

1 **A HEV ORF2 protein-mediated mechanism of hepatitis E-associated kidney**  
2 **disease**

3

4 Birgit Helmchen<sup>\*1</sup>, Anne-Laure Leblond<sup>\*1</sup>, Daniela Lenggenhager<sup>1</sup>, Jasna Jetzer<sup>1</sup>, Maliki  
5 Ankavay<sup>2</sup>, Fritjof Helmchen<sup>3</sup>, Hueseyin Yurtsever<sup>4</sup>, Rossella Parrotta<sup>1</sup>, Marc E. Healy<sup>1</sup>,  
6 Amiskwia Pöschel<sup>5</sup>, Enni Markkanen<sup>5</sup>, Nasser Semmo<sup>6</sup>, Martin Ferrié<sup>7</sup>, Laurence Cocquerel<sup>7</sup>,  
7 Harald Seeger<sup>8</sup>, Helmut Hopfer<sup>9</sup>, Beat Müllhaupt<sup>10</sup>, Jérôme Gouttenoire<sup>2</sup>, Darius Moradpour<sup>2</sup>,  
8 Ariana Gaspert<sup>\*\*1</sup>, Achim Weber<sup>\*\*1,11</sup>

9

10 <sup>1</sup> Department of Pathology and Molecular Pathology, University of Zurich (UZH) and University  
11 Hospital Zurich (USZ), Zurich, Switzerland

12 <sup>2</sup> Division of Gastroenterology and Hepatology, Lausanne University Hospital and University of  
13 Lausanne, Lausanne, Switzerland

14 <sup>3</sup> Brain Research Institute, University of Zurich, Zurich, Switzerland

15 <sup>4</sup> Institute of Pathology, Cantonal Hospital, Aarau, Switzerland

16 <sup>5</sup> Institute of Veterinary Pharmacology and Toxicology, University of Zurich - Vetsuisse Faculty,  
17 Zürich, Switzerland

18 <sup>6</sup> Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University  
19 of Bern, Switzerland

20 <sup>7</sup> Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 9017 - CIIL -  
21 Center for Infection and Immunity of Lille, F-59000 Lille, France

22 <sup>8</sup> Clinic of Nephrology, University Hospital Zurich, Zurich, Switzerland

23 <sup>9</sup> Institute of Medical Genetics and Pathology, University Hospital Basel, University of Basel,  
24 Basel, Switzerland

25 <sup>10</sup> Department of Gastroenterology and Hepatology, University Hospital Zurich, Zurich,  
26 Switzerland

27 <sup>11</sup> Institute of Molecular Cancer Research (IMCR), University of Zurich (UZH), Zurich,  
28 Switzerland

29 \*share first authorship

30 \*\*share last authorship

31

## 32 **Running title**

33 Hepatitis E-associated kidney disease

34

## 35 **Corresponding author: Achim Weber**

36 Department of Pathology and Molecular Pathology, University of Zurich and University Hospital

37 Zurich, Schmelzbergstrasse 12, 8091 Zurich, Switzerland; phone: +41-44-255 2781; e-mail:

38 [achim.weber@usz.ch](mailto:achim.weber@usz.ch)

39

## 40 **Abstract**

41 Hepatitis E virus (HEV) infection, one of the most common forms of hepatitis worldwide, is  
42 often associated with extrahepatic, particularly renal, manifestations. However, the underlying  
43 mechanisms are incompletely understood. Here, we report the development of a *de novo*  
44 immune complex-mediated glomerulonephritis (GN) in a kidney transplant recipient with  
45 chronic hepatitis E. Applying immunostaining, electron microscopy, and mass spectrometry  
46 after laser-capture microdissection, we show that GN developed in parallel with increasing  
47 glomerular deposition of a noninfectious form of HEV open reading frame 2 (ORF2, capsid)  
48 protein secreted in excess. HEV particles or RNA, however, were not detectable. Patients with  
49 acute hepatitis E displayed similar but less pronounced deposits. Our results elucidate an  
50 immunologic mechanism by which this hepatotropic virus causes variable renal manifestations  
51 and establish a link between the HEV ORF2 protein and hepatitis E-associated GN. They  
52 directly provide a tool for etiology-based diagnosis of HEV-associated GN as a distinct entity  
53 and suggest therapeutic implications.

54

55 Hepatitis E virus (HEV) infection, one of the most common causes of acute hepatitis, is a major  
56 global health problem.<sup>1,2</sup> The predominantly enterically transmitted HEV infection has two main  
57 epidemiologic patterns that correlate with geographically prevalent HEV genotypes. In  
58 resource-limited countries, endemic and epidemic HEV-1 and -2 are transmitted from person  
59 to person mainly through contaminated drinking water. In resource-rich countries zoonotic  
60 HEV-3 and -4 infections predominate, transmitted mainly through contaminated meat products.  
61 Despite its high prevalence in industrialized countries, HEV-3 infection has been  
62 underdiagnosed in Europe and North America for many years, in part because of its highly  
63 variable clinical presentation.<sup>1-3</sup> The spectrum ranges from an asymptomatic course to acute,  
64 self-limiting hepatitis to acute-on-chronic liver failure in patients with pre-existing liver disease  
65 and chronic hepatitis in immunocompromised individuals.<sup>4,5</sup>

66 HEV-3 infection in particular has been associated with extrahepatic manifestations, mostly  
67 neurological and renal diseases, whose underlying pathomechanisms are still largely  
68 unknown.<sup>6</sup> It is conceivable that, apart from renal injury generally associated with impaired liver  
69 function, kidney dysfunction in hepatitis E may be caused - solely or additionally - by HEV-  
70 inherent mechanisms. Extrahepatic manifestations develop either directly, i.e. by HEV infection  
71 of the respective organs or indirectly, i.e. by immunologic reactions.<sup>6-8</sup> Histologically confirmed  
72 glomerular diseases reported in patients with hepatitis E including membranoproliferative  
73 glomerulonephritis (MPGN), with or without cryoglobulinemia, and membranous GN, support  
74 an underlying immune-mediated mechanism.<sup>6,9-11</sup> However, a direct pathophysiologic link to  
75 HEV infection, proving a causal relationship with hepatitis E, has not yet been established.<sup>6</sup>

76 Central to the understanding of HEV pathogenesis is the genetic organization and life cycle of  
77 this positive-strand RNA virus whose genome harbors three main open reading frames (ORF)  
78 encoding ORF1 non-structural proteins with viral replicase function, the ORF2 protein,  
79 corresponding to the capsid protein and main antigenic structure,<sup>7</sup> and the ORF3 protein  
80 involved in viral particle secretion.<sup>12</sup> HEV produces different ORF2 isoforms: a non-  
81 glycosylated isoform assembled into infectious particles (ORF2i) and glycosylated isoforms  
82 (ORF2g/c) secreted in large amounts.<sup>13-15</sup>

83 Here, we describe the development of *de novo* immune complex-mediated glomerulonephritis  
84 (GN) in a patient with chronic hepatitis E, and similar but less pronounced deposits in patients  
85 with acute hepatitis E.

86

87 Patients' clinical presentation, histopathologic findings and experimental procedures are  
88 detailed in Supplementary Material. Autopsy findings in patient 1 included liver cirrhosis,  
89 hepatitis with necrosis, and hepatocytes immunohistochemically positive for the HEV ORF2  
90 protein, confirming hepatitis E. Kidney transplant histology showed persistent proliferative and  
91 sclerosing immune complex-mediated GN with a membranoproliferative pattern, consistent  
92 with MPGN with immune complexes (IC-MPGN)(Figure 1b), which had been diagnosed in a  
93 more subtle form in kidney transplant biopsies taken four and three months before death  
94 (Figure 1c). There was no evidence of recurrent IgA nephropathy or antibody-mediated  
95 rejection. Remarkably, the renal allograft as well as retrospectively examined previous biopsies  
96 with GN showed strong immunohistochemical staining for HEV ORF2 protein, decorating the  
97 peripheral capillaries and the mesangium of all glomeruli (Figure 1B, right, and 1C). This  
98 indicated virus replication for at least 4 months, thus establishing the diagnosis of chronic  
99 hepatitis E.<sup>16</sup>

100 HEV RNA was detected by RNA in situ hybridization only in the liver but not in the kidney  
101 transplant, as determined in biopsies performed 4 and 3 months before death and at autopsy  
102 (Supplementary Figure 1a), indicating that the glomerular HEV ORF2 protein was not  
103 associated with HEV virions.

104 Re-evaluation of the patient's previous graft biopsies showed a progressive course: Initial  
105 subtle mesangial expansion, mild hypercellularity, and immune complex deposition,  
106 progressing to a membranoproliferative pattern with endocapillary hypercellularity and  
107 significant subendothelial deposits. Reticular aggregates were found in the cytoplasm of  
108 endothelial cells at the last graft biopsy. Subendothelial electron dense deposits were  
109 confirmed by electron microscopy, which also showed subepithelial and mesangial deposits  
110 but no particles suggestive of virions. Prolonged GN was associated with a markedly increased

111 immunohistochemical (IHC) reactivity for the HEV ORF2 protein (Figure 1c), co-localizing with  
112 IgG and C3 (Supplementary Figure 1b). Immunofluorescence staining confirmed that  
113 colocalization of IgG with HEV ORF2 protein was statistically highly significant ( $p < 10E-10$ ;  
114 Figure 2a and Supplementary Figure 2). This was paralleled by worsening renal function and  
115 increasing proteinuria (Figure 1a). HEV ORF2 immune complexes were not detected in other  
116 organs examined (brain, spleen, heart). Immunoblot analysis using monoclonal antibody (mAb)  
117 1E6 on liver and kidney tissue extracts revealed a band at around 60 kDa, suggesting post-  
118 translational modification of the HEV ORF2 protein preserving the epitope region (aa 437-457)  
119 (Figure 2b). Combining laser-capture microscopy (LCM) and mass spectrometry (MS) allowed  
120 us to further characterize the protein content of the interstitial and glomerular compartments  
121 (Supplementary Table 1). LCM/MS analysis of glomeruli revealed, among other fragments, the  
122 glomeruli marker podocin and HEV ORF2 protein, notably containing the epitope recognised  
123 by mAb 1E6 (Figures 2c-d). In contrast, podocin was not detected in the interstitium, and HEV  
124 ORF2 protein only in traces (4 hits versus 241 in the glomerular compartment; Supplementary  
125 Table 1). This argued for 1) sufficient differential preparation of glomerular versus interstitial  
126 compartments, and 2) principally glomerulus-restricted deposition of HEV ORF2 protein.  
127 Furthermore, unlike in HEV-replicating cells and the liver, IHC with mAbs P1H1, P2H1 and  
128 P2H2, recognizing only infectious HEV ORF2i<sup>17</sup> remained negative in the glomeruli, indicating  
129 that the glomerular deposits lacked the nonglycosylated, infectious HEV ORF2i (Figure 2e).  
130 HEV RNA was detected by RT-qPCR in both frozen and FFPE liver specimens. Among tissue  
131 specimens from kidney, brain, spleen and heart, HEV RNA was detected only in frozen, but  
132 not FFPE specimens (Supplementary Figure 1c). Overall, these findings suggested that the  
133 patient had HEV infection of the liver with concomitant viremia and that HEV ORF2 protein  
134 aggregates were increasingly deposited in glomeruli. However, they do not provide evidence  
135 of a productive HEV infection of kidney cells.

136 These findings prompted us to examine kidney tissue from additional hepatitis E patients. In  
137 three identified cases, all of whom had pre-existing liver cirrhosis and died of acute-on-chronic  
138 liver failure in a context of acute hepatitis E,<sup>5, 18</sup> we found deposits similar as in patient 1, albeit

139 at lower intensities (Table 1). Subtle proliferative glomerular changes with IgG/HEV immune  
140 complexes (visualized by co-immunofluorescence) were detected, consistent with early  
141 hepatitis E-associated GN (Supplementary Figure 3).

142

143 To determine the significance of the glomerular IgG/HEV ORF2 protein deposits we discovered  
144 here for the renal dysfunction observed in association with hepatitis E,<sup>8, 10</sup> it is important to  
145 consider both host and HEV characteristics. Impaired renal function has been reported for  
146 HEV-1 and -3, and associated with both acute and chronic hepatitis E,<sup>4, 8</sup> generally more  
147 transient and milder in the acute form.<sup>6</sup> Its variable presentation includes 1) clinically silent  
148 urinary excretion of HEV ORF2 protein with maintained kidney function<sup>19</sup> 2) transiently impaired  
149 kidney function (with or without proteinuria) with resolution following normalization of  
150 transaminases,<sup>9, 20</sup> and 3) subclinical or overt *de novo* immune complex GN with variable  
151 outcome including kidney failure.<sup>6, 9</sup>

152 The development of GN seems to be associated with an impaired immune status.<sup>21</sup>  
153 Accordingly, patient 1 who developed MPGN with bona fide IgG/HEV ORF2 protein deposits  
154 was immunocompromized. However, reduced immunocompetence can also be assumed for  
155 patients 2-4, who all had liver cirrhosis.<sup>22</sup> The well-documented temporal association between  
156 HEV infection and renal disease, together with the quantitative correlation between ORF2  
157 protein levels and impairment of renal function, argue for a causal relationship between  
158 glomerular deposits and renal dysfunction.<sup>9, 19</sup> In line, we have observed the same type of  
159 immune complexes in both acute and chronic hepatitis E, but more pronounced in the latter, in  
160 accordance with significantly higher HEV ORF2 protein levels found in sera from chronically  
161 as compared to acutely HEV-infected individuals.<sup>23</sup> In patients 2-4, renal dysfunction was due  
162 to hepato-renal syndrome. Glomerular HEV ORF2 protein depositions might have been an  
163 aggravating factor. Nevertheless, it is conceivable that in addition to cases of fully developed  
164 glomerulonephritis<sup>6, 9-11</sup>, as in patient 1, glomerular damage associated with more subtle HEV-

165 ORF2 protein deposits, as in patients 2-4, may represent a general early morphological  
166 correlate and harbinger of impaired glomerular function in the context of HEV infection.

167 Based on our observations, we cannot deduce whether HEV ORF2 protein-associated  
168 immune-mediated glomerular damage also occurs in acute or subclinical hepatitis E in  
169 immunocompetent individuals, in the course of which (transient) impaired renal function has  
170 also been described.<sup>2, 8</sup> However, the host immune status determines the duration of HEV  
171 persistence and thus indirectly also the amount of HEV ORF2 protein formed cumulatively.<sup>4, 23</sup>  
172 If this is the decisive factor determining the extent of glomerular damage, it is expected to be  
173 lower in acute than in chronic hepatitis E.

174 HEV antigens can remain detectable in sera of patients with chronic hepatitis E >100 days  
175 after clearance of HEV RNA, emphasizing that the presence of HEV ORF2 protein does not  
176 necessarily correlate with infectious virions.<sup>23</sup> It has been shown that HEV exists in urine not  
177 only as virions, but also abundantly as free antigen or empty capsid protein, with an obvious  
178 discrepancy between the relatively low levels of HEV RNA compared to high levels of HEV  
179 ORF2 protein in the urine.<sup>7, 19</sup> HEV ORF2 protein trapped in the the glomeruli potentially  
180 explains the latency observed between viral clearance and restitution of kidney function.<sup>9</sup>

181 Considering the genetic organization and the HEV life cycle, it is not surprising that the ORF2  
182 (capsid) protein emerges as the key molecule causing extrahepatic manifestations. Unlike  
183 ORF1 and ORF3 proteins, the ORF2 protein is produced and secreted in significant excess  
184 into the bloodstream, where it exists also in a free form, i.e. not associated with virus particles<sup>13</sup>,  
185 remarkably stable and prone to precipitate.<sup>12, 23</sup> As such, it constitutes the virus' main  
186 immunogenic structure and a potential immunologic decoy.<sup>14, 15</sup> We noticed that the glomerular  
187 HEV ORF2 does not assemble into HEV virions and displays the molecular weight of a  
188 truncated HEV ORF2 protein, similar as described in the urine and the stool.<sup>24, 25</sup> Proteases,  
189 as hallmarks of various inflammatory glomerular diseases, may contribute to the cleavage and  
190 deglycosylation of the HEV ORF2 protein. Together, these attributes predispose this protein to  
191 trigger an immunological reaction.

192 HEV ORF2 protein IHC and IF, corroborated by Western blotting and MS, allowed us to  
193 connect a morphologically variegated and in early stages subtle pattern of glomerular injury  
194 (within the spectrum of MPGN) with a specific etiology. Implementation of HEV ORF2 protein  
195 immunostaining for routine diagnostics is straightforward<sup>26</sup> and allows to delineate hepatitis E-  
196 associated GN from GN of other causes. This approach is in accordance with the proposed  
197 etiology-based classification<sup>27</sup> and defines hepatitis E-associated GN as a distinct entity.<sup>9</sup> HEV  
198 ORF2 protein immunostaining should be helpful especially in cases in which diagnosis is  
199 hampered by limitations of serological testing<sup>1</sup> and in unsuspected cases of hepatitis E in which  
200 extrahepatic manifestations predominate. It may guide therapeutic decisions, especially with  
201 regard to immunosuppressive treatment and/or antiviral therapy.<sup>28</sup>

202 In summary, the discovery of glomerular IgG/HEV ORF2 protein immune complexes may  
203 provide a mechanistic explanation for how this antigen triggers renal disease, in particular  
204 immune complex GN. Our findings potentially explain several still incompletely understood  
205 observations on hepatitis E-associated renal disease, and establish a molecular link between  
206 HEV infection and kidney dysfunction.<sup>6</sup> Finally, they propose ORF2 protein immunostaining as  
207 a diagnostic tool for hepatitis E-associated GN, especially in IC-MPGN of clinically unclear  
208 origin.



209 **Conflict of interest**

210 The authors declare that they have no conflict of interest.

211

212 **Acknowledgments**

213 We would like to thank Angela Broggin, PhD, Rita Bopp, André Fritsche, Carmen Gavrisan,  
214 Marcel Glöckler, Doris Gunterweiler and Michael Reinehr, MD for their excellent assistance  
215 by performing the autopsy, immune stains and electron microscopy analyses and providing  
216 reagents, respectively.

217

218 **List of Abbreviations**

219 HEV, hepatitis E virus; GN, glomerulonephritis; gt, genotype; MPGN, membranoproliferative  
220 glomerulonephritis; ORF, open reading frame; mAb, monoclonal antibody; IHC,  
221 immunohistochemistry; PEN, polyethylene naphthalate; LCM, laser-capture microscopy; MS,  
222 mass spectrometry.

223

224 **Financial Support**

225 This study was supported by grants from the Swiss National Science Foundation (CRSK-  
226 3\_190706 to JG and 310030\_207477 to DM) as well as the Uniscentia Stiftung, Zurich and  
227 the University Hospital Zurich (“USZ Innovations-Pool”) (both to AW).

228

229 **References**

230 [1] Nimgaonkar I, Ding Q, Schwartz RE, Ploss A. Hepatitis E virus: advances and challenges.  
231 *Nat Rev Gastroenterol Hepatol* **15**, 96-110 (2018).

232

233 [2] Dalton HR, Izopet J. Transmission and Epidemiology of Hepatitis E Virus Genotype 3 and  
234 4 Infections. *Cold Spring Harb Perspect Med* **8**, a032144 (2018).

235

236 [3] Horvatits T, Ozga AK, Westholter D, et al. Hepatitis E seroprevalence in the Americas: A  
237 systematic review and meta-analysis. *Liver Int* **38**, 1951-64 (2018).

238

239 [4] Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-  
240 transplant recipients. *N Engl J Med* **358**, 811-7 (2008).

241

242 [5] Lenggenhager D, Pawel S, Honcharova-Biletska H, et al. The histologic presentation of  
243 hepatitis E reflects patients' immune status and pre-existing liver condition. *Mod Pathol* **34**,  
244 233-48 (2021).

245

246 [6] Pischke S, Hartl J, Pas SD, Lohse AW, Jacobs BC, Van der Eijk AA. Hepatitis E virus:  
247 Infection beyond the liver? *J Hepatol* **66**, 1082-95 (2017).

248

249 [7] Geng Y, Zhao C, Huang W, et al. Detection and assessment of infectivity of hepatitis E  
250 virus in urine. *J Hepatol* **64**, 37-43 (2016).

251

252 [8] Lhomme S, Marion O, Abravanel F, Izopet J, Kamar N. Clinical Manifestations,  
253 Pathogenesis and Treatment of Hepatitis E Virus Infections. *J Clin Med* **9**, (2020).

254

255 [9] Kamar N, Weclawiak H, Guilbeau-Frugier C, et al. Hepatitis E virus and the kidney in solid-  
256 organ transplant patients. *Transplantation* **93**, 617-23 (2012).

257

258 [10] Marion O, Abravanel F, Del Bello A, et al. Hepatitis E virus-associated cryoglobulinemia  
259 in solid-organ-transplant recipients. *Liver Int* **38**, 2178-89 (2018).

260

261 [11] Pischke S, Tamanaei S, Mader M, et al. Lack of Evidence for an Association between  
262 Previous HEV Genotype-3 Exposure and Glomerulonephritis in General. *Pathogens* **11**,  
263 (2021).

264

265 [12] Debing Y, Moradpour D, Neyts J, Gouttenoire J. Update on hepatitis E virology:  
266 Implications for clinical practice. *J Hepatol* **65**, 200-12 (2016).

267

268 [13] Montpellier C, Wychowski C, Sayed IM, et al. Hepatitis E Virus Lifecycle and Identification  
269 of 3 Forms of the ORF2 Capsid Protein. *Gastroenterology* **154**, 211-23 e8 (2018).

270

271 [14] Ankavay M, Montpellier C, Sayed IM, et al. New insights into the ORF2 capsid protein, a  
272 key player of the hepatitis E virus lifecycle. *Sci Rep* **9**, 6243 (2019).

273

274 [15] Yin X, Ying D, Lhomme S, et al. Origin, antigenicity, and function of a secreted form of  
275 ORF2 in hepatitis E virus infection. *Proc Natl Acad Sci USA* **115**, 4773-8 (2018).

276

277 [16] European Association for the Study of the Liver. Electronic address eee, European  
278 Association for the Study of the L. EASL Clinical Practice Guidelines on hepatitis E virus  
279 infection. *J Hepatol* **68**, 1256-71 (2018).

280

281 [17] Bentaleb C, Hervouet K, Montpellier C, et al. The endocytic recycling compartment serves  
282 as a viral factory for hepatitis E virus. *Cell Mol Life Sci* **79**, 615 (2022).

283

- 284 [18] Vieira Barbosa J, Mullhaupt B, Brunner F, et al. Autochthonous hepatitis E as a cause of  
285 acute-on-chronic liver failure and death: histopathology can be misleading but transaminases  
286 may provide a clue. *Swiss Med Wkly* **151**, w20502 (2021).
- 287
- 288 [19] Marion O, Capelli N, Lhomme S, et al. Hepatitis E virus genotype 3 and capsid protein in  
289 the blood and urine of immunocompromised patients. *J Infect* **78**, 232-40 (2019).
- 290
- 291 [20] Wallace SJ, Swann R, Donnelly M, et al. Mortality and morbidity of locally acquired  
292 hepatitis E in the national Scottish cohort: a multicentre retrospective study. *Aliment Pharmacol*  
293 *Ther* **51**, 974-86 (2020).
- 294
- 295 [21] Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis--a new look at an old  
296 entity. *N Engl J Med* **366**, 1119-31 (2012).
- 297
- 298 [22] Tuchendler E, Tuchendler PK, Madej G. Immunodeficiency caused by cirrhosis. *Clin Exp*  
299 *Hepatol* **4**, 158-64 (2018).
- 300
- 301 [23] Behrendt P, Bremer B, Todt D, et al. Hepatitis E Virus (HEV) ORF2 Antigen Levels  
302 Differentiate Between Acute and Chronic HEV Infection. *J Infect Dis* **214**, 361-8 (2016).
- 303
- 304 [24] Ying D, He Q, Tian W, et al. Urine is a viral antigen reservoir in hepatitis E virus infection.  
305 *Hepatology* **77**, 1722-34 (2022).
- 306
- 307 [25] Nishiyama T, Umezawa K, Yamada K, et al. The Capsid (ORF2) Protein of Hepatitis E  
308 Virus in Feces Is C-Terminally Truncated. *Pathogens* **11**, 24 (2021).
- 309
- 310 [26] Lenggenhager D, Gouttenoire J, Malehmir M, et al. Visualization of hepatitis E virus RNA  
311 and proteins in the human liver. *J Hepatol* **67**, 471-9 (2017).

312

313 [27] Sethi S, Haas M, Markowitz GS, et al. Mayo Clinic/Renal Pathology Society Consensus  
314 Report on Pathologic Classification, Diagnosis, and Reporting of GN. *J Am Soc Nephrol* **27**,  
315 1278-87 (2016).

316

317 [28] Kamar N, Izopet J, Tripon S, et al. Ribavirin for chronic hepatitis E virus infection in  
318 transplant recipients. *N Engl J Med* **370**, 1111-20 (2014).

319

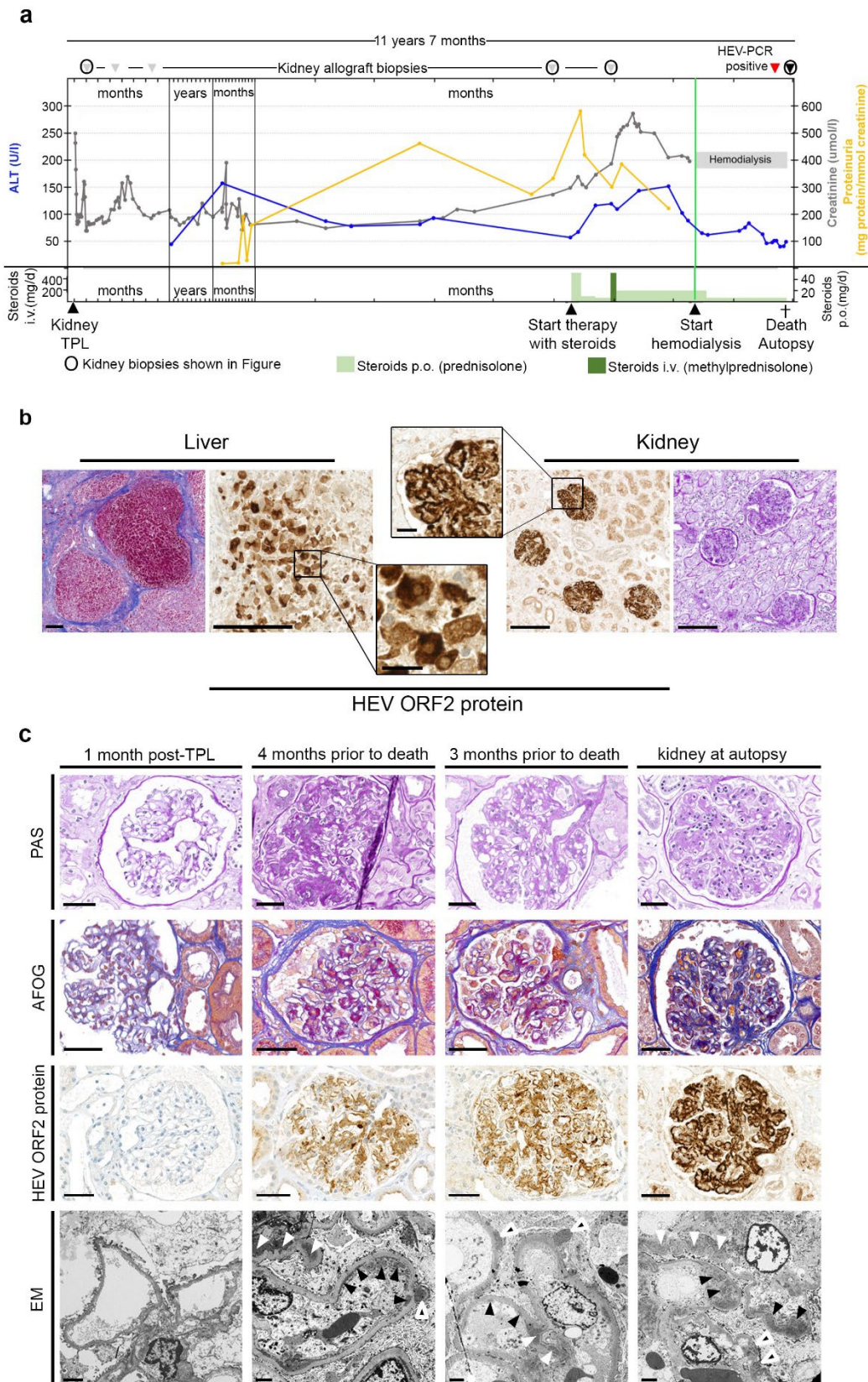
320

321

322

323 **Figures and figure legends**

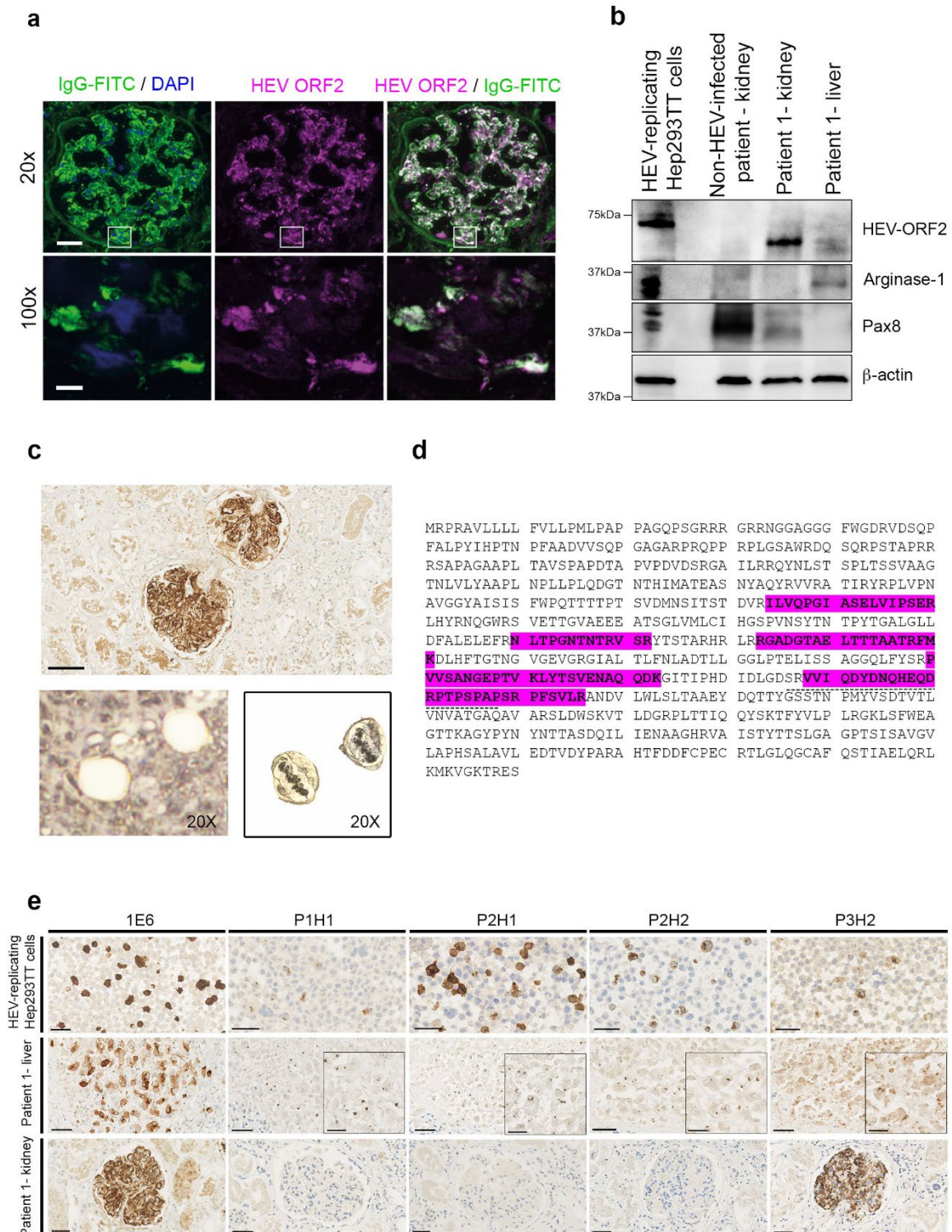
**Figure 1**





325 **Figure 1 Clinical course, autopsy findings and gradual development of immune complex**  
326 **glomerulonephritis with membranoproliferative pattern in a kidney transplant recipient**  
327 **with hepatitis E. (a)** Course of alanine transaminase (ALT, blue), proteinuria (yellow) and  
328 creatinine (grey), time points of therapeutic and diagnostic interventions as well as death /  
329 autopsy. **(b)** Histology of autopsy liver showing cirrhosis (Masson trichrome stain) and hepatitis  
330 with immune reactivity for HEV ORF2 (capsid) protein in hepatocytes (left). Histology of  
331 transplant kidney at autopsy showing glomerulonephritis (periodic acid Schiff [PAS] stain) and  
332 (extracellular) immune reactivity for HEV ORF2 protein in glomeruli (right). Scale bars in  
333 overviews, 200  $\mu\text{m}$ ; scale bars in detail, 25  $\mu\text{m}$ . **(c)** Kidney histology. One month post  
334 transplantation: inconspicuous glomeruli on light microscopy (PAS and acid fuchsin-Orange G  
335 [AFOG] stains), no HEV ORF2 capsid protein deposits, no electron dense deposits on electron  
336 microscopy (EM). Four months prior to death (biopsy 4): glomerulus with mild mesangial and  
337 endocapillary hypercellularity, segmental sclerosis and prominent podocytes (PAS stain).  
338 Mostly mesangial and few glomerular basement membrane protein deposits (AFOG stain).  
339 Moderate mesangial and glomerular basement membrane positivity for HEV ORF2 protein.  
340 Mesangial (white arrowheads), subendothelial (black arrowheads) and subepithelial (black and  
341 white arrowheads) on EM. Three months prior to death (biopsy 5): glomerulus with mild  
342 mesangial and endocapillary hypercellularity (PAS stain). Mostly mesangial and few  
343 glomerular basement membrane protein deposits (AFOG stain). Moderate to strong mesangial  
344 and glomerular basement membrane positivity for HEV ORF2 protein. Mesangial (white  
345 arrowheads), subendothelial (black arrowheads) and subepithelial (black and white  
346 arrowheads) on EM. Kidney at autopsy: glomerulus with mild mesangial and endocapillary  
347 hypercellularity (PAS stain). Mostly mesangial and few glomerular basement membrane  
348 protein deposits (AFOG stain). Strong mesangial and glomerular basement membrane  
349 positivity for HEV ORF2 protein. Mesangial (white arrowheads), subendothelial (black  
350 arrowheads) and subepithelial (black and white arrowheads) on EM. Scale bars in PAS, AFOG,  
351 and HEV ORF2 protein images: 50  $\mu\text{m}$ ; scale bars in EM images: 2  $\mu\text{m}$ .  
352

**Figure 2**



353

354 **Figure 2 Glomerular deposition of HEV ORF2 protein in a kidney transplant recipient**

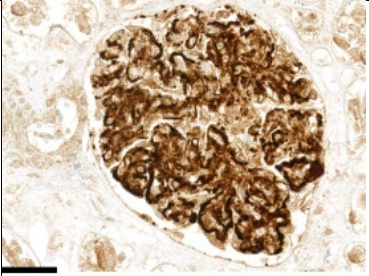
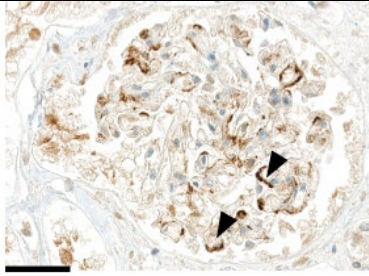
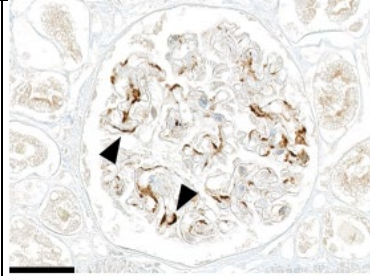
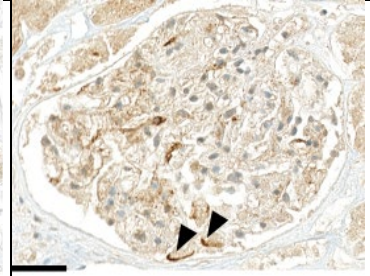
355 **with hepatitis E. (a) Visualization by immunofluorescence staining of a glomerulus from the**

356 **autopsy transplant kidney (patient 1) with IgG (left: green, FITC stain; DAPI counter-stain, blue)**

357 **highlighting the co-localization with HEV ORF2 protein (middle: magenta, Alexa546 stain; right:**



358 white indicating co-localization). Overview at low magnification (top row, scale bar: 50  $\mu\text{m}$ , 20x)  
359 and high resolution images (bottom row, scale bar: 5  $\mu\text{m}$ , 100x) corresponding to the areas  
360 indicated by the white boxes. Highly significant IgG/HEV ORF2 protein co-localization was  
361 found on the scale of entire glomeruli (20x, Pearson's correlation coefficient PPC =  $0.838 \pm$   
362  $0.039$ ; mean  $\pm$  s.d., n = 25 glomeruli;  $p < 10\text{E-}10$ ) as well as for small imaging fields (45 – 85  
363  $\mu\text{m}$  side length) acquired at high resolution (100x, Zeiss ApoTome; Pearson's coefficient  $0.668$   
364  $\pm 0.138$ , n = 16;  $p < 10\text{E-}10$ ). For further glomeruli, please see Supplementary Figure 2. **(b)**  
365 Western blot analysis for HEV ORF2 protein, the liver marker Arginase-1, and kidney marker  
366 Pax8 in HEV-replicating Hep293TT cells, kidney tissue from a non-HEV-infected patient and  
367 patient 1 (Patient 1 - kidney), as well as liver tissue from patient 1 (Patient 1 - liver). **(c)** Laser-  
368 capturing of glomeruli positively stained for HEV ORF2 protein by IHC using 1E6 mAb. Scale  
369 bar: 100  $\mu\text{m}$ . After excision (lower panel, 20x). Laser-captured glomeruli in the LCM cap (20x).  
370 **(d)** Mass spectrometry analysis of the HEV ORF2 sequence derived from laser-captured  
371 glomeruli from the transplant kidney tissue of patient 1. Glomerular fragments of HEV ORF2  
372 protein highlighted in magenta. The dashed line depicts the 1E6 epitope. **(e)**  
373 Immunohistochemistry using 1E6 and P3H2 mAbs recognizing all the isoforms of HEV ORF2  
374 (ORF2i and ORF2g/c) as well as P1H1, P2H1 and P2H2 mAbs recognizing only infectious  
375 HEV ORF2i. HEV-replicating Hep293TT cells, as well as liver (Patient 1 - liver) and kidney  
376 tissue (Patient 1 - kidney). Immunoreactivity using P3H2 staining recapitulates IHC results  
377 using 1E6 staining in the liver and kidney tissue. Immunoreactivity using P1H1, P2H1 and  
378 P2H2 stainings were positive only in Hep293 TT cells expressing ORF2 protein in a replicative  
379 context and liver tissue, but not in kidney tissue. Scale bars: 50  $\mu\text{m}$ .  
380

	Chronic hepatitis E	Acute hepatitis E		
	Patient 1	Patient 2	Patient 3	Patient 4
HEV genotype	HEV-3h_s	HEV-3h_s	HEV-3	HEV-3h_s
Age (years) / Sex	51 / male	59 / male	66 / female	76 / male
History of liver disease	chronic HEV infection	cirrhosis due to NASH	cirrhosis due to NASH/ASH	cirrhosis due to ASH
History of kidney disease	IgA nephropathy, kidney TPL	unknown	unknown	unknown
Immunosuppression (IS)	yes	no	no	no
basis IS / add on IS	tacrolimus, MMF / corticosteroids	-	-	-
Urea max. (mmol/l)	hemodialysis	n.a.	25	n.a.
Creatinine max. (umol/l)	573	188	180	> 200
eGFR min. (ml/min/1.73m <sup>2</sup> )	hemodialysis	33	25	n.a.
Serum albumin min. (g/l)	11	26	26	24
Proteinuria	yes	yes	yes	n.a.
HEV RNA in blood	1.2 x 10 <sup>8</sup> IU/mL	4.0 x 10 <sup>6</sup> IU/mL	4.6 x 10 <sup>4</sup> IU/mL	2.2 x 10 <sup>3</sup> IU/mL
HEV ORF2 immunohistochemistry on autopsy kidney (scale bar: 50 μm)				
Staining intensity, semiquantitative	+++	+	+	+

381

382

**Table 1 Clinical findings, laboratory values and HEV ORF2 IHC on autopsy kidneys, patients 1-4.<sup>18</sup> ASH, alcoholic steatohepatitis; HEV,**

383

hepatitis E virus; MMF, mycophenolate mofetil; NASH, nonalcoholic steatohepatitis; TPL, transplantation. n.a., information not available