

Supplementary Information

Identification of HCV Resistant Variants against Direct Acting Antivirals in Plasma and Liver of Treatment Naïve Patients

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Supplementary Tables

Table S1: Patient characteristics at baseline

Patient	Age (Years)	Sex ^a	Serum viral load (IU/ml)	ALT	Fibrosis
1	55	M	2.60 ×10 ⁶	195	5
2	48	F	4.95 ×10 ⁶	91	3
3	45	M	3.66 ×10 ⁶	79	3
4	29	M	1.63 ×10 ⁶	67	2
5	43	F	2.30 ×10 ⁶	35	1
6	25	F	3.90 ×10 ⁶	48	1
7	47	M	4.60×10 ⁶	68	2
8	36	M	6.98 ×10 ⁶	85	NA
9	54	M	1.71 ×10 ⁶	107	4
10	54	M	4.20 ×10 ⁶	59	3
11	37	F	7.81×10 ⁵	31	3
12	45	F	1.65 ×10 ⁶	43	0
13	53	M	1.84 ×10 ⁶	19	1
14	39	M	5.13 ×10 ⁶	86	5
15	27	M	1.65 ×10 ⁶	61	2
16	21	M	4.81 ×10 ⁵	65	1
17	31	M	2.23 ×10 ⁶	41	NA
18	60	M	6.46 ×10 ⁶	73	3

^a M, male; F, female; NA, not available

Table S2: DPS primer sequence

No	Primer name	Adopter	Taq	MID	Genespecific primer
1	Pt1FNS5bMID1	5'-CGTATCGCCTCCCTCGGCCA	TCAGACGAGTGC	GTGGGGATCCC	GATGATACCCGCTGCTTTGA-3'
2	Pt2RNS5bMID2	5'-CTATGCGCCTTGCCAGCCCGC	TCAG	ACGCTCGACAGGCGGAATTCCTGGTCATAGCCTCCGTGAA-3'	
3	Pt2RNS5bMID3	5'-CTATGCGCCTTGCCAGCCCGC	TCAGAGACGC	ACTGGCGGAATTCCTGGTCATAGCCTCCGTGAA-3'	
4	Pt2RNS5bMID4	5'-CTATGCGCCTTGCCAGCCCGC	TCAGAGCACTG	TAGGGCGGAATTCCTGGTCATAGCCTCCGTGAA-3'	
5	Pt2RNS5bMID5	5'-CTATGCGCCTTGCCAGCCCGC	TCAGATCAGAC	ACGGCGGAATTCCTGGTCATAGCCTCCGTGAA-3'	
6	Pt2RNS5bMID6	5'-CTATGCGCCTTGCCAGCCCGC	TCAGATATCG	GAGGGCGGAATTCCTGGTCATAGCCTCCGTGAA-3'	
7	Pt2RNS5bMID7	5'-CTATGCGCCTTGCCAGCCCGC	TCAGCGTGTCT	ATAGGCGGAATTCCTGGTCATAGCCTCCGTGAA-3'	
8	Pt2RNS5bMID8	5'-CTATGCGCCTTGCCAGCCCGC	TCAG	CTCGGTGTGCGGCGGAATTCCTGGTCATAGCCTCCGTGAA-3'	

MID- multiples identifier

Table S3: Amount of HCV reads, haplotypes, and Simpson’s diversity index per liver (L) or plasma (P) sample of each patient.

Patient	No of reads	No of “cleaned” reads	No reads of haplotypes with frequencies of more than 1%	No haplotypes	Simpson's Diversity Index
1L	16049	15650	13588	4	0.14
1P	17913	17540	15725	2	0.08
2L	18885	18411	16381	4	0.23
2P	18361	18010	16240	6	0.21
3L	14094	13672	8185	11	0.77
3P	27949	27102	23350	2	0.06
4L	27468	26241	21966	6	0.48
4P	29496	28706	23879	5	0.48
5L	29960	28893	23641	6	0.47
5P	31467	30685	24433	5	0.45
6L	25874	24743	19848	2	0.5
6P	26824	25742	21612	4	0.37
7L	29932	27883	22646	5	0.29
7P	27569	26828	21128	4	0.41
8L	24848	24111	21144	3	0.06
8P	21393	20198	16800	5	0.24
9L	17643	16750	10546	14	0.89
9P	21213	20267	15883	6	0.37
10L	13760	12948	9933	7	0.82
10P	26634	25865	21036	4	0.58
11L	16836	16013	13191	4	0.45
11P	24273	23343	19020	7	0.58
12L	14365	13498	12056	3	0.28
12P	22374	21430	18806	6	0.3
13L	17245	16327	14248	4	0.15
13P	23966	22655	19090	5	0.33
14L	33251	31536	24757	7	0.61
14P	36010	34662	28486	7	0.64
15L	28058	27543	24066	5	0.49
15P	37164	36292	31941	4	0.48
16L	32415	30081	22151	4	0.24
16P	22707	22075	19664	2	0.02
17L	28402	27409	21067	10	0.72
17P	25854	24885	21212	5	0.56
18L	8605	8400	7089	5	0.56
18P	23761	23209	19571	3	0.26

Table S4: Detection of HCV-NS5B resistant variants by DPS (% of total cleaned reads).

Patient	S282T	C316N	C316H	C316Y	V321I	D244N	Q309R	D310N	S326G	T329I	A333E
<i>cutoff</i>	0	0.2	0.2	0.2	0	0	0	0	0	0	0.4
1L	0	99.9	0	0	0	0	0.23	0.02	0	0	0
1P	0	99.92	0	0	0	0	0	0	0	0	0
2L	0	0	0	0	0	0	0	0	0	0	0
2P	0	4.38	0	0	0.03	0	0.54	0	0	0	0
3L	0	65.66	3.12	5.63	0	0.02	5.82	0	0	0.02	0
3P	0	99.81	0	0	0	0	0.18	0.03	0	0.07	0
4L	0	99.93	0	0	0.02	0	0.11	0	0	0	0
4P	0	99.88	0	0	0	0.02	0.19	0.04	0.03	0	0
5L	0	20.77	0	0	0	0	0.25	0.22	0.03	0.04	0
5P	0	0	0	0	0.04	0.05	0.21	0.08	0.06	0.07	0
6L	0	99.92	0	0	0.02	0	0.35	0.88	0	0	0
6P	0	99.83	0	0	0	0	0.21	0	0	0	0
7L	0	0.28	0	0	0.02	0.11	0.51	0.02	0.02	0.08	0
7P	0	0	0	0	0.01	0.04	0.62	0.03	0.06	0.09	0
8L	0	0.34	0	99.47	0.03	0.07	99.53	0.07	0.02	0.14	0
8P	0	13.66	0.8	85.26	0	0.04	85.28	0.03	0.08	0.07	0
9L	0	11.55	43.91	1.39	0.02	0.01	2.42	0.2	0.17	0.2	0
9P	0	13.27	0	0	0	0.13	0.48	0.02	0.03	0.16	0
10L	0	73.55	0.64	0	0	0	0.9	0.1	0	0.12	0
10P	0	99.56	0	0	0	0.08	0.4	0	0.05	0.07	0
11L	0	0	0	0	0.03	0	0.68	0	0.07	0.03	0
11P	0	0	0	0	0	0	0.42	0.03	0.06	0.11	0
12L	0	0.3	0.65	0	0	0	0.16	0	0	0	0
12P	0	7.31	0	0	0	0.04	0.28	0	0.03	0	0
13L	0	0	1.7	0	0	0	94.17	0	0	0.01	0
13P	0	0	0	0	0	0	83.41	0	0.11	0.17	0
14L	0	0	97.41	0	0	0	2.53	0.09	0.28	0.05	0
14P	0	0	99.73	0	0.02	0.01	0.88	0.02	0	0.04	0
15L	0	99.93	0	0	0.03	0.09	72.61	0.02	0	0.85	0
15P	0	99.91	0	0	0	0.06	71.42	0	0	0.8	0
16L	0	99.84	0	0	0	0	0.25	0	0.05	0	0
16P	0	97.78	0	0	0	0	0.18	0	0	0	0
17L	0	2.9	0	0	0	0	94.69	0	0.01	0.03	0
17P	0	0.55	0	0	0	0	98.82	0	0	0.08	0
18L	0	0	0	0	0	0	0.23	0	0	0	0
18P	0	0	0	0	0	0	0.39	0	0	0	0

L, liver; P, plasma. Cut-off, position specific error rate determined in control experiments.