Accepted Manuscript

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

Yumiko Nagao, Nao Nishida, Licht Toyo-oka, Atsushi Kawaguchi, Antonio Amoroso, Marco Carrozzo, Michio Sata, Masashi Mizokami, Katsushi Tokunaga, Yasuhito Tanaka

PII: S1542-3565(17)30003-4
DOI: 10.1016/j.cgh.2016.12.029
Reference: YJCGH 55054

To appear in: Clinical Gastroenterology and Hepatology
Accepted Date: 24 December 2016


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Genome-wide Association Study Identifies Risk Variants for Lichen Planus in Patients With Hepatitis C Virus Infection

Short title: GWAS for HCV-related LP

Yumiko Nagao¹,²§, Nao Nishida³,⁴, Licht Toyo-oka⁴, Atsushi Kawaguchi⁵, Antonio Amoroso⁶, Marco Carrozzo⁷, Michio Sata²,⁸, Masashi Mizokami³, Katsushi Tokunaga⁴, Yasuhito Tanaka⁹

¹Department of Organ System Interactions and Information, Saga Medical School, Nabeshima, Saga, 849-8501, Japan,
²Research Center for Innovative Cancer Therapy, Kurume University, Asahi-machi, Kurume, 830-0011, Japan,
³The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, 272-8516, Japan,
⁴Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, 113-0033, Japan,
⁵Center for Comprehensive Community Medicine, Saga Medical School, Nabeshima, Saga, 849-8501, Japan,
⁶Regional Transplantation Center, Piedmont, Molinette Hospital, AOU Citta della Salute e della Scienza di Torino, Turin, Italy,
⁷Oral Medicine Department, Centre for Oral Health Research, Newcastle University, Newcastle upon Tyne, Tyne and Wear NE2 4BW, UK,
⁸Nishinihon Hospital, Hattannda, Kumamoto, 861-8034, Japan,
⁹Department of Virology, Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, 467-8601, Japan.

§Correspondence author
Yumiko Nagao,
Department of Organ System Interactions and Information, Saga Medical School 5-1-1 Nabeshima, Saga 849-8501, Japan, Telephone: +81-952-34-2516, Facsimile: +81-952-34-2516, Email addresses: nagaoyu@cc.saga-u.ac.jp

Financial support: This study was supported by a grant-in-aid from Japan Agency for Medical Research and Development, AMED (H25-kanen-ippan-005 and H28-kanen-16668373).

Conflicts of interest: These authors disclose the following: Y.Nagao belongs to a donation-funded department funded by Nishinihon hospital; Y.Tanaka has received research grants from Bristol-Myers Squibb Company, MSD K.K., Chugai Pharmaceutical Co., Ltd., Janssen Pharmaceutical K.K, Gilead Sciences, and AbbVie Inc. The remaining authors disclose no conflicts.

Specific author contributions: Data acquisition, design of the work and drafting the work: Yumiko Nagao; analysis, interpretation of data, and drafting the work: Nao Nishida; analysis and interpretation of data: Licht Toyo-oka; statistical analysis, interpretation of data and revision of article: Atsushi Kawaguchi; data acquisition: Antonio Amoroso and Marco Carrozzo; collection of samples: Michio Sata; design of the work: Masashi Mizokami; study supervision: Katsushi Tokunaga; study supervision and funding: Yasuhito Tanaka; approved the final version of the submitted work: all the authors.
Abstract

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; 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 (SNPs) in NRP2 (rs884000) and IGFBP4 (rs538399) were associated with risk of HCV-associated lichen planus ($P<1\times10^{-4}$). We also found an association between a SNP 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% CI, 1.95–5.41), 0.40 (95% CI, 0.25–0.63), and 2.15 (95% CI, 1.41–3.28), respectively.

CONCLUSION: 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 SNPs 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.

KEY WORDS: inflammation, risk factor, oral mucosa, autoimmunity
INTRODUCTION

Lichen planus (LP) (see Figure 1 below) 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 lichen planus (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 six 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+ lesional T cell clones may be blocked partially by anti-MHC 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
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 Human Leucocyte Antigens (HLA) class II allele group, DR6, in OLP patients with HCV compared to those without HCV (52% vs. 18%, $P=0.028$, relative risk=4.93). 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 (HCC). In Japan, elderly patients are at a higher risk for HCC, and HCV eradication has a smaller effect on hepatocarcinogenesis in older patients. OLP can appear or be exacerbated during interferon (IFN) therapy for chronic hepatitis C.

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) and inosine triphosphatase (ITPA) 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 ± SD, 67.2 ± 9.6 years; men/women 24/47) and HCV-infected Japanese patients with normal oral mucosa (190 patients; mean age ± SD, 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 PCR assay (COBAS AMPLICOR HCV MONITOR v 2.0 Test, COBAS AmpliPrep/COBAS Taq-Man HCV Test, Roche Molecular Systems, Branchburg, New Jersey, US). Ultrasonographic examination was performed on all patients. Computed tomography (CT), 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.

SNP 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 SNPs were determined using the Genotyping Console v4.1 software (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) (Supplementary Figure S1). 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%, MAF <1% and HWE $P$ value <0.0001. A total of 629,588 SNPs passed
the threshold. The scatter plots for SNP with $P < 0.0001$ in the allelic model were then checked by visual inspection and thirty-five 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 three SNPs (HLA-DR/DQ rs9461799, IGFBP4 rs538399, and NRP2 rs884000) using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) on a LightCycle 480 Real-Time PCR System (Roche, Mannheim, Germany).

### SNP 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, HWE $P$ value $\geq 0.001$ and samples including $>10\%$ missing genotype were eliminated.

### Statistical analysis

For the association tests, $P$ values, ORs and 95% CIs between the SNP and disease phenotype were assessed by chi-squared test with a two-by-two contingency table for the allelic model. To avoid false-positive results due to 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 HCV patients with LP and 190 HCV patients without OLP. A
quantile-quantile plot of the distribution of test statistics for the comparison of
allele frequencies between the two 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 S2). Although no SNPs reached the genome-wide
significant level (i.e. $P < 5 \times 10^{-8}$), the greatest hit association was 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 S1) 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 (i.e. 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 Italian 45 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, two genetic regions included a SNP showing a stronger association in genotype imputation based high-density association mapping than the associations in GWAS (i.e. 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. There is some evidence that HCV-positive OLP patients might be at higher risk of malignant transformation. The relative risk of malignant transformation for OLP patients with HCV, compared to those without HCV infection, was reported to be 3.16. The reported prevalence of HCV infected patients with LP shows wide geographical variation and is high in Japan and Italy. 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 (e.g. immunological 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 (ISDR) of nonstructural protein 5A (NS5A)\textsuperscript{28,29}.

A number of 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 which 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\textsuperscript{30}.

We found no SNP with genome-wide significance ($P < 5 \times 10^{-8}$). However, two SNPs (rs884000 in the \textit{NRP2} locus and rs538399 in the \textit{IGFBP4} locus) showed nominal associations in the GWAS and subsequent high-density association mapping. Moreover, our genetic analysis also supported the association of the \textit{HLA} class II region, including \textit{HLA-DR} and \textit{DQ} genes, with HCV-positive OLP\textsuperscript{18,19}. The two genetic loci found in our study and \textit{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 (\textit{NRP}s), including neuropilin-1 (\textit{NRP1}) and \textit{NRP2}, are related transmembrane receptors that function as 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 (VEGF) family of angiogenesis factors. NRPs function in many key biological processes, including in the cardiovascular, nervous and immune systems. There is substantial evidence that NRPs serve as mediators of developmental and tumor angiogenesis.

Recent evidence suggests that NRP2 is expressed in tumor tissue and plays a role in tumor progression and metastasis. NRP2 is highly expressed on the surface of cancer cells from pancreatic neuroendocrine tumors, colorectal carcinomas, breast cancer, cutaneous melanoma and oral squamous cell carcinoma (SCC). NRP2 expression also correlates with lymph node metastasis in breast cancer and papillary thyroid carcinoma. Cao et al. 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 α5 integrin on endothelial cells to mediate vascular adhesion and extravasation.

The insulin-like growth factor system (IGFs) consists of two peptides (IGF-I and -II), two main receptors (IGF-IR and IGFIIIR), six different IGF binding proteins (IGFBP1-6) and four IGFBP related peptides (IGFBP Rp1-4). IGFs have multiple functions regarding cellular growth, survival and differentiation under different physiological and pathological conditions. IGFBP4 is an important member of the IGF system. IGFBP4 has been reported to play critical role in cardiomyocyte differentiation of embryonic stem cells (ESCs).

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. Ueno et al. initially showed that the expression of IGFBP4 was significantly lower in primary RCC and higher in metastatic RCC, compared to 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, four of 71 LP Japanese patients (5.6%), three women and one man, developed oral SCC. One of the four 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. Another, in whom SVR 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 (Peg-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 Peg-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 and might cause OLP and extrahepatic manifestations. The prevalence of extrahepatic malignant tumors was significantly higher in patients with OLP (29.4%) than in patients without (4.3%). 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.

ACKNOWLEDGEMENTS
We thank Dr. Shintaro Ogawa (Department of Virology, Liver Unit, Nagoya City University Graduate School of Medical Sciences) for disposal of samples. We also thank Dr. Minae Kawashima, Dr. Hiromi Sawai, Ms. Yuko Ogasawara-Hirano, Natsumi Baba, Rieko Shirahashi, Ayumi Nakayama and Megumi Yamaoka-Sageshima (University of Tokyo), and Ms. Yoriko Mawatari, Mayumi Ishii, Takayo Tsuchiura (National Center for Global Health and Medicine) for technical assistance. We also thank Dr. Francesca Bertinetto and Dr. Ennia Dametto (Regional Transplantation Center, Piedmont, Molinette Hospital) for collection of samples.
Figure legends

**Figure 1:** Clinical presentations of lichen planus (Panel A, lower lip; Panel B and D, left buccal mucosa; Panel C, tongue)

**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 HCV patients without OLP. *P* values were calculated using the chi-square test for allele frequencies among 629,553 SNPs.
References


25. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with


Table 1. Characteristics of studied Japanese population (n=261)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>Case with lichen planus</th>
<th>Control with normal oral mucosa</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects</td>
<td>261</td>
<td>71</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>male / female</td>
<td>107 / 154</td>
<td>24 / 47</td>
<td>83 / 107</td>
</tr>
<tr>
<td>Age (mean ± SD), years</td>
<td>61.8 ± 10.4</td>
<td>67.2 ± 9.6</td>
<td>59.8 ± 10.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site of lichen planus</td>
<td>Oral mucosa</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oral &amp; genital mucosa</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oral mucosa &amp; skin</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis of liver diseases</td>
<td>Past history of HCV infection</td>
<td>2</td>
<td>0.77%</td>
<td>1.41%</td>
</tr>
<tr>
<td></td>
<td>AH-C post IFN (SVR)</td>
<td>2</td>
<td>0.77%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>CH-C</td>
<td>170</td>
<td>65.13%</td>
<td>56.34%</td>
</tr>
<tr>
<td></td>
<td>CH-C post IFN (SVR)</td>
<td>23</td>
<td>8.81%</td>
<td>11.27%</td>
</tr>
<tr>
<td></td>
<td>CH-C post IFN (SVR) &amp; NAFLD</td>
<td>1</td>
<td>0.38%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>CH-C post IFN (SVR) &amp; Asymptomatic HBV carrier</td>
<td>1</td>
<td>0.38%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>CH-C &amp; AIH</td>
<td>1</td>
<td>0.38%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>CH-C &amp; ALD</td>
<td>1</td>
<td>0.38%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>CH-C &amp; HCV-related HCC</td>
<td>16</td>
<td>6.13%</td>
<td>8.45%</td>
</tr>
<tr>
<td></td>
<td>CH-C &amp; CH-B &amp; HCC</td>
<td>2</td>
<td>0.77%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>CH-C post IFN (SVR) &amp; HCV-related HCC</td>
<td>1</td>
<td>0.38%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>LC-C</td>
<td>19</td>
<td>7.28%</td>
<td>8.45%</td>
</tr>
<tr>
<td></td>
<td>LC-C &amp; AIH</td>
<td>1</td>
<td>0.38%</td>
<td>1.41%</td>
</tr>
<tr>
<td></td>
<td>LC-C &amp; post IFN (SVR)</td>
<td>1</td>
<td>0.38%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>LC-C &amp; HCV-related HCC</td>
<td>20</td>
<td>7.86%</td>
<td>12.68%</td>
</tr>
</tbody>
</table>

AH-C, acute hepatitis C; CH-C, chronic hepatitis C; IFN, interferon; SVR, sustained virological response; NAFLD, Non-alcoholic fatty liver disease; AIH, Autoimmune hepatitis; ALD, Alcoholic liver disease; LC-C, liver cirrhosis type C; HCC, hepatocellular carcinoma
Table 2. Associations of rs884000 with HCV-related lichen planus

<table>
<thead>
<tr>
<th>rs ID</th>
<th>Physical Position</th>
<th>P-value$^a$</th>
<th>95%CI</th>
<th>Case</th>
<th>Control</th>
<th>Allele A</th>
<th>Allele B</th>
<th>Associated Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs884000</td>
<td>2</td>
<td>206680397</td>
<td>2.84E-06</td>
<td>3.25</td>
<td>1.95</td>
<td>5.41</td>
<td>3</td>
<td>30</td>
</tr>
</tbody>
</table>

$^a$P value of Pearson’s chi-square test for allelic model.

$^b$Odds ratio (OR) of minor allele from two-by-two allele frequency table

Susceptibility to or resistance against HCV related LP was evaluated by the OR for minor allele (i.e. OR > 1 and OR < 1 indicate susceptible and resistant alleles, respectively).
Table 1. Associations of genome-wide association study for HCV-related lichen planus cases and controls: GWAS result_P value<10-4

<table>
<thead>
<tr>
<th>rs ID</th>
<th>Physical Position</th>
<th>P-value</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>95%CI</th>
<th>Case</th>
<th>Control</th>
<th>Allele A</th>
<th>Allele B</th>
<th>Associated Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chr. (build 37/Hg19)</td>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>AB</td>
<td>BB</td>
<td>No Call</td>
<td>MAF</td>
</tr>
<tr>
<td>rs241501</td>
<td>1 49765438</td>
<td>5.49E-05</td>
<td>0.39</td>
<td>0.25</td>
<td>0.62</td>
<td>1 26 44 0 0.020</td>
<td>31 84 75 0 0.38</td>
<td>G  T</td>
<td>AGBL4</td>
</tr>
<tr>
<td>rs568052</td>
<td>55524842 6.37E-05</td>
<td>0.18</td>
<td>0.07</td>
<td>0.46</td>
<td>0 5 66 0 0.04</td>
<td>4 56 130 0 0.17</td>
<td>C  T</td>
<td>PCSK9</td>
<td></td>
</tr>
<tr>
<td>rs10915880</td>
<td>224041034 1.41E-05</td>
<td>4.87</td>
<td>2.24</td>
<td>10.59</td>
<td>3 12 56 0 0.13</td>
<td>1 9 180 0 0.03</td>
<td>A  G</td>
<td>TP53BP2</td>
<td></td>
</tr>
<tr>
<td>rs17363620</td>
<td>5734639 4.59E-05</td>
<td>3.18</td>
<td>1.78</td>
<td>5.67</td>
<td>1 25 45 0 0.19</td>
<td>0 26 163 1 0.07</td>
<td>A  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs16863981</td>
<td>5736321 4.18E-05</td>
<td>3.20</td>
<td>1.79</td>
<td>5.70</td>
<td>1 25 45 0 0.19</td>
<td>0 26 164 0 0.07</td>
<td>A  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs13019074</td>
<td>5737785 8.39E-05</td>
<td>3.09</td>
<td>1.72</td>
<td>5.53</td>
<td>1 24 45 1 0.19</td>
<td>0 26 163 1 0.07</td>
<td>A  C</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs11693312</td>
<td>5745057 2.51E-05</td>
<td>2.92</td>
<td>1.75</td>
<td>4.88</td>
<td>1 32 38 0 0.24</td>
<td>1 35 154 0 0.10</td>
<td>A  G</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs6432151</td>
<td>5760116 4.54E-05</td>
<td>2.73</td>
<td>1.66</td>
<td>4.49</td>
<td>1 34 36 0 0.25</td>
<td>1 40 149 0 0.11</td>
<td>A  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs1437040</td>
<td>5760214 4.54E-05</td>
<td>2.73</td>
<td>1.66</td>
<td>4.49</td>
<td>1 34 36 0 0.25</td>
<td>1 40 149 0 0.11</td>
<td>A  G</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs6710579</td>
<td>5762278 7.94E-05</td>
<td>2.67</td>
<td>1.62</td>
<td>4.39</td>
<td>1 33 36 0 0.25</td>
<td>1 40 148 1 0.11</td>
<td>G  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs7615018</td>
<td>46161631 7.09E-05</td>
<td>0.41</td>
<td>0.27</td>
<td>0.64</td>
<td>0 26 42 0 0.23</td>
<td>30 97 63 0 0.41</td>
<td>C  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs4859240</td>
<td>18247616 9.38E-05</td>
<td>0.40</td>
<td>0.25</td>
<td>0.64</td>
<td>3 21 47 0 0.19</td>
<td>29 81 78 2 0.37</td>
<td>A  G</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs11943343</td>
<td>117407994 9.30E-05</td>
<td>5.06</td>
<td>2.07</td>
<td>12.34</td>
<td>1 12 58 0 0.10</td>
<td>0 8 181 1 0.02</td>
<td>A  G</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs13148375</td>
<td>177557439 6.03E-05</td>
<td>2.88</td>
<td>1.69</td>
<td>4.90</td>
<td>2 27 41 1 0.22</td>
<td>3 28 158 0 0.09</td>
<td>A  C</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs2067833</td>
<td>53330356 3.51E-05</td>
<td>2.33</td>
<td>1.55</td>
<td>3.49</td>
<td>16 30 25 0 0.44</td>
<td>14 67 109 0 0.25</td>
<td>A  G</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs21889877</td>
<td>4216060 3.25E-05</td>
<td>0.23</td>
<td>0.11</td>
<td>0.48</td>
<td>0 8 63 0 0.06</td>
<td>8 63 118 1 0.21</td>
<td>A  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs391583</td>
<td>37178897 4.37E-05</td>
<td>0.36</td>
<td>0.22</td>
<td>0.60</td>
<td>1 20 50 0 0.15</td>
<td>26 76 88 0 0.34</td>
<td>C  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs6995149</td>
<td>37179497 3.50E-05</td>
<td>0.36</td>
<td>0.22</td>
<td>0.59</td>
<td>1 20 50 0 0.15</td>
<td>27 75 88 0 0.34</td>
<td>C  G</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs436802</td>
<td>37180035 2.33E-05</td>
<td>0.34</td>
<td>0.21</td>
<td>0.57</td>
<td>1 19 50 0 0.15</td>
<td>27 75 88 0 0.34</td>
<td>C  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs7865508</td>
<td>3326369 8.15E-05</td>
<td>2.54</td>
<td>1.58</td>
<td>4.09</td>
<td>6 27 38 0 0.27</td>
<td>3 43 143 1 0.13</td>
<td>C  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs7870726</td>
<td>13530105 5.89E-05</td>
<td>3.18</td>
<td>1.77</td>
<td>5.73</td>
<td>4 18 49 0 0.18</td>
<td>1 23 166 0 0.07</td>
<td>C  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs12349442</td>
<td>15212216 5.44E-05</td>
<td>4.70</td>
<td>2.08</td>
<td>10.62</td>
<td>1 14 56 0 0.11</td>
<td>0 10 180 0 0.03</td>
<td>G  T</td>
<td>TTC39B</td>
<td></td>
</tr>
<tr>
<td>rs7087214</td>
<td>106193598 7.40E-05</td>
<td>2.59</td>
<td>1.60</td>
<td>4.19</td>
<td>5 28 38 0 0.27</td>
<td>5 37 148 0 0.12</td>
<td>A  C</td>
<td>CCDC147</td>
<td></td>
</tr>
<tr>
<td>rs8181424</td>
<td>128602101 8.96E-05</td>
<td>2.32</td>
<td>1.51</td>
<td>3.55</td>
<td>9 33 29 0 0.36</td>
<td>2 70 118 0 0.19</td>
<td>C  T</td>
<td>DOCK1</td>
<td></td>
</tr>
<tr>
<td>rs7294533</td>
<td>54612774 6.06E-05</td>
<td>2.75</td>
<td>1.66</td>
<td>4.58</td>
<td>6 22 43 0 0.24</td>
<td>2 35 153 0 0.10</td>
<td>C  T</td>
<td>CBX5</td>
<td></td>
</tr>
<tr>
<td>rs7315138</td>
<td>62389905 6.90E-05</td>
<td>2.24</td>
<td>1.50</td>
<td>3.34</td>
<td>18 28 25 0 0.45</td>
<td>13 76 101 0 0.27</td>
<td>A  T</td>
<td>FAM19A2</td>
<td></td>
</tr>
<tr>
<td>rs202092</td>
<td>30814331 9.82E-05</td>
<td>4.15</td>
<td>1.93</td>
<td>8.93</td>
<td>1 15 55 0 0.12</td>
<td>0 12 177 1 0.03</td>
<td>C  T</td>
<td>KATNAL1</td>
<td></td>
</tr>
<tr>
<td>rs7140779</td>
<td>49946421 3.64E-05</td>
<td>2.50</td>
<td>1.60</td>
<td>3.88</td>
<td>10 27 31 3 0.35</td>
<td>5 56 128 1 0.17</td>
<td>C  T</td>
<td>intergenic region</td>
<td></td>
</tr>
<tr>
<td>rs11624787</td>
<td>53288450 6.27E-05</td>
<td>0.42</td>
<td>0.27</td>
<td>0.65</td>
<td>4 27 40 0 0.25</td>
<td>34 96 57 3 0.44</td>
<td>C  G</td>
<td>FERMT2</td>
<td></td>
</tr>
<tr>
<td>rs538399</td>
<td>17</td>
<td>38610665</td>
<td>6.50E-05</td>
<td>0.40</td>
<td>0.25</td>
<td>0.63</td>
<td>2 25 42</td>
<td>2</td>
<td>0.21</td>
</tr>
<tr>
<td>---------</td>
<td>----</td>
<td>----------</td>
<td>----------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>-------</td>
<td>---</td>
<td>------</td>
</tr>
<tr>
<td>rs3862706</td>
<td>18</td>
<td>57728033</td>
<td>6.09E-05</td>
<td>4.31</td>
<td>2.00</td>
<td>9.28</td>
<td>1 15 53</td>
<td>2</td>
<td>0.12</td>
</tr>
<tr>
<td>rs4812826</td>
<td>20</td>
<td>42972275</td>
<td>8.26E-05</td>
<td>2.18</td>
<td>1.47</td>
<td>3.23</td>
<td>23 37 11</td>
<td>0</td>
<td>0.58</td>
</tr>
<tr>
<td>rs5997363</td>
<td>22</td>
<td>28794069</td>
<td>1.25E-05</td>
<td>7.56</td>
<td>2.64</td>
<td>21.61</td>
<td>2 9 60</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>rs713727</td>
<td>22</td>
<td>49794342</td>
<td>7.44E-05</td>
<td>3.73</td>
<td>1.87</td>
<td>7.43</td>
<td>0 20 51</td>
<td>0</td>
<td>0.14</td>
</tr>
</tbody>
</table>

\(^a\)P value of Pearson’s chi-square test for allelic model.

\(^b\)Odds ratio (OR) of minor allele from two-by-two allele frequency table

Susceptibility to or resistance against HCV related LP was evaluated by the OR for minor allele (i.e. OR > 1 and OR < 1 indicate susceptible and resistant alleles, respectively).
Table 2. Replication study of rs9461799, rs538399, and rs884000 with HCV-related lichen planus in Italian patients

<table>
<thead>
<tr>
<th>rs ID</th>
<th>Chr. (build 37/Hg19)</th>
<th>Physical Position</th>
<th>95% CI</th>
<th>Case</th>
<th>Control</th>
<th>Allele A</th>
<th>Allele B</th>
<th>Associated Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9461799</td>
<td>6</td>
<td>32689529</td>
<td>4.74E-01</td>
<td>1.57</td>
<td>5.42 0.454</td>
<td>12 13 10 3 0.53</td>
<td>1 3 2 0 0.42</td>
<td>T C</td>
</tr>
<tr>
<td>rs538399</td>
<td>17</td>
<td>38610665</td>
<td>9.14E-01</td>
<td>0.91</td>
<td>4.81 0.173</td>
<td>0 13 22 3 0.19</td>
<td>0 2 3 2 0.20</td>
<td>G A</td>
</tr>
<tr>
<td>rs884000</td>
<td>2</td>
<td>206680397</td>
<td>7.95E-01</td>
<td>1.33</td>
<td>11.7 0.152</td>
<td>1 6 30 1 0.11</td>
<td>0 1 5 1 0.08</td>
<td>C A</td>
</tr>
</tbody>
</table>

aP value of Pearson’s chi-square test for allelic model.
bOdds ratio (OR) of minor allele from two-by-two allele frequency table

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