Hepatitis C virus sequence divergence preserves p7 viroporin structural and dynamic features – Supplementary information

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Supplementary figures



Supplementary Figure 1: Phylogenetic tree of HCV p7 sequences. The isolate name and genotype (gt) are indicated. The tree includes all completely annotated HCV isolates deposited in UniProt. Their accession number can be found in the alignment in **Figure S2**. In addition, isolate BAK1 (gt 7b) and the recently described novel genotype 8 (gt 8a) are included. Genotype 8 has no isolate name assigned and the four identified sequences are listed as patient 1-4.¹⁻³ Phylogenetic trees including more and different selections of isolates are published elsewhere.¹⁻⁵ Isolate J used in Montserret *et al.* is referred to as 'Japanese' in UniProt and J in the tree. Isolate J4 is referred to as (HC-)J4 in UniProt and J4 in the tree. Isolate J used to generate the Newick format tree data and TRex was used to plot the initial radial tree.^{6,7}

	10	20	30	40	50	60
J4_(gt_1b/092972)/1-63 ▲	LENLVVLNAAS	AGAHGILS	FLVFFCAAW <mark>Y</mark> I	KGRLAPGAAYA	YG VWPLLLLLA	LPPRAYA
J_(gt_1b/P26662)/1-63	LENLVVL <mark>N</mark> AASV	/A <mark>G</mark> A <mark>H</mark> GLL <mark>S</mark>	FLVFFCAAW <mark>Y</mark> I	KGRLVPGAAYA	.Y <mark>G</mark> VWPLLLLLLA	L PP <mark>R</mark> AYA
EUH1480_(gt_5a/039928)/1-63	CK <mark>N</mark> VIVL <mark>N</mark> AAAA	A <mark>GNHG</mark> FFW	GLLVVCLAW <mark>H</mark> V	KGRLVPGA <mark>TY</mark> L	LL <mark>G</mark> VW <mark>P</mark> LLLVRLL	R <mark>PHR</mark> ALA
EUH1480_OuYang_(gt_5a/039928)/1-63	AKNVIVL <mark>N</mark> AA <mark>S</mark> A	AA <mark>GNHG</mark> FFW	GLLVV <mark>T</mark> LAW <mark>H</mark> V	KGRLVPGA <mark>TY</mark> L	LGVWPLLLVRLL	RPH <mark>R</mark> ALA
H77_(gt_1a/P27958)/1-63 🗛	LENLVIL <mark>N</mark> AASL	.A <mark>GTH</mark> GLV <mark>S</mark>	FLVFFCFAW <mark>Y</mark> L	. <mark>KGR</mark> WVPGAV <mark>Y</mark> A	• <mark>YG</mark> MW <mark>P</mark> LLLLLLA	L P <mark>QR</mark> AYA
1_(gt_1a/P26664)/1-63 A	LENLVILNAAS	.A <mark>GTH</mark> GLVS	FLVFFCFAWYL	.KGKWVPGAVYT	• YGMWPLLLLLLA	L PQRAYA
H_(gt_1a/P27958)/1-63 A	LENLVILNAASL	.A <mark>GTH</mark> GLVS	FLVFFCFAWYL	KGRWVPGAVYA	-YGMWPLLLLLLA	L PORAYA
Glasgow_(gt_la/Q5EG65)/1-63 A	LENLVVLNAASL	AGTHGLVS	FLVFFCFAWFL	RGKWVPGAVYA	-YGMWPLLLLLLA	LPQRAYA
$BK_{(gt_1D/P_{20003})/1-03}$	LENLVVLNSASV	AGAHGILS	FLVFFCAAWYI	KGRLVPGALYA	-YGVWPLLLLLLA	LPPRAYA
$CONI_(gt_1D/Q9WMX2)/1-03 P$		AGANGILS	FLVFFCAAWYI	KGRLVPGAAYA		
$HC-JI_(gt_1D/Q00209)/1-03 P$ Tajwan (at 1b/p20846)/1-63 P			FLVFFCAAWYI			
$HC_{-11} (at 1b/003463)/1-63 A$						
HCR6 (at 1b/0913V3)/1-63 A						
HC-G9 (at $1c/081754$)/1-63		VGTHGTVP	FETEECAAWY			
India (at 1c/0913D4)/1-63	I ENI TVI NAASI	AGTHGTVP	FETEECAAWY	KGKWAPGI VYS		
1FH-1 (gt 2a/099TB8)/1-63		ANCHOLIY	FATEEVAAWHT			
HC-36 (at 2a/P26660)/1-63 A		ASCNGELY	FVIFFVAAWYI	KGRVVPLATYS	TGLWSFGLLLLA	LPOOAYA
HC-J8_(gt_2b/P26661)/1-63 A	LEKLIILHSASA	ASANGPLW	FFIFFTAAWYL	KGRVVPVATYS	LGLWS FLLLVLA	L POOAYA
JPUT971017_(gt_2b/Q9DHD6)/1-63	LEKLIILH <mark>S</mark> ASA	ASANGPLW	FFIFF <mark>T</mark> AAW <mark>Y</mark> L	KGRVVPAATYS	/L <mark>G</mark> LW <mark>S</mark> FLLLVLA	L PQQAYA
BEBE1_(gt_2c/Q68749)/1-63	LEKLVILHAA <mark>S</mark> A	ASSNGLLY	FILFFVAAWCI	KGRAVPMVTY	LL <mark>G</mark> CW <mark>S</mark> FVLLLMA	L PHQAYA
VAT96_(gt_2k/Q9QAX1)/1-63	L <mark>E</mark> KLVILHAA <mark>S</mark> A	AS <mark>SHG</mark> MLC	FIIFFIAAWYI	KGRVTPLVTYS	LGMWSFSLLLLA	L <mark>PQQ</mark> AYA
NZL_1_(gt_3a/Q81258)/1-63	LENLVTLNAVAA	AA <mark>GTH</mark> GIGW	YLVAFCAAW <mark>Y</mark> V	RGKLVPLVTY	TGLWSLALLVLL	L <mark>PQR</mark> AYA
k3a_(gt_3a/Q81495)/1-63 🗛	L <mark>EN</mark> LV <mark>T</mark> L <mark>N</mark> AVAA	AA <mark>GTH</mark> GIGW	YLVAFCAAW <mark>Y</mark> V	RGKLVPLVTYS	. <mark>TG</mark> LW <mark>S</mark> LALLVLL	L PQRAYA
452_(gt_3a/1W5D5)/1-63 🗛	LENLVTLNAVAV	/A <mark>GTH</mark> GIGW	YLVAFCAAW <mark>H</mark> V	RGKLVPLVTYS	. <mark>TG</mark> LW <mark>S</mark> LALLVLL	L PORAYA
Тr-Кj_(gt_3b/Q81487)/1-63 А	M <mark>en</mark> lvml <mark>n</mark> al <mark>s</mark> a	AA <mark>GQQ</mark> GYVW	YLVAFCAAW <mark>H</mark> I	RGKLVPLI <mark>TY</mark> G	_ <mark>TG</mark> LW <mark>P</mark> LALLDLL	L PQRAYA
JK049_(gt_3k/Q68801)/1-63	L <mark>EN</mark> LIVL <mark>N</mark> AISA	AA <mark>GTHG</mark> IWW	SLVAFCVAWHV	RGRIFPIAVYS	EV <mark>G</mark> LW <mark>P</mark> LLLLVLM	L PY <mark>RAY</mark> A
ED43_(gt_4a/039929)/1-63 A	LSNLININAA <mark>S</mark> A	AGAQGFWY	AILFICIVWHV	KGRFPAAAAYA	4C <mark>G</mark> LW <mark>P</mark> CFLLLLM	LPERAYA
SA13_(gt_5a/091936)/1-63 A	LENVIVLNAAAA	A A G T H G F F W	GLLVICFAWHF	KGRLVPGATYL	CLGIWPLLLLLFL	LPQRALA
EUHK2_(gt_6a/039927)/1-63 A	VERLVVLNAASA	AGTAGWWW	AVLFLCCVWYV	KGRLVPACTYM	ALGMWPLLLTILA	LPPRAYA
6a33_(gt_6a/Q512N3)/1-63 A		AGTAGWWW	AVLFLCCVWYV	KGRLVPACTYM	ALGMWPLLL ILA	LPHRAYA
$IN580_(gt_6b/092529)/1-63A$				KGRLVPACTYD	ALGMWPILLVILA	
$VN235_(gt_0a/092330)/1-03P$		ASSUGULY		RGRVVPGATYA.	LHLWPLLLLVLA	
VN004 (at 6b/002532)/1-63 A		AATRGWEC		RGRVVPAVITG		
$VN004_(gt_01/092532)/1-05P$ $VN405_(at_6k/092531)/1-63$			GVVETCAAWYT	PCPAAPTTTVA		
OC69 (at 72/480PD9)/1-63 A		AGTHGTWW		RCRVVPI VTVC		
RAK1(at 7b/A0A1B3TTH6)/1-63A	I ENI VVI NAXSI	AGTHGTHW	FTVAFCAAWXT	KGRYVPSTTYG		
Patient 1(at 8a)/1-63	I ENI TAL NTTSA			KGRAVPAETYC		
Patient 2(gt 8a)/1-63 A		AGTHGLLF	ALLVICAMWYI	KGRAVPAETYE		
Patient_3(qt_8a)/1-63 A	LENLIALNTISA	AGTHGLLF	ALLTICAMWYI	KGRAVPAATYC	FQIWPMLLLILA	
Patient_4(gt_8a)/1-63	LENLVALNTISA	AG <mark>SH</mark> GLLF	ALLVICAMWYI	KGRAVPAFTYC	FQIWPMLLLILA	L PERARA
Conservation			a faile i i		والمراجع المراجع	
conservacion						
8	976996157787	1×73×454	57967867*80	u×u58±757*5	5668×786×7497	5×78×4×

Supplementary Figure 2: Sequence alignment of HCV p7 sequences. The alignment was generated using Jalview.⁸ UniProt reviewed HCV p7 sequences including the novel isolate 7b and genotype 8a. Sequence conservation below is shown based on the 41 p7 isolates from genotypes 1a-c, 2a-c, 2k, 3a-b, 3k, 4a, 5a, 6a-b, 6d, 6g-h, 6k and 7a-b (UniProt identification codes/same order as in alignment: O92972, P26662, O39928, P27958, P26664, P27958, Q5EG65, P26663, Q9WMX2, Q00269, P29846, Q03463, Q913V3, Q81754, Q913D4, Q99IB8, P26660, P26661, Q9DHD6, Q68749, Q9QAX1, Q81258, Q81495, Q1W5D5, Q81487, Q68801, O39929, O91936, O39927, Q5I2N3, O92529, O92530, Q68798, O92532, O92531, A8QPD9 and A0A1B3IIH6, respectively). Sequences are colored according to the ClustalX color-scheme, i.e. hydrophobic: blue, positive charge: red, negative charge: magenta, polar: green, cysteines: pink, glycines: orange, prolines: yellow, aromatic: cyan, unconserved: white.⁹ Isolate names are shown next to the sequences with the corresponding genotype and UniProt accession number in parentheses where available. At least one example from each of the eight recognized genotypes is included. The four recently identified genotype 8 sequences are listed as patient 1-4.3 Å more complete list of HCV p7 isolates can be found at the International Committee on Taxonomy of Viruses.¹⁰ The isolates of particular interest in this study are J4 p7 (gt 1b, UniProt: O92972) and EUH1480 p7 (gt 5a, UniProt: O39928). The EUH1480 isolate with five mutations (T1G, C2A, A12S, C27T and C44S) used by OuYang et al. has also been added.¹¹ The conservation is depicted as a bar graph below the alignment.



Supplementary Figure 3: The hydropathy index of HCV p7 isolates. The index of p7(5a/EUH1480) used in this study and in OuYang *et al.* with five mutations (*) (T1G, A12S, C2A, C27T, and C44S) and p7(5a/EUH1480) wildtype are plotted in red solid and red dashed lines, respectively. Plots of p7(1b/J4) C27S and p7(1b/Japanese) C27S are shown in black solid and black dashed lines, respectively. The Kyte and Doolittle hydropathy scale with default window size 9 was used. The score is proportional to hydrophobicity.^{12,13}

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