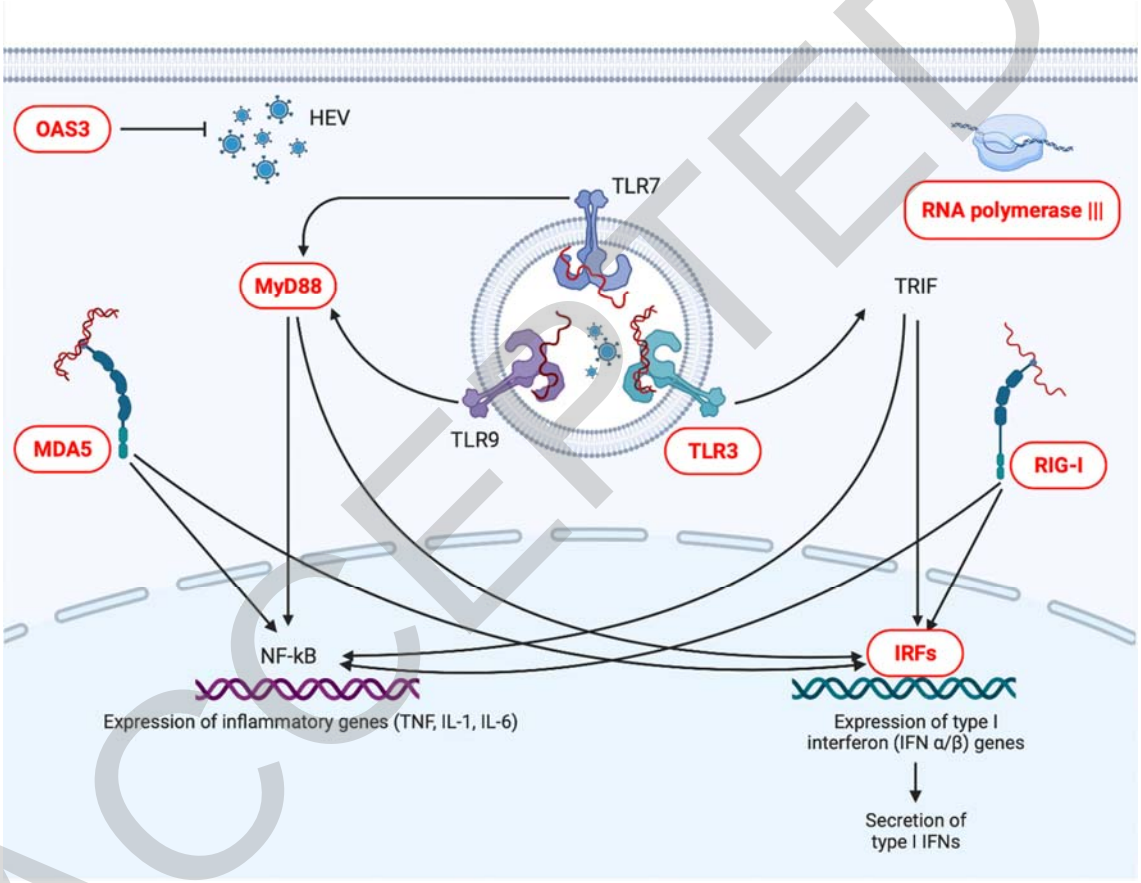

Table 1. Demographic, clinical, laboratory and virological characteristics of study participants with acute hepatitis E virus (HEV) infection.

Group	Symptomatic acute hepatitis E (n = 24)	HEV-associated PTS (n = 12)	Asymptomatic blood donors (n = 16)
Male, n (%)	22 (91.7)	12 (100)	12 (75.0)
Age (years), median (range)	54 (25-80)	53 (39-69)	56 (24-74)
Caucasian origin, n (%)	24 (100)	12 (100)	16 (100)
T2DM, n (%)	5 (20.8)	1 (8.3)	2 (12.5)
Viral load (log_{10} IU/mL), median (range)[1]	5.7 (2.8-7.0)	4.2 (2.6-6.1)	3.1 (1.1-5.3)
HEV genotype, n (%) - 3 - 3h_s - 3a - 3c - NA	 3 (12.5) 9 (37.5) 1 (4.2) 1 (4.2) 10 (41.7)	 2 (16.7) 6 (50.0) 1 (8.3) 3 (25.0)	NA
Peak ALT (U/L), median (range)	2232 (745-5600)	365 (99-3582)	NA
Peak total bilirubin (µmol/L), median (range)	77 (28-469)	14 (10-83)	NA

[1]Viral load was determined by quantitative RT-PCR as described [8].

ALT, alanine aminotransferase; HEV, hepatitis E virus; NA, not available; PTS, Parsonage-Turner syndrome. T2DM, type 2 diabetes mellitus.

Table 2. Pathogenic variants observed in the interferon type I response.

Variant (hg38)	Gene	Impact	Variant ID	Amino acid change	gnomAD AC (#hom), MAF
2:162277580:C>A	IFIH1*	nonsense	rs353375 43	p.Leu509_Glu547*del*	1102 (5), 0.7%
2:162279995:C>G	IFIH1	splicing	rs357446 05	p.Glu627Ter	545 (4), 0.4%
9:32480218:C>A	DDX58	splicing	rs617562 74	NA	2 (0), 0.001 %
4:186083346:C>T	TLR3	missense	rs121434 431	p.Pro554Ser	75 (0), 0.05%
11:614173:C>G	IRF7	splicing	NA	NA	0 (0), 0
12:106457185:C>T	POLR3B	nonsense	NA	p.Arg781Ter	1 (0), 0.0007 %
1:145842346:GC>G	POLR3C	frameshift	rs775151 955	p.Ser512ValfsTer20	228 (0), 0.1%
12:6536586:ACTACATGGTG	GAPD	splicing	rs775048	NA	7 (0), 0.004

AGTG>A	H		305		%
3:38140537:TG>T	MYD88†	frameshift	NA	p.Trp205CysfsTer48	1 (0), 0.0007 %
12:112965870:C>T	OAS3†	nonsense	rs61942233	p.Arg844Ter	1608 (10), 1%

All variants in the table are heterozygous, carried by male patients with a median age of 50 years (range, 41-64 years).

*Two study subjects carried this variant.

†These two variants were observed in the same participant.

AC, allele count; #hom, number of homozygous individuals; gnomAD, genome aggregation database; MAF, minor allele frequency; NA, not available.