


# A mutation in the progesterone receptor predisposes to HEV infection in HIV-positive patients

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## Abstract

**Background and Aims:** Infection with Hepatitis E virus (HEV) can cause chronic liver disease in immunocompromised hosts. In transplant recipients, the use of certain immunosuppressants and food habits has been proposed as risk factors for HEV. In individuals infected with the human immunodeficiency virus (HIV), risk factors for HEV infection are less clear. We aimed to study the association between a mutation in the progesterone receptor (PR) named PROGINs and HEV-infected in HIV-positive individuals.

**Methods:** We evaluated the presence of the SNP PROGINs via KASP in serum samples of 64 HIV-positive individuals and 187 healthy controls. We performed ELISA tests to address the serum levels of IL-10 and IL-12, as well as T-cell stimulation assays in peripheral blood to address immune response in individuals with PROGINs.

**Results:** We found a significant association between the presence of PROGINs mutation and HEV seroprevalence in individuals infected with HIV (30% in HIV+/HEV+ versus 2% in HIV+/HEV, respectively,  $P = .009$ ). Moreover, we found that HIV+/HEV+ individuals expressing the PROGINs mutation had lower serum levels of IL-10 and higher levels of IL-12. The presence of the mutation led to a reduced response upon stimulation of CD4+ and CD8+ T cells compared to those without the mutation, suggesting an immune modulation associated with PROGINs.

**Conclusions:** Our study identified a mutation in the PR that provides significant insights into mechanisms of HEV infection in immunosuppressed individuals.

## KEYWORDS

hepatitis E, human immunodeficiency virus, progesterone receptor, SNP

## 1 | INTRODUCTION

Hepatitis E virus (HEV) is a RNA virus considered endemic in many areas of Asia and Africa, with sporadic outbreaks related to genotype 1 infection reported in people that consume contaminated food

**Abbreviations:** HEV, hepatitis E virus; HIV, human immunodeficiency virus; RNA, ribonucleic acid; IL, interleukin; PR, progesterone receptor; WT, wild type; ELISA, enzyme-linked immunosorbent assay; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PCR, polymerase chain reaction; PBMC, peripheral blood mononuclear cells; CD, cluster differentiation; EDTA, Ethylenediamine tetraacetic acid.

or water.<sup>1</sup> When isolated cases occur in immunocompetent hosts, HEV infection is usually self-limited or subclinical unless the host is a pregnant woman, in which the reported mortality is as high as 50%.<sup>2</sup> The mechanisms related to fulminant hepatic failure during HEV infection in pregnancy remain unclear although hormonal alterations have shown to play a role.<sup>3,4</sup> In recent years, immunocompromised individuals have been shown to develop chronic infection or reactivation of HEV, mainly due to infection with genotype 3.<sup>5,6</sup> This effect is more evident in patients receiving a solid organ

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transplant, but can occur in other forms of immunosuppression such as bone marrow transplantation.<sup>7,8</sup> In immunocompromised individuals, infection with human immunodeficiency virus (HIV) correlation with long-term complications by HEV infection is less well established.<sup>9,10</sup> Most studies have found a higher seroprevalence of HEV in HIV-infected patients, but there is less convincing evidence of chronicity.<sup>9,11,12</sup> Interestingly nonetheless, several studies found an increased seroprevalence of HEV in cirrhotics with HIV, compared to HIV-infected non-cirrhotic patients, implicating the virus as a potential risk factor for progression of liver disease to advanced fibrosis stages in this setting.<sup>13,14</sup> Overall, there is a paucity of knowledge in regard to risk factors that affect prevalence or predispose to HEV infection in individuals with HIV.<sup>9,10</sup> Recent studies have described novel factors implicated in infection and mortality of HEV infection during pregnancy.<sup>3,15</sup> In this regard, a mutation in the progesterone receptor (PR) has been reported to be associated with acute viral hepatitis and liver failure from HEV in pregnant women.<sup>4</sup> This mutation, called PROGINS (progesterone receptor G insert), consists of two single nucleotide polymorphisms and a G-intron insert, and it has been reported to render the PR less functional.<sup>16</sup> Therefore, during pregnancy, progesterone could have a differential effect on peripheral lymphocytes expressing PR in individuals with the mutation predisposing to HEV infection. Since lymphocytes, particularly CD4+ lymphocytes, are most affected during HIV infection, it is possible that a second “immune hit” could predispose certain patients to other viral infections. In this study, we addressed whether the PR mutation PROGINS represents a risk factor for HEV infection in HIV-positive individuals.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

#### 2.1.1 | HIV-positive patients

HIV-positive patients from the Department of Infectious Diseases of the Erasmus Medical Center between June 2006 and June 2011 were selected as described before.<sup>17</sup> The median age of patients was 42 years, 73% were males and 80% were on antiretroviral therapy. EDTA-plasma samples stored at  $-80^{\circ}\text{C}$  were used for the detection of HEV-specific antibodies and HEV-RNA. *Healthy Controls*: Samples present in our serum biobank who voluntarily donated serum and blood for research purposes. They remain anonymous and we do not keep information regarding age or gender.

We obtained approval for our study by the Medical Ethical Review board of the Erasmus Medical Center (reference number MEC- 2011-438). Informed consent was requested in those where PBMC samples were obtained.

### 2.2 | HEV-specific antibody detection

For both HEV-specific IgM and HEV-specific IgG detection in serum or EDTA-plasma samples, the commercially available enzyme-linked

### Key points

- Hepatitis E infection is more frequent in HIV-positive patients and the only known risk factor for hepatitis E in HIV is CD4 counts
- We found that the presence of the PROGINS mutation is associated with HEV infection in HIV-positive patients
- Individuals with PROGINS mutations showed a distinctive immune response with altered serum levels of IL-10 as well as decreased T-cell stimulation
- Our manuscript provides new insight into mechanisms of HEV infection in immunosuppression

immunosorbent assay (ELISA) (Wantai) was used according to the manufacturer's instructions.

### 2.3 | Serum IL-10 and IL-12 detection

Cytokine levels were determined using the human IL-10 high sensitivity and the human IL-12p70 ELISA kit (eBioscience).

### 2.4 | Host genotyping

Competitive allele-specific PCR assays (KASP™, LGC Genomics, Huddleston, UK) were employed for the detection of the reference SNP rs1042838. The PROGINS allele was defined by the exon 4 missense SNP rs1042838, Val660Leu as previously described.<sup>4</sup> Serum samples stored at  $-80^{\circ}\text{C}$  were used for DNA extraction and genotyping procedures which were carried out at LGC genomics. Purified genomic DNA of  $\geq 5$  ng was used for genotyping. Genotypes were assigned using all of the data from HIV samples simultaneously and healthy controls on a second instance. Genotype sequences were derived from NCBI.

### 2.5 | Stimulation and intracellular cytokine analysis of T cells and NK cells

#### 2.5.1 | T-cell stimulation

Peripheral blood mononuclear cells (PBMCs) from healthy individuals and patients (obtained from fresh blood and later frozen) were cultured in a 96-well plate. Cells were stimulated with PMA (50 ng/mL) and ionomycin (400 ng/mL, both from Sigma) for 1 hour, with or without progesterone 1 or 10  $\mu\text{g}$ , or progesterone 10  $\mu\text{g}$  alone (Sigma Aldrich), at  $37^{\circ}\text{C}$ . Brefeldin A was added and the cells were incubated for another 4 hours. Samples were fixed, permeabilized and stained with IL-2 APC, CD4 PE-Cy7, CD8 eFluor450, CD3 FITC (all eBioscience). Cytokine-producing cells were detected by flow cytometry (FACS Canto-II, BD). *NK cell stimulation*: PBMCs from healthy individuals were cultured in a 24-well plate. Cells

were stimulated with IL-12 and IL-18 alone or with pre-treatment with progesterone 1 or 10 µg, or progesterone 10 µg alone. After 18 hours, brefeldin A was added to the cultures and the cells were incubated for an additional 4 hours. Cellular activation and surface markers were measured using anti-CD3 PacificBlue (OKT3, eBioscience), anti-CD56 PE (MY31, BD) and anti-CD69 APC (L78, BD), followed by fixation with 2% formaldehyde, and permeabilization with anti-IFN-γ FITC (BD). Activated and cytokine-producing NK cells were assessed by flow cytometry and analysed using FlowJo version 10.1 (Tree Star Inc).

## 2.6 | Statistical analysis

Baseline characteristics were compared between HEV IgG positive and negative patients expressing PROGINs mutation or wild-type progesterone receptor (PR), using Fisher exact test (between HEV+ and - and presence or absence of PROGINs). Quantitative comparisons were performed using nonparametric Wilcoxon test or Mann-Whitney test (for values that did not show a normal distribution). For comparison of stimulation of T or NK cells, paired *t*-test was used to evaluate median of responses. A *P* value <.05 was considered statistically significant.

## 3 | RESULTS

### 3.1 | PROGINs mutation in HIV+ and healthy controls

A total of 64 HIV+ patients and 187 healthy controls were evaluated for the presence of PROGINs. Of those HIV+, 26 were seropositive for HEV (HIV+/HEV+) and 38 (age-matched) were HEV seronegative. The median age for HIV+/HEV+ individuals was 47 years (IQR 42-53) and their median CD4 count 350/mm<sup>3</sup>. Median age for HIV+/HEV- individuals was 40 years (IQR 33-46) with a median CD4 count of 280/mm<sup>3</sup> (Table 1). We found 8 out of 26 (30%) HIV+/HEV+ individuals

to express the PROGINs mutation compared to 1 out of 38 (2%) of HIV+/HEV- individuals (*P* = .009, Figure 1A). Baseline characteristics of the individuals expressing wild-type PR or the PROGINs mutation were similar, with a median of 47 years for both groups and a median CD4 count of 340/mm<sup>3</sup> for those with wild-type PR, and 487/mm<sup>3</sup> for those with PROGINs mutation (Table 1). There was a higher percentage of males among individuals expressing the PROGINs mutation compared to wild-type PR (86% vs 66%), but the difference was not significant, suggesting that the higher seroprevalence of HEV in HIV-positive individuals with the PROGINs mutation cannot be explained by age, gender or CD4 count. A similar analysis of the PROGINs mutation in 187 healthy controls available in our serum bank showed that 26 out of 187 controls (14%) carried the PROGINs mutation. Interestingly, in a subset of 70 of the healthy controls, eight (11%) were positive for HEV IgG and only two of those expressed PROGINs mutation (Table S1).

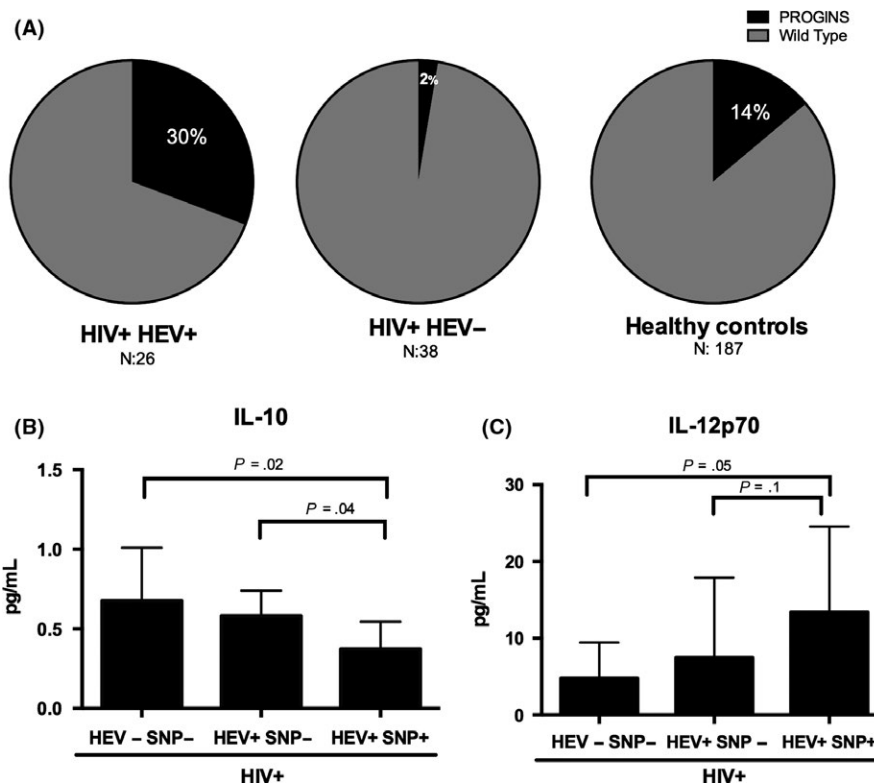
### 3.2 | Cytokine levels in the presence or absence of PROGINs in HIV

We further analysed whether the presence of the PROGINs mutation had an impact on the modulation of immune parameters in HIV-positive individuals. We performed ultra-sensitive ELISA assays to determine the levels of IL-10 and IL-12 in serum of 5 HIV+/HEV- samples with wild-type PR, 5 HIV+/HEV+ samples with wild-type PR and 5 HIV+/HEV+ samples that carried the PROGINs mutation. These samples were obtained in duplicate at two different time points with confirmation of liver enzymes being under normal limits (to rule out external inflammatory stimuli). We found that levels of IL-10 were significantly lower in samples that expressed the PROGINs mutation compared to those with wild-type PR, either HEV+ or HEV- (*P* = .04 and .02, respectively; Figure 1B). Levels of IL-12 were elevated in those with PROGINs mutation, although this trend was not significant (*P* = .05), likely due to the low number of samples (Figure 1C).

	HIV+ HEV+ N:26 N (%)	HIV+ HEV- N:38 N (%)	Controls N:187 N (%)
PROGINs mutation rs1042838	8 (30%)	1 (2%)	26 (14%)
HEV serostatus	HEV+	HEV-	
Median age (IQR)	47 (42-53)	40 (33-46)	
Male percentage	70%	70%	
CD4+ T cell count <sup>a</sup> (IQR)	350 (230-580)	280 (200-410)	
Mutation status	PROGINs+	PROGINs-	
Median age (IQR)	47 (40-63)	47 (41-53)	
Male percentage	87%	66%	
CD4+ T-cell count (IQR)	487 (279-592)	340 (121-470)	

**TABLE 1** Patient characteristics

<sup>a</sup>CD4 count/mm<sup>3</sup>.  
IQR, Interquartile Range.



**FIGURE 1** Presence and effect of PROGINs in HIV/HEV-seropositive individuals and controls. (A) Pie chart showing frequency of PROGINs as a percentage in HIV+/HEV+ (N:26); HIV+/HEV- individuals (N:38) and healthy controls (N:187). Dark colouring indicates presence of PROGINs, grey colouring indicates wild-type progesterone receptor. (B) Serum levels of IL-10 (in 10 individuals, 5 each group) measured with ultra-sensitive ELISA. Y-axis represents levels of IL-10 in pg/ml; X-axis represents the different groups. (C) Serum level of IL-12 fraction p70 in HIV+ individuals (in 10 individuals, 5 each group) measured by ELISA. Y-axis represents levels of IL-12p70 in pg/ml; X-axis represents the different groups. SNP = single nucleotide polymorphism rs1042838 (indicative of PROGINs)

### 3.3 | Role of PROGINs in immune functional response

To further understand the role of PROGINs in immune modulation under stress, we performed functional assays in CD4<sup>+</sup>, CD8<sup>+</sup> T cells and NK cells. Since PBMCs from HIV-positive individuals with the mutation were not available, we performed the assays (in duplicate) with samples from five healthy controls who carried wild-type PR and five samples with the PROGINs mutation. Upon stimulation with PMA/ionomycin, we found lower cytokine production in CD4<sup>+</sup> and CD8<sup>+</sup> T cells from samples carrying the PROGINs mutation compared to those with wild-type PR, and this reduced response was more evident when we used synthetic progesterone (Figure S1A,B). This effect was statistically significant in CD4<sup>+</sup> T cells, but not in CD8<sup>+</sup> T cells, and was observed in multiple independent experiments. In contrast to the effect on T-cell function, we found no differential response upon stimulation of NK cells with IL-12 and IL-18 regardless of the presence of the mutation or not (Figure S1C).

## 4 | DISCUSSION

In this study, we describe for the first time a mutation in the progesterone receptor associated to HEV seroprevalence in HIV-infected patients. The presence of the mutation led to lower IL-10 levels in serum, and a decreased functionality of CD4<sup>+</sup> and CD8<sup>+</sup> T cells upon polyclonal stimulation.

We show that the PROGINs SNP is found at a frequency of 14% in a large cohort of healthy volunteers, and the same frequency was

observed in all HIV-positive samples combined (9 out of 64, 14%). However, stratification of PROGINs frequency between HEV-positive and HEV-negative individuals within the HIV-infected group demonstrated a staggering difference between both populations with 30% of HIV+/HEV+ expressing PROGINs versus 2% in those HIV+/HEV-. These findings suggest that the presence of the mutation is a potential risk factor for HEV in HIV-infected individuals. Altered immunity in HIV patients is well described, even in those with viral control by effective antiretroviral therapy.<sup>18</sup> Our findings suggest that additional immunomodulation in patients carrying the PROGINs mutation may enhance susceptibility to HEV infection or development of an immune response to it.

Our experiments in healthy volunteers expressing the PROGINs mutation revealed a decreased response to stimulation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Although it is difficult to prove causality, it is reasonable to speculate that weaker T-cell responses in patients carrying the PROGINs mutation may result in higher HEV seroprevalence in this group. The suggestion that the PROGINs mutation acts as a factor related to an altered immunity based on a differential response to progesterone in T cells is relevant, and may also be pertinent for other diseases in which the PROGINs mutation has been evaluated and found to be a potential risk factor, such as breast and ovarian cancer.<sup>19,20</sup> Importantly, in contrast to T cells, we found that NK cells from individuals with PROGINs mutations have a similar functionality to those with wild-type progesterone receptors.

It was previously reported that the PROGINs mutation allows for infection of HEV during pregnancy via modulation of immune responses.<sup>4</sup> In this study, it was hypothesized that differential IL-10 modulation due to the presence of the PROGINs mutation could

affect Th1/Th2 regulation during pregnancy hence allowing for HEV infection.<sup>4</sup> However, in our study, we found reduced serum IL-10 levels in those with PROGINS mutations, suggesting that IL-10 mediated inhibition of T- or NK-cell responses is unlikely to contribute to the enhanced HEV seroprevalence in the HIV setting.

None of the HEV-seropositive individuals had detectable HEV RNA in blood, and we cannot therefore address this association to the development of chronic HEV infection. In order for the PROGINS mutation to be the dominant risk factor for HEV, one would have to assume complete exposure to HEV in the entire population. Although this is somewhat unlikely, it is possible that most individuals are exposed to risk factors associated to HEV such as pork consumption and ingestion of charcuterie.<sup>9,14</sup>

Overall, our findings show for the first time an association with the PR mutation PROGINS and infection with HEV in HIV-positive patients, likely related to alterations in the immune milieu. A larger study involving individuals receiving organ transplant and other forms of immunosuppression is warranted.

## CONFLICT OF INTEREST

The authors do not have any disclosures to report.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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