of *LEPR* in cirrhotic liver, and more than half of the mutations were recurrently mutated in 2 or more patients. Notably, more than 90% of those nucleotide alterations that accumulated in the Ig domain of *LEPR* were nonsynonymous mutations. Furthermore, we revealed that several nonsynonymous mutations that appeared in the Ig domain of *LEPR* impaired signaling to STAT3 in response to leptin, causing dysregulation of leptin signaling in the cells with those mutations. Sequencing the *LEPR* gene in patients with severe early-onset obesity revealed that the extracellular region of the LEPR has a variety of mutations in those patients.[35] A functional study of missense mutations in the *LEPR* found in severely obese patients also revealed that mutated LEPR has impaired signaling to STAT3, which is consistent with their inability to activate pathways involved in the reduction of food intake.[36] Together, these findings suggested that somatic mutations in the *LEPR* gene might provide the genetic basis for developing metabolic dysregulation in hepatocytes during hepatocarcinogenesis.

In the present study, we showed for the first time that *db/db* mice with disruption of the Lepr gene were more susceptible to developing hepatic inflammation as well as TAA-mediated tumorigenesis than wild-type mice. Consistent with our findings, a previous study reported an increased incidence of hepatocyte hyperplasia in leptin-deficient *ob/ob* mice, a model for nonalcoholic fatty liver disease.[37] Taken together, it is strongly suggested that dysregulation of LEPR signaling has a role in hepatic tumor development, but the mechanism of how the leptin signaling deficiency contributes to an enhanced inflammatory response and tumorigenesis is currently unknown. It should be noted that both *ob/ob* mice and *db/db* mice are characterized by hepatic steatosis, and steatosis is well recognized as a common histopathologic feature of the chronic HCV-infected liver. Epidemiological studies have revealed that fatty liver disease may be a common underlying pathology in patients with HCC,[38,39] and steatosis is an important cofactor in accelerating the development of hepatic fibrosis and inflammatory activity,[40,41] contributing to the progression of HCC in HCV-related chronic liver disease.[42] We found no correlation between the prevalence of *LEPR* mutations and the histological feature of fatty changes in HCV-positive cirrhotic liver tissues. On the other hand, previous studies have shown that leptin can oppose the action of insulin-induced signaling by reducing the phosphorylation of insulin receptor substrate 1 in human hepatic cells.[43,44] In addition, it was shown that leptin suppresses HCC via activation of the immune response, suggesting the tumor-suppressing function of leptin-mediated signaling.[45] Thus, we speculate that dysregulation of leptin signaling in the liver might be involved in the neoplastic process of patients with HCV-related chronic liver damage. Because somatic mutations in *LEPR* are limited to a small proportion of cells in cirrhotic liver tissue and the TAA-mediated liver inflammation model does not fully recapitulate HCV-associated chronic liver disease, further analysis is required to determine whether dysregulation of LEPR-mediated signaling caused by *LEPR* mutations contributes to the enhanced inflammatory response or tumorigenesis in patients with HCV-related chronic liver damage.

In conclusion, we showed that various somatic mutations latently accumulate in the nontumorous cirrhotic liver of patients with HCV infection. The findings that the *LEPR* gene was recurrently mutated in cirrhotic liver provide a novel putative link between the inflammation-mediated genetic aberrations, the dysregulation of leptin signaling, and the development of HCC in patients with HCV-related chronic liver disease. The gene catalogue identified in the HCV-infected chronically damaged liver might contain the putative driver gene associated with tumor initiation as well as the gene that provides the genetic basis for the development of HCC. Thus, further studies are required to identify the genetic alterations that contribute to tumor development in chronically inflamed liver underlying chronic HCV infection.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2013.09.025.

References

1. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–867.
2. Chiba T, Marusawa H, Ushijima T. Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. Gastroenterology 2012;143:550–563.
3. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396:643–649.
4. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
5. Hussain SP, Schwank J, Staib F, et al. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene 2007;26:2166–2176.
6. Loeb LA, Bielas JH, Beckman RA. Cancers exhibit a mutator phenotype: clinical implications. Cancer Res 2008;68:3551–3557.
7. Barrett MT, Sanchez CA, Prevo LJ, et al. Evolution of neoplastic cell lineages in Barrett oesophagus. Nat Genet 1999;22:106–109.
8. Leedham SJ, Graham TA, Oukrif D, et al. Clonality, founder mutations, and field cancerization in human ulcerative colitis-associated neoplasia. Gastroenterology 2009;136:542–550.
9. **Hussain SP, Hofseth LJ**, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3:276–285.
10. Matsumoto Y, Marusawa H, Kinoshita K, et al. Helicobacter pylori infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Nat Med 2007;13:470–476.
11. Komori J, Marusawa H, Machimoto T, et al. Activation-induced cytidine deaminase links bile duct inflammation

to human cholangiocarcinoma. Hepatology 2008; 47:888–896.
12. Endo Y, Marusawa H, Kou T, et al. Activation-induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. Gastroenterology 2008;135:889–898.
13. Ikeda K, Marusawa H, Osaki Y, et al. Antibody to hepatitis B core antigen and risk for hepatitis C-related hepatocellular carcinoma: a prospective study. Ann Intern Med 2007;146:649–656.
14. Endo Y, Marusawa H, Kinoshita K, et al. Expression of activation-induced cytidine deaminase in human hepatocytes via NF-kappaB signaling. Oncogene 2007; 26:5587–5595.
15. Kou T, Marusawa H, Kinoshita K, et al. Expression of activation-induced cytidine deaminase in human hepatocytes during hepatocarcinogenesis. Int J Cancer 2007; 120:469–476.
16. Wei X, Walia V, Lin JC, et al. Exome sequencing identifies GRIN2A as frequently mutated in melanoma. Nat Genet 2011;43:442–446.
17. Wang L, Tsutsumi S, Kawaguchi T, et al. Whole-exome sequencing of human pancreatic cancers and characterization of genomic instability caused by MLH1 haploinsufficiency and complete deficiency. Genome Res 2012;22:208–219.
18. **Wang K, Kan J**, Yuen ST, et al. Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. Nat Genet 2011;43: 1219–1223.
19. **Fujimoto A, Totoki Y**, Abe T, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet 2012;44:760–764.
20. **Guichard C, Amaddeo G, Imbeaud S**, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012;44:694–698.
21. Nasu A, Marusawa H, Ueda Y, et al. Genetic heterogeneity of hepatitis C virus in association with antiviral therapy determined by ultra-deep sequencing. PLoS One 2011;6:e24907.
22. Nishijima N, Marusawa H, Ueda Y, et al. Dynamics of hepatitis B virus quasispecies in association with nucleos(t)ide analogue treatment determined by ultra-deep sequencing. PLoS One 2012;7:e35052.
23. Varela I, Tarpey P, Raine K, et al. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature 2011; 469:539–542.
24. **Clément K, Vaisse C**, Lahlou N, et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 1998;392:398–401.
25. Laurent-Puig P, Zucman-Rossi J. Genetics of hepatocellular tumors. Oncogene 2006;25:3778–3786.
26. **Morita S, Matsumoto Y**, Okuyama S, et al. Bile acid-induced expression of activation-induced cytidine deaminase during the development of Barrett's oesophageal adenocarcinoma. Carcinogenesis 2011;32:1706–1712.
27. Lee GH, Proenca R, Montez JM, et al. Abnormal splicing of the leptin receptor in diabetic mice. Nature 1996; 379:632–635.
28. Becker FF. Thioacetamide hepatocarcinogenesis. J Natl Cancer Inst 1983;71:553–558.
29. Schnur J, Nagy P, Sebestyén A, et al. Chemical hepatocarcinogenesis in transgenic mice overexpressing mature TGF beta-1 in liver. Eur J Cancer 1999; 35:1842–1845.
30. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. Nature 2007;446:153–158.
31. **Yan XJ, Xu J, Gu ZH**, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet 2011; 43:309–315.
32. Schwartz MW, Woods SC, Porte D, et al. Central nervous system control of food intake. Nature 2000;404:661–671.
33. Tartaglia LA. The leptin receptor. J Biol Chem 1997; 272:6093–6096.
34. **Peelman F, Iserentant H**, De Smet AS, et al. Mapping of binding site III in the leptin receptor and modeling of a hexameric leptin.leptin receptor complex. J Biol Chem 2006;281:15496–15504.
35. Farooqi IS, Wangensteen T, Collins S, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. N Engl J Med 2007;356:237–247.
36. **Kimber W, Peelman F**, Prieur X, et al. Functional characterization of naturally occurring pathogenic mutations in the human leptin receptor. Endocrinology 2008; 149:6043–6052.
37. Yang S, Lin HZ, Hwang J, et al. Hepatic hyperplasia in noncirrhotic fatty livers: is obesity-related hepatic steatosis a premalignant condition? Cancer Res 2001; 61:5016–5023.
38. Marrero JA, Fontana RJ, Su GL, et al. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. Hepatology 2002;36:1349–1354.
39. Kodama Y, Brenner DA. c-Jun N-terminal kinase signaling in the pathogenesis of nonalcoholic fatty liver disease: multiple roles in multiple steps. Hepatology 2009;49:6–8.
40. Hourigan LF, Macdonald GA, Purdie D, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 1999;29:1215–1219.
41. Lonardo A, Adinolfi LE, Loria P, et al. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. Gastroenterology 2004; 126:586–597.
42. Ohata K, Hamasaki K, Toriyama K, et al. Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. Cancer 2003;97:3036–3043.
43. Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. Science 1996;274:1185–1188.
44. Wang Y, Kuropatwinski KK, White DW, et al. Leptin receptor action in hepatic cells. J Biol Chem 1997; 272:16216–16223.

45. Elinav E, Abd-Elnabi A, Pappo O, et al. Suppression of hepatocellular carcinoma growth in mice via leptin, is associated with inhibition of tumor cell growth and natural killer cell activation. J Hepatol 2006;44:529–536.

Author names in bold designate shared co-first authorship.

Received February 11, 2013. Accepted September 10, 2013.

Reprint requests
Address requests for reprints to: Hiroyuki Marusawa, MD, PhD, Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. e-mail: maru@kuhp.kyoto-u.ac.jp; fax: (81) 75-751-4303.

Acknowledgments
Data profiling: Sequence reads with Genome Analyzer were deposited in the DNA Data Bank of Japan Sequence Read Archive (http://trace.ddbj.nig.ac.jp/dra/index_e.shtml) under accession no. DRA000867.

The authors thank Dr K. Terasawa, Dr T. Fujiwara, Dr K. Takahashi, and Dr Y. Ueda for helpful suggestions; Dr N. Nishijima, Dr A. Takai, Dr A. Nasu, Dr Y. Endo, Y. Nakagawa, and C. Kakimoto for help with the analyses; and Dr H. Kokuryu and Dr T. Kusaka at Kyoto Katsura Hospital for their support.

Conflicts of interest
The authors disclose no conflicts.

Funding
Supported by Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research, KAKENHI on Innovative Areas, and Health and Labour Sciences Research Grants for Research on Intractable Disease and Research on Hepatitis from the Ministry of Health, Labour and Welfare, Japan.

Supplementary Methods

Patients

The study group comprised patients who underwent living donor liver transplantation or potentially curative resection of primary HCC at Kyoto University Hospital from 2000 to 2010. The selection of patients enrolled in this study was based on the availability of a sufficient amount of tissue for analysis. Patients included 17 men and 9 women, with a mean age at the time of surgery of 54.9 ± 7.7 years (mean ± SD; range, 37–76 years). Among them, whole exome sequencing was applied to 7 tumors, 4 nontumorous cirrhotic livers, and matched peripheral lymphocytes from 4 patients (Supplementary Table 1, patients 1–4). Furthermore, we performed selected exome sequencing of 22 nontumorous cirrhotic livers, 10 tumors, and matched peripheral lymphocytes from 22 other affected patients (Supplementary Table 1, patients 5–26). All patients were positive for serum anti-HCV and/or HCV RNA. Written informed consent for the use of resected tissue was obtained from all patients in accordance with the Declaration of Helsinki, and the Kyoto University Graduate School and Faculty of Medicine Ethics Committee approved the study.

Sequence Data Analysis and Variant Filtering

Using NextGENe v2.2 software (SoftGenetics, State College, PA), the obtained reads were aligned with the reference sequences of the Human Genome Build 37.3. Reads with 96% or more bases matching a particular position of the reference sequences were aligned. Furthermore, reads with a median quality value score of more than 20 and no more than 3 uncalled nucleotides were allowed anywhere in one read. Only sequences that passed the quality filters were analyzed, and each position of the genome was assigned a coverage depth representing the number of times the nucleotide position was sequenced. To identify somatic mutations, we used a number of scores to provide an empirical estimation of the likelihood that a given mutation was real and not an artifact of sequencing or alignment errors.

In the whole exome sequencing analysis, candidates of somatic mutations were selected according to the variant filtering process (Supplementary Figure 1). We defined nucleotide alterations that appeared in more than 20% of reads as somatic mutations.[1–3] When detecting the genes commonly mutated in both tumor and nontumorous liver tissues of the same subjects, we also selected potential nucleotide alterations that appeared between 5% and 20% of the total reads in nontumorous liver tissues for further evaluation. We excluded potential somatic mutations that represented more than 5% of the reads in peripheral lymphocytes of the same patient as common variants in each individual. Candidate nucleotide alterations were tested using standard Sanger sequencing on an Applied Biosystems 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) to validate the presence of each mutation.

In selected exome sequencing analysis, candidates of somatic mutations were selected according to the variant filtering process (Supplementary Figure 2). We defined somatic mutations with more than 20% of reads as high-frequency mutations and those that appeared between 1% and 20% of total reads as low-frequency mutations. We excluded potential somatic mutations that represented more than 1% of the reads in peripheral lymphocytes of the same patient. In cases in which we could not obtain lymphocyte DNA, candidates of somatic mutations found in the lymphocytes of 2 or more different patients were excluded in consideration of possible Japanese polymorphisms.

We compared our variants against common and germline polymorphisms present in the dbSNP135 to discard known germline single nucleotide polymorphisms.

All sequence reads were deposited in the DNA Data Bank of Japan Sequence Read Archive (accession no. DRA000867).

Score

SoftGenetics developed the overall mutation score to provide an empirical estimation of the likelihood that a given mutation is real and not an artifact of sequencing or alignment errors. The overall mutation score of NextGENe can be used like Phred scores, in which the scores are logarithmically linked to error probabilities. The overall mutation score of NextGENe is obtained as the product of the "coverage score," which is calculated from the depth of coverage at the position of the mutation and with a value ranging from 0 (where depth of coverage is 1) to an unlimited number, multiplied by each of the 4 types of additional penalty scores, such as the read balance score, allele balance score, mismatch score, and wrong allele score, with values less than 1 but positive (the calculating formula for each score is not shown). These scores are described in the NextGENe User Manual in detail (http://www.softgenetics.com/NextGENe.html).

Overall mutation score. SoftGenetics developed the overall mutation score to provide an empirical estimation of the likelihood that a given mutation is real and not an artifact of sequencing or alignment errors. A low overall mutation score, however, does not mean that the mutation is more than likely a false mutation. The low score implies only that the mutation cannot be called a true mutation with absolute certainty. As a general guideline, if the coverage is high (500 to several thousand reads) and the data are bidirectional, then scores that are ≤ 5 indicate that the mutation is most likely false, whereas scores of ≥ 25 indicate that the mutation is most likely true.

Mismatch score. Several variations from the reference sequence that occur very close together often indicate a region where mutation calls are less reliable. The mismatch score penalizes a specific mutation if other mismatched bases are found nearby. The software first looks for mismatches that occur in a minimum percentage of reads in the 10-base pair region that is found on either side of the mutation that is being scored.

Wrong allele score. Mismatches that are different from the consensus are referred to as wrong mismatches. These wrong mismatches most likely result from sequencing errors. For example, A, C, G, T, and insertions represent wrong mismatches when a deletion was called at a position.

Cell Culture and Transfection

The complementary DNA encoding the wild-type and the mutated LEPR were generated by reverse-transcription polymerase chain reaction from the messenger RNA of the liver tissues, followed by polymerase chain reaction amplification using Phusion high-fidelity DNA polymerase (Finnzymes, Espoo, Finland) and the following oligonucleotide primers: 5′-CGCGGATCCATGATTTGTCAAAAATTC-3′ (sense) and 5′- AAGGAAAAAAGCGGCCGCTTACACAGTTAGGTCA CACA-3′ (antisense). The resulting polymerase chain reaction fragments were inserted into the *Bam*HI-*Not*I sites of pcDNA3 for HEK293 and the *Bam*HI-*Apa*I sites of lentivirus for HepG2, as described previously.[4]

HEK293 and HepG2 cells were maintained in Dulbecco's modified Eagle medium (Gibco BRL, Rockville, MD) containing 10% fetal bovine serum. For transfection of plasmids into HEK293 cells, we used Lipofectamine2000 transfection reagent (Invitrogen, Carlsbad, CA). At 40 hours after transfection, the cells were serum starved for 8 hours and then either left unstimulated or stimulated with 100 ng/mL recombinant human leptin (Sigma-Aldrich, St Louis, MO) for 10 minutes. Expression of either wild-type or mutant LEPR in HepG2 cells was performed using a lentiviral vector–mediated wild-type and mutated LEPR expression system as described previously.[5] In brief, LEPR complementary DNA fragments were inserted into the viral vectors, followed by the production of lentiviral stocks in HEK293 cells. HepG2 cells were cultured in virus-containing medium for 48 hours, starved for 8 hours, treated with 100 ng/mL recombinant human leptin (Sigma-Aldrich) for 10 minutes, and then subjected to immunoblotting, immunostaining, quantitative reverse-transcription polymerase chain reaction, or a cell proliferation (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide [MTT]) assay.

Immunoblotting Analysis

Immunoblotting was performed using anti-STAT3 and anti–phospho-STAT3 antibody (Cell Signaling Technology, Danvers, MA) according to the manufacturer's protocol.

Animal Experiments

C57BL/KsJ-*db/db* mice (*db/db* mice), which possess homozygous deletion of the *Lepr*, *Ob-R* gene, and misty mice, which are wild-type with a normal *Lepr*, were purchased from Japan SLC (Shizuoka, Japan). TAA (Sigma-Aldrich) was prepared at a concentration of 0.02% and administered in drinking water to mice for 24 weeks or 30 weeks beginning at 5 weeks of age. These mice were then killed for analysis of the development of liver tumors. All animal experiments were approved by the Ethics Committee for Animal Experiments and performed under the Guidelines for Animal Experiments of Kyoto University.

Supplementary References

1. **Wang K, Kan J**, Yuen ST, et al. Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. Nat Genet 2011;43:1219–1223.
2. Varela I, Tarpey P, Raine K, et al. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature 2011;469:539–542.
3. **Yan XJ, Xu J, Gu ZH**, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet 2011; 43:309–315.
4. Endo Y, Marusawa H, Kinoshita K, et al. Expression of activation-induced cytidine deaminase in human hepatocytes via NF-kappaB signaling. Oncogene 2007; 26:5587–5595.
5. **Morita S, Matsumoto Y**, Okuyama S, et al. Bile acid-induced expression of activation-induced cytidine deaminase during the development of Barrett's oesophageal adenocarcinoma. Carcinogenesis 2011;32:1706–1712.
6. Aly HH, Watashi K, Hijikata M, et al. Serum-derived hepatitis C virus infectivity in interferon regulatory factor-7-suppressed human primary hepatocytes. J Hepatol 2007;46:26–36.

Supplementary Table 1. Clinical Features of 4 Patients Who Underwent Whole Exome Sequencing and 22 Patients Who Underwent Selected Exome Sequencing

Patient no.	Age (*y*)	Sex	Body mass index (kg/m^2)	α-Fetoprotein (*ng/mL*)	Des-γ-carboxy prothrombin (*mAU/mL*)	HCC	Histological grade
Whole exome sequencing							
1	51	Male	23.3	16	185	M	Well
2	58	Female	22.3	103	7	M	Mod
3	55	Female	26.7	779	881	M	Mod
4	53	Male	22.3	34	85	S	Mod
Selected exome sequencing							
5	65	Male	25.2	17	7	M	Mod
6	49	Female	21.6	149	107	M	Mod
7	40	Male	25.7	24	50	M	Mod
8	50	Male	25.0	16	23	M	Mod
9	57	Female	23.4	8	30	M	Mod
10	56	Female	22.8	5	929	M	Mod
11	53	Male	18.6	30	31	M	Mod
12	65	Female	29.7	6	1877	S	Mod
13	57	Male	19.0	19	167	S	Well
14	76	Male	21.8	75,363	37,784	M	Poor
15	64	Male	18.7	177	8	—	—
16	57	Male	25.5	45	68	—	—
17	54	Female	25.9	<3	10	—	—
18	50	Male	22.3	585	61	—	—
19	60	Female	21.3	434	72	—	—
20	57	Male	25.0	15	8310	—	—
21	56	Male	19.0	15	383	—	—
22	49	Female	21.8	38	227	—	—
23	59	Male	25.6	6	12	—	—
24	49	Male	23.2	4	320	—	—
25	37	Male	22.2	4	13	—	—
26	51	Male	20.5	3	90	—	—

M, multiple; Well, well-differentiated HCC; Mod, moderately differentiated HCC; S, solitary; Poor, poorly differentiated HCC.

Supplementary Table 2. Overview of Whole Exome Sequencing Data From 4 Patients With HCC Who Had HCV Infection

	Tumor (n = 7)	Nontumor (n = 4)	Lymphocytes (n = 4)
Total reads	44,323,036	41,920,372	38,661,394
Aligned reads	40,046,800	33,742,449	31,595,571
Aligned sequence (*base pairs*)	2,824,088,514	2,384,058,470	2,221,753,713
Median read depth	40.2	31.9	27.4
Coverage			
1×	31,560,125	32,343,635	30,935,484
8×	24,724,702	23,432,758	23,549,909
20×	17,707,636	15,000,474	16,272,508
30×	13,599,418	11,752,775	12,527,511

NOTE. Whole exome sequencing was performed on tumor tissues, nontumorous cirrhotic liver tissues, and matched peripheral lymphocytes from each patient. Total reads, aligned reads, aligned sequences (base pairs), median read depth, and number of target regions, which were 1×, 8×, 20×, and 30× or more coverage depth read, are shown.

Supplementary Table 3. List of 970 Nucleotide Positions in 768 Genes That Were Mutated at a Frequency of More Than 20% of Reads in 7 HCC Tumors From 4 Patients

Gene	Reference position	Chromosome	Coding sequence	Coverage	Allele change	Amino acid change	Functional predictions by SIFT	Patient no.
AGRN	875083	1	26	20	A < C	NS	D	2
LOC728661	1487244	1	8	18	G < T	NS	N	3
CDC2L2	1540787	1	3	43	T < C	NS	N	4
PANK4	2331358	1	18	32	T < C	NS	N	4
KIAA0562	3645675	1	7	67	T < C	S	N	4
CHD5	5928578	1	24	54	C < T	S	N	2
PTCHD2	11319504	1	7	23	G < C	NS	N	4
PLOD1	11750469	1	4	22	G < T	NS	N	4
PRAMEF1	12595752	1	3	170	G < A	S	N	4
PRAMEF1	12596087	1	3	93	C < T	NS	D	4
PRAMEF11	12625168	1	5	48	G < A	S	N	4
PRAMEF11	12628397	1	3	38	C < T	S	D	4
PRAMEF11	12628415	1	3	36	C < T	S	N	4
HNRNPCL1	12647885	1	1	143	T < C	S	N	4
PRAMEF7	12717626	1	1	27	A < G	S	N	4
PRAMEF9[a]	13064237	1	1	26	G < A	NS	N	2
PRAMEF9[a]	13064255	1	1	35	G < A	NS	D	2
PRAMEF18	13117381	1	1	27	G < A	NS	N	4
ARHGEF10L	17547108	1	1	109	T < G	S	N	4
PLA2G2D	20082054	1	3	56	T < C	NS	N	4
HSPG2	21856574	1	5	77	C < A	NS	N	4
CELA3A	21973988	1	6	105	T < G	NS	N	4
CELA3A	21976308	1	7	49	G < A	S	N	4
LOC100289113	22086886	1	1	28	A < C	NS	D	1
LUZP1	23059855	1	1	48	T < C	S	N	4
TRIM63	26025003	1	5	90	T < C	NS	N	4
SLC9A1	27120757	1	1	56	A < G	S	N	4
PHC2	33310033	1	8	132	C < T	S	N	4
CSMD2	33528214	1	51	83	T < C	NS	N	4
SLC2A1	42884612	1	8	74	T < C	S	N	4
TIE1	43269564	1	14	55	T < C	S	N	4
MAST2	45983460	1	17	45	T < G	NS	N	4
LRP8	53222315	1	9	143	G < T	S	N	4
ANGPTL3	62554389	1	2	24	A < T	NS	D	3
LEPR	65548341	1	4	31	C < A	S	N	3
RPE65	68386987	1	12	33	A < C	NS	N	1
ZNF644	90894104	1	2	18	G < A	NS	N	3
RBM15	110372981	1	1	17	A < C	S	N	1
RBM15	110373546	1	1	39	T < C	NS	N	3
CHI3L2	111273982	1	9	79	C < T	NS	N	4
CSDE1	114765324	1	8	29	C < A	NS	N	3
CSDE1	114765325	1	8	29	C < A	NS	N	3
IGSF3	116648924	1	2	69	G < A	S	NO	2
NBPF20	122618548	1	15	62	G < A	S	N	4
NBPF20	122618618	1	15	140	C < T	NS	N	4
NBPF20	122618624	1	15	174	A < T	NS	N	4
PDE4DIP	122663887	1	31	88	C < T	S	N	4
PDE4DIP	122667176	1	28	71	C < T	NS	N	4
NBPF10	123083515	1	1	83	A < G	NS	N	4
NBPF10	123092695	1	8	17	C < T	NS	N	3
NBPF10	123094578	1	10	100	A < C	NS	N	3
NBPF10	123094595	1	10	217	A < G	NS	N	4
NBPF10	123158473	1	86	408	G < C	NS	N	4
ANKRD35	123351469	1	10	14	A < T	NS	N	3
GPR89C	123673973	1	1	23	T < G	NS	D	4
BCL9	124884100	1	6	18	G < A	S	N	3
NBPF14	125797806	1	18	56	C < T	NS	N	1
NBPF14	125799375	1	16	76	T < C	S	N	2
NBPF14	125799402	1	16	71	G < A	S	N	2

Supplementary Table 3. Continued

Gene	Reference position	Chromosome	Coding sequence	Coverage	Allele change	Amino acid change	Functional predictions by SIFT	Patient no.
NBPF15	126071852	1	4	159	A < G	S	N	4
HRNR	129676605	1	2	214	G < A	S	N	4
HRNR	129676984	1	2	52	G < C	NS	NO	3
HRNR	129677003	1	2	88	C < T	NS	N	4
FLG[a]	129766583	1	2	89	C < G	NS	N	2
FLG	129767962	1	2	102	C < T	NS	N	1
FLG	129768306	1	2	39	T < C	NS	N	4
FLG	129768312	1	2	59	C < G	NS	N	4
FLG	129771039	1	2	449	G < A	NS	N	2
FLG	129771228	1	2	298	C < G	NS	N	4
FLG	129773236	1	2	135	T < C	NS	N	2
FLG	129773862	1	2	222	G < C	NS	N	4
FLG	129774814	1	2	232	T < C	NS	N	4
PGLYRP3	130769598	1	2	118	C < T	S	N	4
CLK2	132724561	1	8	17	C < T	NS	D	3
CLK2	132724562	1	8	17	G < T	S	N	3
MSTO1	133072971	1	11	33	T < G	S	N	3
GON4L	133214185	1	27	50	C < A	NS	D	2
IQGAP3	134016387	1	12	66	C < G	NS	N	4
PEA15	137673244	1	3	25	A < T	NS	N	3
HSPA6	138985040	1	1	34	C < T	NS	D	4
NUF2	140800188	1	8	38	C < A	NS	NO	3
FAM78B	143529898	1	2	204	C < G	S	N	4
F5	147009112	1	10	162	C < T	NS	N	4
FAM5C[a]	167558142	1	7	33	G < A	NS	N	1
ZBTB41	174618823	1	10	13	A < C	NS	NO	2
KIF21B[a]	178450152	1	18	56	T < C	S	N	1
TMEM9	178602981	1	4	127	A < G	S	N	4
ELF3	179471218	1	2	54	C < G	S	N	4
PPP1R12B	180023641	1	21	22	C < A	NS	N	3
KDM5B	180267325	1	1	13	G < C	NS	D	1
CHI3L1	180642801	1	5	141	T < C	NS	D	4
FAM71A	189989294	1	1	133	T < C	NS	N	3
MIA3	200015587	1	13	40	T < C	S	N	4
JMJD4	205110357	1	6	53	C < T	S	N	4
OBSCN[a]	205602418	1	8	22	T < C	NS	D	1
RHOU	206063445	1	2	52	C < G	S	N	4
GNPAT	208576822	1	2	47	C < T	NS	D	3
LYST	213162183	1	3	14	G < T	NS	N	2
ADSS	221776216	1	7	22	A < C	NS	D	3
ADSS	221776218	1	7	22	C < T	S	N	3
KIF26B	223037622	1	11	110	C < T	S	N	4
LOC391343	227830117	2	1	41	T < G	NS	NR	4
LOC391343	227830313	2	1	16	G < C	S	NR	3
PXDN	228577682	2	17	144	G < C	NS	N	4
ODC1	237355517	2	10	27	G < A	S	N	4
APOB	247947498	2	16	24	A < C	NS	N	3
APOB	247947499	2	16	24	A < T	NS	N	3
ALK	256154817	2	15	59	A < G	S	N	4
FSHR	275890251	2	10	21	G < T	NS	NO	3
C2orf63	282104344	2	10	39	G < A	NS	N	4
CYP26B1	299058969	2	6	23	G < A	S	N	1
CCDC142	301407728	2	4	37	G < A	NS	NO	2
ST3GAL5	312787861	2	3	17	T < C	NS	N	4
KIAA1310	319723935	2	13	14	G < T	NS	N	3
ACTR1B	320724551	2	6	276	C < T	S	N	2
CHST10	323459632	2	5	57	G < C	S	N	4
MAP4K4	324943015	2	26	37	G < C	NS	D	3
SLC9A4	325569668	2	3	69	A < T	NS	D	3
TGFBRAP1	328335511	2	10	51	T < C	NS	N	4

Supplementary Table 3. Continued

Gene	Reference position	Chromosome	Coding sequence	Coverage	Allele change	Amino acid change	Functional predictions by SIFT	Patient no.
RGPD3	329534289	2	1	37	A < G	S	N	4
LIMS1	331725649	2	1	96	G < A	NS	D	4
10-Sep	332632987	2	6	104	G < A	S	N	4
LOC645529	336733665	2	3	118	T < C	NS	NR	4
POTEF	353150970	2	13	98	T < A	NS	N	4
POTEF	353185302	2	1	71	T < C	NS	N	4
TUBA3E	353260275	2	3	94	C < T	NS	N	2
ACTBL3	354657198	2	1	108	T < G	S	NR	4
THSD7B	360341133	2	11	14	G < C	S	N	2
GALNT5	380364795	2	7	19	T < G	NS	D	3
SCN9A	389348565	2	11	38	T < C	NS	N	3
SCN9A	389352545	2	9	39	T < C	S	N	4
ABCB11	391996481	2	23	27	G < A	NS	NO	3
OLA1	397151333	2	9	14	A < T	S	N	3
TTN	401781960	2	95	24	T < G	NS	D	3
TTN	401781961	2	95	24	C < T	S	N	3
SESTD1	402189020	2	14	28	G < T	NS	D	3
DUSP19	406151282	2	1	41	C < G	NS	D	3
ZNF804A	408011183	2	4	20	A < G	S	N	3
LOC200726	429716908	2	1	28	C < T	NS	NR	3
ERBB4	434456096	2	28	29	C < T	NS	N	3
RNF25	441737431	2	8	18	G < A	S	N	3
C2orf24	442244994	2	8	51	G < A	NS	N	4
C2orf24	442245306	2	8	31	A < G	NS	N	4
TUBA4A	442323330	2	4	103	C < T	NS	D	4
OBSL1	442639564	2	4	59	G < A	S	N	4
SERPINE2	447057057	2	5	24	G < A	S	N	3
DOCK10	447917888	2	20	54	G < A	NS	NO	1
DIS3L2	455310852	2	10	90	G < A	NS	N	1
ALPP	455451136	2	1	40	C < T	NS	D	4
LRRFIP1	460828821	2	11	16	A < G	S	N	1
HDAC4	462131420	2	20	77	G < A	S	N	4
ITPR1	469948734	3	21	68	A < C	S	N	4
WNT7A	479128207	3	3	47	C < T	S	N	4
ZFYVE20	480358270	3	5	13	C < T	NS	N	3
OXNAD1	481544487	3	1	101	C < T	S	N	4
RARB	490854079	3	5	14	T < C	S	N	3
EOMES	492992244	3	4	84	C < T	NS	N	1
SCN10A	504030094	3	9	56	C < T	S	N	4
SCN11A[a]	504168069	3	15	110	T < A	NS	NO	1
CX3CR1	504539250	3	1	36	T < C	NS	N	3
CTNNB1	506498027	3	2	39	G < A	NS	D	1
CCR5	511646366	3	1	20	C < A	NS	NO	3
COL7A1	513857189	3	21	50	T < C	S	N	4
RBM6	515335643	3	16	16	G < A	NS	D	3
RBM5	515386440	3	22	46	G < A	NS	D	3
GLYCTK	517558462	3	4	64	C < A	S	N	2
KBTBD8	532186608	3	2	48	G < T	NS	NO	3
FOXP1	536153715	3	13	189	A < C	NS	D	3
FOXP1	536153718	3	13	190	C < T	S	N	3
LOC100288801	540845478	3	1	220	G < A	NS	N	4
LOC100288801[a]	540846776	3	2	228	G < A	S	N	2
EPHA3	554308286	3	2	14	T < C	S	N	3
DCBLD2	560650518	3	16	37	G < A	NS	N	3
DCBLD2	560650520	3	16	35	A < C	NS	NO	3
BOC	575123882	3	6	56	C < T	S	N	4
GPR156	582017947	3	9	64	A < T	S	N	2
HEG1	586864008	3	6	41	T < C	NS	N	3
MCM2[a]	589457039	3	5	53	A < G	NS	N	1
RUVBL1	589951418	3	6	33	T < A	NS	D	3