Supplementary Table 3. Continued

Gene	Reference position	Chromosome	Coding sequence	Coverage	Allele change	Amino acid change	Functional predictions by SIFT	Patient no.
ABCA7	2506887468	19	25	15	A < G	S	N	4
REXO1	2507659336	19	3	14	C < T	S	N	4
FAM108A1	2507714358	19	2	13	T < C	NS	N	4
PIP5K1C	2509476658	19	13	27	G < A	NS	N	3
MAP2K2	2509934448	19	6	47	C < T	NS	D	4
ACER1	2512145698	19	3	26	T < C	NS	N	4
LASS4	2514105354	19	7	55	T < C	S	N	4
MUC16	2514735984	19	51	113	T < C	NS	N	4
MUC16	2514735995	19	51	104	C < T	NS	N	4
MUC16	2514783318	19	5	66	C < T	NS	N	4
MUC16	2514793493	19	3	59	C < T	NS	N	4
MUC16	2514797580	19	3	46	T < C	S	N	3
ICAM3	2516182922	19	2	22	T < C	NS	N	4
MAST1	2518717997	19	26	18	G < A	S	N	3
CYP4F12	2521526643	19	5	51	T < C	NS	N	4
OR10H2	2521572582	19	1	89	C < T	S	N	4
AP1M1	2522077812	19	11	81	C < G	NS	D	3
CPAMD8	2522819358	19	17	55	G < A	NS	N	3
KIAA1683	2524110904	19	2	62	C < G	NS	N	4
ISYNA1	2524280086	19	7	57	T < C	S	N	4
KIAA0892	2525185534	19	7	61	G < A	S	N	3
ZNF536	2533672403	19	3	28	T < C	S	N	4
GPI	2537503303	19	7	71	C < G	S	N	4
CD22	2538462717	19	5	17	C < G	S	N	3
C19orf15	2541461368	19	1	88	T < C	NS	N	4
MAP4K1	2541732174	19	14	25	G < A	NS	N	4
CAPN12	2541857821	19	18	71	A < G	S	N	4
LGALS4[a]	2541932950	19	3	30	C < A	NS	D	1
ECH1	2541939937	19	9	29	C < T	NS	D	4
PLEKHG2	2542544839	19	12	22	C < T	S	N	3
FCGBP	2543017507	19	21	76	G < A	S	N	4
FCGBP	2543053201	19	6	13	G < T	S	N	1
SNRPA	2543896811	19	2	60	A < G	S	N	4
CYP2F1	2544255597	19	1	31	G < A	S	N	4
ERF	2545386691	19	4	32	G < A	S	N	4
PSG3	2545867483	19	4	156	C < A	S	N	4
PSG8	2545901763	19	2	60	C < A	NS	D	3
CEACAM20	2547650657	19	7	58	T < C	NS	NR	4
ERCC2	2548501717	19	6	71	T < G	S	N	4
EMP3	2551464282	19	2	40	G < T	NS	NO	1
TMEM143	2551479358	19	6	19	A < C	NS	D	3
PTH2	2552559200	19	2	22	G < C	NS	N	3
SHANK1	2553853011	19	2	18	T < C	NS	N	1
ZNF808[a]	2555691982	19	3	39	G < A	NS	N	1
ZNF765	2556544681	19	3	19	C < G	NS	N	1
ZNF765	2556544684	19	3	19	T < C	S	N	1
ZNF761	2556586232	19	2	16	G < A	S	NR	4
LILRB3[a]	2557359732	19	3	208	G < C	NS	N	2
LILRA1	2557740721	19	5	61	T < C	NS	N	2
KIR2DL4	2557949666	19	3	17	C < G	NS	N	3
KIR3DL1	2557963191	19	3	109	A < G	S	N	4
KIR2DS4	2557982650	19	3	37	T < G	S	NR	2
KIR2DS4	2557982701	19	3	36	G < T	NS	NR	2
KIR2DS4	2557982728	19	3	21	G < C	NS	NR	2
RDH13	2558201492	19	1	28	C < T	NS	N	4
RDH13	2558201493	19	1	29	G < T	NS	N	4
ZFP28	2559692350	19	3	33	C < A	S	N	3
ZNF550[a]	2560701027	19	1	67	C < T	NS	D	1
ZSCAN22	2561483213	19	2	17	T < G	NS	N	3
KIR2DS1	2561871082	19	3	153	A < G	S	NR	2

Supplementary Table 3. Continued

Gene	Reference position	Chromosome	Coding sequence	Coverage	Allele change	Amino acid change	Functional predictions by SIFT	Patient no.
SIGLEC1	2565630685	20	3	19	C < T	NS	N	4
PAK7	2571487871	20	4	131	C < T	NS	N	4
FLRT3	2576252200	20	1	17	T < G	NS	D	3
CST9L	2585490888	20	2	216	T < G	NS	N	4
BPI	2595650275	20	11	148	A < G	NS	N	4
LBP	2595672219	20	2	23	G < A	S	N	4
LBP[a]	2595691968	20	10	25	G < T	S	N	1
KIAA1219	2595847726	20	10	14	T < G	NS	D	3
PTPRT	2599404902	20	31	37	C < A	NS	D	3
SEMG2	2602544774	20	2	42	A < G	S	N	4
ZNF335	2603273210	20	21	32	A < G	S	N	4
PCK1	2614832083	20	3	52	A < G	S	N	4
CTSZ	2616266012	20	5	51	A < G	S	N	4
OGFR	2620085220	20	4	20	G < A	S	N	4
KCNQ2	2620689776	20	14	14	C < G	NS	N	4
LOC100132288	2622007697	21	2	24	C < T	NS	NR	4
LOC100288017	2623267208	21	1	18	G < A	NS	NR	2
POTED	2623681322	21	1	60	G < A	NS	N	2
KRTAP13-2	2640442822	21	1	81	A < T	NS	D	3
C21orf66	2642816546	21	12	14	A < C	NS	N	1
C21orf66	2642816547	21	12	14	C < T	NS	N	1
WRB	2649461227	21	2	73	G < T	NS	NO	4
WRB	2649461228	21	2	73	A < T	NS	D	4
DSCAM	2650145634	21	27	116	G < C	S	N	4
PRDM15	2651870131	21	31	65	G < A	S	N	4
PFKL	2654380692	21	4	19	C < T	S	N	4
KRTAP10-6	2654660390	21	1	137	G < A	S	N	4
KRTAP12-2	2654734983	21	1	59	C < T	NS	N	4
KRTAP12-2	2654735333	21	1	71	G < A	S	N	4
KRTAP12-2	2654735334	21	1	69	C < T	NS	N	4
COL6A2	2656200935	21	27	63	C < G	S	N	3
FTCD	2656222648	21	2	55	T < A	NS	D	2
CECR5	2658218162	22	6	14	T < G	NS	D	3
CECR2	2658624712	22	16	25	C < T	S	N	4
LOC100288065	2658662354	22	4	63	A < G	NS	N	4
TBX1	2660347982	22	4	51	C < T	S	N	4
ZNF280B[a]	2663338671	22	1	78	G < A	S	N	2
C22orf30	2672604693	22	3	17	C < T	NS	NO	3
ISX	2675974809	22	2	60	G < A	S	N	1
HMGXB4	2676157665	22	4	42	A < T	S	N	3
APOL1	2677147183	22	2	27	C < T	NS	N	1
TMPRSS6	2677959079	22	17	144	G < A	S	N	4
TMPRSS6	2677959089	22	17	133	A < G	NS	N	4
SSTR3	2678099174	22	1	18	G < A	S	N	4
APOBEC3A	2679853734	22	3	53	C < T	S	N	4
L3MBTL2	2682109341	22	5	24	C < T	S	N	4
NAGA	2682959967	22	3	46	C < T	S	N	4
TTLL12	2684071796	22	5	53	C < T	S	N	3
SCUBE1	2684110469	22	15	48	C < G	S	N	4
LOC100289317	2686220100	22	1	25	A < G	NS	NR	4
CELSR1	2687425845	22	1	58	A < G	NS	N	4
MAPK8IP2	2691494874	22	11	52	G < A	NS	N	4
CD99	2693971425	X	6	16	G < A	NS	D	4
PRKX	2694923444	X	2	88	G < A	S	N	4
ARHGAP6[a]	2702537703	X	4	81	G < A	NS	D	2
DMD	2723711682	X	37	92	T < C	NS	N	4
WAS	2739828502	X	11	13	G < T	NS	N	3
GATA1	2739931290	X	2	14	A < C	NS	N	3
GAGE12E	2740547782	X	1	62	G < T	NS	D	4
PAGE1[a]	2740686656	X	3	37	T < C	S	N	1

Supplementary Table 3. Continued

Gene	Reference position	Chromosome	Coding sequence	Coverage	Allele change	Amino acid change	Functional predictions by SIFT	Patient no.
USP27X	2740875896	X	1	39	G < C	NS	NR	3
TSPYL2	2744246047	X	6	76	A < G	NS	N	3
FAM120C	2745238526	X	14	66	C < A	NS	D	2
ITIH5L	2745914425	X	8	22	G < A	NS	N	3
MSN	2752987418	X	9	47	G < A	S	N	2
OPHN1	2755314567	X	20	47	T < C	NS	N	3
DGAT2L6	2757452592	X	5	20	C < T	S	N	3
LPAR4	2765991350	X	1	46	G < T	NS	D	3
LPAR4	2765991352	X	1	43	C < T	S	N	3
PCDH11X	2779854213	X	7	171	C < A	NS	N	3
SYTL4	2787924130	X	9	24	G < A	S	N	3
SYTL4	2787924131	X	9	24	T < C	NS	D	3
NXF5	2789077402	X	3	81	T < C	NS	D	1
NXF2	2789554971	X	10	19	C < T	S	N	3
CLDN2	2794152808	X	1	26	C < A	NS	N	3
CLDN2	2794152809	X	1	26	C < T	NS	N	3
TRPC5	2799176319	X	1	24	G < A	NS	D	3
TRPC5	2799176320	X	1	24	G < T	NS	N	3
RHOXF2B	2807087244	X	4	29	A < G	NS	N	3
PLAC1	2821580905	X	1	20	A < G	NS	N	3
RBMX	2823837181	X	8	40	G < C	NS	D	4
SLITRK4	2830598721	X	1	56	T < C	NS	N	3
NSDHL	2839816887	X	6	109	A < G	NS	D	3
MPP1	2841799005	X	5	36	T < C	S	N	3
MPP1	2841799006	X	5	36	T < C	NS	N	3
RBMY1D	2863471281	Y	11	23	T < C	S	N	1

SIFT, Sorting Intolerant From Tolerant; N, nonsynonymous mutation; D, deleterious; N, neutral; S, synonymous mutation; NO, nonsense mutation; NR, no record found.
[a]These genes were commonly mutated in the synchronously developed HCCs from patients 1 and 2.

Supplementary Table 4. Functional Relevance of Mutations Detected in HCC Tumors

Patient 1					
HCC 1	No. of mutated genes	51			
	Amino acid change (+)	38 (74.5%)			
	Functional loss[a] (+)	20 (39.2%)			
	KEGG pathway				
	Pathways in cancer	CTNNB1	PDGFRB	TP53	
	Wnt signaling pathway	FBXW11			
	PI3K-Akt signaling pathway	ITGB8			
	Others	ALDH1A1	HDAC9	SORBS2	RYR3
HCC 2	No. of mutated genes	79			
	Amino acid change (+)	58 (73.4%)			
	Functional loss[a] (+)	23 (29.1%)			
	KEGG pathway				
	Viral carcinogenesis	HDAC9	RB1	TP53	
	Wnt signaling pathway	FBXW11			
	PI3K-Akt signaling pathway	ITGB8			
	Others	ALDH1A1	NXF5		
Patient 2					
HCC 1	No. of mutated genes	39			
	Amino acid change (+)	20 (51.3%)			
	Functional loss[a] (+)	10 (25.6%)			
	KEGG pathway				
	Metabolic pathways	DBH			
	Others	AGRN			
HCC 2	No. of mutated genes	70			
	Amino acid change (+)	40 (57.1%)			
	Functional loss[a] (+)	20 (28.6%)			
	KEGG pathway				
	Metabolic pathways	ADSSL1	FTCD	RDH16	
	Others	ABCA13	BTRC	VWF	C4A
		GRM4			
Patient 3					
HCC 1	No. of mutated genes	30			
	Amino acid change (+)	20 (66.7%)			
	Functional loss[a] (+)	6 (20.0%)			
	KEGG pathway				
	Metabolic pathways	CYP1A2			
HCC 2	No. of mutated genes	276			
	Amino acid change (+)	208 (75.3%)			
	Functional loss[a] (+)	90 (32.6%)			
	KEGG pathway				
	Metabolic pathways	ACSM4	ADSS	UGT2B28	DHRS4L2
		GALNT5	ME1	POLE	NSDHL
		PIK3C2G			
	PI3K-Akt signaling pathway	COL6A6	HGF	ANGPT1	LPAR4
	Neuroactive ligand receptor	GLRA2			
	Others	CDK9	CA2	ABCC12	AP1M1
		GNPAT	GLYAT	RUVBL1	GDF9
		MYL12A	MLL3	SLC18A2	MAP4K4
		PRPF8	PIP4K2A	SLC9A4	NUP37
		VCP	TTN		

Supplementary Table 4. Continued

Patient 4					
HCC	No. of mutated genes	364			
	Amino acid change (+)	177 (48.6%)			
	Functional loss[a] (+)	46 (12.6%)			
	KEGG pathway				
	Metabolic pathways	ACSM5	ALPP	PNLIPRP1	
	MAPK signaling pathway	HSPA6	MAP2K2		
	PI3K-Akt signaling pathway	FLT4			
	Others	ECH1	CHI3L1	FURIN	CD99
		KCNJ12	ITGAE	TMPRSS4	REXO1L1
		RBMX	PLOD3	TUBA4A	PGA3

[a]The number of mutated genes predicted to be "damaging (deleterious)" by Sorting Intolerant From Tolerant (SIFT) functional impact predictions (http://provean.jcvi.org/index.php). The genes categorized in multiple pathways are shown in only one representative pathway.

Supplementary Table 5. List of 448 Indels in 409 Genes at a Frequency of >20% of Reads in 7 HCC Tumors From 4 Patients

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
7813482	ERRFI1	1	3	17	insA	3
12628605[a]	PRAMEF11	1	3	27	insC	2
12718307	PRAMEF7	1	2	47	insT	3
17358674[a]	PADI6	1	9	41	delG	4
17358674[a]	PADI6	1	9	62	delGT	2
26696284	ARID1A	1	2	19	delC	4
31395890[a]	SERINC2	1	9	17	insG	3
46770747	CYP4B1	1	8	42	delAT	4
46770748	CYP4B1	1	8	42	delT	4
52949277[a]	LOC100133211	1	1	42	delG	4
53189215	MAGOH	1	3	25	insA	3
54095320[a]	CDCP2	1	4	21	insC	4
62557547	ANGPTL3	1	4	17	insT	3
78876012	ELTD1	1	10	13	insA	3
89014597	GBP1	1	4	17	insA	2
90670304	BARHL2	1	2	20	insC	3
108974106	CLCC1	1	6	84	insG	4
122705624[a]	PDE4DIP	1	14	80	delG	4
122713730[a]	PDE4DIP	1	6	465	delT	1
131401399	DENND4B	1	12	43	insG	3
131474740	NUP210L	1	34	13	insG	3
131951749	SHE	1	3	112	insA	2
133072970	MSTO1	1	11	33	insA	3
133795656	CCT3	1	2	34	insT	3
133844355[a]	RHBG	1	9	40	delC	4
134043234[a]	TTC24	1	3	12	delC	3
136505109	IFI16	1	7	31	insT	3
146999551	F5	1	13	23	insT	3
151907797	GPR52	1	1	18	insA	2
162596811	C1orf25	1	10	26	insT	3
163815430	TPR	1	15	43	delT	3
174374155	CFHR4	1	5	18	insC	3
177507300	NR5A2	1	5	20	insT	3
201685738	NVL	1	6	32	insA	3
203543660	ACBD3	1	2	18	insG	3
208315870[a]	ARV1	1	3	146	delCT	1
208315871[a]	ARV1	1	3	144	delT	1
213162772	LYST	1	3	19	insG	3
214770350	RYR2	1	11	22	insG	3
219641707	PLD5	1	2	102	insA	1
224245114	AHCTF1	1	14	19	insG	3
230467928	RNASEH1	2	8	13	insT	3
236404886	ADAM17	2	19	29	insG	3
251086730[a]	LOC375190	2	8	40	insC	4
254371933	IFT172	2	38	18	insT	3
258505433[a]	SRD5A2	2	1	28	insG	2
267355164	SLC8A1	2	1	15	insG	3
282849120	EFEMP1	2	1	14	insA	3
287714203	PAPOLG	2	15	20	delT	3
288274645	USP34	2	15	14	insC	3
301408194	CCDC142	2	2	27	insC	3
302628924	C2orf3	2	3	22	insA	3
312276713	RETSAT	2	4	55	delC	2
314781292	RGPD2	2	5	27	insT	3
317672121[a]	LOC391405	2	4	43	delA	4
318709414	TRIM43	2	1	22	insA	3
325574912	SLC9A4	2	6	27	insT	3
329490991	RGPD3	2	20	29	insT	3
331565693	GCC2	2	22	18	insC	2
332910299	RGPD5	2	21	25	insA	3
333616906	RGPD7	2	8	21	delA	3

Supplementary Table 5. Continued

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
335725611	SLC20A1	2	8	14	insC	3
336700192	RABL2A	2	4	76	delG	1
340887331	DDX18	2	7	18	insT	3
350555029	IWS1	2	11	62	delG	3
355383031[a]	ZNF806	2	3	50	delC	4
355383457[a]	ZNF806	2	3	56	insA	4
355383669[a]	ZNF806	2	3	52	delA	4
361736116	NXPH2	2	2	48	insT	3
385464478	KCNH7	2	10	41	insT	3
387807820	COBLL1	2	2	23	insA	3
388452944	SCN2A	2	26	57	insT	3
400689016	TTC30A	2	1	32	insA	3
401650351	TTN	2	270	19	insG	3
401670059	TTN	2	242	25	insT	3
401800064	TTN	2	64	26	insA	3
402189048	SESTD1	2	14	31	insG	3
403038177	CWC22	2	11	16	insA	3
418958541	DNAH7	2	34	27	insC	3
439213578	XRCC5	2	13	22	delC	3
446013793	ACSL3	2	14	26	insT	3
446670417	SCG2	2	1	17	insA	3
446671148	SCG2	2	1	30	insC	3
456401277[a]	SAG	2	10	76	delA	2
463724350	AQP12B	2	1	26	delC	2
479793553[a]	GRIP2	3	10	55	insG	4
504388108	TTC21A	3	6	13	insA	3
509772719	ZNF852	3	3	20	delTC	4
509772720	ZNF852	3	3	19	delC	4
511646368	CCR5	3	1	24	insT	3
515483759[a]	SLC38A3	3	2	18	insG	4
538148646	GLT8D4	3	6	14	insC	3
538243406[a]	FLJ10213	3	1	12	insA	2
538564462	PDZRN3	3	10	24	insG	3
540846731[a]	LOC100288801	3	2	39	delG	2
540918687	ZNF717	3	4	18	delC	1
570208682	HHLA2	3	4	18	insT	3
570487430	DZIP3	3	10	45	insT	3
574780156	CD200R1	3	4	24	insA	3
587083495	ZNF148	3	6	14	insG	3
591946859[a]	LOC644974	3	6	36	delC	3
595459401	TOPBP1	3	26	16	insT	3
608303870	PLSCR2	3	4	59	insG	3
611616867	C3orf16	3	5	22	delCT	3
611616868	C3orf16	3	5	21	delT	3
612474548	SELT	3	4	38	insT	4
631967149	PHC3	3	10	13	insT	3
648156647	DGKG	3	2	44	insC	3
652237997[a]	CLDN16	3	1	271	delG	2
658673909	PAK2	3	12	16	insT	3
660166378	ZNF595	4	4	17	insA	1
662098266[a]	POLN	4	23	65	delG	3
696277331	FLJ16686	4	3	53	delC	1
725715810	TMPRSS11F	4	7	30	insA	3
728128121	LOC100129410	4	3	13	insC	2
752860649	UNC5C	4	14	17	insT	3
767688857[a]	EGF	4	24	16	insC	1
779934903	KIAA1109	4	39	17	insT	3
782346647	ANKRD50	4	3	37	insT	3
827268418	NEK1	4	4	17	insT	3
841124368	CDKN2AIP	4	3	16	insA	3
853638502	KIAA0947	5	14	24	insT	3

Supplementary Table 5. Continued

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
889026155[a]	CARD6	5	3	32	insT	2
891727995	PAIP1	5	2	16	insG	2
901348948	MAP3K1	5	13	24	insC	3
909928153	ADAMTS6	5	3	23	insG	3
914509435	SERF1B	5	3	15	insG	3
914509482	SERF1B	5	3	49	insA	3
915509411[a]	GTF2H2	5	13	31	insT	2
919186632	HEXB	5	11	43	insA	3
922917852	SCAMP1	5	7	37	insA	1
928534269	EDIL3	5	7	25	insA	3
931867232	CCNH	5	7	26	insT	3
956697390	EPB41L4A	5	11	13	insT	1
966610635	ZNF474	5	1	39	delT	1
972596345	SLC12A2	5	8	35	insT	3
980635083[a]	SMAD5	5	6	105	insC	1
985314450[a]	LOC100288105	5	1	14	delC	4
985640033[a]	PCDHB9	5	1	32	insA	1
985844899[a]	PCDHGA8	5	1	27	delC	3
992330379	SCGB3A2	5	1	14	delA	3
994446878[a]	TIGD6	5	1	136	delT	1
994476149	HMGXB3	5	6	14	delA	3
998157358	GRIA1	5	11	19	insC	3
1020539380	FAM153B	5	4	23	insC	3
1039337531	C6orf114	6	1	30	insA	3
1052252527	BTN2A2	6	2	44	insG	3
1054107191[a]	ZNF187	6	1	33	insG	4
1056096293[a]	FLJ45422	6	2	18	insT	2
1057247419[a]	MICA	6	5	27	delG	3
1082305754	DST	6	45	18	insT	3
1088830738	EYS	6	6	19	insT	3
1093406718	COL19A1	6	5	16	insA	3
1113248546	MDN1	6	15	14	insC	3
1113280524	MDN1	6	2	40	insA	3
1131602437[a]	FOXO3	6	2	64	insG	3
1133380782	SLC22A16	6	4	20	insA	3
1135037748	C6orf225	6	1	17	delC	3
1153093327	SAMD3	6	7	14	delC	3
1154647636	LOC643854	6	1	26	insT	3
1154648098	LOC643854	6	1	20	insC	3
1159216432	BCLAF1	6	2	13	delT	2
1161156444	PBOV1	6	1	36	insG	3
1182019086	RSPH3	6	6	43	insA	3
1200500350	RSPH10B2	7	19	23	insG	3
1206053594	VWDE	7	19	26	insA	4
1221518316	TAX1BP1	7	13	14	insA	3
1222659823[a]	KIAA0644	7	1	90	delC	4
1222659922[a]	KIAA0644	7	1	26	insC	3
1226974977	BBS9	7	7	19	insT	1
1228643735[a]	DPY19L1	7	18	22	delAT	4
1228643736[a]	DPY19L1	7	18	50	delT	1
1262731853	TYW1B	7	8	142	delA	4
1262954278	TRIM74	7	2	24	insA	2
1265555276	TRIM73	7	2	84	insT	2
1266437261[a]	FLJ37078	7	14	43	insC	2
1266593512[a]	ZP3	7	8	51	insG	1
1266763110[a]	POMZP3	7	5	83	delA	4
1278946055	C7orf62	7	1	20	insC	3
1283360469	HEPACAM2	7	4	29	insT	3
1283589759	CALCR	7	9	26	insT	3
1290893801[a]	ZAN	7	30	28	insG	3
1291366094	MOGAT3	7	2	24	insA	3

Supplementary Table 5. Continued

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
1291722996[a]	EMID2	7	13	20	insG	4
1292538685	LOC100289561	7	1	14	insA	3
1295252926	MLL5	7	12	21	insG	3
1298402792	NRCAM	7	1	17	insT	3
1319055841[a]	KCP	7	10	62	insC	1
1319073009	KCP	7	1	30	delC	2
1333766379	LOC441294	7	1	46	insA	4
1334380185	CTAGE4	7	1	39	insA	3
1334381975	ARHGEF5L	7	1	19	insA	1
1339923632[a]	KRBA1	7	12	76	insC	2
1339973995[a]	SSPO	7	9	44	insC	1
1340003537[a]	SSPO	7	60	15	insC	4
1340012514	SSPO	7	76	23	delA	2
1340015859	SSPO	7	83	14	delC	2
1340525483	C7orf29	7	1	24	delC	1
1341211228[a]	ATG9B	7	10	49	insC	1
1341434558	SMARCD3	7	10	21	delC	3
1342197228	GALNTL5	7	5	71	delT	4
1342442397[a]	MLL3	7	14	208	insT	4
1356372261[a]	XKR5	8	6	55	delAG	1
1374409954[a]	NEFL	8	3	38	delG	4
1380219728[a]	UBXN8	8	7	83	insT	1
1380304215	TEX15	8	1	23	insA	3
1388426070[a]	PLEKHA2	8	11	28	delC	2
1395399601[a]	PRKDC	8	31	17	insG	1
1398930064	PXDNL	8	14	27	insA	3
1410692513[a]	YTHDF3	8	4	24	insG	1
1415952398	C8orf34	8	2	32	insG	3
1445261384	LAPTM4B	8	2	16	insC	3
1490189877[a]	JRK	8	1	12	delCA	3
1490189878[a]	JRK	8	1	19	delA	2
1491176363	ZNF623	8	1	29	insT	3
1492082552[a]	RECQL4	8	14	20	delG	3
1498992866	LOC645969	9	1	155	insT	4
1527437913[a]	C9orf144B	9	4	20	delC	4
1543290663[a]	FOXD4L5	9	1	39	delG	1
1546032104	TRPM3	9	22	19	insT	3
1552818643	VPS13A	9	48	29	insG	3
1574648314	COL15A1	9	13	29	insC	3
1586295095	MUSK	9	1	62	insT	3
1608846803[a]	ABO	9	6	117	insC	4
1620006324	GDI2	10	7	14	insG	3
1620254092	IL2RA	10	4	14	insC	3
1621795546[a]	ITIH5	10	14	23	delC	1
1633127998	NSUN6	10	2	26	insA	3
1647389817	ITGB1	10	13	22	insA	3
1652560241[a]	LOC340947	10	2	25	delT	1
1653671683[a]	LOC642424	10	3	117	delT	1
1657313101	AGAP4	10	7	23	delT	2
1658942495	FAM25G	10	3	48	insC	3
1662197526	LOC100287932	10	6	22	insA	4
1662338998[a]	AGAP6	10	1	50	insC	2
1666373362	PCDH15	10	19	56	insC	3
1673760921	TMEM26	10	6	25	insT	3
1685560504	FAM149B1	10	7	26	insT	3
1701949711	PANK1	10	3	30	insA	3
1708407108	CCNJ	10	3	18	insC	3
1708510568	ZNF518A	10	1	30	insC	1
1708668598	DNTT	10	2	18	insA	3
1709332414	C10orf12	10	1	18	insG	3
1728973932[a]	PNLIPRP2	10	3	52	insG	1

Supplementary Table 5. Continued

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
1733216553	BRWD2	10	8	28	insT	3
1737221823	ZRANB1	10	1	17	insA	3
1738045786	MMP21	10	7	33	insG	3
1748226082	C11orf21	11	4	63	insG	1
1750053615	RRM1	11	14	17	insT	3
1753342600	SYT9	11	4	18	insG	3
1760006709[a]	SPON1	11	5	72	insC	4
1764016157	SAAL1	11	7	40	delT	4
1771005317	LUZP2	11	12	34	insA	3
1782417168	TRAF6	11	6	24	delG	3
1782519665	RAG2	11	1	21	insA	3
1792247476[a]	CREB3L1	11	12	40	insG	1
1802120506	TCN1	11	7	13	insA	3
1802663567[a]	MS4A14	11	2	61	delTT	4
1802663568[a]	MS4A14	11	2	22	delT	3
1803663946[a]	TMEM216	11	3	54	insA	4
1804797590	AHNAK	11	3	12	insG	3
1805556025	SLC22A10	11	1	17	insC	3
1810263379[a]	UNC93B1	11	7	53	insG	3
1810284280[a]	ALDH3B1	11	2	63	insC	2
1810287509[a]	ALDH3B1	11	6	18	insC	1
1810293595[a]	ALDH3B1	11	9	28	insC	4
1814065554	LOC729523	11	1	22	delT	3
1826743977	DLG2	11	5	23	insT	3
1832107207	LOC642446	11	1	33	delT	4
1837197723[a]	CWC15	11	5	152	insT	1
1837299118[a]	SFRS2B	11	1	36	insC	4
1850549218	ATM	11	49	24	insT	3
1852355678	ZC3H12C	11	2	25	insC	3
1854201323[a]	DIXDC1	11	7	16	insC	1
1860877259[a]	TREH	11	15	28	insG	2
1861246651[a]	SLC37A4	11	3	37	delC	1
1861288156	VPS11	11	2	13	insC	4
1867800518	EI24	11	9	14	insC	4
1867851321	CHEK1	11	5	44	insC	3
1888645169[a]	PRB3	12	4	34	delG	4
1888731023[a]	PRB1	12	3	136	delC	1
1891856090	ATF7IP	12	11	19	insG	3
1893735417[a]	MGST1	12	2	12	delAA	3
1893735418[a]	MGST1	12	2	18	delA	3
1898574937	SLCO1B1	12	7	17	insC	3
1902256413	BCAT1	12	5	22	insG	3
1913975525	KIF21A	12	10	20	insT	3
1914378775	SLC2A13	12	10	17	insA	3
1927092176	KRT6C	12	1	15	insG	2
1930622534	SUOX	12	3	14	insG	3
1931678522	TMEM194A	12	9	23	insG	3
1932337710	OS9	12	12	17	insA	3
1959863488	LRRIQ1	12	26	12	delA	3
1962616654	C12orf50	12	3	28	insA	3
1978598568[a]	TDG	12	3	14	insA	3
1986789153	LOC100287839	12	9	35	insC	3
1997115077	RSRC2	12	10	28	insG	3
1999523126	UBC	12	1	29	delT	3
2009256491	ZMYM5	13	5	14	insC	3
2012756904	SACS	13	9	20	insT	3
2012761230	SACS	13	9	23	insT	3
2017859185	FLT1	13	4	36	insA	3
2022550582	STARD13	13	5	85	delT	1
2026525487	CSNK1A1L	13	1	13	insC	2
2038965626	RCBTB1	13	8	17	insG	3

Supplementary Table 5. Continued

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
2046563396	PRR20	13	2	28	delC	2
2063234131	KLF12	13	4	47	insT	3
2066482633	MYCBP2	13	75	17	insT	3
2066508358	MYCBP2	13	62	14	insC	3
2066632819	MYCBP2	13	22	23	delC	3
2066717508	MYCBP2	13	2	33	delAA	3
2066717509	MYCBP2	13	2	33	delA	3
2088603872	GPR18	13	1	20	insT	3
2105948032	NDRG2	14	1	34	delG	1
2106009532	FLJ10357	14	18	14	delG	3
2108927297	DHRS4L2	14	6	41	insA	4
2109139875[a]	MDP-1	14	6	13	delA	1
2117359342	AKAP6	14	1	20	insA	3
2117747539	AKAP6	14	12	21	insA	3
2137979011	DDHD1	14	10	22	insC	3
2148241015[a]	GPHB5	14	1	18	insG	4
2158414589[a]	C14orf169	14	1	19	insC	3
2159993929[a]	FAM164C	14	1	14	insA	1
2160606560	TTLL5	14	4	17	insA	3
2179419547	SERPINA12	14	2	54	insC	3
2179491154	SERPINA4	14	3	14	insG	3
2181450460	PAPOLA	14	5	33	insC	3
2202211427[a]	CHRFAM7A	15	4	191	delCA	1
2202211428[a]	CHRFAM7A	15	4	252	delA	4
2203996021[a]	CHRNA7	15	6	166	delTG	1
2203996022[a]	CHRNA7	15	6	50	delG	2
2204534873	SCG5	15	5	24	insC	3
2212460825	CASC5	15	10	14	insA	3
2220067652	SLC12A1	15	5	21	insA	3
2237036677	LOC100287371	15	3	32	insG	3
2243652079[a]	NR2E3	15	6	34	delC	1
2251295853	KIAA1024	15	1	14	insT	3
2252413491	ARNT2	15	14	24	insC	3
2256610001	ZSCAN2	15	2	14	insC	3
2257065252	PDE8A	15	4	20	delT	3
2261248094	FANCI	15	2	19	insC	3
2261584966	C15orf42	15	7	21	insT	3
2270957952[a]	LOC145814	15	4	23	insC	4
2271092254[a]	SYNM	15	1	19	insG	3
2274046312[a]	C16orf35	16	12	89	insG	4
2274304546	AXIN1	16	1	20	delC	3
2277509768[a]	NLRC3	16	7	81	delG	1
2285935013[a]	LOC729978	16	4	20	delAT	4
2285935014[a]	LOC729978	16	4	44	delT	1
2292434768	NOMO2	16	24	22	insG	3
2294397443	ACSM2A	16	9	23	delA	3
2294883814	DNAH3	16	53	18	insC	3
2304906998	HSD3B7	16	6	48	delC	2
2332868773	CLEC18C	16	3	24	insA	3
2333553555[a]	HYDIN	16	68	29	delA	4
2338969142[a]	CNTNAP4	16	1	82	insT	1
2351412465[a]	LOC100289580	16	2	67	delC	2
2354387432	PRPF8	17	4	11	insG	3
2356396572[a]	P2RX5	17	3	13	delG	1
2359357840	C17orf100	17	1	14	insG	2
2360272579[a]	SENP3	17	6	20	delA	4
2361527508[a]	PIK3R6	17	16	42	insG	1
2363416732	C17orf48	17	3	19	insA	3
2371198121	LGALS9C	17	9	16	insA	4
2376394518[a]	SEBOX	17	1	29	insG	4
2376430014[a]	SLC46A1	17	4	15	delA	1

Supplementary Table 5. Continued

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
2382300534	CCL7	17	2	24	insT	3
2383802642[a]	MMP28	17	4	28	insC	4
2384283858	TBC1D3C	17	13	31	insG	1
2388631071	KRT10	17	1	14	delC	3
2392844842[a]	PLCD3	17	10	24	insC	1
2393016586[a]	MAP3K14	17	4	16	insG	2
2407377091	CLTC	17	3	28	insT	3
2409792732	MED13	17	2	24	insA	3
2411313186[a]	WDR68	17	5	42	delG	1
2412151377	DDX5	17	8	23	insT	3
2434290839	MYOM1	18	8	16	insA	3
2448603454	RBBP8	18	14	22	insC	3
2451555232	LOC100287386	18	2	31	insA	1
2471234235	SLC14A2	18	4	39	delC	3
2492044315	CDH19	18	11	24	insA	3
2501962862	ZNF516	18	2	27	delG	2
2508173565[a]	SPPL2B	19	7	26	insC	2
2510788089	UHRF1	19	14	13	insC	3
2514792389	MUC16	19	3	18	insA	3
2514803399	MUC16	19	3	29	insT	3
2518236406	ZNF799	19	4	25	insA	3
2521463907[a]	CYP4F8	19	4	79	insC	1
2522001621[a]	HSH2D	19	5	71	delA	2
2538892348	C19orf55	19	9	17	delG	2
2543188059	ZNF780B	19	2	24	insC	3
2543756504[a]	LTBP4	19	24	14	insG	1
2544255517[a]	CYP2F1	19	1	53	insC	4
2544853028	CEACAM5	19	4	26	insT	3
2547650400[a]	CEACAM20	19	8	54	delT	1
2547930257[a]	CBLC	19	8	18	insC	3
2552076265[a]	DHDH	19	4	55	insG	2
2552600822	ALDH16A1	19	10	73	insC	2
2554469302[a]	LOC147645	19	10	37	insG	4
2555437083[a]	ZNF480	19	1	51	delG	1
2555750854	ZNF83	19	1	26	insG	3
2559350849	ZSCAN5C	19	1	50	insA	2
2560590155	ZNF749	19	3	34	insA	1
2560866399	ZNF671	19	4	14	insA	3
2561351770[a]	ZNF274	19	4	78	insG	2
2562070563	DEFB126	20	2	20	delCC	3
2562070564	DEFB126	20	2	20	delC	3
2567847490[a]	CHGB	20	4	28	delGA	2
2567847491[a]	CHGB	20	4	70	delA	2
2580083985	CSRP2BP	20	4	17	insG	3
2583130413[a]	NCRNA00153	20	7	49	insG	1
2606534089	DDX27	20	4	21	insA	3
2608270909	MOCS3	20	1	23	insG	3
2640900547[a]	KRTAP7-1	21	1	27	delA	4
2643647262[a]	SON	21	12	40	insA	1
2643647273[a]	SON	21	12	33	delA	4
2654166830	TRAPPC10	21	21	24	insT	3
2656193953[a]	LOC100288508	21	5	14	insC	1
2670991039	HORMAD2	22	2	23	insG	3
2681753989	DNAJB7	22	1	66	insA	1
2683020374	CYP2D6	22	5	18	insG	4
2701397266	WWC3	X	7	15	insG	3
2708217926	RBBP7	X	2	16	insC	3
2709924320	CDKL5	X	4	26	insC	3
2711314268	CXorf23	X	3	22	delG	3
2713575280	PHEX	X	19	29	insG	3
2736210225	KDM6A	X	17	17	insC	3

Supplementary Table 5. Continued

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
2736225869	KDM6A	X	24	16	insA	3
2737802282	SLC9A7	X	7	21	insC	3
2739406485	SSX1	X	6	45	insT	3
2739444455[a]	SSX9	X	2	11	delC	2
2741301867[a]	DGKK	X	22	55	insG	1
2743920170	SSX2B	X	6	35	insC	3
2745406637	WNK3	X	16	40	insA	3
2755460714	OPHN1	X	8	18	insC	3
2757668459	KIF4A	X	28	24	insG	3
2758547967	NONO	X	6	22	insT	3
2761842615	RLIM	X	3	18	insG	3
2771580011	HDX	X	5	18	insA	3
2779112744	PCDH11X	X	2	18	insT	3
2788398590	CENPI	X	20	19	insC	3
2789376503	TCEAL6	X	1	24	insG	2
2789554171	NXF2	X	7	14	insT	3
2789554906	NXF2	X	10	29	insA	3
2802179110	IL13RA2	X	4	18	insT	3
2823639589	ARHGEF6	X	18	16	insA	3
2840930428[a]	LCAP	X	1	57	insC	2
2841794077	MPP1	X	7	14	insG	3

[a]These indels commonly occurred in more than one HCC.

Supplementary Table 6. List of 81 Nucleotide Positions in 77 Genes With Indels at a Frequency of >20% of Reads in 4 Nontumorous Tissues From 4 Patients

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
36247083	THRAP3	1	4	18	delG	1
75174421	SLC44A5	1	16	19	delT	1
114430296	TRIM33	1	20	23	delC	1
133132499	YY1AP1	1	7	37	insT	1
133844355	RHBG	1	9	48	delC	4
201121914	CAPN2	1	3	14	delC	1
201173637	TP53BP2	1	13	22	delG	1
247664301	C2orf43	2	4	18	delA	2
319394229	SNRNP200	2	37	20	delA	1
322653290	AFF3	2	14	35	delA	1
331834049	RANBP2	2	20	16	delG	1
332901065	RGPD5	2	20	22	delT	1
374789589	NEB	2	4	37	insT	4
382950536	LY75	2	5	19	delA	4
401635744	TTN	2	274	25	delA	1
409835043	FAM171B	2	8	17	delT	1
412064501	COL3A1	2	14	21	insA	4
454784716	PTMA	2	4	14	delT	1
463724350[a]	AQP12B	2	1	27	delC	3
463734336	AQP12A	2	2	14	delG	2
503335742	DLEC1	3	4	20	delT	1
503335743	DLEC1	3	4	20	delA	1
735406533	CNOT6L	4	10	21	delG	1
785877214	LARP2	4	14	23	delA	1
798021293	SCOC	4	1	18	insC	1
810971969	TRIM2	4	5	18	delC	1
883256725	PRLR	5	3	29	delG	4
939146286	ANKRD32	5	16	15	insC	1
985314450[a]	LOC100288105	5	1	27	delC	3
1033746568	BMP6	6	5	24	delC	1
1068664364	KIAA0240	6	4	17	insT	4
1193244025	FAM120B	6	1	43	insA	1
1222659823	KIAA0644	7	1	463	delC	4
1282877394	CDK6	7	3	21	delA	1
1289880816	CYP3A4	7	12	45	delG	1
1333766765	LOC441294	7	1	13	delA	4
1340012514	SSPO	7	76	53	delA	3
1356372261	XKR5	8	6	130	delA	4
1490189877[a]	JRK	8	1	29	delC	3
1490189878[a]	JRK	8	1	15	delA	2
1492082552[a]	RECQL4	8	14	43	delG	3
1505961686	MPDZ	9	2	28	insG	4
1526015264	NFX1	9	3	36	delT	1
1573925487	GABBR2	9	17	59	insT	4
1580509237	ABCA1	9	4	24	insT	1
1637516682	ARMC3	10	18	19	delT	1
1637516683	ARMC3	10	18	19	delT	1
1657313100[a]	AGAP4	10	7	19	delT	2
1657313101[a]	AGAP4	10	7	14	delT	3
1807397040	SYVN1	11	7	15	insA	1
1832107207	LOC642446	11	1	18	delT	4
1855967268	ZW10	11	8	40	delC	1
1861246651	SLC37A4	11	3	143	delC	4
1884320486	ATN1	12	4	15	delA	1
1929584670	KIAA0748	12	6	31	delC	4
1955959709	PPFIA2	12	18	52	delA	4
1994379801	CIT	12	17	28	delG	1
1995110709	DYNLL1	12	2	14	delG	1
1997144069	KNTC1	12	2	31	delC	4
2105624179	RNASE4	14	1	15	delC	1

Supplementary Table 6. Continued

Reference position	Gene	Chromosome	Coding sequence	Coverage	Allele change	Patient no.
2162358340	C14orf133	14	13	15	delT	1
2243652079	NR2E3	15	6	129	delC	4
2256057594	ADAMTSL3	15	12	26	delT	1
2277509768[a]	NLRC3	16	7	12	delG	2
2302633200	EIF3C	16	4	18	delG	4
2303380808	SULT1A4	16	3	24	delA	1
2351412465	LOC100289580	16	2	103	delC	4
2356396572	P2RX5	17	3	40	delG	4
2376621991	SPAG5	17	3	13	delC	1
2386619109	CCDC49	17	5	14	delT	1
2413869089	APOH	17	5	24	delC	1
2501962862	ZNF516	18	2	29	delG	3
2507200605	MUM1	19	8	36	delG	1
2538892348	C19orf55	19	9	20	delG	2
2565046537	UBOX5	20	2	15	delG	1
2587599923	ZNF337	20	4	19	delT	1
2598525448	ZHX3	20	1	19	delT	1
2625038622	NRIP1	21	1	24	delG	1
2661518554	FAM108A5	22	2	13	delG	3
2748277559	SPIN2B	X	1	13	delG	2
2792445004	TEX13A	X	2	18	delC	3

[a]These indels commonly occurred in more than one HCC.

Supplementary Table 7. List of 40 Somatic Mutations With Amino Acid Changes Commonly Detected in Both the Tumor (at a Frequency of More Than 20% of Reads) and Matched Nontumorous Cirrhotic Liver (at a Frequency of More Than 5% of Reads) of the Same Patient

					Tumor		Nontumor	
Gene	Reference position	Chromosome	Reference nucleotide	Mutation nucleotide	Mutation frequency (%)	Patient no.	Mutation frequency (%)	Patient no.
LEPR	65548341	1	C	A	25.8	3	15.0	3
							21.9	1
ZNF408	1792629936	11	T	A	20.4	2	16.0	2
							15.8	4
HRNR	129676984	1	G	C	28.9	3	5.4	3
PXDN	228577682	2	G	C	45.1	4	47.2	4
POTEF	353150970	2	T	A	41.8	4	31.0	4
ALPP	455451136	2	C	T	32.5	4	37.5	4
GPR125	682521774	4	C	A	38.1	2	40.0	2
HERC6	746068457	4	T	A	36.5	4	44.9	4
EGFLAM	886579974	5	T	G	23.3	3	5.3	3
C4A	1057829599	6	T	G	25.0	2	11.5	2
WISP3	1134999625	6	T	G	43.3	4	64.3	4
C7orf10	1234451360	7	T	A	25.0	3	8.3	3
PVRIG	1290339880	7	C	T	23.5	1	21.3	1
MUC17	1291200140	7	G	A	21.2	4	12.5	4
PLOD3	1291376235	7	G	C	48.2	4	51.7	4
COL27A1	1589933932	9	A	G	56.8	4	54.6	4
AGAP9	1658906463	10	T	G	36.7	4	16.2	4
POLL	1713935693	10	G	T	44.8	4	38.6	4
MUC5AC	1747183167	11	G	A	43.9	4	43.8	4
MRGPRX3	1764064669	11	T	C	40.0	4	42.5	4
TMEM133	1843211533	11	A	C	59.5	4	83.3	4
TMEM123	1844621025	11	G	A	27.3	2	7.3	2
TMPRSS4	1860336319	11	C	T	54.4	4	41.3	4
DHRS4L2	2108914889	14	G	T	20.5	3	11.5	3
GOLGA6C	2247104814	15	A	T	21.7	4	9.6	4
PRSS22	2276813235	16	C	T	50.0	4	36.7	4
FAM38A	2351390771	16	C	T	21.4	4	54.3	4
GGT6	2357265990	17	G	A	92.3	4	41.7	4
COX10	2366897810	17	C	T	55.2	4	36.3	4
KIAA0100	2376657621	17	A	C	47.8	4	60.0	4
TBC1D3B	2384202011	17	C	T	63.0	4	27.4	4
TBC1D3D	2385938140	17	A	G	45.9	4	21.0	4
ERBB2	2387531879	17	A	G	66.7	4	54.6	4
CSH2	2411602334	17	C	T	90.9	4	79.5	4
QRICH2	2423941144	17	T	G	50.0	4	60.4	4
MOCOS	2461870479	18	T	C	72.0	4	62.2	4
CPAMD8	2522819358	19	G	A	21.8	3	15.2	3
MAP4K1	2541732174	19	G	A	36.0	4	54.3	4
PSG8	2545901763	19	C	A	28.3	3	9.5	3
KRTAP12-2	2654734983	21	C	T	59.3	4	43.5	4

NOTE. The first 2 genes listed were recurrently mutated in the nontumorous inflamed livers of 2 patients.

Supplementary Table 8. Overview of Selected Exome Sequencing Data From 22 Patients With HCV Infection

		Aligned reads	Aligned sequence (*base pairs*)	Median read depth
TP53	Tumor	29,334	2,035,570	1476.2
	Nontumor	31,848	2,200,641	1575.3
	Lymphocytes	36,690	2,539,944	1917.2
CTNNB1	Tumor	90,022	6,215,000	2344.3
	Nontumor	75,785	5,282,450	1991.2
	Lymphocytes	100,430	7,013,325	2710.8
LEPR	Tumor	34,328	2,390,335	538.3
	Nontumor	60,128	4,219,089	1025.6
	Lymphocytes	86,830	6,085,511	1423.0

NOTE. Selected exome sequencing of *TP53*, *CTNNB1*, and *LEPR* was performed for 22 nontumorous cirrhotic liver tissues, 10 HCC tissues, and matched peripheral lymphocytes from each patient. Aligned reads, aligned sequences (base pairs), and median read depth are shown for each sample.

Supplementary Table 9. Clinical Features and Overview of Deep Sequencing Data of Patients Who Underwent Deep Sequencing of the *LEPR* Gene

	Chronic hepatitis (n = 15)	Normal liver (n = 9)
Age (*y*)	59.3	55.9
Sex (male/female)	6/9	7/2
Aligned reads	4290	3956
Aligned sequence (*base pairs*)	1,044,737	1,275,068
Median read depth	2838	3440
No. of mutations in the *LEPR* gene	0	0

NOTE. We determined the sequences of the *LEPR* gene in the liver of 15 noncirrhotic patients with HCV-associated chronic hepatitis. In addition, normal liver tissues were obtained from 9 liver donors at the time of the operation. Age, sex, aligned reads, aligned sequences (base pairs), median read depth, and numbers of mutations are shown.

Supplementary Table 10. Mean Body Weights and Serum Levels of Insulin, Triglyceride, Total Cholesterol, and Alanine Aminotransferase of C57BL/KsJ-*db/db* (*db/db*) Mice and Misty (Control) Mice After 4 Weeks of Treatment With TAA

	db/db	Control
Body weight (*g*)	46.5 ± 0.6	23.5 ± 0.4
Insulin (*ng/mL*)	30.6 ± 28.3	1.6 ± 0.2
Triglyceride (*mg/dL*)	95.0 ± 5.0	50.0 ± 20.0
Total cholesterol (*mg/dL*)	215.0 ± 15.0	95.0 ± 15.0
Alanine aminotransferase (*IU/L*)	1325.0 ± 1085.0	75.0 ± 35.0

NOTE. All data are presented as mean ± SD.

Supplementary Table 11. Categorization of the Mutated Genes Detected by Whole Exome Sequencing of the AID-Expressing Hepatocyte Cell Line Using the Kyoto Encyclopedia of Genes and Genomes Database

Pathway				
Metabolic pathways	ATP6V0A4	DMGDH	HSD17B3	PGD
	ATP6V1C2	GALNT1	HYAL2	PHGDH
	BCMO1	GATM	NDST1	POLR3B
	CPS1	HKDC1	PAH	
PI3K-Akt signaling pathway	BCL2L11	IBSP NOS3	PRKCZ	TEK
	COL27A1			
MAPK signaling pathway	FLNB	SP1	CACNA1F	PTPN7
Cytokine-cytokine receptor interaction	LEPR	TNFRSF8	TNFRSF10A	
Transcriptional misregulation in cancer	EYA1	GZMB	JMJD7-PLA2G4B	
Proteoglycans in cancer	FLN	ITGB3	TIMP3	VTN
PPAR signaling pathway	CPT1B	CYP4A22	PPARD	
Cell cycle	E2F2	ESPL1	MCM7	
Pathways in cancer	FLT3	TRAF4	PDGFA	
Hedgehog signaling pathway	GLI3	LRP2	CSNK1A1L	
Others	95 genes			

NOTE. The genes categorized in multiple pathways are shown in only one representative pathway. Constitutive AID expression resulted in the accumulation of nucleotide alterations in various genes, including LEPR, of the cultured hepatocyte-derived cells. Whole exome sequencing was performed on DNA derived from established non-neoplastic human primary hepatocyte cells[6] with constitutive AID expression. AID expression in the cultured hepatocytes was performed using a lentiviral system.[5] After 8 weeks of AID expression, the DNA was extracted and subjected to whole exome sequencing as described in Materials and Methods. Overall, a total of 460 nucleotide positions in 380 different genes were defined as mutated in the AID-expressing cultured hepatocytes through the variant filtering process. Among them, pathway analyses by the Kyoto Encyclopedia of Genes and Genomes revealed that many genes, including LEPR, were categorized into well-known signaling pathways: the metabolic pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, cytokine-cytokine receptor interaction pathway, and transcriptional misregulation in cancer pathway. Only categorized genes are shown.

Chronic Rejection Associated with Antiviral Therapy for Recurrent Hepatitis C after Living-Donor Liver Transplantation

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Background. Chronic rejection (CR) has been reported to be associated with antiviral therapy for recurrent hepatitis C in liver transplant (LT) recipients. The aims of this study were to clarify the details of antiviral therapy-associated CR after living-donor liver transplantation (LDLT) and to identify the factors associated with CR.

Methods. A retrospective chart review was performed on 125 recipients who had received antiviral therapy for recurrent hepatitis C after LDLT between January 2001 and September 2012. The characteristics of patients who developed CR during or within 6 months after antiviral therapy were compared with those of 76 patients who did not develop CR despite receiving antiviral therapy for more than 1 year.

Results. Seven of 125 (6%) patients developed CR during or within 6 months after the end of antiviral therapy. CR was diagnosed after a median (range) of 9 (1–16) months of antiviral therapy. In five patients, rejection progressed rapidly and resulted in death within 3 months after diagnosis. Analysis revealed two significant factors associated with CR: reduction of the immunosuppressant dose during antiviral therapy and a low fibrosis score as the indication for antiviral therapy.

Conclusions. CR developed in association with antiviral therapy for recurrent hepatitis C after LDLT. This complication may be prevented by ensuring that the immunosuppressant dose is not reduced during antiviral therapy.

Keywords: Chronic rejection, Hepatitis C, Liver transplantation, Living donor, Antiviral therapy.

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Hepatitis C virus (HCV) infection, which leads to liver cirrhosis and hepatocellular carcinoma, is the most common indication for liver transplantation (LT) in Japan, the United States, and western Europe. Most patients who undergo LT for HCV-related liver disease develop recurrent viral infection, and 70% to 90% suffer from histologically proven recurrent hepatitis (*1–6*). The progression of recurrent hepatitis C is often rapid. Without appropriate antiviral therapy, 10% to 25% of patients develop cirrhosis within 5 years after transplantation, and this explains the relatively poor prognosis for HCV-positive recipients compared with HCV-negative recipients (*7*). Interferon (IFN)–based combination therapy is commonly administered to prevent the progression of hepatitis C after LT (*8, 9*), but its efficacy in LT recipients is limited. The mean (range) sustained virologic response (SVR) rate in patients with recurrent hepatitis C after LT is only 30% (8%–50%) (*10*). One reason for the low SVR rate is the high rate of treatment withdrawal, particularly because of the unique adverse effects of IFN therapy for transplant recipients, including chronic rejection (CR) (*11, 12*).

CR is characterized by progressive ductopenia, with atrophy and loss of the bile ducts in the portal tracts and by arteriopathy with foamy cell infiltration (*13–15*). A cholestatic liver enzyme pattern suggests the diagnosis of CR. If bile duct enlargement and/or hepatic artery changes are excluded by imaging studies as potential causes of abnormal liver function tests, then CR is confirmed or excluded by liver biopsy examination. The incidence of CR after LT is

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approximately 3% to 5%. This event does not simply represent end-stage acute cellular rejection (ACR), although the two may be temporally related. The pathogenesis of CR is not completely understood, although its association with donor-specific human leukocyte antigen antibodies was recently reported (*16*). Additional immunosuppressive therapy is unlikely to be beneficial for CR patients, particularly those with late disease in which bile duct loss affects more than 50% of the portal tracts, and retransplantation is required (*15*).

Several studies have suggested an association of CR with IFN-based antiviral therapy (*17–20*). Two recent reports found that CR was associated with antiviral therapy for recurrent hepatitis C after LT (*11, 12*). Stanca et al. (*12*) reported that 12 of 70 LT recipients with HCV infection treated with pegylated IFN (peg-IFN) and ribavirin developed CR. Their study indicated that ACR and CR are not strongly associated and that CR progresses rapidly, terminating in graft failure. Fernandez et al. (*11*) reported that 7 of 79 (9%) patients developed CR during antiviral therapy. They found that the use of cyclosporine in immunosuppression therapy, achievement of an SVR, and ribavirin discontinuation were factors associated with CR development.

Although the details of patients with antiviral therapy-associated CR after deceased-donor liver transplantation (DDLT) have been reported (*11, 12*), no study of antiviral therapy-associated CR in patients receiving living-donor liver transplantation (LDLT) has been published thus far. The features specific to LDLT, including blood-relative donors, posttransplantation liver regeneration, and ABO-incompatible LT, might result in characteristic differences between LDLT and DDLT patients.

We aimed to clarify the details of antiviral therapy-associated CR after LDLT and to identify the factors associated with CR.

RESULTS

Patient Characteristics and Treatment Outcomes

The study included 125 HCV-infected LT patients treated with standard IFN and/or peg-IFN in combination with ribavirin for recurrent hepatitis C after LDLT. Of these, 69 (55%) were men (median [range] age at the beginning of therapy, 57 [32–70] years). Most patients were infected with HCV genotype 1b (n=101 [81%]). The HCV genotype for the remaining patients was 2a (n=14), 2b (n=6), 3a+3b (n=1), and indeterminate (n=2). Genotype was not examined in one patient. The median (range) serum HCV RNA load at the beginning of antiviral therapy after LDLT was 3980 (31 to <69,000) kIU/mL. The median (range) donor age was 42 (19–65) years. Seventy-three (58%) donors were men, and 84 (67%) were blood relatives of the recipients. The graft type was the right lobe for 108 (86%) patients and the left lobe for 17 (14%) patients. The blood type combination was incompatible for 27 (22%) patients. Thirty-six (29%) patients had histologically diagnosed ACR before antiviral therapy, 16 of whom had moderate or severe ACR. No patient had shown ACR findings in the liver biopsy examination immediately before antiviral therapy. The median (range) time to treatment initiation after LDLT was 8.9 (1.1–72.4) months. Before treatment, necroinflammatory activity of levels A1, A2, and A3 based on the METAVIR score was found in 82 (66%), 40 (32%), and 3 (2%) patients, respectively. Fibrosis scores of F0, F1, F2, and F3 were found in 19 (15%), 82 (66%), 19 (15%), and 5 (4%) patients, respectively. Tacrolimus-based immunosuppression was administered to 117 (94%) patients and cyclosporine was administered to 7 (6%) patients. Mycophenolate mofetil (MMF) without calcineurin inhibitor (CNI) was administered to one patient because of renal failure at the beginning of antiviral therapy. In the patients who received tacrolimus, the mean (range) serum trough level at therapy initiation was 6.2 (2.0–12.7) ng/mL. In addition to CNIs, MMF and prednisolone were administered at the start of the antiviral treatment to 39 (31%) and 21 (17%) patients, respectively.

Of the 123 patients in whom the final treatment outcomes could be evaluated, 54 (44%) patients achieved SVR, 12 (10%) relapsed, 30 (24%) were nonresponders, and 27 (22%) withdrew from treatment. The remaining two patients were still undergoing treatment during the analysis.

Characteristics of Patients with Antiviral Therapy-Associated CR

Seven of 125 (6%) patients developed CR during or within 6 months after the end of antiviral therapy. The characteristics and clinical courses of these seven patients are shown in Table 1. Although four patients had a history of ACR before antiviral therapy was initiated (three of whom had moderate or severe ACR), three had no previous ACR episodes. The METAVIR score-based fibrosis level before antiviral therapy was F0 in three of the seven patients, F1 in three patients, and F2 in one patient, indicating that the antiviral therapy had been initiated at an early stage of fibrosis. The median (range) time from transplantation to initiation of antiviral therapy in these seven recipients was 9 (2–72) months. Tacrolimus was administered to five patients and cyclosporine was administered to one patient when the antiviral therapy was initiated. One patient did not receive a CNI because of renal failure (patient 7). Four patients received MMF, and one patient received prednisolone in combination with tacrolimus and MMF. The trough levels of tacrolimus and cyclosporine were within the therapeutic range. Standard amounts of immunosuppressant were therefore used for all patients, except for patient 7 who received MMF only. Immunosuppressant doses were reduced during therapy in five of seven patients. The tacrolimus dose was reduced for two patients (patients 2 and 3), as a result of which the blood trough level of tacrolimus decreased by approximately 2 ng/mL. In patient 3, MMF (500 mg/day) was also stopped during treatment. In patient 4, the MMF dose was reduced from 1000 to 250 mg per day, and prednisolone treatment (2.5 mg/day) was also terminated during treatment. In patient 5, MMF (1000 mg/day) was stopped immediately after initiation of antiviral therapy. Patient 6 received no CNI, and MMF dose was reduced from 500 to 250 mg per day during treatment. Three patients received standard IFN, and four received peg-IFN. Ribavirin was not administered to three patients immediately before the diagnosis of CR because of anemia.

CR was diagnosed after a median (range) of 9 (1–16) months of antiviral therapy. Two patients were diagnosed