Supplementary information:

Genotype-specific acquisition, evolution and adaptation of characteristic mutations in
hepatitis E virus

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| Organism                        | Mutations                  | Method of introducing mutations |
|--------------------------------|----------------------------|---------------------------------|
| Hepatitis E virus              | Mutations                  | Engineered                      |
| HEV ORF1                       | Mutagenesis                | Site directed                   |
| HEV ORF2                       | Site directed mutations    | Wild type                       |
| HEV genotype 1                 | Transductions              | Induced                         |
| HEV genotype 3                 | Mutants                    |                                 |
| HEV genotype 4                 | Codon                      |                                 |

Table S1. Search terms used in this study.
Table S2. Schematic illustration of the study

Table S3. The GenBank accession numbers of HEV sequences used in this study.
| GT1/ORF1 | GT1/ORF2 | GT3/ORF1 | GT3/ORF2 | GT4/ORF1 | GT4 ORF2 |
|---------|---------|---------|---------|---------|---------|
| AY204877 | AY204877 | AB089824 | AB089824 | AB161717 | AB161717 |
| AAB94808 | AAB61825 | BAC44897 | BAC44899 | AB197673 | AB197673 |
| NC_001434 | NC_001434 | AB291951 | AB291951 | BAE02700 | BAE02701 |
| BAG09237 | BAG09239 | BAH08575 | BAH08577 | AJ272108 | AJ272108 |
| NP_056779. | M80581 | AF060669 | AF060669 | AB108537 | AB108537 |
| M80581 | M80581 | AF060668 | AF060668 | BAC77167 | BAC77168 |
| L08816 | AF185822 | AB369687 | AB369687 | AB193177 | AB193177 |
| AAB82002 | D11092 | BAG32124 | BAG32126 | AB074917 | AB074917 |
| AF185822 | JF443717 | BAG32124 | AAD15813 | AB197674 | AB197674 |
| D11092 | M73218 | AB074918 | AB074918 | BAB93538 | BAB96557 |
| JF443717 | P29326 | APO03430 | APO03430 | BAD95600 | BAB96559 |
| M73218 | AFB71096 | BAB93541 | BAB96562 | BAE02703 | BAE02704 |
| AFB71094. | D10330 | BAB93539 | BAB96564 | AB074915 | AB074915 |
| P29324 | BAA01867 | BAB93194 | BAB63941 | AB200239 | AB200239 |
| D10330 | NP_056788 | AB074920 | AB091394 | AB161718 | AB161718 |
| AAA45725 | X99441 | BAC65250 | BAC65250 | AB080575 | AB080575 |
| BAA01865 | AAA65490 | BAE92012 | BAC65252 | AB091395 | AB091395 |
| X99441 | L25595 | AB301710 | AB301710 | AB097812 | AB097812 |
| Q40610 | D11093 | AB630971 | AB630971 | AB091395 | AB091395 |
| AAA96139 | Q68985 | AB437318 | AB437318 | BAC75630 | BAC65254 |
| L25595 | CAA66937 | AB437317 | AB437317 | BAD95603 | BAC5695|
| D11093 | X98292 | AB189075 | AB189075 | BAC65253 | BACD5604 |
| AAA65488 | AAC35764 | AB189074 | AB189074 | BAC05694 | LC22745 |
| CAA67802 | JF443718 | AB189073 | AB189073 | LC22745 | B291964 |
| JF443718 | P29324 | AAD10627 | AAD10627 | BAK52326 | BAH59609 |
| AFB71097 | AAD4593 | BAF92625 | BAF92627 | BAVAS29763 | BAH08615 |
| AAG16764 | AAG16766 | BAD74172 | BAK52238 | BAC79251 | BAC52976 |
| AFB71100 | AB73523 | BAD74175 | BAD74177 | BAD95597 | B291965 |
| ABE87252 | AAM66330 | BAD74181 | AB291961 | BAH08614 | B093347 |
| Q9WC28 | JF443722. | BAH08584 | BAH08584 | KC166976 | AB291965 |
| AAL50055 | JF443723. | BAH08605 | BAH08605 | AB099347 | AB161719 |
| AAM66329 | JF443717 | BAH08605 | BAH08605 | AB291964 | AB193176 |
| JF443718 | AAD10627 | BAK5236 | BAH59609 | AB193176 | BAD95598 |
| AFB71097 | AAD4593 | BAF92625 | BAF92627 | BAVAS29763 | BAH08615 |
| AAG16764 | AAG16766 | BAD74172 | BAK52238 | BAC79251 | BAC52976 |
| AFB71100 | AB73523 | BAD74175 | BAD74177 | BAD95597 | B291965 |
| ABE87252 | AAM66330 | BAD74181 | AB291961 | BAH08614 | B093347 |
| Q9WC28 | JF443722. | BAH08584 | BAH08584 | KC166976 | AB291965 |
| AAL50055 | JF443723. | BAH08605 | BAH08605 | AB099347 | AB161719 |
| AAM66329 | JF443717 | BAH08605 | BAH08605 | AB291964 | AB193176 |
| JF443718 | JF443722. | FJ457024 | FJ457024 | B291961 | B291961 |
| AFB71109 | JF443724 | KC166967 | KC166967 | AB291956 | B291956 |
| AAO72991 | AC70099 | AB369689 | AB291954 | B291954 | B291954 |
| JF443723 | JF443725 | B291962 | B291962 | BAH08586 | BAD34958 |
| AFB71112 | AFB71120 | AB291957 | AB291957 | BAH08589 | BAH08589 |
| FJ457024 | AFB71123 | B291956 | B291956 | BAH08610 | BAH08610 |
| JF443724 | JF443724 | AFB71120 | AFB71120 | B291954 | B291954 |
| AFB71115 | AFB71108 | BAH08590 | BAH08590 | B229074 | B229074 |
| ACJ70098 | BAN5759S | BAG32130 | BAG32130 | AGT96614 | AB604239 |
| JF443725 | BAF66974 | BAH08593 | BAH08593 | B229071 | B229071 |
| AFB71118 | JF443721 | BAH08578 | BAH08578 | B229072 | B229072 |
| AFB71103 | JF443720 | KC166968 | KC166968 | AB229079 | AB229079 |
### A. Twenty main mutations analyzed in the present study.

| Mutations                          | Effect                                           | Reference(s) |
|------------------------------------|--------------------------------------------------|--------------|
| Y1320H; G1634R/K, K1383N, D1384G; K1398R; V1479I; Y1587F | Ribavirin treatment failure                      | [1-3]        |
| L477T; L613T                       | Influence the immunoreactivity of HEV by affecting the neutralization epitope | [4]          |
| F179S, A317T, T735I, L1110F, V1120I, F1439Y, C1483W, N1530T, P259S | Fulminant hepatic failure                        | [5-7]        |
| V1213A                             | Chronic hepatitis                                | [8]          |

### B. Other studies analyzed to see the effect of specific mutations.

| Studies for HEV mutation evaluations                                                                 | Reference(s) |
|------------------------------------------------------------------------------------------------------|--------------|
| H443L; C457A; C459A; C471A; C472A; C481A; C483A; H497L; H590L                                        | [9, 10]      |
| Insertion/deletion                                                                                | [11]         |
| Mutational analysis                                                                               | [12]         |
| Insertion/deletion of a 24 bp RdRp-derived fragment                                                | [1]          |
| N809A; H812L; G816A/V; G817A/V                                                                    | [13, 14]     |
| L1110F; V1120I                                                                                    | [15]         |
| Effect of mutagenesis on enzyme activity                                                          | [16]         |
| K1383N                                                                                            | [1]          |
| F51L                                                                                              | [11, 17]     |
| T59A                                                                                              | [17, 18]     |
| S390L                                                                                             | [17, 18]     |
| N137Q; N310Q; N311Q                                                                              | [19]         |
| N562Q/D/P/Y                                                                                      | [19]         |
| A5145C; A5178C; A5190C; G5676T; T5690G                                                             | [20]         |
| CGC5148–5150AGA                                                                                   | [20]         |
| A5108Δ; T5109C; C5112U; TCT5116–5118AGC; T5121C                                                   | [20]         |
| S80A (V66G)                                                                                      | [20]         |
| G6574C; C6570G; G7106T/A; G7097A; C7144A                                                          | [21-23]      |
| C1816, U3148, C5907                                                                              | [24, 25]     |
| 186 bp insertion                                                                                  | [26]         |
| 90 bp insertion                                                                                   | [27]         |
| 171 bp insertion                                                                                  | [28]         |
| 174 bp insertion                                                                                  | [28]         |
| 117 bp insertion                                                                                  | [29]         |
| 246 bp deletion                                                                                   | [30]         |
| Recombination                                                                                     | [31]         |
| Mutations in HEV                                                                                   | [32, 33]     |
| Topic                                           | Reference |
|------------------------------------------------|-----------|
| Deletion                                        | [34]      |
| HEV adaptations                                 | [35, 36]  |
| HEV mutations (review)                          | [37]      |
| Ribavirin induced mutagenesis                   | [3]       |
| Role of asparagine at position at 562           | [38]      |
| Role of truncated ORF2 region in immoreactivity | [39]      |
| HEV pathogenesis in pregnancy                   | [40]      |
### Table S5. Missense SNPs in ORF1 and ORF2 regions of HEV predicted to be deleterious using nsSNP Analyzer, PROVEAN, PMUT and SNPs & GO.

| Mutations | Provean | PMUT | SNPs&Go | nSSNP analyser | Provean | PMUT | SNPs&Go | nSSNP analyser |
|-----------|---------|------|---------|---------------|---------|------|---------|---------------|
| **ORF1**  |         |      |         |               |         |      |         |               |
| GT1       |         |      |         |               |         |      |         |               |
| GT3       |         |      |         |               |         |      |         |               |
| GT4       |         |      |         |               |         |      |         |               |
| F179S     | -       | -    | -       | -             | -       | -    | -       | -             |
| A317T     | -       | -    | -       | -             | -       | -    | -       | -             |
| T735I     |   ✓     | ✓    | ✓       | ✓             |   ✓     | ✓    | ✓       | ✓             |
| L1110F    | -       | -    | -       | -             | -       | -    | -       | -             |
| V1120I    | -       | -    | -       | -             | -       | -    | -       | -             |
| V1213A    | -       | -    | -       | -             | -       | -    | -       | -             |
| Y1320H    |   ✓     | ✓    | ✓       | ✓             |   ✓     | ✓    | ✓       | ✓             |
| K1383N    |   ✓     | ✓    | ✓       | ✓             |   ✓     | ✓    | ✓       | ✓             |
| D1384G    |   ✓     | ✓    | ✓       | ✓             |   ✓     | ✓    | ✓       | ✓             |
| K1398N    | -       | -    | -       | -             | -       | -    | -       | -             |
| F1439Y    | -       | -    | -       | -             | -       | -    | -       | -             |
| V1479I    | -       | -    | -       | -             | -       | -    | -       | -             |
| C1483W    |   ✓     | ✓    | ✓       | ✓             |   ✓     | ✓    | ✓       | ✓             |
| N1530T    |   ✓     | ✓    | ✓       | ✓             |   ✓     | ✓    | ✓       | ✓             |
| Y1587F    |   ✓     | ✓    | ✓       | ✓             |   ✓     | ✓    | ✓       | ✓             |
| G1634R    | -       | -    | -       | -             | -       | -    | -       | -             |
| G1634K    | -       | -    | -       | -             | -       | -    | -       | -             |
| **ORF2**  |         |      |         |               |         |      |         |               |
| GT1       |         |      |         |               |         |      |         |               |
| GT3       |         |      |         |               |         |      |         |               |
| GT4       |         |      |         |               |         |      |         |               |
| P259S     | -       | -    | -       | -             | -       | -    | -       | -             |
| L477T     |   ✓     | ✓    | ✓       | ✓             |   ✓     | ✓    | ✓       | ✓             |
| L613T     | -       | -    | -       | -             | -       | -    | -       | -             |
| Mutations | I-Mutant | I-Mutant | I-Mutant |
|-----------|----------|----------|----------|
| **ORF1**  |          |          |          |
| F179S     | ✓        | ✓        | ✓        |
| A317T     | ✓        | ✓        | ✓        |
| T735I     | ✓        | ✓        | ✓        |
| L1110F    | ✓        | ✓        | ✓        |
| V1120I    | ✓        | ✓        | ✓        |
| V1213A    | ✓        | ✓        | ✓        |
| Y1320H    | ✓        | ✓        | ✓        |
| K1383N    | ✓        | ✓        | ✓        |
| D1384G    | ✓        | ✓        | ✓        |
| K1398N    | ✓        | ✓        | ✓        |
| F1439Y    | ✓        | ✓        | ✓        |
| V1479I    | ✓        | ✓        | ✓        |
| C1483W    | ✓        | ✓        | ✓        |
| N1530T    | ✓        | ✓        | ✓        |
| Y1587F    | ✓        | ✓        | ✓        |
| G1634R    | ✓        | ✓        | ✓        |
| G1634K    |          |          |          |
| **ORF2**  |          |          |          |
| P259S     | -        | -        | -        |
| L477T     | ✓        | ✓        | ✓        |
| L613T     | ✓        | ✓        | ✓        |

**Table S6.**

Stability analysis of the mutations by using I-Mutant web server. Tick mark in the table shows that a mutation is found to be destabilizing by the server.
Reference.

1. Debing, Y., et al., Hepatitis E virus mutations associated with ribavirin treatment failure result in altered viral fitness and ribavirin sensitivity. Journal of hepatology, 2016. 65(3): p. 499-508.
2. Debing, Y., et al., A mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. Gastroenterology, 2014. 147(5): p. 1008-1011. e7.
3. Todt, D., et al., In vivo evidence for ribavirin-induced mutagenesis of the hepatitis E virus genome. Gut, 2016. 65(10): p. 1733-1743.
4. Zhang, H., et al., The Leu477 and Leu613 of ORF2-encoded protein are critical in forming neutralization antigenic epitope of hepatitis E virus genotype 4. Cellular & molecular immunology, 2008. 5(6): p. 447.
5. Parvez, M.K. and M.S. Al-Dosari, Evidence of MAPK–JNK1/2 activation by hepatitis E virus ORF3 protein in cultured hepatoma cells. Cytotechnology, 2015. 67(3): p. 545-550.
6. Devhare, P., et al., Analysis of helicase domain mutations in the hepatitis E virus derived from patients with fulminant hepatic failure: effects on enzymatic activities and virus replication. Journal of virology, 2014. 184: p. 103-110.
7. Graff, J., et al., Mutations within potential glycosylation sites in the capsid protein of hepatitis E virus prevent the formation of infectious virus particles. Journal of virology, 2008. 82(3): p. 1185-1194.
8. Graff, J., et al., The open reading frame 3 gene of hepatitis E virus contains a cis-reactive element and encodes a protein required for infection of macaques. Journal of virology, 2005. 79(11): p. 6680-6689.
24. Inoue, J., et al., *Analysis of the full-length genome of genotype 4 hepatitis E virus isolates from patients with fulminant or acute self-limited hepatitis* E. Journal of medical virology, 2006. **78**(4): p. 476-484.

25. Inoue, J., et al., *Nucleotide substitutions of hepatitis E virus genomes associated with fulminant hepatitis and disease severity*. The Tohoku journal of experimental medicine, 2009. **218**(4): p. 279-284.

26. Johne, R., et al., *An ORF1-rearranged hepatitis E virus derived from a chronically infected patient efficiently replicates in cell culture*. Journal of viral hepatitis, 2014. **21**(6): p. 447-456.

27. Legrand-Abravanel, F., *Hepatitis E Virus Genotype 3 Diversity*, France-Volume 15, Number 1—January 2009-Emerging Infectious Disease journal-CDC. 2009.

28. Shukla, P., et al., *Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus–host recombinant*. Proceedings of the National Academy of Sciences, 2011. **108**(6): p. 2438-2443.

29. Nguyen, H., et al., *A naturally occurring human/hepatitis E recombinant virus predominates in serum but not in faeces of a chronic hepatitis E patient and has a growth advantage in cell culture*. Journal of General Virology, 2012. **93**(3): p. 526-530.

30. Ray, R., et al., *Indian hepatitis E virus shows a major deletion in the small open reading frame*. Virology, 1992. **189**(1): p. 359-362.

31. Purdy, M.A., J. Lara, and Y.E. Khudyakov, *The hepatitis E virus polyproline region is involved in viral adaptation*. PloS one, 2012. **7**(4): p. e35974.

32. Dalton, H.R., et al., *Locally acquired hepatitis E in chronic liver disease*. Lancet, 2007. **369**(9569): p. 1260.

33. Navaneethan, U., M. Al Mohajer, and M.T. Shata, *Hepatitis E and pregnancy: understanding the pathogenesis*. Liver international, 2008. **28**(9): p. 1190-1199.