

Fig. 2. Serum TL1A levels in PBC patients with different histological scores. (A) Serum TL1A levels tended to be higher in PBC patients with a cholangitis activity (CA) score of 2 as compared with those with a CA score of 0 or 1. There was no significant difference in serum TL1A levels among patients with different histological scores for bile duct loss (B), fibrosis (C) or hepatitis (D). Statistical differences were analysed using a two-tailed Mann–Whitney *U*-test. n.s.; not significant.

UDCA + PSL (2; 3%) during observation (median: 37.6 months; range: 8–148 months). The daily dose of UDCA was 600 mg ($n = 67$), 900 mg ($n = 7$) and 1200 mg ($n = 2$). Serum ALT, ALP and total IgM levels were significantly decreased in both early- and late-stage PBC patients receiving UDCA treatments (data not shown). On the other hand, serum TL1A levels were significantly decreased only in PBC patients classified as early stage ($P = 0.04$, Fig. 3B) at study entry, and not those categorized as late stage ($P = 0.52$, Fig. 3C). Thus, we noted a trend towards decreased serum TL1A levels ($P = 0.13$) under UDCA treatment in the total PBC patient population (Fig. 3A). By the end of the observation period, however, serum TL1A levels were sustained at significantly higher levels in early- and late-stage patients as compared with healthy controls (early stage: $P = 0.06$; late stage: $P = 0.002$).

Immunohistochemical analysis of TL1A in liver tissue

In the non-diseased liver, TL1A was localized in blood vessels, Kupffer cells, infiltrating mononuclear cells and

intrahepatic bile ducts, but not in hepatocytes (Fig. 4A). In PBC and CHC patients, the localization of TL1A-positive cells was similar to that of non-diseased controls, however, the number of TL1A-positive cells was increased in both PBC (Fig. 4C, 4D) and CHC (Fig. 4B) patients as compared with non-diseased controls. There was no apparent difference between the PBC and CHC patients.

mRNA expression of TL1A in liver tissues and its association with histological activity

The expression of TL1A mRNA in the livers of PBC ($P = 0.17$) and CHC patients ($P = 0.06$) was higher as compared with those of controls (Fig. 5A), although this difference was not statistically significant. Compared with controls, the expression of TL1A mRNA was significantly increased only in late-stage CHC ($P = 0.03$, Fig. 5B). In addition, the expression of TL1A mRNA tended to be higher in late-stage than early-stage patients for both PBC ($P = 0.07$) and CHC ($P = 0.16$).

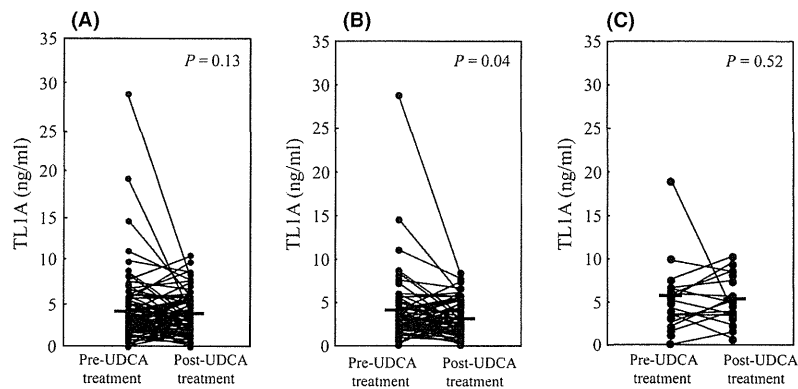


Fig. 3. Effect of UDCA treatment on serum TL1A levels in PBC patients. (A) In the PBC patient group as a whole ($n = 76$), serum TL1A levels tended to be decreased by UDCA treatment (pre: 4.5 ± 4.5 ng/ml, post: 3.6 ± 2.5 ng/ml). Serum TL1A levels were significantly decreased in early-stage PBC patients ($n = 60$) after UDCA treatment (pre: 4.0 ± 4.2 ng/ml, post: 3.0 ± 2.1 ng/ml) (B), but not in late-stage PBC patients ($n = 16$) (pre: 5.5 ± 4.3 ng/ml, post: 5.2 ± 2.3 ng/ml) (C). Horizontal lines represent mean values. Statistical analysis was performed using a two-tailed Wilcoxon's single-rank test.

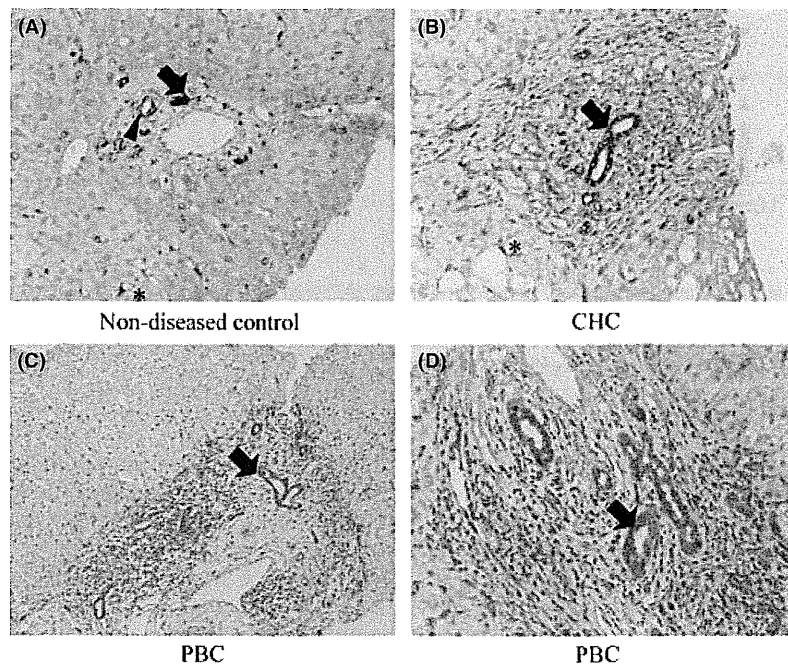


Fig. 4. Immunohistochemical staining of TL1A in the liver specimens of PBC and CHC patients. (A) TL1A was localized in intrahepatic bile ducts (arrow), blood vessels (arrowhead) and Kupffer cells (asterisk), but not in hepatocytes in non-diseased controls. In addition to these TL1A-positive cells, TL1A was primarily localized to mononuclear cells infiltrating the portal tract in both CHC (B) and PBC (C and D) liver sections. Original magnification: 400 \times .

The significance of DcR3 in PBC patients

At the time of enrolment, the number of patients with detectable serum DcR3 levels was significantly higher in patients with PBC (34%, $P = 1.0 \times 10^{-5}$), CHC (19%, $P = 0.02$) and AIH (47%, $P = 2.0 \times 10^{-5}$), as compared with healthy controls (2%). Serum DcR3 levels in PBC ($P = 0.004$), CHC ($P = 0.005$) and AIH ($P = 0.002$) patients were significantly higher than those

in healthy controls (Fig. 6A). Serum DcR3 levels were also significantly higher in both early- and late-stage PBC (early stage: $P = 0.009$; late stage: $P = 0.003$) patients and CHC (early stage: $P = 0.008$; late stage: $P = 0.01$) patients as compared with healthy controls (Fig. 6B).

Serum DcR3 levels, when detectable, tended to be lower in PBC patients receiving UDCA treatment ($P = 0.15$, Fig. 7A). When these patients were stratified

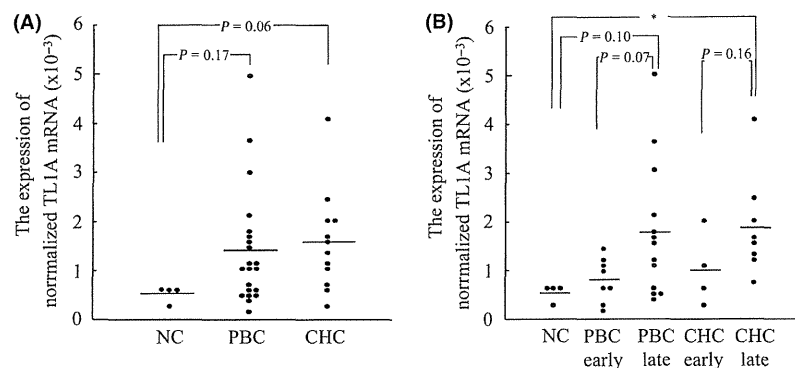


Fig. 5. Expression of TL1A mRNA in the liver specimens of PBC and CHC patients. The TL1 mRNA expression in the liver specimens of non-diseased controls ($n = 4$) as well as PBC ($n = 21$) and CHC ($n = 12$) patients was normalized to GAPDH mRNA expression. (A) The normalized TL1A mRNA expression in both PBC (0.0014 ± 0.0012) and CHC patients (0.0016 ± 0.0010) was higher than that in non-diseased controls (0.0005 ± 0.0002). (B) The normalized TL1A mRNA expression was higher in the late stage than in the early stage of both PBC (early: $n = 8$, 0.0008 ± 0.0004 ; late: $n = 13$, 0.0017 ± 0.0014) and CHC patients (early: $n = 4$, 0.0009 ± 0.0008 ; late: $n = 8$, 0.0019 ± 0.0010). A significant increase in TL1A mRNA was observed only in late-stage CHC patients. Horizontal lines represent mean values for each group. Statistical differences were analysed using a two-tailed Student's *t*-test; * $P < 0.05$. NC: non-diseased controls.

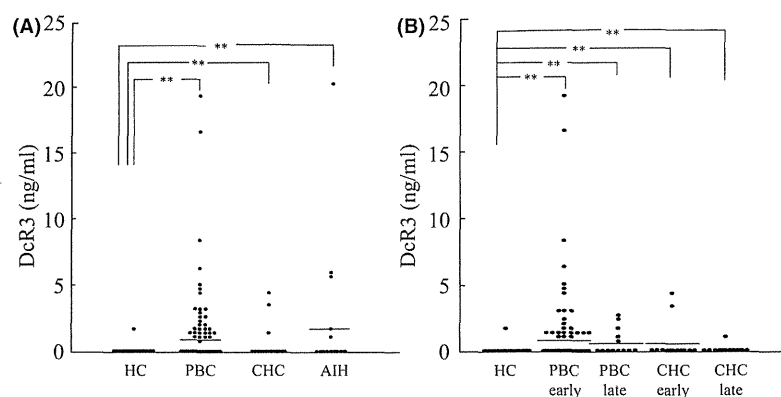


Fig. 6. Serum DcR3 levels in PBC, CHC and AIH patients. (A) Serum DcR3 levels were significantly higher in patients with PBC ($n = 110$, 0.92 ± 2.3 ng/ml), CHC ($n = 26$, 0.41 ± 1.1 ng/ml) and AIH ($n = 19$, 2.0 ± 4.8 ng/ml) as compared with healthy controls ($n = 46$, 0.04 ± 0.25 ng/ml). (B) Serum DcR3 levels were higher in both the early- and late-stage PBC (early: $n = 96$, 0.94 ± 2.4 ng/ml; late: $n = 14$, 0.71 ± 1.0 ng/ml) and CHC (early: $n = 13$, 0.62 ± 1.5 ng/ml; late: $n = 13$, 0.20 ± 0.41 ng/ml) patients as compared with healthy controls. Horizontal lines represent mean values for each group. Statistical differences were analysed using a two-tailed Mann–Whitney *U*-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

by clinical stage at the end of the observation period, serum DcR3 levels were found to be significantly decreased only in patients with the non-jaundice stage ($P = 0.004$, Fig. 7B), whereas these levels were significantly increased in patients with the jaundice stage who progressed to hepatic failure ($P = 0.04$, Fig. 7C).

Discussion

In this study, we report for the first time that serum TL1A levels were significantly increased in both early- and late-stage PBC patients, and that these levels were significantly decreased after UDCA treatment in early-stage PBC patients. Interestingly, serum TL1A levels

were also increased in other liver diseases such as CHC and AIH, although a significant increase was observed only in the late-stage CHC patients. These results may indicate that TL1A is involved not only in the early pathogenesis of PBC but also in CHC and AIH as a common denominator of chronic liver inflammation.

Previous reports have shown that serum TL1A levels are increased in various chronic inflammatory diseases such as IBD (12, 42) and RA (14). In these diseases, serum TL1A levels were higher in patients with active disease than those with inactive disease or in remission. In addition, serum TL1A levels were significantly higher in the late or terminal stages of these conditions as compared with their early stage. Previous studies have also

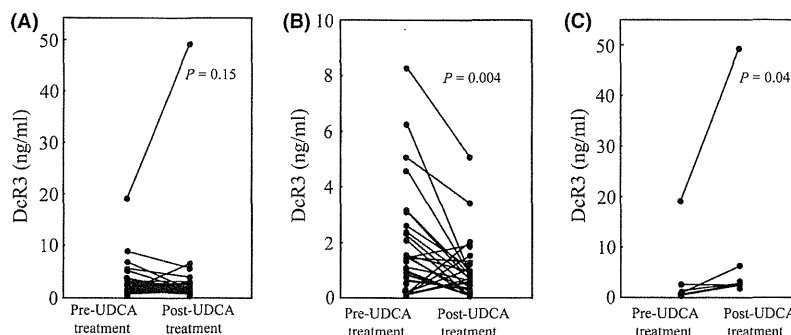


Fig. 7. Effect of UDCA treatment on serum DcR3 levels in PBC patients. In PBC patients who had detectable DcR3 levels by ELISA, serum DcR3 levels before and after UDCA treatment were compared by a two-tailed Wilcoxon's single-rank test. (A) In the group as a whole ($n = 39$), UDCA treatment tended to lower serum DcR3 levels (pre: 2.1 ± 3.4 ng/ml, post: 2.4 ± 7.9 ng/ml). (B) Serum DcR3 levels were significantly decreased in non-jaundice-stage patients ($n = 33$) after UDCA treatment (pre: 1.7 ± 1.9 ng/ml, post: 0.90 ± 1.0 ng/ml). (C) Serum DcR3 levels were significantly increased in jaundice-stage patients ($n = 6$) who progressed to hepatic failure after UDCA treatment (pre: 3.8 ± 7.7 ng/ml, post: 10.7 ± 19.1 ng/ml).

shown that TL1A is localized in macrophages and CD4⁺ or CD8⁺ lymphocytes infiltrating the intestinal lamina propria in Crohn's disease, and plasma cells infiltrating the lamina propria in ulcerative colitis (13). TL1A is also localized in macrophages and plasma cells in the synovial tissue in RA (7). In addition, upregulation of TL1A expression has been demonstrated in involved tissues (13, 15). These results indicate that TL1A is involved in the pathogenesis of these chronic inflammatory diseases. In this study, serum TL1A levels tended to correlate with cholangitis but not fibrosis in PBC patients, whereas the TL1A levels correlated with fibrosis in CHC patients. These results may indicate that TL1A is differentially involved in the liver inflammation between PBC and CHC, although there is no apparent difference in TL1A-positive cells as determined by immunohistochemical staining of the liver tissues.

Our immunohistochemical data identified TL1A in intrahepatic small bile ducts as well as infiltrating mononuclear cells, Kupffer cells and blood vessels in the PBC liver. It is noteworthy that the intrahepatic small bile ducts immunohistochemically expressed TL1A; however, the mechanism of TL1A production by these ducts *in vivo* is poorly understood. *In vitro*, TL1A is induced in endothelial cells by TNF- α and IL-1, and in monocytes and dendritic cells by stimulation with TLR ligands (11). Cultured biliary epithelial cells (BECs) constitutively express TL1A (data not shown) and various human TLRs, and produce inflammatory cytokines and chemokines when stimulated with TLR ligands *in vitro* (43, 44). TL1A also induces CD4⁺ T cells to produce IFN- γ through IL-12 and IL-18 signalling *in vitro* (19), and modulates Th-1 and Th-17 effector responses in animal models of experimental antigen-induced arthritis (16), experimental autoimmune encephalomyelitis (17) and chronic ileitis and colitis (19, 20). Previous reports have shown a significant role for both Th-1 and Th-17 cytokines in the pathogenesis of PBC (45).

Although the mechanism of TL1A induction in BEC is as yet poorly understood, we hypothesize that TL1A, which is induced in BEC, blood vessels and mononuclear cells by inflammatory cytokines and TLR signalling, may be involved in the pathogenesis of PBC via modulation in Th-1 and Th-17 effector responses.

We also showed that serum TL1A levels were significantly decreased in early-stage PBC patients after UDCA treatment. This is consistent with previous reports in which significant decreases in serum TL1A levels were observed in RA patients after anti-TNF monoclonal antibody therapy (14). These results may indicate that serum TL1A serves as a serological marker for disease activity in PBC as well as RA. Although serum ALP and ALT levels decreased significantly to baseline levels in most of the late- and early-stage PBC patients in this study, serum TL1A levels remained significantly higher in late-stage PBC patients as compared with healthy controls, even after UDCA treatment. These results might indicate that UDCA treatment is not sufficient to suppress TL1A-mediated inflammation, particularly in late-stage PBC. Neutralizing anti-TL1A antibodies attenuates inflammation in mouse models of chronic colitis and collagen-induced arthritis (16, 20), suggesting that TL1A might be potentially a new therapeutic target for PBC.

DcR3 binds to TL1A, FasL and LIGHT, and inhibits these ligands-mediated apoptosis or lymphokine production (6). In this study, we demonstrated that serum DcR3 levels were significantly increased at the time of enrolment in both early- and late-stage PBC patients as well as CHC and AIH patients. Under UDCA treatment, serum DcR3 levels were significantly decreased in non-jaundice-stage PBC patients, whereas they were significantly increased in jaundice-stage PBC patients who progressed to hepatic failure. This observation is consistent with previous findings that serum DcR3 is significantly increased in patients with cirrhosis (22) and

autoimmune diseases such as IBD (12, 28), SLE (21) and RA (14). Through downregulation of Th-1 and/or Th-17 immune responses, DcR3 protects against the development and progression of autoimmune diseases in mouse models of conditions such as cyclophosphamide-induced diabetes (31, 32) and experimental autoimmune encephalomyelitis (46). Therefore, it is speculated that the decrease in DcR3 levels during treatment in PBC patients might be because of secondary effect with the decrease in TL1A levels under UDCA treatment. DcR3 is also significantly induced in bacterial infections (29) and is a marker of mortality and multiple-organ dysfunction (47). These reports may indicate that the marked increase in serum DcR3 levels in jaundice-stage PBC patients is caused by bacterial infections and/or multiple-organ dysfunction.

In conclusion, we showed for the first time that serum TL1A and DcR3 levels were increased in PBC patients and that these levels changed in association with disease progression and UDCA treatment, indicating that TL1A and DcR3 might be involved in the pathogenesis of PBC. Further studies are needed to elucidate the exact role of TL1A and DcR3 in the pathogenesis of PBC and to explore its potential as a possible therapeutic target.

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Conflict of interest: None.

References

- Hirschfield GM, Liu X, Xu C, et al. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med* 2009; **360**: 2544–55.
- Hirschfield GM, Liu X, Han Y, et al. Variants at IRF5-TNPO3, 17q12-21 and MMEL1 are associated with primary biliary cirrhosis. *Nat Genet* 2010; **42**: 655–7.
- Liu X, Invernizzi P, Lu Y, et al. Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis. *Nat Genet* 2010; **42**: 658–60.
- Mells GF, Floyd JA, Morley KI, et al. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat Genet* 2011; **43**: 329–32.
- Nakamura M, Nishida N, Kawashima M, et al. Genome-wide association study identifies TNFSF15 and POU2AF1 as susceptibility loci for primary biliary cirrhosis in the Japanese population. *Am J Hum Genet* 2012; **91**: 721–8.
- Migone TS, Zhang J, Luo X, et al. TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. *Immunity* 2002; **16**: 479–92.
- Cassatella MA, Pereira-da-Silva G, Tinazzi I, et al. Soluble TNF-like cytokine (TL1A) production by immune complexes stimulated monocytes in rheumatoid arthritis. *J Immunol* 2007; **178**: 7325–33.
- Shih DQ, Kwan LY, Chavez V, et al. Microbial induction of inflammatory bowel disease associated gene TL1A (TNFSF15) in antigen presenting cells. *Eur J Immunol* 2009; **39**: 3239–50.
- Yue TL, Ni J, Romanic AM, et al. TL1, a novel tumor necrosis factor-like cytokine, induces apoptosis in endothelial cells. Involvement of activation of stress protein kinases (stress-activated protein kinase and p38 mitogen-activated protein kinase) and caspase-3-like protease. *J Biol Chem* 1999; **274**: 1479–86.
- Haridas V, Shrivastava A, Su J, et al. VEGI, a new member of the TNF family activates nuclear factor-kappa B and c-Jun N-terminal kinase and modulates cell growth. *Oncogene* 1999; **18**: 6496–504.
- Bayry J. Immunology: TL1A in the inflammatory network in autoimmune diseases. *Nat Rev Rheumatol* 2010; **6**: 67–8.
- Bamias G, Kaltsa G, Siakavellas SI, et al. High intestinal and systemic levels of decoy receptor 3 (DcR3) and its ligand TL1A in active ulcerative colitis. *Clin Immunol* 2010; **137**: 242–9.
- Bamias G, Martin C 3rd, Marini M, et al. Expression, localization, and functional activity of TL1A, a novel Th1-polarizing cytokine in inflammatory bowel disease. *J Immunol* 2003; **171**: 4868–74.
- Bamias G, Siakavellas SI, Stamatelopoulou KS, et al. Circulating levels of TNF-like cytokine 1A (TL1A) and its decoy receptor 3 (DcR3) in rheumatoid arthritis. *Clin Immunol* 2008; **129**: 249–55.
- Bamias G, Evangelou K, Vergou T, et al. Upregulation and nuclear localization of TNF-like Cytokine 1A (TL1A) and its receptors DR3 and DcR3 in psoriatic skin lesions. *Exp Dermatol* 2011; **20**: 725–31.
- Bull MJ, Williams AS, Mecklenburgh Z, et al. The Death Receptor 3-TNF-like protein 1A pathway drives adverse bone pathology in inflammatory arthritis. *J Exp Med* 2008; **205**: 2457–64.
- Pappu BP, Borodovsky A, Zheng TS, et al. TL1A-DR3 interaction regulates Th17 cell function and Th17-mediated autoimmune disease. *J Exp Med* 2008; **205**: 1049–62.
- Meylan F, Davidson TS, Kahle E, et al. The TNF-family receptor DR3 is essential for diverse T cell-mediated inflammatory diseases. *Immunity* 2008; **29**: 79–89.
- Bamias G, Mishina M, Nyce M, et al. Role of TL1A and its receptor DR3 in two models of chronic murine ileitis. *Proc Natl Acad Sci USA* 2006; **103**: 8441–6.
- Takedatsu H, Michelsen KS, Wei B, et al. TL1A (TNFSF15) regulates the development of chronic colitis by modulating both T-helper 1 and T-helper 17 activation. *Gastroenterology* 2008; **135**: 552–67.
- Lee CS, Hu CY, Tsai HF, et al. Elevated serum decoy receptor 3 with enhanced T cell activation in systemic lupus erythematosus. *Clin Exp Immunol* 2008; **151**: 383–90.
- Wu Y, Han B, Sheng H, et al. Clinical significance of detecting elevated serum DcR3/TR6/M68 in malignant tumor patients. *Int J Cancer* 2003; **105**: 724–32.
- Roth W, Isenmann S, Nakamura M, et al. Soluble decoy receptor 3 is expressed by malignant gliomas and

- suppresses CD95 ligand-induced apoptosis and chemotaxis. *Cancer Res* 2001; **61**: 2759–65.
24. Ge Z, Sanders AJ, Ye L, et al. Aberrant expression and function of death receptor-3 and death decoy receptor-3 in human cancer. *Exp Ther Med* 2011; **2**: 167–72.
 25. Chang YC, Hsu TL, Lin HH, et al. Modulation of macrophage differentiation and activation by decoy receptor 3. *J Leukoc Biol* 2004; **75**: 486–94.
 26. Hsu TL, Chang YC, Chen SJ, et al. Modulation of dendritic cell differentiation and maturation by decoy receptor 3. *J Immunol* 2002; **168**: 4846–53.
 27. Huang ZM, Kang JK, Chen CY, et al. Decoy receptor 3 suppresses TLR2-mediated B cell activation by targeting NF- κ B. *J Immunol* 2012; **188**: 5867–76.
 28. Funke B, Autschbach F, Kim S, et al. Functional characterisation of decoy receptor 3 in Crohn's disease. *Gut* 2009; **58**: 483–91.
 29. Kim S, McAuliffe WJ, Zaritskaya LS, et al. Selective induction of tumor necrosis receptor factor 6/decoy receptor 3 release by bacterial antigens in human monocytes and myeloid dendritic cells. *Infect Immun* 2004; **72**: 89–93.
 30. Chen J, Zhang L, Kim S. Quantification and detection of DcR3, a decoy receptor in TNFR family. *J Immunol Methods* 2004; **285**: 63–70.
 31. Wang YL, Chou FC, Sung HH, et al. Decoy receptor 3 protects non-obese diabetic mice from autoimmune diabetes by regulating dendritic cell maturation and function. *Mol Immunol* 2010; **47**: 2552–62.
 32. Sung HH, Juang JH, Lin YC, et al. Transgenic expression of decoy receptor 3 protects islets from spontaneous and chemical-induced autoimmune destruction in nonobese diabetic mice. *J Exp Med* 2004; **199**: 1143–51.
 33. Ka SM, Hsieh TT, Lin SH, et al. Decoy receptor 3 inhibits renal mononuclear leukocyte infiltration and apoptosis and prevents progression of IgA nephropathy in mice. *Am J Physiol Renal Physiol* 2011; **301**: F1218–30.
 34. Ka SM, Sytwu HK, Chang DM, et al. Decoy receptor 3 ameliorates an autoimmune crescentic glomerulonephritis model in mice. *J Am Soc Nephrol* 2007; **18**: 2473–85.
 35. Han B, Moore PA, Wu J, et al. Overexpression of human decoy receptor 3 in mice results in a systemic lupus erythematosus-like syndrome. *Arthritis Rheum* 2007; **56**: 3748–58.
 36. Silveira MG, Brunt EM, Heathcote J, et al. American Association for the Study of Liver Diseases endpoints conference: design and endpoints for clinical trials in primary biliary cirrhosis. *Hepatology* 2010; **52**: 349–59.
 37. Nakamura M, Kondo H, Mori T, et al. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. *Hepatology* 2007; **45**: 118–27.
 38. Manns MP, Czaja AJ, Gorham JD, et al. Diagnosis and management of autoimmune hepatitis. *Hepatology* 2010; **51**: 2193–213.
 39. Nakanuma Y, Zen Y, Harada K, et al. Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: interobserver agreement. *Pathol Int* 2010; **60**: 167–74.
 40. Takii Y, Nakamura M, Ito M, et al. Enhanced expression of type I interferon and toll-like receptor-3 in primary biliary cirrhosis. *Lab Invest* 2005; **85**: 908–20.
 41. Harada K, Van de Water J, Leung PS, et al. In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: predominance of the Th1 subset. *Hepatology* 1997; **25**: 791–6.
 42. Bamias G, Kaltsa G, Siakavellas SI, et al. Differential expression of the TL1A/DcR3 system of TNF/TNFR-like proteins in large vs. small intestinal Crohn's disease. *Dig Liver Dis* 2012; **44**: 30–6.
 43. Yokoyama T, Komori A, Nakamura M, et al. Human intrahepatic biliary epithelial cells function in innate immunity by producing IL-6 and IL-8 via the TLR4-NF- κ B and -MAPK signaling pathways. *Liver Int* 2006; **26**: 467–76.
 44. Shimoda S, Harada K, Niuro H, et al. Biliary epithelial cells and primary biliary cirrhosis: the role of liver-infiltrating mononuclear cells. *Hepatology* 2008; **47**: 958–65.
 45. Harada K, Shimoda S, Sato Y, et al. Periductal interleukin-17 production in association with biliary innate immunity contributes to the pathogenesis of cholangiopathy in primary biliary cirrhosis. *Clin Exp Immunol* 2009; **157**: 261–70.
 46. Chen SJ, Wang YL, Kao JH, et al. Decoy receptor 3 ameliorates experimental autoimmune encephalomyelitis by directly counteracting local inflammation and downregulating Th17 cells. *Mol Immunol* 2009; **47**: 567–74.
 47. Chen CY, Yang KY, Chen MY, et al. Decoy receptor 3 levels in peripheral blood predict outcomes of acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2009; **180**: 751–60.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Correlation between TL1A levels and biochemical parameters in sera of PBC patients. The correlations between levels of TL1A and those of ALP (A), ALT (B) or total IgM (C) in sera of PBC patients were analysed by Spearman's rank test; the correlation coefficient and *P*-value are shown.

Fig. S2. Serum TL1A levels in antinuclear antibody-positive and -negative PBC patients. Serum TL1A levels were compared between PBC patients who were positive and negative for anti-gp210 (A) and anticentromere (B) antibodies (anti-gp210 negative vs. positive: *n* = 68, 5.4 ± 5.9 ng/ml vs. *n* = 41, 4.1 ± 3.3 ng/ml; anticentromere negative vs. positive: *n* = 79, 5.0 ± 5.4 ng/ml vs. *n* = 30, 4.4 ± 3.7 ng/ml). Statistical differences were analysed using a two-tailed Mann–Whitney *U*-test.

Genetic polymorphisms of *OCT-1* confer susceptibility to severe progression of primary biliary cirrhosis in Japanese patients

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Abstract

Background To identify the genetic factors involved in the pathogenesis of primary biliary cirrhosis (PBC), we focused on the organic cation transporter 1 (*OCT1/SLC22A1*), which is closely associated with phosphatidylcholine synthesis in hepatocytes.

Methods We selected four (rs683369, rs2282143, rs622342 and rs1443844) *OCT-1* single nucleotide polymorphisms (SNPs), and genotyped these SNPs using the TaqMan probe method in 275 Japanese PBC patients and 194 gender-matched, healthy volunteers as controls.

Results The Chi-square test revealed that the rs683369 variant allele (G) was associated with insusceptibility to PBC development [$P = 0.009$, odds ratio (OR) 0.60, 95 % confidence interval (CI) 0.40–0.88] in an allele model, and

that the rs683369 variant allele (G) was associated with jaundice-type progression in a minor allele dominant genotype model ($P = 0.032$, OR 3.10, 95 % CI 1.05–9.14). The *OCT-1* rs2282143 variant (T) and rs622342 variant (C) were also associated with jaundice-type progression in a minor allele recessive genotype model ($P = 0.0002$, OR 10.58, 95 % CI 2.36–47.54, and $P = 0.006$, OR 7.84, 95 % CI 1.39–44.36, respectively). Furthermore, the association of *OCT-1* rs683369 and rs622342 with susceptibility to jaundice-type progression was confirmed by a replication study with a distinct set of PBC patients who underwent liver transplantation.

Conclusions The present study is the first report on the association of *OCT-1* genetic polymorphisms with the overall development and jaundice-type progression of PBC.

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Keywords PBC · OCT-1 · Genetic polymorphism · Phosphatidylcholine · Hepatic failure · Progression

Introduction

Primary biliary cirrhosis (PBC) is a chronic and slowly progressive autoimmune liver disease characterized histopathologically by destruction of the intrahepatic small bile ducts with lymphocyte-predominant portal inflammation, resulting in cholestasis, further hepatic damage, fibrosis, cirrhosis, and eventually hepatic failure [1]. In addition to antimitochondrial antibodies (AMA), antinuclear antibodies (ANA) such as anti-gp210, anti-sp100, and anticentromere antibodies are detected in approximately 50–90 % of PBC patients [2–7].

At present, a majority of PBC patients undergoing treatment with ursodeoxycholic acid (UDCA) have a normal life expectancy without the need for additional therapeutic approaches. However, one-third of PBC patients show severe progression and require additional treatments [8]. Ultimately, a few percent of PBC patients who show resistance to UDCA undergo liver transplantation or die within a decade of diagnosis [9]. The patterns of PBC progression thus differ strikingly among individuals, but the genetic or environmental factors influencing these differences are largely unknown.

PBC is a multifactorial disorder, with multiple genetic and environmental factors contributing to its etiology [1, 10]. Since the concordance rate of PBC in monozygotic twins is very high (63 %) and familial clustering of PBC has been reported at high frequencies (1.0–7.1 %) [11, 12], strong genetic factors have been implicated in the pathogenesis of PBC. Many candidate gene-based association studies with single nucleotide polymorphisms (SNPs) have identified a number of susceptibility genes for PBC. However, most of these have not been replicated in different ethnicities except for *CTLA4* [13]. In addition, there have been several reports describing weak associations between PBC progression and genetic polymorphisms in *TNF alpha*, *eNOS*, *apo-E*, *SLC4A2/AE2*, *Keratin*, *CYP2E1*, *CTLA4*, *ITGAV*, and *IL-1* [14–21]. However, these results require further replication studies in multiple ethnicities.

Recent genome-wide association studies have identified more than 20 non-HLA susceptibility loci for PBC, including *TNFSF15*, *POU2AF1*, *IL12A*, *IL12RB2*, *STAT4*, *IRF5*, *IKZF3*, *MMEL1*, *SPIB*, *DENND1B*, *CD 80*, *IL7R*, *CXCR5*, *TNFRSF1A*, *CLEC16A*, *NFKB1*, *RAD51L1*, *MAP3K7IP1*, *PLCL2*, *RPS6KA4*, and *TNFAIP2* [22–27]. These results indicate the importance of IL-12 and TNF/TLR–NFκB signaling as well as B cell differentiation pathways in the development of PBC. However, these

genome-wide association studies have not yet identified genetic loci associated with the progression of PBC.

Multidrug resistance protein 3 (MDR3) is a member of the superfamily of ATP-binding cassette (ABC) transporters, in which genetic polymorphisms are involved in cholestatic liver diseases such as intrahepatic cholestasis of pregnancy [28–30]. We previously reported that genetic polymorphism in MDR3 is associated with severe progression of PBC (i.e., jaundice-type progression) [31]. MDR3 functions as a transporter that is responsible for phosphatidylcholine (PC) secretion into bile. PC reduces bile acid toxicity by forming micelles with bile acid [32–35]. On the other hand, choline is used for the synthesis of PC in hepatocytes [36].

Organic cation transporter 1 (OCT-1) is a member of the solute carrier (SLC) transporter family, which transports choline into hepatocytes [37, 38]. Approximately 650 SNPs have been identified in the *OCT-1* gene, of which 69 are accompanied by amino acid changes. Among these non-synonymous 69 SNPs, 12 have been found to be associated with alterations in OCT-1 expression and/or function in vitro [39]. OCT-1 also transports metformin into hepatocytes, and the genetic polymorphisms of *OCT-1* are known to influence the antihyperglycemic action of metformin in vivo [40]. These findings indicate that the transport of choline into hepatocytes is influenced by *OCT-1* genetic polymorphisms leading to altered synthesis of PC, which causes insufficient protection of bile ducts due to bile acid toxicity. Thus, we hypothesized that *OCT-1* genetic polymorphisms are potentially associated with greater severity of both bile duct damage and progression of PBC.

Methods

Subjects

The study subjects comprised 275 unrelated Japanese patients with PBC [39 males, 236 females, age 32–78 years, median 57 year, mean \pm standard deviation (SD) 56.4 ± 10.4 years at the time of entry], and 194 gender-matched, unrelated, healthy Japanese volunteers as controls (Table 1). The PBC patients were registered in the PBC cohort study of the National Hospital Organization Study Group for Liver Disease in Japan (NHOSLJ) from August 1982 to August 2011. The time of entry was defined as the time of initial diagnosis of PBC. The observation period was defined as the time from the date of entry until the date of death, liver transplantation, last contact, death from non-liver-associated diseases, or end of follow-up, whichever came first.

Table 1 Characteristics of control subjects and PBC patients at each stage at the end of observation

Characteristics	Control subjects	PBC patients	Stage I	Stage II	Stage III
Total number of patients	194	275	194	66	15
Mean age (years)	39.8 ± 9.28	65.0 ± 11.7	63.5 ± 11.05	70.4 ± 9.62	59.8 ± 9.39
Observation period (months)	–	69.7 ± 52.5	63.8 ± 46.85	83.3 ± 59.9	85.7 ± 73.1
Male/female (%)	17/177 (8.8/91.2)	39/236 (14.1/85.9)	22/172 (11.3/88.7)	11/55 (16.7/83.3)	6/9 (40.0/60.0)
Anti-gp210 antibodies+ (%)	–	87 (31.6 %)	47 (24.2 %)	26 (39.4 %)	14 (93.3 %)
Anti-centromere antibodies+ (%)	–	66 (24.0 %)	38 (19.6 %)	25 (37.9 %)	3 (20 %)

PBC, primary biliary cirrhosis

Patients were diagnosed with PBC if they met at least two of the following internationally accepted criteria [41]: biochemical evidence of cholestasis, based mainly on alkaline phosphatase elevation; presence of serum anti-mitochondrial antibodies; histological evidence of non-suppurative destructive cholangitis; and destruction of interlobular bile ducts.

Patients with acute or autoimmune hepatitis (AIH; alanine aminotransferase >200 IU/l, aspartate aminotransferase >200 IU/l), given maintenance doses of prednisolone (PSL) higher than 5 mg/body for concomitant AIH, persistent hepatitis virus B or C infection, alcoholic liver disease, and other chronic liver diseases were excluded from this study.

Of the 275 patients in the study, 78 (26.4 %) had concomitant autoimmune diseases, as follows: Sjögren's syndrome ($n = 38$), systemic sclerosis ($n = 4$), Hashimoto's thyroiditis ($n = 8$), CREST syndrome ($n = 3$), rheumatoid arthritis ($n = 12$), Raynaud's disease ($n = 7$), interstitial pneumonitis ($n = 2$), polymyositis ($n = 1$), mixed connective tissue disease ($n = 1$), Basedow's disease ($n = 1$), and sarcoidosis ($n = 1$).

Patients were treated for PBC during the observation period as follows: UDCA (300–900 mg/day) alone ($n = 170$), bezafibrate (200–400 mg/day) alone ($n = 3$), PSL (≤ 5 mg/day) alone ($n = 3$), UDCA + bezafibrate ($n = 64$), UDCA and/or bezafibrate + maintenance PSL (≤ 5 mg/day) ($n = 29$), or no medication ($n = 1$).

Histological examination

Four- μ m-thick formalin-fixed and paraffin-embedded sections of needle liver biopsy specimens (length ≥ 20 mm, a total of 210 samples from 210 different PBC patients) were routinely stained with H&E, Azan-Mallory, reticulin silver impregnation, and rhodamine. The histological variables included fibrosis (0–4), portal inflammation (0–3), interface hepatitis (0–3), lobular inflammation (0–3), copper-associated protein deposition (0–1), chronic non-suppurative destructive cholangitis (0–2), granuloma (0–2), ductal paucity (0–3), and ductal proliferation (0–3). Upon completion of the evaluation of each of these variables, a

numerical necroinflammatory grade (A0–A3) and a histological stage using Scheuer's classification (stage 1–4) were determined [3, 7, 42]. Each biopsy specimen was analyzed by two independent observers (M.I. and Y.T.). In case of initial disagreement in the assessment, consensus was achieved by further review.

Clinical staging of PBC

PBC patients were assigned to one of the following three clinical stages based on liver biopsy findings and/or clinical manifestations: clinical stage I (early stage)—Scheuer's stage 1 or 2 or unknown histological stage without any signs indicating portal hypertension or liver cirrhosis; clinical stage II (late stage without jaundice)—Scheuer's stage 3 or 4 or any histological stage with signs indicating portal hypertension or liver cirrhosis, but without jaundice (total bilirubin <2 mg/dl); and clinical stage III (late stage with jaundice)—any Scheuer's stage with persistent jaundice (total bilirubin \geq mg/dl). Clinical stages I + II were also defined as “non-jaundice stages,” while stage III was defined as the “jaundice stage.” Clinical stage I was defined as the “early stage,” while stages II + III were defined as “late stages.” In addition, the progression to clinical stage II was defined as “non-jaundice-type progression,” while the progression to clinical stage III was defined as “jaundice-type progression.”

Replication study in patients who underwent liver transplantation

A replication study for progression to clinical stage III (jaundice-type progression) was performed using a different set of 35 PBC patients [3 males (8.5 %), 32 females (91.5 %); age at liver transplantation 34–69 years, median 51.0 years, mean \pm SD 51.3 \pm 8.18 years] who underwent orthotopic liver transplantation in Kyushu University Hospital during the period from September 1999 to August 2007. The observation period from the initial diagnosis of PBC to liver transplantation ranged from 1 to 23 years (median 9.5 years, mean \pm SD 9.2 \pm 5.3 years).

Preparation of genomic DNA

Genomic DNA was extracted from whole blood samples using the NucleoSpin Blood L Kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's protocol. Genomic DNA was also extracted from livers removed from PBC patients who underwent liver transplantation using the QuickGene DNA Whole Blood Kit S (Fujifilm, Tokyo, Japan) according to the manufacturer's protocol.

Selection of SNPs.

Four SNPs—rs683369, rs2282143, rs622342, and rs1443844—were selected in the *OCT-1* gene (HGNC: 10963), referred to as *SLC22A1*, using the data available on the International HapMap website (<http://www.HapMap.org/>). These SNPs were previously reported to be associated with function and/or expression. The gene structure and positions of the four chosen SNP sites in the *OCT-1* gene are shown in Fig. 1.

Genotyping of four SNPs in OCT-1

We genotyped four candidate SNPs using TaqMan probes. In brief, the polymorphic region was amplified by PCR using a real-time PCR System (BIORAD, CA, USA) from 10 ng of genomic DNA in a 10- μ l reaction mixture containing Premix EX Taq™ (Takara Biotechnology, Japan) and TaqMan® SNP Genotyping Assays (Applied Biosystems, CA, USA). The genotype was determined by detecting the fluorescence of FAM and VIC, according to the manufacturer's protocol. The fluorescence of FAM corresponded to the major allele while that of VIC corresponded to the minor allele.

Ethics board approval

The study protocol was approved by the Committee for Ethical Issues on Human Genome and Gene Analysis at the Clinical Research Center of the National Hospital

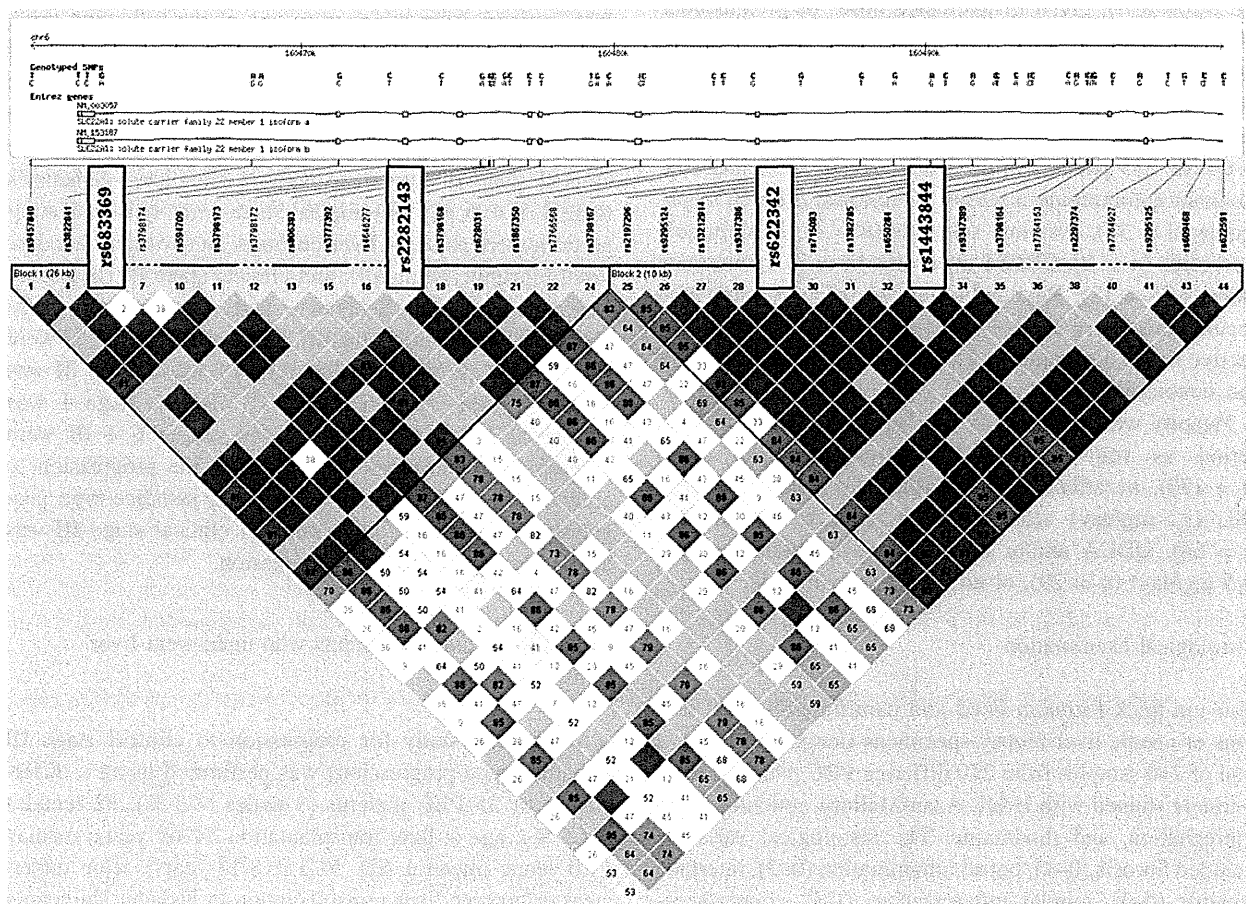


Fig. 1 Linkage disequilibrium (LD) plots for tag SNPs in *OCT1* using Haploview software version 4.2. The significant tag SNPs associated with the progression of PBC in the present study is highlighted in the blue boxes. Each diamond represents pairwise LD

strength with color (D'/LOD method) and value (r^2). Red diamonds indicate high LD, blue indicate moderate, and white indicate low. Empty diamonds show r^2 value of 1.0. Haplotype blocks highlighted with inverted triangles were calculated using solid spine algorithm

Organization (NHO) Nagasaki Medical Center (Approval number 15005), Kyushu University Hospital (Approval number 449-00) and at every hospital participating in the clinical study. Written informed consent was obtained from each subject.

Statistical analysis

Data obtained are indicated as means ± SDs. Age and gender of PBC patients and control subjects were evaluated by the unpaired Student’s *t* test and Chi-square test, respectively, using Prism 4 (GraphPad Software Inc., San Diego, CA, USA). Clinicopathological parameters were compared between subgroups of PBC patients using the unpaired Student’s *t* test and Chi-square test, again with Prism 4. Expected allele frequencies were calculated from respective single allele frequencies according to the Hardy–Weinberg equilibrium. The observed and expected allele frequencies were compared by Chi-square test using SNP Alyze 6.0 standard software. The frequencies and distributions of alleles and genotypes were compared between PBC patients and control subjects, as well as between subgroups of PBC patients using the Chi-square test with Prism 4. A *P* value of <0.05 was considered to be statistically significant.

Results

Clinical course of PBC patients

At the beginning of the study, 182 liver biopsy specimens were classified as Scheuer’s stage 1–2, while 28 were classified as Scheuer’s stage 3–4. Based on our criteria for clinical staging, 238, 35, and 2 PBC patients were at clinical stage I (early stage), clinical stage II (late stage without jaundice), and clinical stage III (late stage with jaundice), respectively, at the time of study entry. During the observation period (13–306 months, median 54.0 months, mean ± SD 69.7 ± 52.5 months), of the 238

patients originally at clinical stage I, 37 progressed to clinical stage II while seven progressed to clinical stage III. Six of the 35 patients initially at clinical stage II progressed to clinical stage III. The two patients at clinical stage III at the time of entry received liver transplantations. At the end of the observation period, therefore, 194 patients were at clinical stage I and 66 were at clinical stage II. Of the 13 patients who progressed to clinical stage III during the observation period, six patients received liver transplantations, three remained alive without receiving liver transplantations, and four died of end-stage hepatic failure.

Comparison of clinicopathological parameters among PBC patients and between PBC patients and control subjects

There was a significant difference in mean age (*P* = 0.0001) but no difference in gender (*P* = 0.475) between PBC patients and control subjects (Table 2). The demographics of PBC patients at each stage are shown in Table 2. There was a significant difference in mean age (*P* = 0.0002) and observation period (*P* = 0.0039) between early-stage and late-stage PBC patients. Specifically, the observation period in early-stage PBC patients was approximately 2 years shorter than that in late-stage PBC patients. This finding implies that a few patients with early-stage PBC might progress to more advanced stages (clinical stage II or III) in the future. In addition, the frequency of males was significantly higher in jaundice-stage (clinical stage III) PBC patients as compared with non-jaundice-stage (clinical stages I + II) patients. There were no significant differences in age or observation period between non-jaundice-stage (clinical stages I + II) and jaundice-stage (clinical stage III) PBC patients (Table 2). These results imply that male PBC patients constitute a high-risk group for progression to the jaundice stage.

The following alleles are wild-types (major types): “C” at rs683369 SNP, “C” at rs2282143 SNP, “A” at rs622342 SNP, and “A” at rs1443844 SNP. The other alleles are variants (minor types). The distribution of SNPs in *OCT-1* among PBC patients corresponded well to the

Table 2 Comparison of demographics between control subjects and PBC patients, early-stage and late-stage PBC patients, and non-jaundice-stage and jaundice-stage PBC patients

Characteristics	Control subjects	PBC	<i>P</i> value	Early stage (stage I)	Late stage (stages II + III)	<i>P</i> value	Non-jaundice stage (stages I + II)	Jaundice stage (stage III)	<i>P</i> value
Total number of patients	194	275		194	81		260	15	
Mean age (years)	39.8 ± 9.28	65.0 ± 11.7	0.0001	63.5 ± 11.05	68.4 ± 10.38	0.0002	65.4 ± 11.10	59.8 ± 9.39	0.070
Observation period (months)	–	–	–	63.8 ± 46.85	83.7 ± 62.12	0.0039	68.7 ± 51.69	85.7 ± 73.13	0.223
Male/female (%)	17/177 (8.8/91.2)	39/236 (14.1/85.9)	0.074	22/172 (11.3/88.7)	17/64 (20.9/79.1)	0.065	33/227 (12.7/87.3)	6/9 (40.0/60.0)	0.011

Hardy–Weinberg equilibrium, implying that our sample had a homogeneous genetic background.

OCT-1 genetic polymorphisms that confer susceptibility to the development and progression of PBC in an allele model

A Chi-square test revealed that the frequency of the “G” variant allele at rs683369 SNP was significantly decreased in PBC patients as compared to healthy control subjects (16.0 vs. 10.0 %; $P = 0.009$, OR 0.60, 95 % CI 0.40–0.88), as shown in Table 3. As shown in Table 5, this test also showed that the frequency of the following alleles tended to be higher in jaundice-stage PBC patients as compared to non-jaundice-stage PBC patients: the “G” variant allele at rs683369 SNP (20.0 vs. 9.0 %; $P = 0.054$), the “T” variant allele at rs2282143 SNP (30.0 vs. 18.0 %; $P = 0.084$), and the “C” variant allele at rs622342 SNP (27.0 vs. 14.0 %; $P = 0.068$). There was no allele that showed significant increase in late-stage as compared to early-stage PBC patients (Table 4).

OCT1 genetic polymorphisms that confer susceptibility to the development and progression of PBC in minor allele dominant or recessive genotype models

The distributions and frequencies of genotypes at the four SNP sites were compared between patients with early- and late-stage PBC and those at the non-jaundice and jaundice

stages, as well as between PBC patients and healthy control subjects. A Chi-square test revealed several findings. First, the frequency of the “C/G or G/G” genotypes at rs683369 SNP was significantly higher in healthy control subjects as compared to PBC patients (18.9 vs. 27.3 %; $P = 0.031$, OR 0.62, 95 % CI 0.40–0.96) (Table 3). Moreover, as shown in Table 3, “G/G” genotypes at rs683369 SNP were significantly more frequent in healthy control subjects as compared to PBC patients (0.7 vs. 3.7 %; $P = 0.025$, OR 0.20, 95 % CI 0.04–0.95). Second, the frequency of the “C/G or G/G” genotypes at rs683369 SNP were significantly higher in jaundice-stage PBC patients as compared to non-jaundice-stage PBC patients (40.0 vs. 17.7 %; $P = 0.032$, OR 3.10, 95 % CI 1.05–9.14) (Table 5). Third, the frequency of the “T/T” genotype at rs2282143 SNP was significantly higher in jaundice-stage PBC patients as compared to non-jaundice-stage PBC patients (20.0 vs. 2.4 %; $P = 0.0002$, OR 10.58, 95 % CI 2.36–47.54) (Table 5). Finally, as shown in Table 5, the frequency of the “C/C” genotype at rs622342 SNP was significantly higher in jaundice-stage PBC patients as compared to non-jaundice-stage PBC patients (13.3 vs. 1.9 %; $P = 0.006$, OR 7.84, 95 % CI 1.39–44.4).

Replication study in PBC patients undergoing liver transplantation

OCT-1 rs683369, rs2282143, and rs622342 SNPs were significantly associated with the progression of PBC. In

Table 3 Allele and genotype comparisons of tag SNPs in three inheritance models: control subjects vs. PBC patients

Gene symbol	Tag SNP (major > minor)	Genotype	Number of genotypes (%)		Inheritance model	P value	OR	95 % CI
			Control subjects	PBC				
<i>OCT-1/SLC22a1</i>	rs683369 (C > G)	MAF	0.16	0.10	Allele	0.009	0.60	0.40–0.88
		C/C	141 (72.6)	223 (81.1)	Dominant	0.031	0.62	0.40–0.96
		C/G	46 (23.7)	50 (18.2)				
		G/G	7 (3.7)	2 (0.7)	Recessive	0.025	0.20	0.04–0.95
	rs2282143 (C > T)	MAF	0.16	0.17	Allele	0.502	1.13	0.79–1.58
		C/C	138 (71.1)	184 (66.9)	Dominant	0.331	1.22	0.81–1.82
		C/T	48 (24.7)	82 (29.8)				
		T/T	8 (4.2)	9 (3.3)	Recessive	0.526	0.73	0.27–1.93
	rs622342 (A > C)	MAF	0.18	0.15	Allele	0.270	0.82	0.57–1.16
		A/A	133 (68.6)	199 (72.4)	Dominant	0.372	0.83	0.55–1.24
		A/C	53 (27.3)	69 (25.1)				
		C/C	8 (4.1)	7 (2.5)	Recessive	0.339	0.61	0.21–1.70
rs1443844 (A > G)	MAF	0.29	0.30	Allele	0.905	1.02	0.76–1.35	
	A/A	97 (50.0)	135 (49.1)	Dominant	0.359	1.19	0.82–1.70	
	A/G	79 (40.7)	115 (41.8)					
	G/G	18 (9.3)	25 (9.1)	Recessive	0.944	0.98	0.51–1.84	

Allele, allele model; Dominant, minor allele dominant model; Recessive, minor allele recessive model

Abbreviations: MAF, minor allele frequency; OR, odds ratio; CI, confidence interval

Table 4 Allele and genotype comparisons of tag SNPs in three inheritance models: PBC progression in early- vs. late-stage patients

Gene symbol	Tag SNP (major > minor)	Genotype	Number of genotypes (%)		Inheritance model	P value	OR	95 % CI
			Early stage	Late stage				
<i>OCT-1/SLC22a1</i>	rs683369 (C > G)	MAF	0.10	0.10	Allele	0.976	1.01	0.55–1.87
		C/C	157 (80.9)	66 (81.5)	Dominant	0.914	0.96	0.50–1.88
		C/G	36 (18.6)	14 (17.3)				
		G/G	1 (0.5)	1 (1.2)	Recessive	0.522	2.41	0.14–39.07
	rs2282143 (C > T)	MAF	0.17	0.20	Allele	0.389	1.23	0.77–1.95
		C/C	132 (68.0)	52 (64.2)	Dominant	0.537	1.19	0.69–2.04
		C/T	57 (29.4)	25 (30.9)				
		T/T	5 (2.6)	4 (4.9)	Recessive	0.316	1.96	0.51–7.51
	rs622342 (A > C)	MAF	0.15	0.16	Allele	0.685	1.11	0.67–1.84
		A/A	141 (72.7)	58 (71.6)	Dominant	0.856	1.05	0.59–1.88
		A/C	49 (25.3)	20 (24.7)				
		C/C	4 (2.1)	3 (3.7)	Recessive	0.401	1.83	0.40–8.35
rs1443844 (A > G)	MAF	0.30	0.29	Allele	0.744	0.94	0.63–1.40	
	A/A	95 (49.0)	40 (49.4)	Dominant	0.950	0.98	0.58–1.65	
	A/G	80 (41.2)	35 (43.2)					
	G/G	19 (9.8)	6 (7.2)	Recessive	0.649	0.74	0.28–1.91	

Allele, allele model; Dominant, minor allele dominant model; Recessive, minor allele recessive model
Abbreviations: MAF, minor allele frequency; OR, odds ratio; CI, confidence interval

Table 5 Allele and genotype comparisons of tag SNPs in three inheritance models: PBC progression in non-jaundice vs. jaundice-stage patients

Gene symbol	Tag SNP (major > minor)	Genotype	Number of Genotypes (%)		Inheritance model	P value	OR	95 % CI
			Non-jaundice stage	Jaundice stage				
<i>OCT-1/SLC22a1</i>	rs683369 (C > G)	MAF	0.09	0.20	Allele	0.054	2.46	0.95–6.31
		C/C	214 (82.3)	9 (60.0)	Dominant	0.032	3.10	1.05–9.14
		C/G	44 (16.9)	6 (40.0)				
		G/G	2 (0.8)	0 (0.0)	Recessive	0.733	3.34	0.15–72.58
	rs2282143 (C > T)	MAF	0.18	0.30	Allele	0.084	2.02	0.89–4.55
		C/C	175 (67.3)	9 (60.0)	Dominant	0.558	1.37	0.47–3.98
		C/T	79 (30.3)	3 (20.0)				
		T/T	6 (2.4)	3 (20.0)	Recessive	0.0002	10.58	2.36–47.54
	rs622342 (A > C)	MAF	0.14	0.27	Allele	0.068	2.16	0.92–5.02
		A/A	190 (73.1)	9 (60.0)	Dominant	0.271	1.81	0.62–5.27
		A/C	65 (25.0)	4 (26.7)				
		C/C	5 (1.9)	2 (13.3)	Recessive	0.006	7.84	1.39–44.36
rs1443844 (A > G)	MAF	0.30	0.23	Allele	0.413	0.70	0.29–1.65	
	A/A	126 (48.5)	9 (60.0)	Dominant	0.384	0.63	0.22–1.81	
	A/G	110 (42.3)	5 (33.3)					
	G/G	24 (9.2)	1 (6.7)	Recessive	0.737	0.70	0.08–5.57	

Allele, allele model; Dominant, minor allele dominant model; Recessive, minor allele recessive model
Abbreviations: MAF, minor allele frequency; OR, odds ratio; CI, confidence interval

order to verify the reproducibility of this result, we performed a replication study in PBC patients who underwent liver transplantation. A Chi-square test revealed that the frequencies of the “C/G or G/G” genotypes at rs683369

SNP were significantly higher in liver transplantation cases as compared to non-jaundice-stage PBC patients (34.3 vs. 17.7 %; $P = 0.021$, OR 2.42, 95 % CI 1.12–5.23) (Table 6). This was also the case for the “C/C” genotype at

Table 6 Replication study in PBC patients who underwent liver transplantation

Gene symbol	Tag SNP (major > minor)	Genotype	Number of Genotypes (%)		Inheritance model	P value	OR	95 % CI
			Non-jaundice stage	Liver transplantation				
<i>OCT-1/SLC22a1</i>	rs683369 (C > G)	MAF	0.09	0.20	Allele	0.0058	2.46	1.28–4.74
		C/C	214 (82.3)	23 (65.7)	Dominant	0.021	2.42	1.12–5.23
		C/G	44 (16.9)	10 (28.6)				
		G/G	2 (0.8)	2 (5.7)	Recessive	0.017	7.82	1.06–57.40
	rs2282143 (C > T)	MAF	0.18	0.19	Allele	0.825	1.08	0.56–2.04
		C/C	175 (67.3)	22 (62.9)	Dominant	0.599	1.22	0.58–2.53
		C/T	79 (30.3)	13 (37.1)				
		T/T	6 (2.4)	0 (0.0)	Recessive	0.364	–	–
	rs622342 (A > C)	MAF	0.14	0.24	Allele	0.033	1.90	1.05–3.46
		A/A	190 (73.1)	23 (65.7)	Dominant	0.361	1.42	0.67–3.00
		A/C	65 (25.0)	7 (20.0)				
		C/C	5 (1.9)	5 (14.3)	Recessive	0.0001	8.50	2.32–31.08
rs1443844 (A > G)	MAF	0.30	0.29	Allele	0.890	0.92	0.53–1.59	
	A/A	126 (48.5)	16 (45.7)	Dominant	0.760	1.12	0.54–2.26	
	A/G	110 (42.3)	18 (51.4)					
	G/G	24 (9.2)	1 (2.9)	Recessive	0.203	0.29	0.04–2.21	

Allele, allele model; Dominant, minor allele dominant model; Recessive, minor allele recessive model

Abbreviations: MAF, minor allele frequency; OR, odds ratio; CI, confidence interval

Table 7 Combined analysis of jaundice-stage patients in the initial cohort and PBC patient who underwent liver transplantation in the replication cohort

Gene symbol	Tag SNP (major > minor)	Genotype	Number of genotypes (%)		Inheritance model	P value	OR	95 % CI
			Non-jaundice stage	Jaundice stage				
<i>OCT-1/SLC22a1</i>	rs683369 (C > G)	MAF	0.09	0.20	Allele	0.0016	2.46	1.39–4.36
		C/C	214 (82.3)	32 (64.0)	Dominant	0.0034	2.62	1.35–5.06
		C/G	44 (16.9)	16 (32.0)				
		G/G	2 (0.8)	2 (4.0)	Recessive	0.063	5.38	0.74–39.11
	rs2282143 (C > T)	MAF	0.18	0.22	Allele	0.286	1.33	0.79–2.25
		C/C	175 (67.3)	31 (62.0)	Dominant	0.467	1.26	0.67–2.36
		C/T	79 (30.3)	16 (32.0)				
		T/T	6 (2.4)	3 (6.0)	Recessive	0.154	2.70	0.65–11.19
	rs622342 (A > C)	MAF	0.14	0.25	Allele	0.0084	1.98	1.18–3.31
		A/A	190 (73.1)	32 (64.0)	Dominant	0.192	1.52	0.81–2.89
		A/C	65 (25.0)	11 (22.0)				
		C/C	5 (1.9)	7 (14.0)	Recessive	0.0001	8.30	2.52–27.36
rs1443844 (A > G)	MAF	0.30	0.27	Allele	0.498	0.85	0.52–1.37	
	A/A	126 (48.5)	25 (50.0)	Dominant	0.842	0.94	0.51–1.72	
	A/G	110 (42.3)	23 (46.0)					
	G/G	24 (9.2)	2 (4.0)	Recessive	0.222	0.41	0.09–1.79	

Allele, allele model; Dominant, minor allele dominant model; Recessive, minor allele recessive model

Abbreviations: MAF, minor allele frequency; OR, odds ratio; CI, confidence interval

rs622342 SNP (14.3 vs. 1.9 %; $P = 0.0001$, OR 8.50, 95 % CI 2.32–31.08) (Table 6). When the NHOSLJ cohort was combined with the replication cohort, “C/G or G/G” genotypes at rs683369 SNP were significantly higher in jaundice-stage PBC

patients ($P = 0.0034$, OR 2.62, 95 % CI 1.35–5.06) as compared to non-jaundice-stage PBC patients (Table 7). This was also the case for “C/C” genotype at rs622342 SNP ($P = 0.0001$, OR 8.30, 95 % CI 2.52–27.36).

Multivariate analysis

Since the presence of anti-gp210 antibodies is a strong risk factor for the progression to jaundice-stage in PBC, the three *OCT-1* SNPs (“C/G” or “G/G” genotype at rs683369, “T/T” genotype at rs2282143 and “C/C” genotype at rs622342), which revealed significant risk factors for the progression to jaundice-stage in the present study, were independently evaluated by multivariate analysis in conjunction with anti-gp210 antibodies-status and male sex (Table 8). While the positive anti-gp210 antibodies status revealed the most significant risk factor for the progression to jaundice-stage in each analysis ($P < 0.0001$, OR 12.84–13.75), only “C/C” genotype at rs622342 remained a significant risk factor for the progression to jaundice-stage ($P = 0.010$, OR 10.144, 95 % CI 1.75–58.71). The “C/G” or “G/G” genotype at rs683369 and “T/T” genotype at rs2282143 showed a trend of risk for jaundice-stage progression.

Discussion

The present study is the first to demonstrate the association of *OCT-1* genotypes with the progression of PBC in the Japanese population. The results showed that the rs683369 genotype “C/G or G/G” and the rs622342 genotype “C/C” in *OCT-1* were closely associated with the susceptibility to severe progression (especially jaundice-type) of PBC in the Japanese population. Conversely, genotypes “C/C” in rs683369 and “A/A or A/C” in rs622342 were associated with insusceptibility to the progression of PBC. These findings suggest that *OCT-1* is one of the genetic determinants for the predisposition to severe progression of PBC in the Japanese population. However, it remains to be confirmed whether this association is reproducible in a

larger number of Japanese PBC patients, as well as in other ethnic populations.

OCT-1 is one of the most abundant transporters responsible for the uptake of choline from sinusoidal blood across the basolateral membrane of hepatocytes. Following the synthesis of PC from choline in hepatocytes, PC is secreted into bile ducts via MDR3 [32, 33, 38, 43]. Secreted PC reduces the cytotoxic effects of bile acids by combining with them to form micelles [34–37]. In addition, some reports have shown that the amount of *OCT-1* protein in hepatocytes is decreased during cholestasis [39, 44, 45]. It has been reported that 69 of approximately 650 SNPs identified in the *OCT-1* gene are accompanied by amino acid changes. Approximately 15 % of these non-synonymous 69 SNPs are reportedly associated with alterations in *OCT-1* expression and/or function in vitro [39]. Collectively, these reports indicate that genetic polymorphisms of *OCT-1* are potentially involved in the pathogenesis of cholestatic liver diseases, including PBC. Our own results are consistent with this suggestion, demonstrating for the first time that variant genotypes of rs683369 and rs622342 contribute to the more severe progression of PBC (i.e., jaundice-type progression).

In addition to choline, several drugs, including metformin, amantadine, and levodopa are substrates of *OCT-1* [46–48]. Several reports have investigated the relation between *OCT-1* genetic polymorphisms and the effects of these drugs. For example, the effect of metformin is decreased in patients with the rs622342 SNP genotype “C/C” [40]. Since metformin must be transported into hepatocytes in order to exert its antihyperglycemic effect, it is possible that this transport does not occur at sufficient levels in patients with the rs622342 SNP genotype “C/C”. Thus, it is reasonable to speculate that patients with the rs622342 SNP genotype “C/C” who have altered function of *OCT-1* show more severe PBC progression due to decreased supply of choline into hepatocytes, resulting in insufficient secretion of PC into bile ducts. In fact, an insufficient supply of choline into hepatocytes is observed in PBC livers at the mRNA and protein levels (manuscript in preparation). *OCT-1* rs683369 is a non-synonymous SNP (*OCT-1* Phe160Leu) that influences the mRNA expression of *OCT-1*, but not its transporter activity or its affinity to its substrates (e.g., metformin, 1-methyl-4-phenylpyridinium) [39, 49, 50]. On the other hand, the rs622342 SNP is located in an intron, and its exact influence on *OCT-1* expression and function is unknown. *OCT-1* rs683369 and rs622342 SNPs are in linkage disequilibrium (linkage disequilibrium coefficient, $D' = 0.826$, $r^2 = 0.365$). In addition, new genetic polymorphisms that influence the expression, location, and function of *OCT-1* have recently been reported in East Asian populations. However, the degree of their linkage

Table 8 Multivariate analysis for the progression to jaundice stage

Factor	<i>P</i> value	OR	95 % CI
rs683369 (risk genotype) ^a	0.087	2.29	0.89–5.92
gp210 positive	0.0001	12.84	3.71–44.51
Sex (male)	0.312	1.74	0.59–5.08
rs2282143 (risk genotype) ^a	0.062	4.97	0.92–26.73
gp210 positive	0.0001	12.97	3.73–45.06
Sex (male)	0.335	1.71	0.57–5.10
rs622342 (risk genotype) ^a	0.010	10.14	1.75–58.71
gp210 positive	0.0001	13.75	3.86–49.04
Sex (male)	0.799	1.17	0.34–3.99

Abbreviations: OR, odds ratio; CI, confidence interval

^a rs683369 (C/G, G/G), rs2282143 (T/T), rs622342 (C/C)

disequilibrium with the rs683369 and rs622342 SNPs is still unknown [51]. Collectively, these findings suggest the possibility that other still unidentified genetic polymorphisms in linkage disequilibrium with the rs683369 and/or rs622342 SNPs contribute to the severe progression of PBC.

Since the presence of anti-gp210 antibodies is the strongest risk factor so far identified for the progression to jaundice-stage in PBC [2, 5, 52], the risk of three *OCT-1* SNPs (“C/G” or “G/G” genotype at rs683369, “T/T” genotype at rs2282143 and “C/C” genotype at rs622342) for the progression to jaundice-stage were evaluated by multivariate analysis in conjunction with anti-gp210 antibodies-status. While the positive anti-gp210 antibodies-status revealed the most significant risk factor for the progression to jaundice-stage, only the “C/C” genotype at rs622342 remained a significant risk factor for this progression. The “C/G” or “G/G” genotype at rs683369 and “T/T” genotype at rs2282143 showed a trend of risk for jaundice-stage progression. These results indicate that the three SNPs (*OCT-1* rs683369, rs2282143 and rs622342) might be risk factors for jaundice-stage progression, independent from anti-gp210 antibodies-status.

In conclusion, our results clearly indicate that *OCT-1* genetic polymorphisms are closely associated with the severe progression (i.e., jaundice-type progression) of PBC. This implies that the genotyping of *OCT-1* could be potentially useful for DNA-based diagnosis in Japanese patients with PBC as a genetic biomarker for predicting the progression and prognosis.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. *N Engl J Med.* 2005;353:1261–73.
2. Nakamura M, Kondo H, Mori T, et al. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. *Hepatology.* 2007;45:118–27.
3. Gershwin ME, Ansari AA, Mackay IR, et al. Primary biliary cirrhosis: an orchestrated immune response against epithelial cells. *Immunol Rev.* 2000;174:210–25.
4. Invernizzi P, Podda M, Battezzati PM, et al. Autoantibodies against nuclear pore complexes are associated with more active and severe liver disease in primary biliary cirrhosis. *J Hepatol.* 2001;34:366–72.
5. Nakamura M, Shimizu-Yoshida Y, Takii Y, et al. Antibody titer to gp210-C terminal peptide as a clinical parameter for monitoring primary biliary cirrhosis. *J Hepatol.* 2005;42:386–92.
6. Wiesierska-Gadek J, Penner E, Battezzati PM, et al. Correlation of initial autoantibody profile and clinical outcome in primary biliary cirrhosis. *Hepatology.* 2006;43:1135–44.
7. Worman HJ, Courvalin J-C. Antinuclear antibodies specific for primary biliary cirrhosis. *Autoimmun Rev.* 2003;2:211–7.
8. Poupon R. Primary biliary cirrhosis: a 2010 update. *J Hepatol.* 2010;52(5):745–58.
9. Corpechot C, Carrat F, Bahr A, et al. The effect of ursodeoxycholic acid therapy on the natural course of primary biliary cirrhosis. *Gastroenterology.* 2005;128(2):297–303.
10. Selmi C, Invernizzi P, Zuin M, et al. Genes and (auto)immunity in primary biliary cirrhosis. *Genes Immun.* 2005;6:543–56.
11. Selmi C, Invernizzi P, Zuin M, et al. Genes and (auto)immunity in primary biliary cirrhosis. *Genes Immun.* 2005;6(7):543–56.
12. Brind AM, Bray GP, Portmann BC, et al. Prevalence and pattern of familial disease in primary biliary cirrhosis. *Gut.* 1995;36(4):615–7.
13. Aiba Y, Nakamura M, Joshita S, et al. Genetic polymorphisms in CTLA4 and SLC4A2 are differentially associated with the pathogenesis of primary biliary cirrhosis in Japanese patients. *J Gastroenterol.* 2011;46(10):1203–12.
14. Tanaka A, Quaranta S, Mattalia A, et al. The tumor necrosis factor- α promoter correlates with progression of primary biliary cirrhosis. *J Hepatol.* 1999;30(5):826–9.
15. Selmi C, Zuin M, Biondi ML, et al. Genetic variants of endothelial nitric oxide synthase in patients with primary biliary cirrhosis: association with disease severity. *J Gastroenterol Hepatol.* 2003;18(10):1150–5.
16. Poupon R, Ping C, Chrétien Y, et al. Genetic factors of susceptibility and of severity in primary biliary cirrhosis. *J Hepatol.* 2008;49(6):1038–45.
17. Zhong B, Strnad P, Selmi C, et al. Keratin variants are over-represented in primary biliary cirrhosis and associate with disease severity. *Hepatology.* 2009;50(2):546–54.
18. Kimura Y, Selmi C, Leung PS, et al. Genetic polymorphisms influencing xenobiotic metabolism and transport in patients with primary biliary cirrhosis. *Hepatology.* 2005;41(1):55–63.
19. Juran BD, Atkinson EJ, Schlicht EM, et al. Primary biliary cirrhosis is associated with a genetic variant in the 3' flanking region of the CTLA4 gene. *Gastroenterology.* 2008;135(4):1200–6.

20. Donaldson P, Agarwal K, Craggs A, et al. HLA and interleukin 1 gene polymorphisms in primary biliary cirrhosis: associations with disease progression and disease susceptibility. *Gut*. 2001;48(3):397–402.
21. Inamine T, Nakamura M, Kawauchi A, et al. A polymorphism in the integrin α V subunit gene affects the progression of primary biliary cirrhosis in Japanese patients. *J Gastroenterol*. 2011;46(5):676–86.
22. Mellis GF, Floyd JA, Morley KI, et al. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat Genet*. 2011;43(4):329–32.
23. Hirschfield GM, Liu X, Xu C, et al. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med*. 2009;360:2544–55.
24. Hirschfield GM, Liu X, Han Y, et al. Variants at IRF5-TNPO3, 17q12-21 and MMEL1 are associated with primary biliary cirrhosis. *Nat Genet*. 2010;42(8):655–7.
25. Tanaka A, Invernizzi P, Ohira H, et al. Replicated association of 17q12-21 with susceptibility of primary biliary cirrhosis in a Japanese cohort. *Tissue Antigens*. 2011;78(1):65–8.
26. Liu X, Invernizzi P, Lu Y, et al. Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis. *Nat Genet*. 2010;42(8):658–60.
27. Nakamura M, Nishida N, Kawashima M, et al. Genome-wide association study identifies TNFSF15 and POU2AF1 as susceptibility loci for primary biliary cirrhosis in the Japanese population. *Am J Hum Genet*. 2012;91:721–8.
28. Pauli-Magnus C, Lang T, Meier Y, et al. Sequence analysis of bile salt export pump (*ABCB11*) and multidrug resistance p-glycoprotein 3 (*ABCB4*, *MDR3*) in patients with intrahepatic cholestasis of pregnancy. *Pharmacogenetics*. 2004;14:91–102.
29. de Vree JML, Jacquemin E, Sturm E, et al. Mutations in the *MDR3* gene cause progressive familial intrahepatic cholestasis. *Proc Natl Acad Sci USA*. 1998;95:282–7.
30. Jacquemin E, de Vree JML, Cresteil D, et al. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. *Gastroenterology*. 2001;120:1448–58.
31. Ohishi Y, Nakamura M, Iio N, et al. Single-nucleotide polymorphism analysis of the multidrug resistance protein 3 gene for the detection of clinical progression in Japanese patients with primary biliary cirrhosis. *Hepatology*. 2008;48(3):853–62.
32. Smit JJ, Schinkel AH, Oude Elferink RP, et al. Homozygous disruption of the murine *mdr2* P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell*. 1993;75:451–62.
33. Oude Elferink RP, Ottenhoff R, van Wijland M, et al. Regulation of biliary lipid secretion by *mdr2* P-glycoprotein in the mouse. *J Clin Invest*. 1995;95:31–8.
34. Ali S, Zakim D. The effects of bilirubin on the thermal properties of phosphatidylcholine bilayers. *Biophys J*. 1993;65:101–5.
35. Oude Elferink RP, Paulusma CC. Function and pathophysiological importance of ABCB4 (*MDR3* P-glycoprotein). *Eur J Physiol*. 2007;453:601–10.
36. Michel V, Yuan Z, Ramsuvar S, et al. Choline transport for phospholipid synthesis. *Exp Biol Med (Maywood)*. 2006;231(5):490–504.
37. Sinclair CJ, Chi KD, Subramanian V, et al. Functional expression of a high affinity mammalian hepatic choline/organic cation transporter. *J Lipid Res*. 2000;41(11):1841–8.
38. Zeisel SH, Da Costa KA, Franklin PD, et al. Choline, an essential nutrient for humans. *FASEB J*. 1991;5(7):2093–8.
39. Nies AT, Koepsell H, Winter S, et al. Expression of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) is affected by genetic factors and cholestasis in human liver. *Hepatology*. 2009;50(4):1227–40.
40. Becker ML, Visser LE, van Schaik RH, et al. Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus. *Pharmacogenomics J*. 2009;9(4):242–7.
41. Lindor KD, Gershwin ME, Poupon R, et al. Primary biliary cirrhosis. *Hepatology*. 2009;50(1):291–308.
42. Scheuer PJ. Primary biliary cirrhosis. *Proc R Soc Med*. 1967;60:1257–60.
43. van Helvoort A, Smith AJ, Sprong H, et al. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. *Cell*. 1996;87:507–17.
44. Jin HE, Hong SS, Choi MK, et al. Reduced antidiabetic effect of metformin and down-regulation of hepatic Oct1 in rats with ethynyl estradiol-induced cholestasis. *Pharm Res*. 2009;26(3):549–59.
45. Denk GU, Soroka CJ, Mennone A, et al. Down-regulation of the organic cation transporter 1 of rat liver in obstructive cholestasis. *Hepatology*. 2004;39(5):1382–9.
46. Becker ML, Visser LE, van Schaik RH, et al. OCT1 polymorphism is associated with response and survival time in anti-Parkinsonian drug users. *Neurogenetics*. 2011;12(1):79–82.
47. Klaassen CD, Aleksunes LM. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev*. 2010;62(1):1–96.
48. Jonker JW, Schinkel AH. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). *J Pharmacol Exp Ther*. 2004;308(1):2–9.
49. Sakata T, Anzai N, Shin HJ, et al. Novel single nucleotide polymorphisms of organic cation transporter 1 (SLC22A1) affecting transport functions. *Biochem Biophys Res Commun*. 2004;313(3):789–93.
50. Shu Y, Leabman MK, Feng B, et al. Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. *Proc Natl Acad Sci USA*. 2003;100(10):5902–7.
51. Chen L, Takizawa M, Chen E, et al. Genetic polymorphisms in organic cation transporter 1 (OCT1) in Chinese and Japanese populations exhibit altered function. *J Pharmacol Exp Ther*. 2010;335(1):42–50.
52. Nakamura M, Yasunami M, Kondo H, et al. Analysis of HLA-DRB1 polymorphisms in Japanese patients with primary biliary cirrhosis (PBC): the HLA-DRB1 polymorphism determines the relative risk of antinuclear antibodies for disease progression in PBC. *Hepatol Res*. 2010;40:494–504.

<原 著>

硬化性胆管炎の全国調査

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要旨：われわれは今回、2005 年以降に診断された PSC 並びに自己免疫性膵炎を合併していない IgG4 関連硬化性胆管炎 (IgG4-related sclerosing cholangitis ; IgG4-SC) を対象として、アンケートによる全国調査を行った。PSC 197 例、IgG4-SC 43 例が集計された。PSC と比較して IgG4-SC は有意に男性、高齢者に多かった。PSC の年齢分布は前回全国調査同様若年者・高齢者に 2 つのピークがあった。IgG4-SC の診断に対する血清 IgG4 値の感度・特異度は 89.5%・87.6% であった。病変部位は PSC では「肝内外」、IgG4-SC では「肝内のみ」が最も多かった。PSC における炎症性腸疾患の合併は 34% にとどまっており、前回全国調査同様比較的低率であった。PSC の 3 年生存率は 85.0%、IgG4-SC の 3 年生存率は 90.0% で、IgG4-SC の方が予後良好であった。

索引用語： 原発性硬化性胆管炎 IgG4 関連 自己免疫性膵炎

はじめに

原発性硬化性胆管炎 (primary sclerosing cholangitis ; PSC) は肝内外の胆管に多発性・びまん性の狭窄が生じ、胆汁うっ滞を来す慢性肝疾患であり、病理学的には胆管壁の線維性肥厚を特徴とする¹⁾。われわれは、過去 1997 年・2003 年の 2 回にわたって原発性硬化性胆管炎についての全国調査を行い、本邦の PSC 症例の年齢分布は欧米とは異なり二峰性であり、高齢者において欧米にはみられないピークが存在すること、および炎症性腸疾患の合併が少なく、その反面高齢者に自己免疫性膵炎 (autoimmune pancreatitis ; AIP) の合併がみられることを報告した²⁾³⁾。しかしその後、主として本邦の研究者によって、IgG4 関連硬化性胆管炎 (IgG4-related sclerosing cholangitis ; IgG4-SC) の疾患概念が報告・確立される^{4)~7)}に伴い、過去 2 回の全国調査において PSC として報告された症例の中に IgG4-SC が混入

しており、ことに AIP を合併した症例は実際には IgG4-SC である可能性が指摘された。この両疾患は治療法が大きく異なることから、PSC と IgG4-SC との臨床像の類似点・相違点を把握し、治療開始前に両疾患を適切に鑑別することは極めて重要である。しかし 2003 年当時には未だ IgG4-SC という疾患概念が広く知られておらず、診断基準も存在しなかったことから、前回の全国調査時には PSC と IgG4-SC とを鑑別することは困難であった。

しかし、その後 IgG4-SC についての報告が相次ぎ、PSC との画像診断および病理学的な差異についての知見も集積された^{8)~10)}。2012 年には「IgG4 関連硬化性胆管炎臨床診断基準 2012」が公表され¹¹⁾、統一した基準によって PSC と IgG4-SC との鑑別診断が可能となった。これに伴い、われわれは本邦における PSC、および PSC との鑑別が困難である AIP を合併していない IgG4-SC の実態、および両者の臨床像の相違を把握するため、改めて全国調査を行った。

対象と方法

本調査は前々回・前回の全国調査と同様、日本全国の各施設に PSC および IgG4-SC についてのアンケートを送付し、調査対象に該当する症例が存在する場合にはその臨床情報を記入・送付いただく方式をとった。アンケートの送付先は、日本胆道学会評議員、厚生労働省「難治性の肝・胆道疾患に関する調査研究」班

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表 1 PSC と IgG4-SC の臨床像の比較

	PSC	IgG4-SC	P value
sex (male : female)	106 : 91	33 : 10	0.006
age* ¹	48.1 [4.0-86.3]	69.3 [47.6-87.4]	<0.001
<i>Symptoms at presentation</i>			
none	100 (55%)	22 (54%)	NS
jaundice	46 (25%)	9 (22%)	NS
cholangitis	37 (20%)	7 (17%)	NS
skin itching	31 (17%)	8 (20%)	NS
<i>Laboratory data*¹</i>			
TP	7.5 [4.8-9.8]	7.7 [6-11.8]	NS
Alb	3.9 [1.3-4.9]	3.5 [2.2-4.8]	<0.001
T.Bil	1.0 [0.2-29.4]	0.9 [0.4-27.2]	NS
AST	55 [10-751]	44 [17-426]	NS
ALT	60 [7-927]	42 [7-260]	NS
ALP (xUNL)	2.25 [0.32-17.0]	2.05 [0.30-13.74]	NS
γGTP	236 [11-2975]	265.5 [17-1344]	NS
IgG	1623.5 [508-4456]	2303 [680-6615]	<0.001
IgG4	48.9 [3.0-369]	519.5 [22.2-2470]	<0.001
IgA	284 [43.2-1597]	272.5 [53-963]	NS
IgM	119 [24-599]	80.5 [20-247]	<0.001
IgE	177 [4-1816]	703 [20-3550]	NS
CEA	1.99 [0.3-28]	2.4 [0.7-14.7]	NS
CA19-9	19.85 [0.6-6957.2]	31.2 [2-4862]	NS
<i>Detection of autoantibodies*²</i>			
ANA	58/105 (36%)	14/17 (45%)	NS
pANCA	2/88 (2%)	1/11 (8%)	NS
cANCA	3/48 (6%)	0/4 (0%)	NS

*¹ 年齢, 血液検査値は, 中央値 [最小値 - 最大値] を表す.

*² 自己抗体の検出は, 陽性数/陰性数 (陽性率) を表す.

員, および「IgG4 関連全身硬化性疾患の診断法の確立と治療方法の開発に関する研究」班班員の所属する施設とし, 全国で計 144 施設であった.

調査対象は, 本邦において IgG4-SC の疾患概念がおおむね広く認識されたと推定される 2005 年 1 月以降に各施設で診断された PSC, および AIP を合併していない IgG4-SC 症例とした. AIP を合併した IgG4-SC については, 臨床的に PSC との鑑別はさほど困難ではないと考えられるため, 調査対象から外した. PSC の診断には Mayo Clinic による診断基準を本邦の実情にあわせて改訂した基準¹²⁾, IgG4-SC の診断には 2012 年に公表された診断基準¹¹⁾を用いた. AIP の合併・非合併の診断は各施設における画像診断によって行った.

統計解析には IBM® SPSS® Statistics, version 19 を用いた. PSC と IgG4-SC の間の比較では, 連続量では Mann-

Whitney's U test, 非連続量ではカイ二乗検定を用い, 多重比較を考慮して $P < 0.01$ を有意とした. 生存曲線の作成には Kaplan-Meier 法を用いた. 本調査は帝京大学医学部倫理委員会の審査・承認を得た上で実施された.

結 果

全国 144 施設のうち, 46 施設からアンケートが返送された. その結果, PSC 197 例, IgG4-SC 43 例が集計された.

診断時情報

PSC および IgG4-SC の診断時所見を表 1 に示す. 男女比は, PSC では男性 106 例・女性 91 例とやや男性が多かったのに対し, IgG4-SC は男性 33 例に対して女性 10 例であり, PSC と比較して IgG4-SC は有意に男性に多かった ($p = 0.006$). 診断時年齢の中央値は PSC 48.1

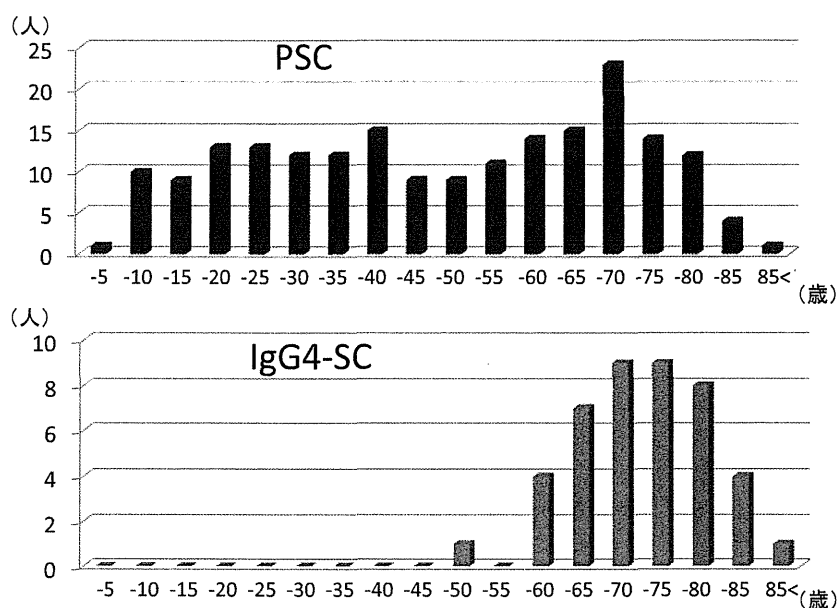


図1 PSC・IgG4-SC 症例の年齢分布

歳, IgG4-SC 69.3 歳で, IgG4-SC 症例は有意に高齢であった ($p < 0.001$). 診断時年齢の分布を図 1 に示す. PSC では 20 歳から 40 歳にかけてなだらかなピークがあり, 40 歳から 60 歳にかけて症例数が減少した後, 高齢層で再び増加して 65 歳から 70 歳にかけて症例数が最高となるという, 二峰性の年齢分布をとった. 一方, IgG4-SC では 45 歳以下での発症例はなく, 発症のピークは 65 歳から 75 歳であり年齢分布は一峰性となった. 診断時症状では両者の間に有意な差異はみられず, 症状がなかった症例が PSC 55%, IgG4-SC 54% と最も高頻度であった (表 1).

診断時の血液所見では (表 1), PSC で有意に高値だったのがアルブミンと IgM, IgG4-SC で有意に高値だったのが IgG と IgG4 であった. アルブミンは PSC では中央値が 3.9g/dl であり, 3.5g/dl を下回っている症例が 193 例中 62 例 (32%) であったのに対し, IgG4-SC では中央値 3.5g/dl で, 3.5g/dl を下回っている症例が 38 例中 16 例 (42%) であった (図 2). IgM は PSC で有意に高値であり, 基準値範囲内 ($\leq 200\text{mg/dl}$) の症例は, PSC では IgM が測定されていた 169 例中 129 例 (76%) であったのに対し, IgG4-SC では 36 例中 35 例 (97%) であり, IgG4-SC で IgM が基準値を超えていたのは 1 例のみであった (図 3). IgG4 は IgG4-SC で有意に高値であったが, 135mg/dl をカットオフとし

た場合, PSC で 135mg/dl を超えていた症例が 15 例 (12%) 存在した一方, IgG4-SC で 135mg/dl 以下だった症例は 4 例 (11%) であった (図 4, 表 2). このことから, カットオフ 135mg/dl とした場合の血清 IgG4 値の IgG4-SC 診断に対する感度・特異度は, それぞれ 89.5%, 87.6% となった. IgE については, 平均値は PSC で 177mg/dl, IgG4-SC で 703mg/dl であり, IgG4-SC で高値ではあったものの, 測定されていた症例が PSC では 73 例, IgG4-SC では 19 例と少なかったこともあり, $P = 0.056$ となり有意水準には達しなかった. ALP 値の分布は両者ともほぼ同等であり, 基準値上限の 2 倍以内であった症例が PSC 86 例 (全体の 44%)・IgG4-SC 20 例 (47%), 基準値範囲内であった症例も PSC で 27 例 (14%)・IgG4-SC で 9 例 (21%) 存在した (図 5).

画像・病理所見

病変部位については, 簡略化して「肝内のみ」「肝内外」「肝外のみ」の 3 つの選択肢から回答するという形式とした. その結果, PSC では「肝内外」との回答が 52% と最も多かったのに対し, IgG4-SC では「肝内のみ」との回答が 58% を占め, 最も多かった (図 6). 生検による病理組織診断は, PSC 197 例中 125 例 (64%), IgG4-SC 43 例中 31 例 (72%) で施行されており, 肝生検は PSC 97 例 (49%)・IgG4-SC 16 例 (37%), 胆道生検は PSC 44 例 (22%), IgG4-SC 23 例 (53%) で行