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Inborn errors of type I interferon immunity in patients with symptomatic acute hepatitis E

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Abstract

Background and Aims: The clinical spectrum of human infection by HEV ranges from asymptomatic to severe acute hepatitis. Furthermore, HEV can cause diverse neurological manifestations, especially Parsonage-Turner syndrome. 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 3 groups of study participants with PCR-proven acute HEV infection: (1) 24 patients with symptomatic acute hepatitis E; (2) 12 patients with HEV-associated Parsonage-Turner syndrome; and (3) 16 asymptomatic blood donors (controls). For variant calling and annotation, we used GATK4 best practices followed by Variant Effect Predictor (VEP) and Annovar. For variant classification, we implemented the American College of Medical Genetics and Genomics/Association for Molecular Pathology Bayesian

Abbreviations:ACMG, American College of Medical Genetics and Genomics; ALT, alanine aminotransferase; AMP, Association for Molecular Pathology; gnomAD, Genome Aggregation Database; IAV, influenza A virus; IFN, interferon; *IRF7*, interferon regulatory factor 7; MAF, minor allele frequency; OAS, oligoadenylate synthetase; PoP, probability of pathogenicity; PTS, Parsonage-Turner syndrome; VEP, Variant Effect Predictor.

Jacques Fellay and Montserrat Fraga share the last authorship.

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well as liver histology when available. Patients under immunosuppressive treatment or who had traveled 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. All research was conducted in accordance with both the Declarations of Helsinki and Istanbul.

Data and sample collection

The following data were 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 the 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 (Supplemental Methods, <http://links.lww.com/HEP/I129>); (3) laboratory data including peak ALT, peak total bilirubin, and international normalized ratio (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 (Supplemental 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), and 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 $0.2 \leq$ 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 American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) guidelines^[23] in R (<https://www.r-project.org>) (see full description in the Supplemental 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 (Supplemental Methods, <http://links.lww.com/HEP/I129>, rule BS2 and BS2_moderate). Variants with PoP ≥ 0.9 were considered 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, that is., 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 predefined 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.

Acute HEV infection documented by PCR (n = 52)		
Symptomatic		Asymptomatic
Severe acute hepatitis E (n = 24)	Parsonage-Turner syndrome (n = 12)	Blood donors (n = 16)

FIGURE 1 Study population. A total of 52 participants with acute 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.

Of the 24 patients with symptomatic acute hepatitis, 22 (91.7%) were male, and the median age was 54 years (range, 25–80 y). The majority of these patients were hospitalized (17/24, 70.8%). Median viral load was 5.7 log₁₀ IU/mL (range, 2.8–7.0 log₁₀ 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, HE, 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 y). Damage outside of the brachial plexus was noted in 4/12 (33.3%) patients. Median viral load was 4.2 log₁₀ IU/mL (range, 2.6–6.1 log₁₀ IU/mL). Viral genotyping was possible in 9/12 (75.0%) patients, revealing genotype 3 in all and subtype 3h_s in 6, that is, 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 y). 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 the time of blood donation.

Variant classification

In total, 13,768,097 variants in patients who are symptomatic passed the quality control criteria and were annotated. A total of 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. A total of 2279 variants had an intermediate PoP (between 0.1 and 0.9), resulting in their classification as variants of unknown significance. Five hundred sixty-six variants exceeded the pathogenicity threshold (≥ 0.9) and were considered 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 was calculated for the 3 groups (symptomatic acute hepatitis, PTS, and

TABLE 1 Demographic, clinical, laboratory, and virological characteristics of study participants with acute 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 (y), median (range)	54 (25–80)	53 (39–69)	56 (24–74)
White 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) ^a	5.7 (2.8–7.0)	4.2 (2.6–6.1)	3.1 (1.1–5.3)
HEV genotype, n (%)			
3	3 (12.5)	2 (16.7)	NA
3h_s	9 (37.5)	6 (50.0)	—
3a	1 (4.2)	1 (8.3)	—
3c	1 (4.2)	—	—
NA	10 (41.7)	3 (25.0)	—
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

^aViral load was determined by quantitative RT-PCR as described.^[8]

Abbreviations: ALT, alanine aminotransferase; NA, not available; PTS, Parsonage-Turner syndrome. T2DM, type 2 diabetes mellitus.

asymptomatic controls) separately (Supplemental Table S1, <http://links.lww.com/HEP/I129>). We observed comparable distributions of variants and variant types across the 3 groups (Supplemental Table S1, <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 2 symptomatic cohorts (severe hepatitis group and PTS group) separately (Supplemental Tables S2, <http://links.lww.com/HEP/I129>, S3A, B, <http://links.lww.com/HEP/I129> as well as S4A, B, <http://links.lww.com/HEP/I129>). (1) Homozygous, compound heterozygous, and X-linked variants: 1 homozygous variant in *OCM* and 1 X-linked variant in *TSPAN7* were observed in 2 symptomatic acute hepatitis subjects. One X-linked variant in *CXorf58* was found in 1 patient with PTS. No compound heterozygous variant was observed. (2) Gene enrichment: 9 genes with at least 2 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 2 groups, using all genes with at least 1 damaging

variant as input, that is, a total of 348 genes for the symptomatic acute hepatitis group and 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; Supplemental Table S5, <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 subpathway of type I IFN production. No pathway was significantly enriched in the PTS group (Figure 2, right). 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 1 of the 9 genes identified as from type I IFN response (Table 2, Figure 3). These genes can be classified into 2 groups: (1) genes encoding intracellular viral sensors: *IFIH1*, *DDX58*, *TLR3*, *POLR3B*, and *POLR3C* that recognize virus

Severe Acute Hepatitis

Parsonage-Turner Syndrome

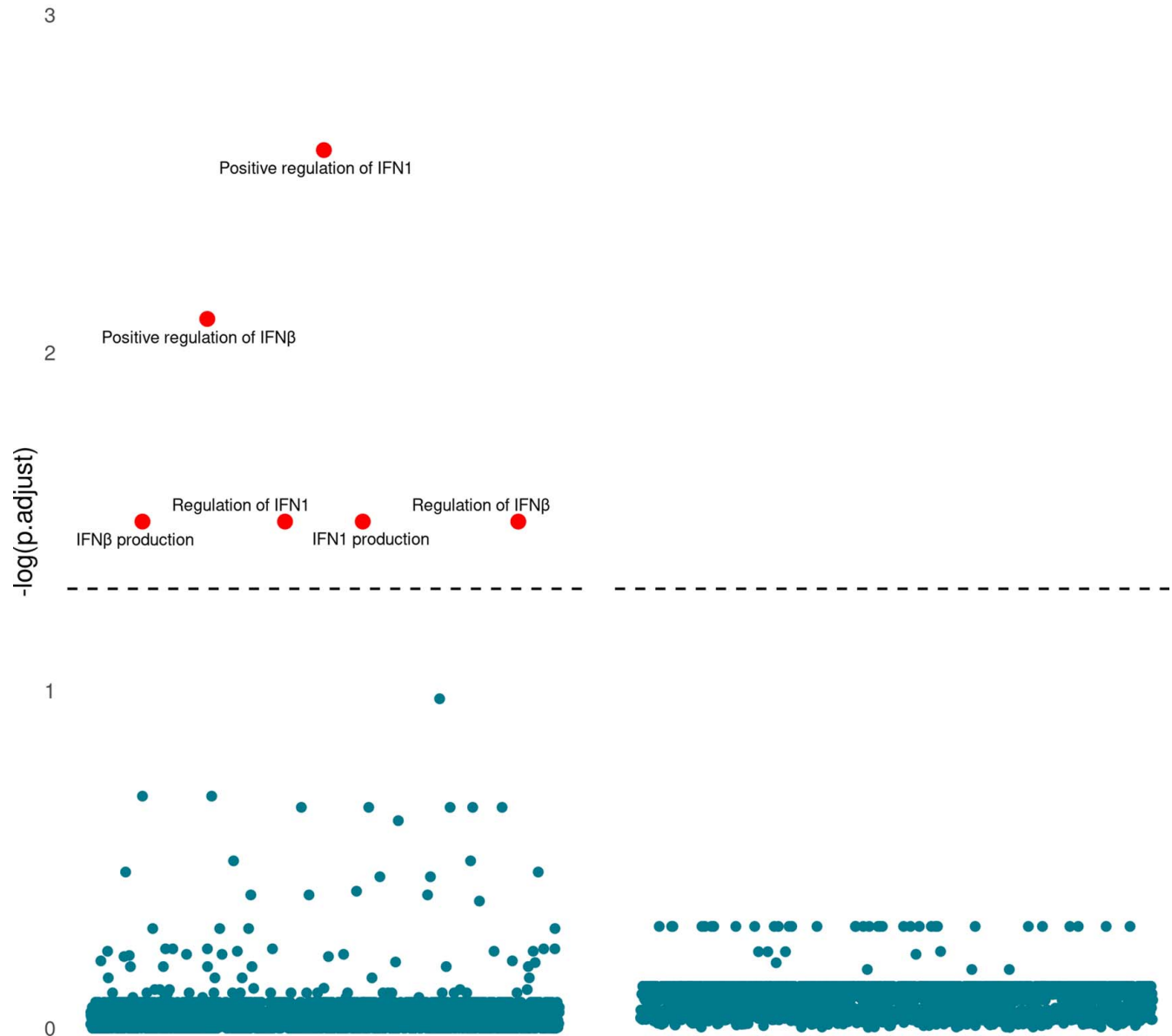


FIGURE 2 Results of pathway enrichment analyses. Left: Deleterious variants in the interferon I pathway are significantly enriched in the group of patients with severe acute hepatitis E. Pathway enrichment was analyzed using 348 genes in the severe hepatitis group. Each dot represents a pathway in Gene Ontology. Right: Pathogenic variants in the group of patients with Parsonage-Turner syndrome 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. Abbreviations: IFN, interferon; PTS, Parsonage-Turner syndrome.

infection and trigger an antiviral innate immune response. (2) Genes encoding regulators or effectors of the IFN response: interferon regulatory factor 7 (*IRF7*), *MYD88*, *OAS3*, and *GAPDH*.

We identified 2 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 the 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

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 ^a	Nonsense	rs35337543	p.Leu509_Glu547del	1102 (5), 0.7%
2:162279995:C > G	IFIH1	Splicing	rs35744605	p.Glu627Ter	545 (4), 0.4%
9:32480218:C > A	DDX58	Splicing	rs61756274	NA	2 (0), 0.001%
4:186083346:C > T	TLR3	Missense	rs121434431	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	rs775151955	p.Ser512Val fsTer20	228 (0), 0.1%
12:6536586:ACTACATGGTGAGTG > A	GAPDH	Splicing	rs775048305	NA	7 (0), 0.004%
3:38140537:TG > T	MYD88 ^b	Frameshift	NA	p.Trp205CysfsTer48	1 (0), 0.0007%
12:112965870:C > T	OAS3 ^b	Nonsense	rs61942233	p.Arg844Ter	1608 (10), 1%

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

^aTwo study subjects carried this variant.

^bThese 2 variants were observed in the same participant.

Abbreviations: AC, allele count; #hom, number of homozygous individuals; gnomAD, Genome Aggregation Database; MAF, minor allele frequency; NA, not available; OAS3, 2'-5'-oligoadenylate synthetase 3.

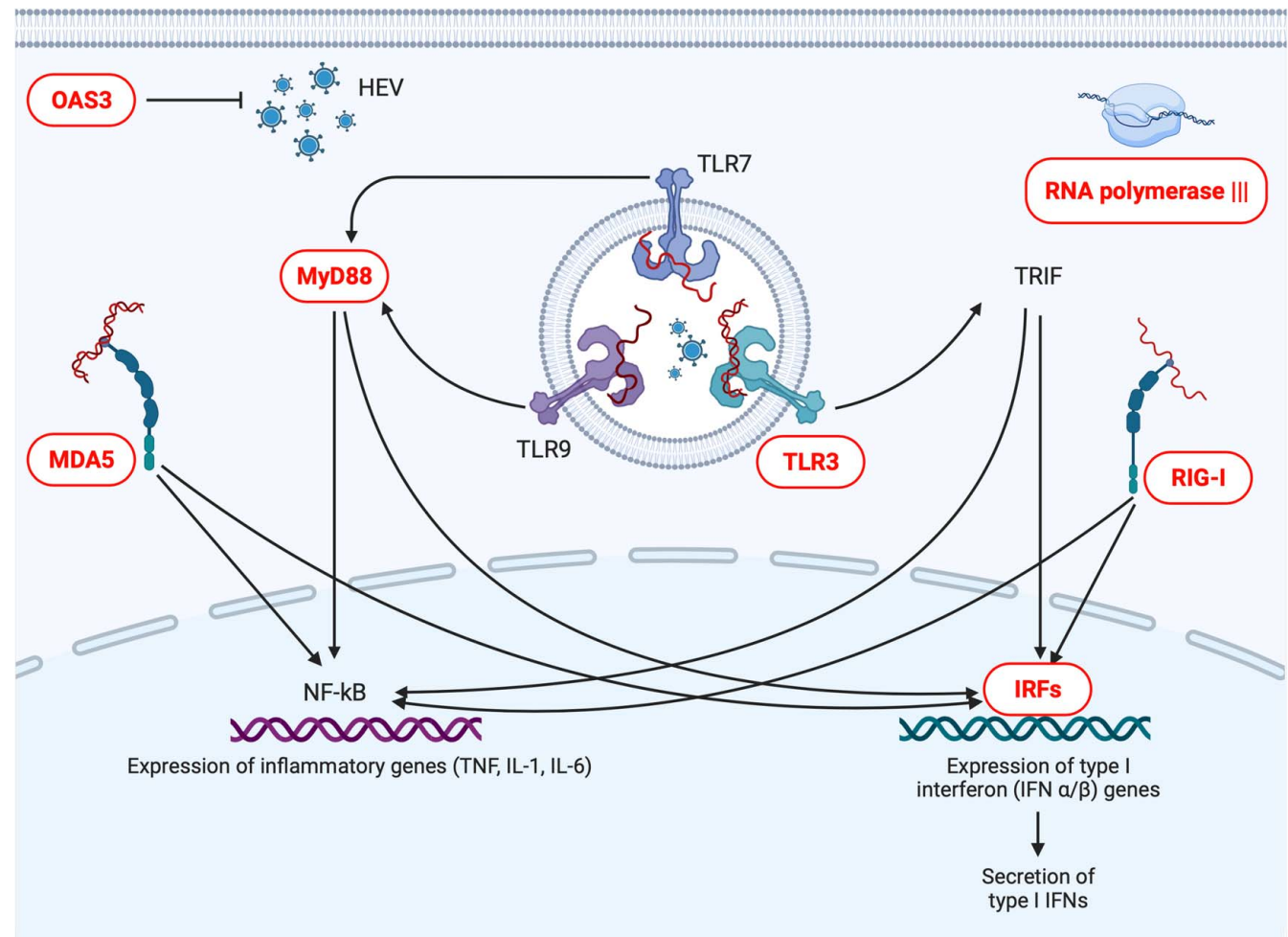


FIGURE 3 Inborn errors of type I interferon response underlie HEV-induced symptomatic acute hepatitis. Molecules in red represent key elements in the antiviral immunity against HEV. Our findings reveal that deleterious variants in these pivotal proteins predisposed to symptomatic acute hepatitis after HEV infection in patients without any pre-existing liver disease. Figure created with BioRender.com. Abbreviations: IFN, interferon; OAS, oligoadenylate synthetase.

base in the 5' direction (probability = 0.65). Consequently, the reading frame is shifted by 1 bp, 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 2 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-bp 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 IFN 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 2 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 inter-individual differences observed in response to diverse infections.^[28] Previous host genetic studies of HEV infection were limited by a 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 pre-existing 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 a control group.

Our variant prioritization strategy, based on careful quality control and annotation followed by an 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 1 X-linked variant in symptomatic acute hepatitis subjects and 1 homozygous variant in 1 patient with PTS; (2) 26 genes with more than 1 damaging variant in patients with severe hepatitis 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-based 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 patients with PTS 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 (Supplemental Table S6, <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 dsRNA to launch a type I IFN response, with the purpose of limiting the replication of RNA viruses.^[30] We have shown that the 2 *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 2 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 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 on 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 an HEV-infected individual presenting with severe hepatitis meets the conclusion of *in vitro* data suggesting a role for this pattern recognition receptor 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 pattern recognition receptors, the signal is conveyed by phosphorylation of IFN 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 IFN-stimulated gene products, 2'-5'-OAS are known as enzymes which, on 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 for 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 of 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 on 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 inhibitors.^[50] The study demonstrates that impaired IFN response facilitates HEV production in primary human hepatocytes and may contribute to increased viral pathogenicity, especially in patients treated with Janus kinase inhibitors. We here provide direct evidence, based on the 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 pre-existing 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 symptoms 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 (Supplemental 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-CoV-2, IAV, and respiratory syncytial virus.

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.

DATA AVAILABILITY STATEMENT

The authors' data are deposited: <https://zenodo.org/records/10286145>.

AUTHOR CONTRIBUTIONS

Ali Saadat, Jérôme Gouttenoire, Darius Moradpour, Jacques Fellay, and Montserrat Fraga designed the study. All authors acquired the data. Ali Saadat, Jérôme Gouttenoire, Darius Moradpour, Jacques Fellay, and Montserrat Fraga analyzed and interpreted the data. Ali Saadat, Jérôme Gouttenoire, Darius Moradpour, Jacques Fellay, and Montserrat Fraga drafted the article. All authors critically revised the article for intellectual content and approved the final version.

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CONFLICTS OF INTEREST

The authors have no conflicts to report.

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