Genome-Wide Association Study Identifies Risk Variants for Lichen Planus in Patients With Hepatitis C Virus Infection

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BACKGROUND & AIMS: **There is a close relationship between hepatitis C virus (HCV) infection and lichen planus, a chronic inflammatory mucocutaneous disease. We performed a genome-wide association study (GWAS) to identify genetic variants associated with HCV-related lichen planus.**

METHODS: **We conducted a GWAS of 261 patients with HCV infection treated at a tertiary medical center in Japan from October 2007 through January 2013; a total of 71 had lichen planus and 190 had normal oral mucosa. We validated our findings in a GWAS of 38 patients with HCV-associated lichen planus and 7 HCV-infected patients with normal oral mucosa treated at a medical center in Italy.**

RESULTS: **Single-nucleotide polymorphisms in *NRP2* (rs884000) and *IGFBP4* (rs538399) were associated with risk of HCV-associated lichen planus ($P < 1 \times 10^{-4}$). We also found an association between a single-nucleotide polymorphism in the HLA-DR/DQ genes (rs9461799) and susceptibility to HCV-associated lichen planus. The odds ratios for the minor alleles of rs884000, rs538399, and rs9461799 were 3.25 (95% confidence interval, 1.95–5.41), 0.40 (95% confidence interval, 0.25–0.63), and 2.15 (95% confidence interval, 1.41–3.28), respectively.**

CONCLUSIONS: **In a GWAS of Japanese patients with HCV infection, we replicated associations between previously reported polymorphisms in HLA class II genes and risk for lichen planus. We also identified single-nucleotide polymorphisms in *NRP2* and *IGFBP4* loci that increase and reduce risk of lichen planus, respectively. These genetic variants might be used to identify patients with HCV infection who are at risk for lichen planus.**

Keywords: Inflammation; Risk Factor; Oral Mucosa; Autoimmunity.

Lichen planus (LP) (Figure 1) is a common, chronic inflammatory mucocutaneous disease that affects mainly middle-aged adults, the prevalence being greater among women. The oral mucosa, skin, genital mucosa, and nails are commonly involved, in any combination. The clinical features of oral LP (OLP) are generally polymorphic and usually consist of bilateral and/or multiple symmetrical lesions, such as reticular, plaque-like, papular, atrophic, erosive, and bullous, and these are categorized into 6 types.[1] In particular, erosive and atrophic forms of OLP manifest painful symptoms, with weight loss and poor quality of life, and have the potential of malignant transformation.[2,3]

OLP is a T-cell-mediated autoimmune disease in which autocytotoxic $CD8^+$ T cells trigger apoptosis of oral epithelial cells.[4] The cytotoxic activity of $CD8^+$

Abbreviations used in this paper: CI, confidence interval; GWAS, genome-wide association study; HCV, hepatitis C virus; IFN, interferon; IGF, insulin-like growth factor; LP, lichen planus; OLP, oral lichen planus; OR, odds ratio; RCC, renal cell carcinoma; SCC, squamous cell carcinoma; SNP, single-nucleotide polymorphism.

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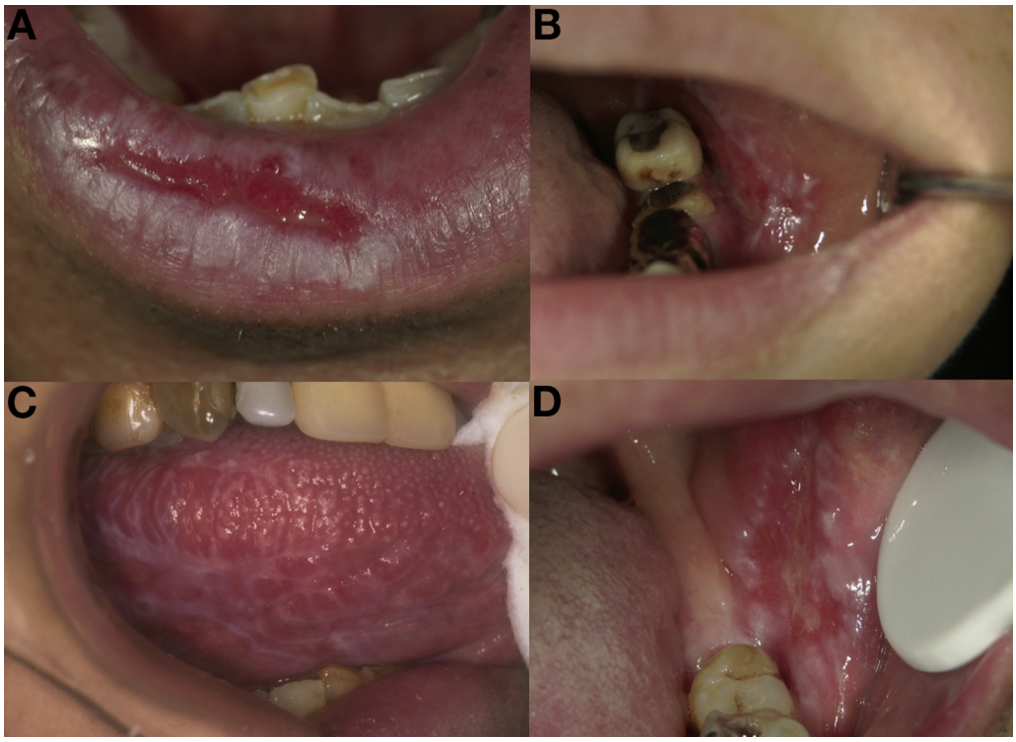

Figure 1. Clinical presentations of lichen planus. (*A*) Lower lip, (*B* and *D*) left buccal mucosa, (*C*) tongue.

lesional T-cell clones may be blocked partially by anti–major histocompatibility complex class I monoclonal antibody.[5]

The cause of OLP is unknown, but it seems to be triggered by stress; genetics; allergic reactions to medicines or dental materials; and by viral infections, such as with hepatitis C virus (HCV).[6] It has been shown that chronic HCV infection, in addition to causing liver disease, is responsible for several extrahepatic manifestations and immune abnormalities, including hematologic, renal, and mucocutaneous diseases.[7–9] Replication of HCV in the oral mucosa[10] and presence of HCV-specific T cells in OLP specimens[11,12] could be involved in the pathogenesis of OLP. Three recent independent meta-analyses provide robust evidence that LP and HCV are associated.[13–15]

In particular, the relationship between OLP and HCV has been suggested by studies from Japan and Mediterranean countries, indicating a strong geographic relationship.[16,17] The differences with respect to geographic area could be associated with the different genetic susceptibility of the hosts. HCV-associated OLP may be subdivided into distinct subtypes, because studies have shown an increased frequency of the HLA class II allele group, *DR6*, in patients with OLP with HCV compared with those without HCV (52% vs 18%; $P = .028$; relative risk, 4.93).[18,19] The *HLA-DR6* allele group is frequently observed in Italian patients with OLP and hepatitis C.

HCV infection is a major public health problem because it causes chronic hepatitis, cirrhosis, and hepatocellular carcinoma. In Japan, elderly patients are at a higher risk for hepatocellular carcinoma, and HCV eradication has a smaller effect on hepatocarcinogenesis in older patients.[20] OLP can appear or be exacerbated during interferon (IFN) therapy for chronic hepatitis C.[21,22]

The genome-wide association study (GWAS) has become a powerful tool for investigating the human genetic basis of various diseases. Various genome-based host variants, such as *IFNL3* (also known as *IL28B*)[23–25] and inosine triphosphatase (*ITPA*)[26] genes, have been found to be valuable markers for treatment response to hepatitis C and predicting spontaneous viral clearance.

The purpose of this study was to identify the host genetic factors for HCV-related LP in the Japanese and Italian populations.

Materials and Methods

Ethical Considerations

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committee of Saga Medical School, Kurume Medical School, and each participating medical center. Written informed consent for participation in the study was obtained from each patient and all samples were anonymized. All the applied methods in this study were carried out in accordance with the approved guidelines.

Study Group and Samples

Genomic DNA samples were collected from HCV-infected Japanese patients with LP (71 patients; mean age ± standard deviation, 67.2 ± 9.6 years; men/women 24/47) and HCV-infected Japanese patients with normal

oral mucosa (190 patients; mean age ± standard deviation, 59.8 ± 10.0 years; men/women 83/107) who consulted Kurume University School of Medicine, Fukuoka, Japan from October 30, 2007, to January 22, 2013 (Table 1). OLP had been diagnosed clinically and histopathologically. The sites of LP in the subjects included oral mucosa (n = 65), oral and genital mucosa (n = 1), oral mucosa and skin (n = 3), and skin (n = 2).

Moreover, a replication study was performed using Italian individuals (38 patients with LP and 7 patients with normal oral mucosa) who consulted Regional Transplantation Center, Piedmont, Molinette Hospital, Turin, Italy.

Genomic DNA was extracted from the peripheral blood of a total of 261 Japanese HCV-infected patients using the QIAamp DNA Blood Midi kit (Qiagen, Tokyo, Japan) in the Department of Virology, Liver Unit, Nagoya City University Graduate School of Medical Sciences. One microgram of purified genomic DNA was dissolved in 100 μL of TE buffer (pH 8.0) (Wako, Osaka, Japan), followed by storage at -20°C until use.

Evaluation of Liver Diseases in Japanese Patients

A total of 261 subjects were tested for their liver function. Anti-HCV was measured using a chemiluminescent enzyme immunoassay kit (Lumipulse II HCV, Fujirebio, Tokyo, Japan) and HCV RNA in serum was analyzed by quantitative polymerase chain reaction assay (COBAS Amplicor HCVv Monitor v 2.0 Test, COBAS AmpliPrep/COBAS Taq-Man HCV Test, Roche Molecular Systems, Branchburg, NJ). Ultrasonographic examination was performed on all patients. Computed tomography, liver biopsy, and endoscopic examination for esophageal or gastric varices were performed on some patients. We used other possible predictors of liver cirrhosis progression, including serum albumin, total bilirubin, prothrombin time, and platelet count.

Single-Nucleotide Polymorphism Genotyping and Data Cleaning

We genotyped 261 HCV-infected patients with or without LP using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Inc, Santa Clara, CA), in accordance with the manufacturer's instructions. The genotype calls for 900K single-nucleotide polymorphisms (SNPs) were determined using the Genotyping Console v4.1 software (Affymetrix, Inc, Santa Clara, CA) (with Birdseed v1 algorithm) and all samples passed a heterozygosity check. No duplication or related samples were identified by identity by descent testing. A principal component analysis was performed using 261 studied samples together with HapMap samples (including 43 JPT, 40 CHB, 91 YRI, and 91 CEU samples)

Table 1. Characteristics of Studied Japanese Population (n = 261)

Characteristics		Total		Cases with lichen planus		Control subjects with normal oral mucosa		*P* value
Subjects, n		261		71		190		
Sex	Male/female	107/154		24/47		83/107		NS
Age	Mean ± SD, *y*	61.8 ± 10.4		67.2 ± 9.6		59.8 ± 10.0		< .0001
Site of lichen planus, n	Oral mucosa			65				
	Oral and genital mucosa			1				
	Oral mucosa and skin			3				
	Skin			2				
Diagnosis of liver diseases	Past history of HCV infection	2	0.77%	1	1.41%	1	0.53%	
	AH-C post-IFN (SVR)	2	0.77%	0	0.00%	2	1.05%	
	CH-C	170	65.13%	40	56.34%	130	68.42%	
	CH-C post-IFN (SVR)	23	8.81%	8	11.27%	15	7.89%	
	CH-C post-IFN (SVR) and NAFLD	1	0.38%	0	0.00%	1	0.53%	
	CH-C post-IFN (SVR) and asymptomatic HBV carrier	1	0.38%	0	0.00%	1	0.53%	
	CH-C and AIH	1	0.38%	0	0.00%	1	0.53%	
	CH-C and ALD	1	0.38%	0	0.00%	1	0.53%	
	CH-C and HCV-related HCC	16	6.13%	6	8.45%	10	5.26%	
	CH-C and CH-B and HCC	2	0.77%	0	0.00%	2	1.05%	
	CH-C post-IFN (SVR) and HCV-related HCC	1	0.38%	0	0.00%	1	0.53%	
	LC-C	19	7.28%	6	8.45%	13	6.84%	
	LC-C and AIH	1	0.38%	1	1.41%	0	0.00%	
	LC-C and post-IFN (SVR)	1	0.38%	0	0.00%	1	0.53%	
	LC-C and HCV-related HCC	20	7.66%	9	12.68%	11	5.79%	

AH-C, acute hepatitis C; AIH, autoimmune hepatitis; ALD, alcoholic liver disease; CH-C, chronic hepatitis C; HCC, hepatocellular carcinoma; LC-C, liver cirrhosis type C; NAFLD, nonalcoholic fatty liver disease; NS, not significant; SD, standard deviation; SVR, sustained virologic response.

(Supplementary Figure 1). The cluster of studied samples showed overlap with that of HapMap-JPT. The average sample call rate for the 261 studied samples was 98.82% (95.26%–99.55%). Low-quality genotype data were excluded by the following thresholds for quality control: SNP call rate <95%, minor allele frequency <1%, and Hardy-Weinberg equilibrium P value < .0001. A total of 629,588 SNPs passed the threshold. The scatter plots for SNP with $P < .0001$ in the allelic model were then checked by visual inspection and 35 SNPs were excluded from further analysis. Finally, a total of 629,553 SNPs were used for further statistical analyses.

A replication study was conducted in Italian individuals for 3 SNPs (*HLA-DR/DQ* rs9461799, *IGFBP4* rs538399, and *NRP2* rs884000) using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA) on a LightCycle 480 Real-Time PCR System (Roche, Mannheim, Germany).

Single-Nucleotide Polymorphism Imputation

Unobserved genotypes were imputed using the phased genotype data of 1000 Genomes Project reference data (Integrated Phase 3, June 2014 released) with standard software packages, such as IMPUTE version 2 (IMPUTE2) with default parameters. GTOOL was used for data format conversion from PLINK format to IMPUTE2 format. A 1-Mb window size centered on each candidate SNP was applied to impute. After imputation, the results of association test for imputed data were obtained using PLINK 1.07. SNPs with >1% missing genotype data, Hardy-Weinberg equilibrium P value ≥ .001 and samples including >10% missing genotype were eliminated.

Statistical Analysis

For the association tests, P values, odds ratios (ORs), and 95% confidence intervals (CIs) between the SNP and disease phenotype were assessed by chi-square test with a 2-by-2 contingency table for the allelic model. To avoid false-positive results caused by multiple testing, the significance level for the GWAS was set at $P = 5 \times 10^{-8}$. As sensitive analysis, logistic regression analysis with additive genetic model and gender and age as covariates was implemented.

Results

Genome-Wide Association Analysis

Figure 2 (Manhattan Plot) shows a genome-wide view of single point associations of 629,553 SNPs, based on allele frequencies in a comparison of 71 patients with HCV with LP and 190 patients with HCV without OLP. A quantile-quantile plot of the distribution of test statistics for the comparison of allele frequencies between the 2 groups showed that the inflation factor lambda was 1.027 for all the tested SNPs, and 1.024 when SNPs in the HLA region (chr6: 29,645,000–33,365,000, GRCh37 hg19) were excluded (Supplementary Figure 2). Although no SNPs reached the genome-wide significant level (ie, $P < 5 \times 10^{-8}$), the greatest hit association was

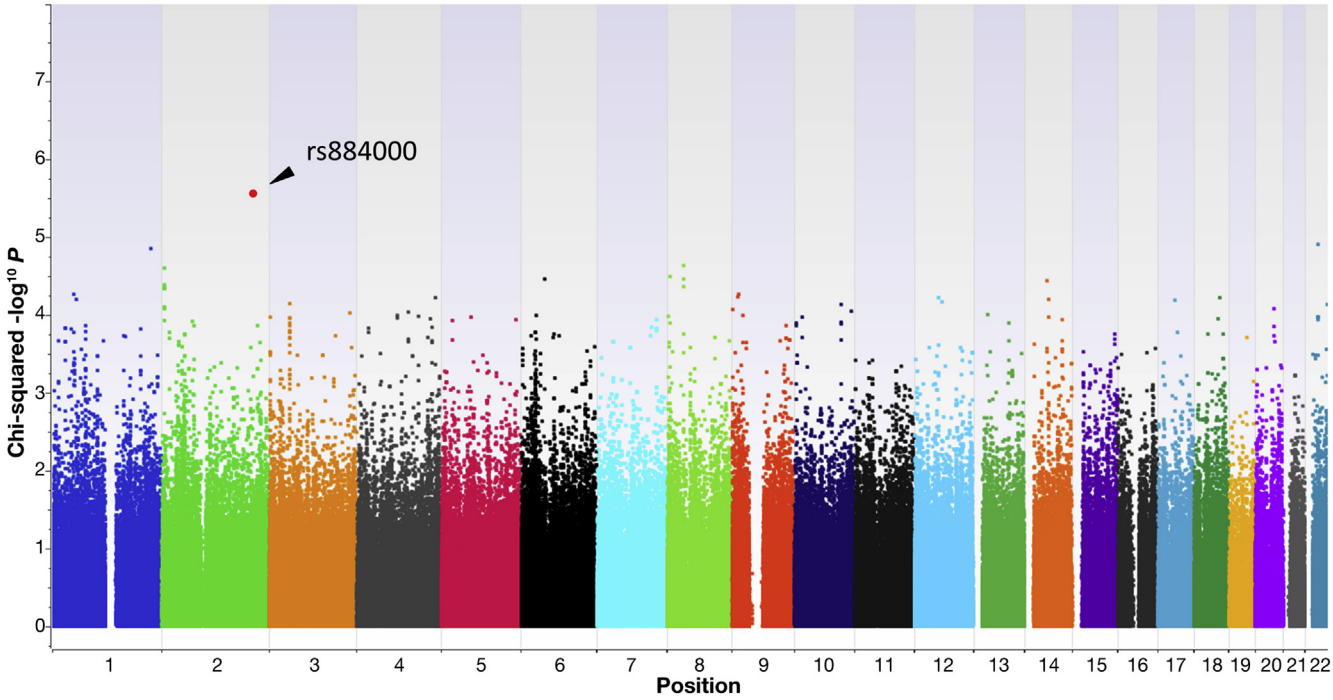

Figure 2. Genome-wide view of the single-point association data based on allele frequencies in a comparison of 71 HCV Japanese patients with LP and 190 patients with HCV without OLP. P values were calculated using the chi-square test for allele frequencies among 629,553 SNPs.

observed for rs884000, which is located about 17.5 kb downstream from the *NRP2* (neuropilin-2) gene, showing $P = 2.84 \times 10^{-6}$ (OR, 3.25; 95% CI, 1.95–5.41) (Table 2, Supplementary Table 1) and $P = 8.60 \times 10^{-6}$ (OR, 3.70; 95% CI, 2.10–6.68) when age and gender were adjusted. From the HLA class II region (Chr6: 32,256,456–33,258,648, GRCh37 hg19), including the *HLA-DR* gene, which has been reported as a disease susceptibility gene for LP in the Italian population, the top hit association was observed at rs9461799 showing $P = 3.99 \times 10^{-4}$ (OR, 2.15; 95% CI, 1.41–3.28) (Supplementary Figure 3) and age- and gender-adjusted $P = 1.39 \times 10^{-3}$ (OR, 2.20; 95% CI, 1.37–3.62). Here, susceptibility to or resistance against HCV related LP was evaluated by the OR for the minor allele (ie, OR >1 and OR <1 indicate susceptible and resistant alleles, respectively).

We conducted a replication analysis of candidate SNPs associated with OLP or LP in 45 Italian subjects (Supplementary Table 2). The replication analysis in Italian subjects did not reach significant associations, but showed the same trend of ORs as shown in Japanese subjects.

High-Density Association Mapping Based on Genotype Imputation

Genotype imputation was carried out based on genome-wide SNP typing data using the phased genotype data of the 1000 Genomes Project reference data with IMPUTE2 software packages under default parameters. Among the genetic regions including SNPs with $P < 1 \times 10^{-4}$ in the GWAS, 2 genetic regions included a SNP showing a stronger association in genotype imputation based high-density association mapping than the associations in GWAS (ie, rs884000 and rs538399) (Supplementary Figure 4). The SNP rs538399, showing $P = 6.50 \times 10^{-5}$ (OR, 0.40; 95% CI, 0.25–0.63) in the GWAS, is an intron variant of the insulin-like growth factor binding protein 4 (*IGFBP4*) gene.

Discussion

One of the most important issues concerning OLP is its increased potential for malignant transformation into oral squamous cell carcinoma (SCC).[2,3] There is some evidence that HCV-positive patients with OLP might be at higher risk of malignant transformation.[27] The relative risk of malignant transformation for patients with OLP with HCV, compared with those without HCV infection, was reported to be 3.16.[27]

The reported prevalence of HCV-infected patients with LP shows wide geographic variation and is high in Japan and Italy.[16,17] We consider that the pathogenesis of OLP in HCV infection is not directly related to the virus itself, but the response generated by host factors (eg, immunologic and genetic factors and insulin resistance). Previous reports indicated that there are no differences between HCV-infected patients with LP and those without in terms of viral factors, such as viral load, genotype/subtype, and mutations leading to aa substitutions in the HCV core region (70 and/or 91) and IFN-sensitivity-determining region of nonstructural protein 5A.[28,29]

Several reports document the impact of IFN on HCV-associated OLP. As regards the effects of IFN therapy on LP lesions, there are reports of improvements in lesions, reports of LP manifestation triggered by IFN, and reports of exacerbation of LP. Especially, worsening pain and/or inflammation in OLP in patients receiving IFN therapy are particular problems that may result in the inability to complete IFN therapy. It remains difficult to predict the onset or exacerbation of OLP among HCV infected-patients. Most recently, we reported successful treatment of HCV-infected OLP by IFN-free therapy with direct-acting antivirals.[30]

We found no SNP with genome-wide significance ($P < 5 \times 10^{-8}$). However, 2 SNPs (rs884000 in the *NRP2* locus and rs538399 in the *IGFBP4* locus) showed nominal associations in the GWAS and subsequent high-density association mapping. Moreover, our genetic analysis also supported the association of the HLA class II region, including *HLA-DR* and *DQ* genes, with HCV-positive OLP.[18,19] The 2 genetic loci found in our study and *HLA* genes could be useful as predictors for onset of OLP among HCV-infected patients. The replication study in Italian subjects showed the trend similar to Japanese results.

Neuropilins (NRPs), including *NRP1* and *NRP2*, are related transmembrane receptors that function as

Table 2. Associations of rs884000 With HCV-Related Lichen Planus

rs ID	Chr.	Physical position (build 37/Hg19)	*P* value[a]	OR[b]	95% CI		Cases					Control subjects					Allele A	Allele B	Associated gene
					Low	High	AA	AB	BB	No call	MAF	AA	AB	BB	No call	MAF			
rs884000	2	206680397	2.84E-06	3.25	1.95	5.41	3	30	38	0	0.25	3	30	157	0	0.09	G	T	*NRP2*

NOTE. Susceptibility to or resistance against HCV-related LP was evaluated by the OR for minor allele (ie, OR >1 and OR <1 indicate susceptible and resistant alleles, respectively).
MAF, minor allele frequency.
[a]P value of Pearson chi-square test for allelic model.
[b]OR of minor allele from 2-by-2 allele frequency table.

mediators of neuronal guidance and angiogenesis. NRPs bind members of the class 3 semaphorin family (Sema3A, Sema3B, and Sema3C), regulators of neuronal guidance, and of the vascular endothelial growth factor family of angiogenesis factors.[31] NRPs function in many key biologic processes, including in the cardiovascular, nervous, and immune systems.[32] There is substantial evidence that NRPs serve as mediators of developmental and tumor angiogenesis.[31]

Recent evidence suggests that *NRP2* is expressed in tumor tissue and plays a role in tumor progression and metastasis.[33,34] *NRP2* is highly expressed on the surface of cancer cells from pancreatic neuroendocrine tumors,[35] colorectal carcinomas,[33] breast cancer,[36] cutaneous melanoma,[37] and oral SCC.[38] *NRP2* expression also correlates with lymph node metastasis in breast cancer[36] and papillary thyroid carcinoma.[34] Cao et al[39] showed that *NRP2* promotes metastasis of renal cell carcinoma (RCC) and pancreatic cancer in mouse and zebrafish models and showed a mechanism through which *NRP2* expressed on cancer cells interacts with α-5 integrin on endothelial cells to mediate vascular adhesion and extravasation.

The insulin-like growth factor system (IGFs) consists of 2 peptides (IGF-I and -II), 2 main receptors (IGF-IR and IGFIIR), 6 different IGF binding proteins (IGFBP1-6), and 4 IGFBP-related peptides (IGFBP Rp1-4). IGFs have multiple functions regarding cellular growth, survival, and differentiation under different physiologic and pathologic conditions.[40] IGFBP4 is an important member of the IGF system. IGFBP4 has been reported to play a critical role in cardiomyocyte differentiation of embryonic stem cells.[41]

Several cancer cell lines, including from multiple myeloma, neuroblastoma, and mesothelioma, and cancers of the lung, gastric, thyroid, breast, prostate, and colon have been reported to express IGFBP4.[42] Ueno et al[43] initially showed that the expression of IGFBP4 was significantly lower in primary RCC and higher in metastatic RCC, compared with normal human kidney tissue, and that IGFBP4 transfectants promoted cell growth (*in vitro* and *in vivo*), invasion, and motility in primary RCC.

In the present study, 4 of 71 Japanese patients with LP (5.6%), 3 women and 1 man, developed oral SCC. One of the 4 subjects was a 84-year-old woman who suffered from HCV-related liver cirrhosis. She developed oral verrucous carcinoma, arising OLP-coexisting vulvo-vaginal gingival syndrome and esophageal SCC.[44] Another, in whom sustained virologic response was obtained by IFN therapy, was a 73-year-old man who suffered from chronic hepatitis C and hypertension. He developed tongue cancer and Graves ophthalmopathy during pegylated IFN plus ribavirin therapy. The third patient was a 57-year-old woman who suffered from chronic hepatitis C during IFN therapy. She developed tongue cancer arising from OLP. The fourth was a 67-year-old woman who suffered from chronic hepatitis C. She developed tongue cancer arising from OLP after treatment with pegylated IFN plus ribavirin. Only she had the risk allele at rs88400 in *NRP2* but she did not have the resistance allele at rs538399 in *IGFBP4*. No patients in the control group developed oral cancer.

We reported previously that insulin resistance might be involved in the development of multiple primary cancers in patients with oral SCC and HCV infection[45] and might cause OLP and extrahepatic manifestations.[46,47] The prevalence of extrahepatic malignant tumors was significantly higher in patients with OLP (29.4%) than in patients without (4.3%).[47] Two SNPs (rs884000 on *NRP2* and rs538399 on *IGFBP4*) may play a role in the malignant transformation of OLP.

Our study had some limitations. The sample size was relatively smaller than that for the conventional GWAS for diagnostic criteria. However, to the best of our knowledge, our GWAS for HCV-related LP is the first such report.

In conclusion, we identified novel associations of rs884000 in *NRP2*, rs538399 on *IGFBP4*, and supported the association of the *HLA-DR/DQ* genes, with HCV-positive LP in the Japanese and Italian population. Our data suggest that these genes may be involved in the development of LP and malignant transformation and useful as a predictive marker for the onset of OLP with IFN therapy among HCV infected-patients.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2016.12.029.

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Conflicts of interest
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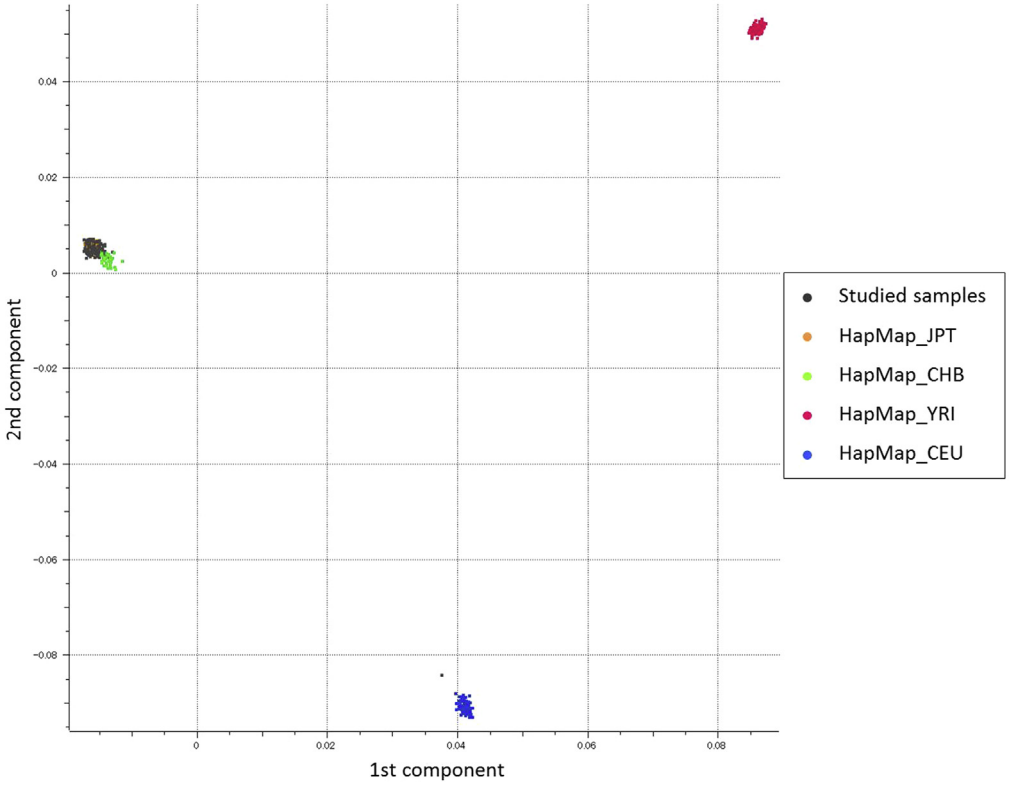
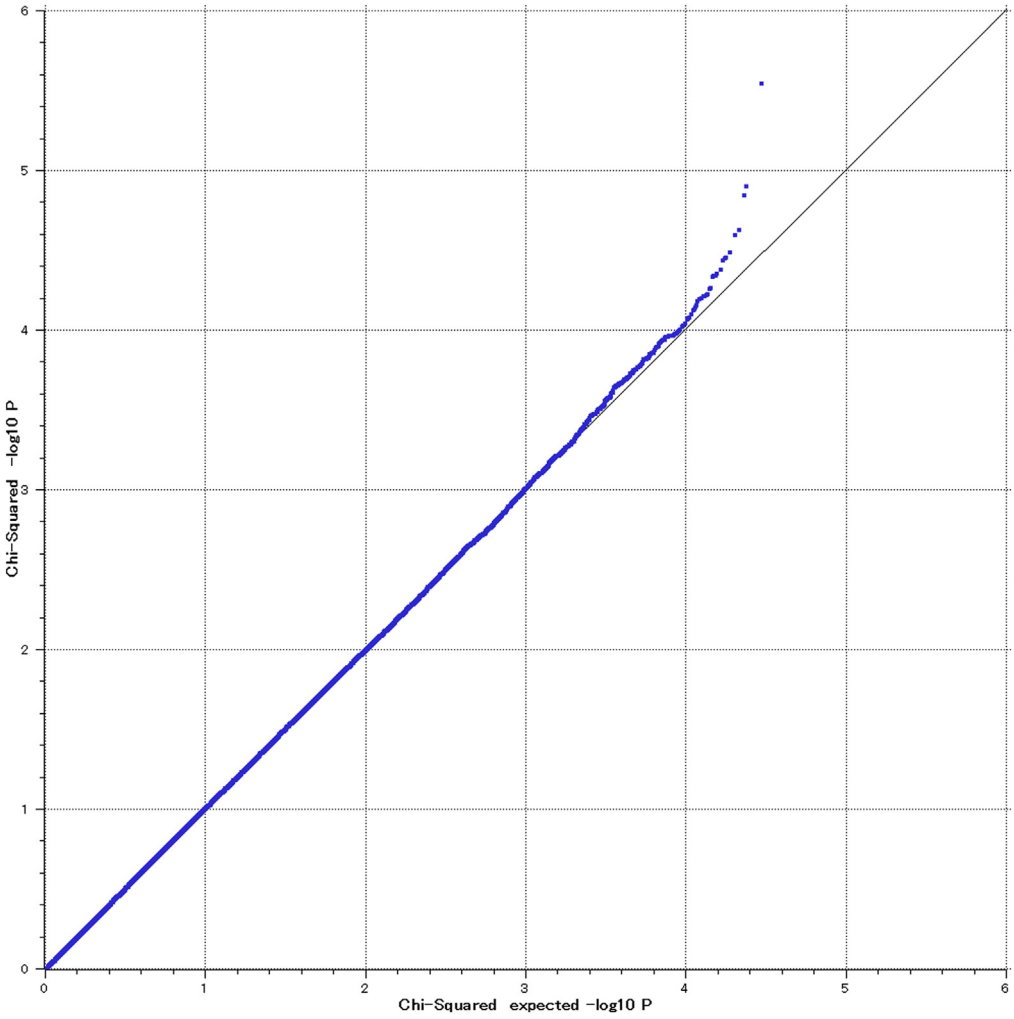
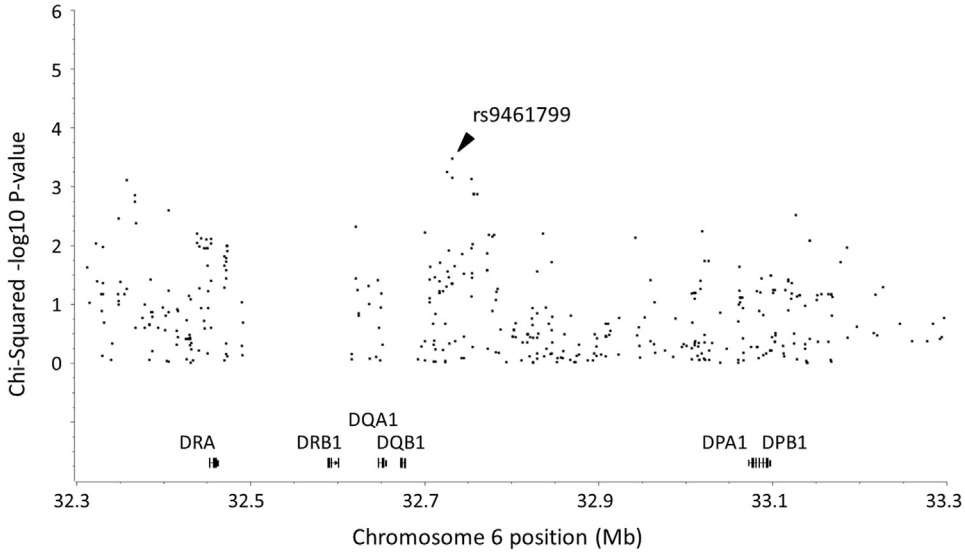


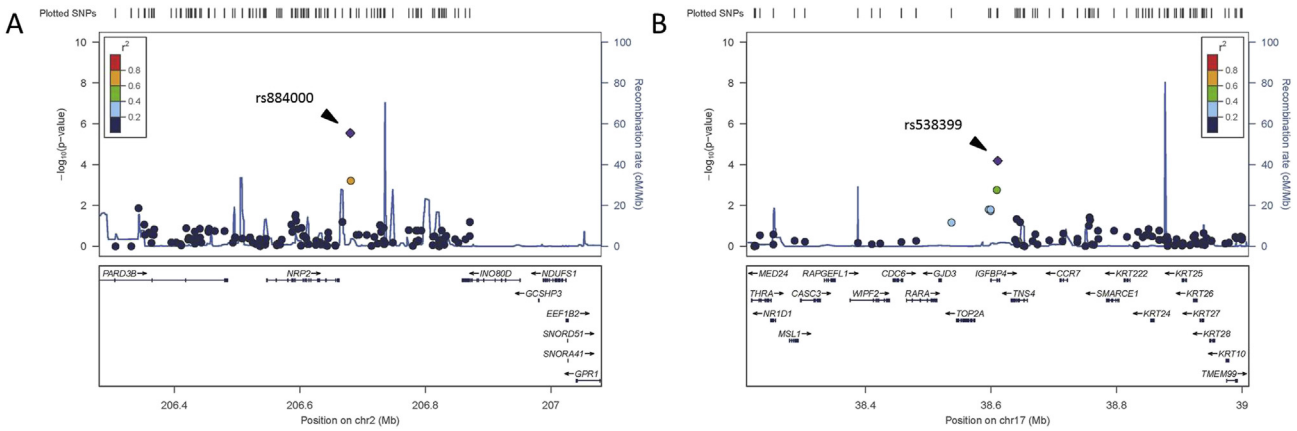
Supplementary Figure 1. Principal component analysis of 261 studied samples together with HapMap samples (including 43 JPT, 40 CHB, 91 YRI, and 91 CEU samples).



Supplementary Figure 2. Quantile-Quantile plot of the allele-based Chi-square test statistics for the GWAS results. The dots represent the *P* values of each SNP that passed the quality control filters. The inflation factor λ was estimated to be 1.027 for all the tested.



Supplementary Figure 3. Regional Manhattan plot of the HLA class II region (Chr6: 32,256,456 - 33,258,648, GRCh37 hg19) in the GWAS.



Supplementary Figure 4. High density association mapping based on genotype imputation around (*A*) rs884000, and (*B*) rs538399.

Supplementary Table 1. Associations of GWAS for HCV-Related LP Cases and Control Subjects

rs ID	Chr.	Physical position (build 37/Hg19)	*P* value[a]	OR[b]	95% CI		Cases					Control subjects					Allele A	Allele B	Associated gene
					Low	High	AA	AB	BB	No call	MAF	AA	AB	BB	No call	MAF			
rs241501	1	49765438	5.49E-05	0.39	0.25	0.62	1	26	44	0	0.20	31	84	75	0	0.38	G	T	AGBL4
rs568052	1	55524842	6.37E-05	0.18	0.07	0.46	0	5	66	0	0.04	4	56	130	0	0.17	C	T	PCSK9
rs10915880	1	224041034	1.41E-05	4.87	2.24	10.59	3	12	56	0	0.13	1	9	180	0	0.03	A	G	TP53BP2
rs17363620	2	5734639	4.59E-05	3.18	1.78	5.67	1	25	45	0	0.19	0	26	163	1	0.07	A	T	Intergenic region
rs16863981	2	5736321	4.18E-05	3.20	1.79	5.70	1	25	45	0	0.19	0	26	164	0	0.07	A	T	Intergenic region
rs13019074	2	5737785	8.39E-05	3.09	1.72	5.53	1	24	45	1	0.19	0	26	163	1	0.07	A	C	Intergenic region
rs11693312	2	5745057	2.51E-05	2.92	1.75	4.88	1	32	38	0	0.24	1	35	154	0	0.10	A	G	Intergenic region
rs6432151	2	5760116	4.54E-05	2.73	1.66	4.49	1	34	36	0	0.25	1	40	149	0	0.11	A	T	Intergenic region
rs1437040	2	5760247	4.54E-05	2.73	1.66	4.49	1	34	36	0	0.25	1	40	149	0	0.11	A	G	Intergenic region
rs6710579	2	5762278	7.94E-05	2.67	1.62	4.39	1	33	36	1	0.25	1	40	148	1	0.11	G	T	Intergenic region
rs7610518	3	46161631	7.09E-05	0.41	0.27	0.64	3	26	42	0	0.23	30	97	63	0	0.41	C	T	XCR1
rs4859240	3	182476614	9.38E-05	0.40	0.25	0.64	3	21	47	0	0.19	29	81	78	2	0.37	A	G	Intergenic region
rs11943343	4	117407994	9.30E-05	5.06	2.07	12.34	1	12	58	0	0.10	0	8	181	1	0.02	A	G	Intergenic region
rs13148375	4	177557439	6.03E-05	2.88	1.69	4.90	2	27	41	1	0.22	3	28	158	1	0.09	A	C	Intergenic region
rs2067833	6	53330356	3.51E-05	2.33	1.55	3.49	16	30	25	0	0.44	14	67	109	0	0.25	A	G	Intergenic region
rs2189887	8	5501607	3.25E-05	0.23	0.11	0.48	0	8	63	0	0.06	8	63	118	1	0.21	A	T	Intergenic region
rs391583	8	37178897	4.37E-05	0.36	0.22	0.60	1	20	50	0	0.15	26	76	88	0	0.34	C	T	Intergenic region
rs6995149	8	37179494	3.50E-05	0.36	0.22	0.59	1	20	50	0	0.15	27	75	88	0	0.34	C	G	Intergenic region
rs436802	8	37180035	2.33E-05	0.34	0.21	0.57	1	19	50	1	0.15	27	75	88	0	0.34	C	T	Intergenic region
rs7865508	9	332639	8.51E-05	2.54	1.58	4.09	6	27	38	0	0.27	3	43	143	1	0.13	C	T	DOCK8
rs7870726	9	13530105	5.89E-05	3.18	1.77	5.73	4	18	49	0	0.18	1	23	166	0	0.07	C	T	Intergenic region
rs12343442	9	15212216	5.44E-05	4.70	2.08	10.62	1	14	56	0	0.11	0	10	180	0	0.03	G	T	TTC39B
rs7087214	10	106193598	7.40E-05	2.59	1.60	4.19	5	28	38	0	0.27	5	37	148	0	0.12	A	C	CCDC147
rs8181424	10	128602101	8.96E-05	2.32	1.51	3.55	9	33	29	0	0.36	2	70	118	0	0.19	C	T	DOCK1
rs7294533	12	54612774	6.06E-05	2.75	1.66	4.58	6	22	43	0	0.24	2	35	153	0	0.10	C	T	CBX5
rs7315138	12	62388915	6.90E-05	2.24	1.50	3.34	18	28	25	0	0.45	13	76	101	0	0.27	A	T	FAM19A2
rs202092	13	30814313	9.82E-05	4.15	1.93	8.93	1	15	55	0	0.12	0	12	177	1	0.03	C	T	KATNAL1
rs7140779	14	49946421	3.64E-05	2.50	1.60	3.88	10	27	31	3	0.35	5	56	128	1	0.17	C	T	Intergenic region
rs11624787	14	53288450	6.27E-05	0.42	0.27	0.65	4	27	40	0	0.25	34	96	57	3	0.44	C	G	FERMT2
rs538399	17	38610665	6.50E-05	0.40	0.25	0.63	2	25	42	2	0.21	26	99	64	1	0.40	C	T	IGFBP4
rs3862706	18	57728033	6.09E-05	4.31	2.00	9.28	1	15	53	2	0.12	0	12	178	0	0.03	G	T	Intergenic region
rs4812826	20	42972275	8.26E-05	2.18	1.47	3.23	23	37	11	0	0.58	28	93	69	0	0.39	A	G	R3HDML
rs5997363	22	28794069	1.25E-05	7.56	2.64	21.61	2	9	60	0	0.09	0	5	185	0	0.01	A	G	TTC28
rs713727	22	49794342	7.44E-05	3.73	1.87	7.43	0	20	51	0	0.14	1	14	175	0	0.04	C	T	Intergenic region

NOTE. GWAS result *P* value $< 10^{-4}$. Susceptibility to or resistance against HCV-related LP was evaluated by the OR for minor allele (ie, OR >1 and OR <1 indicate susceptible and resistant alleles, respectively).
MAF, minor allele frequency.

[a]*P* value of Pearson chi-square test for allelic model.

[b]OR of minor allele from 2-by-2 allele frequency table.

Supplementary Table 2. Replication Study of rs9461799, rs538399, and rs884000 with HCV-Related LP in Italian Patients

rs ID	Chr.	Physical position (build 37/Hg19)	*P* value[a]	OR[b]	95% CI		Cases					Control subjects					Allele A	Allele B	Associated gene
					Low	High	AA	AB	BB	No call	RAF	AA	AB	BB	No call	RAF			
rs9461799	6	32689529	4.74E-01	1.57	5.42	0.454	12	13	10	3	0.53	1	3	2	0	0.42	T	C	*HLA* class II
rs538399	17	38610665	9.14E-01	0.91	4.81	0.173	0	13	22	3	0.19	0	2	3	2	0.20	G	A	*IGFBP4*
rs884000	2	206680397	7.95E-01	1.33	11.7	0.152	1	6	30	1	0.11	0	1	5	1	0.08	C	A	*NRP2*

NOTE. Susceptibility to or resistance against HCV-related LP was evaluated by the OR for minor allele (ie, OR >1 and OR <1 indicate susceptible and resistant alleles, respectively).

RAF, risk allele frequency.

[a]*P* value of Pearson chi-square test for allelic model.

[b]OR of minor allele from 2-by-2 allele frequency table.