Table 1. Demographics and clinical characteristics of PBC, CHC and AIH patients at study enrolment

	PBC n = 110	CHC n = 26	AIH n = 19
Age, mean ± SD (years)	59.8 ± 12.0	56.4 ± 10.6	58.6 ± 15.4
Women, n (%)	92 (83.6)	15 (57.7)	15 (78.9)
Early stage, n (%)	96 (87.3)	13 (50.0)	–
Late stage, n (%)	14 (12.7)	13 (50.0)	–
ALT, mean ± SD (IU/L)	55 ± 42	79 ± 50	196 ± 317
ALP, mean ± SD (IU/L)	684 ± 509	336 ± 224	461 ± 280
IgM, mean ± SD (mg/dl)	390 ± 307	119 ± 47	277 ± 375
No mediation, n (%)	90 (81.8)	22 (84.6)	7 (36.8)
UDCA alone treatment, n (%)	20 (18.2)	1 (3.8)	1 (5.3)
Pegylated interferon-α treatment, n (%)	0 (0)	1 (3.8)	0 (0)
Prednisolone alone treatment, n (%)	0 (0)	0 (0)	9 (47.4)

SD, standard deviation; ALT, alanine aminotransferase; ALP, alkaline phosphatase; UDCA, ursodeoxycholic acid

Serum TL1A levels are increased in both early- and late-stage PBC

Serum TL1A levels were significantly higher in PBC patients compared with healthy controls ($P = 8.0 \times 10^{-5}$, Fig. 1A). Serum TL1A levels were also significantly higher in CHC and AIH patients compared with healthy controls ($P = 0.04$ and $P = 0.003$ respectively). There were no significant differences in TL1A levels among PBC, CHC and AIH patients (Fig. 1A).

Figure 1B shows that serum TL1A levels in healthy controls were comparable with those in early-stage CHC patients ($P = 0.85$), but were significantly higher in patients with early-stage PBC ($P = 2.0 \times 10^{-4}$). Serum TL1A levels were significantly higher in both late-stage PBC and CHC patients as compared with healthy controls (PBC: $P = 0.002$; CHC: $P = 0.002$). Serum TL1A levels were significantly higher in late-stage than early-stage CHC patients ($P = 0.002$), a distinction not observed in PBC patients ($P = 0.15$).

Association of TL1A with biochemical parameters, prevalence of antinuclear antibodies and histological scores in PBC patients

There were no significant associations between serum TL1A levels and levels of either ALP ($P = 0.59$), alanine aminotransferase (ALT) ($P = 0.79$) or total immunoglobulin M (IgM) ($P = 0.32$) at the time of subject enrolment (Fig. S1). Serum TL1A levels were similar between PBC patients who were negative and positive for anti-gp210 antibodies and anticentromere antibodies (Fig. S2). Although serum TL1A levels showed a non-significant increase in chronic cholangitis of moderate grade (CA2) but not of minimal or mild grade (CA0–1) ($P = 0.06$), serum TL1A levels were not associated with histological scores reflecting bile duct loss, fibrosis or the grade of hepatitic change (HA) (Fig. 2).

Serum TL1A is decreased in response to UDCA treatment in patients with early-stage PBC

To evaluate the effect of UDCA on serum TL1A levels in PBC patients, we followed the levels of serum TL1A, ALP, ALT and total IgM in 76 PBC patients receiving various UDCA treatments. Specifically, these patients received UDCA alone (58; 76%), UDCA + bezafibrate (14; 18%), UDCA + bezafibrate + PSL (2; 3%) or

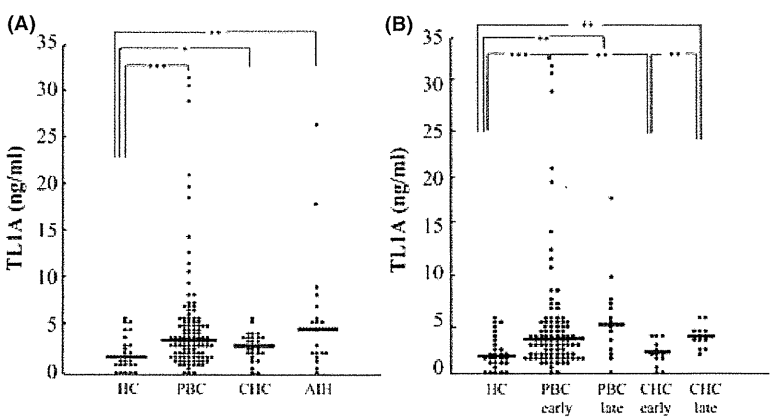

Fig. 1. Serum TL1A levels in PBC, CHC and AIH patients. (A) Serum TL1A levels were significantly higher in patients with PBC (n = 110, 4.9 ± 5.0 ng/ml), CHC (n = 26, 3.0 ± 1.5 ng/ml) and AIH (n = 19, 5.9 ± 6.4 ng/ml) as compared with healthy controls (n = 29, 2.2 ± 1.7 ng/ml). (B) Serum TL1A levels were significantly higher in both early-stage (n = 96, 4.7 ± 5.1 ng/ml) and late-stage PBC patients (n = 14, 5.7 ± 4.4 ng/ml) as compared with healthy controls, whereas serum TL1A levels were significantly higher in late-stage (n = 13, 3.8 ± 1.1 ng/ml) but not in early-stage CHC patients. Serum TL1A levels in early-stage PBC patients (n = 96, 4.7 ± 5.1 ng/ml) were significantly higher as compared with those of CHC patients (n = 13, 2.1 ± 1.3 ng/ml). Horizontal lines represent mean values for each group. Statistical differences were analysed using a two-tailed Mann–Whitney U-test; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. HC; healthy controls.

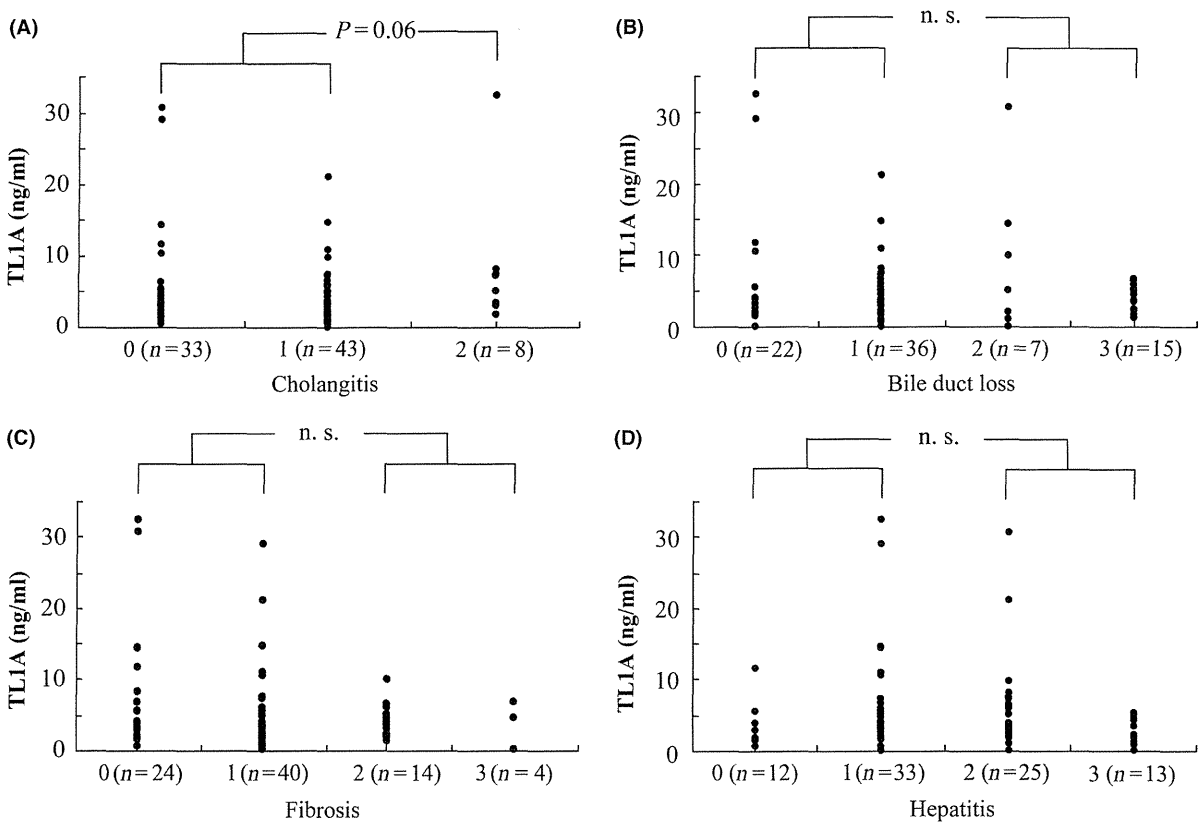

Fig. 2. Serum TL1A levels in PBC patients with different histological scores. (A) Serum TL1A levels tended to be higher in PBC patients with a cholangitis activity (CA) score of 2 as compared with those with a CA score of 0 or 1. There was no significant difference in serum TL1A levels among patients 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).

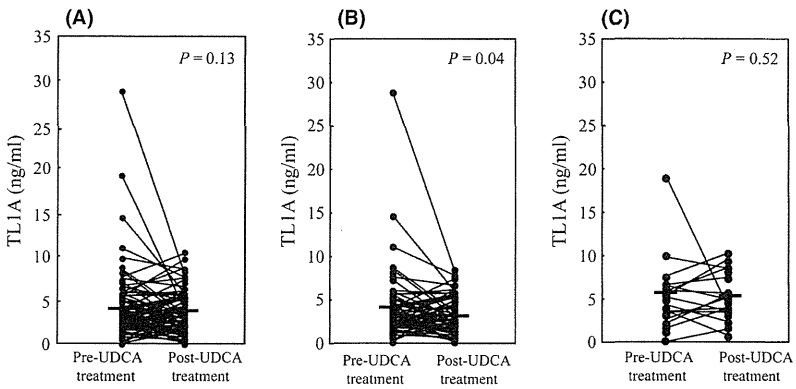

Fig. 3. Effect of UDCA treatment on serum TL1A levels in PBC patients. (A) In the PBC patient group as a whole (n = 76), serum TL1A levels tended to be decreased by UDCA treatment (pre: 4.5 ± 4.5 ng/ml, post: 3.6 ± 2.5 ng/ml). Serum TL1A levels were significantly decreased in early-stage PBC patients (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.

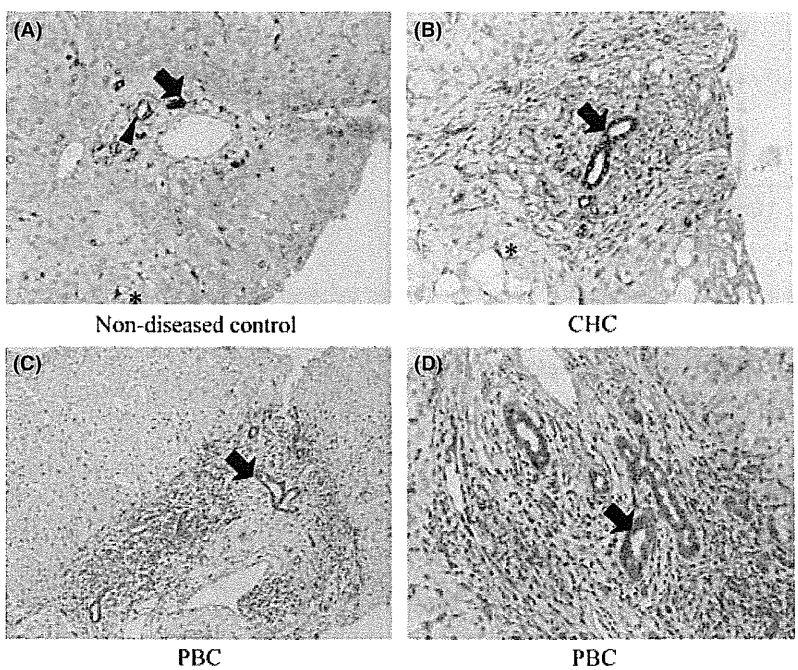

Fig. 4. Immunohistochemical staining of TL1A in the liver specimens of PBC and CHC patients. (A) TL1A was localized in intrahepatic bile ducts (arrow), blood vessels (arrowhead) and Kupffer cells (asterisk), but not in hepatocytes in non-diseased controls. In addition to these TL1A-positive cells, TL1A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

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

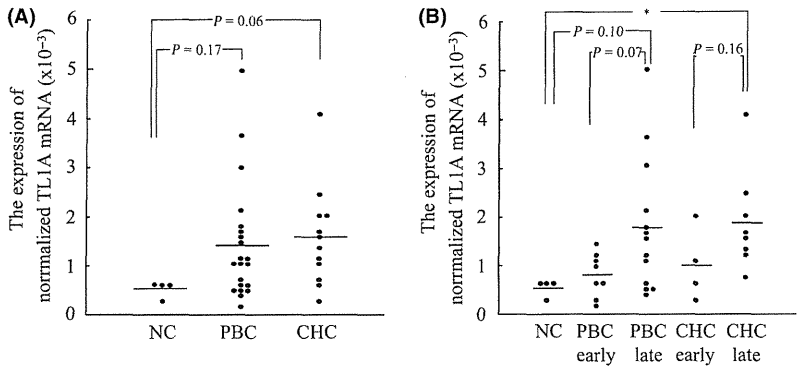

Fig. 5. Expression of TL1A mRNA in the liver specimens of PBC and CHC patients. The TL1 mRNA expression in the liver specimens of non-diseased controls (n = 4) as well as PBC (n = 21) and CHC (n = 12) patients was normalized to GAPDH mRNA expression. (A) The normalized TL1A mRNA expression in both PBC (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.

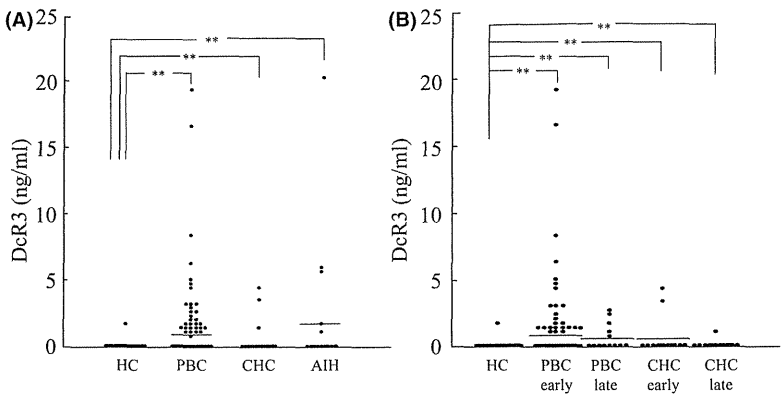

Fig. 6. Serum DcR3 levels in PBC, CHC and AIH patients. (A) Serum DcR3 levels were significantly higher in patients with PBC (n = 110, 0.92 ± 2.3 ng/ml), CHC (n = 26, 0.41 ± 1.1 ng/ml) and AIH (n = 19, 2.0 ± 4.8 ng/ml) as compared with healthy controls (n = 46, 0.04 ± 0.25 ng/ml). (B) Serum DcR3 levels 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

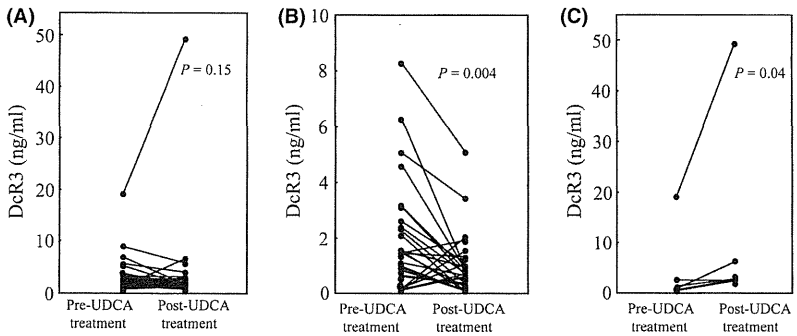

Fig. 7. Effect of UDCA treatment on serum DcR3 levels in PBC patients. In PBC patients who had detectable DcR3 levels by ELISA, serum DcR3 levels before and after UDCA treatment were compared by a two-tailed Wilcoxon's single-rank test. (A) In the group as a whole (n = 39), UDCA treatment tended to lower 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.

Acknowledgements

Financial support: This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science to Y. Aiba (#25860576) and M. Nakamura (#23591006); by a Grant-in-Aid for Clinical Research from the National Hospital Organization to M. Nakamura and by a grant from the Research Program of Intractable Disease provided by the Ministry of Health, Labour and Welfare of Japan.

Conflict of interest: None.

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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.

J Gastroenterol
DOI 10.1007/s00535-013-0795-0

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

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

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Received: 13 December 2012 / Accepted: 14 March 2013

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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Published online: 24 April 2013

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 ± 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 ($\leq$5 mg/day) alone ($n = 3$), UDCA + bezafibrate ($n = 64$), UDCA and/or bezafibrate + maintenance PSL ($\leq$5 mg/day) ($n = 29$), or no medication ($n = 1$).

Histological examination

Four-μm-thick formalin-fixed and paraffin-embedded sections of needle liver biopsy specimens (length $\geq$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 ± SD 51.3 ± 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 ± SD 9.2 ± 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-μl 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

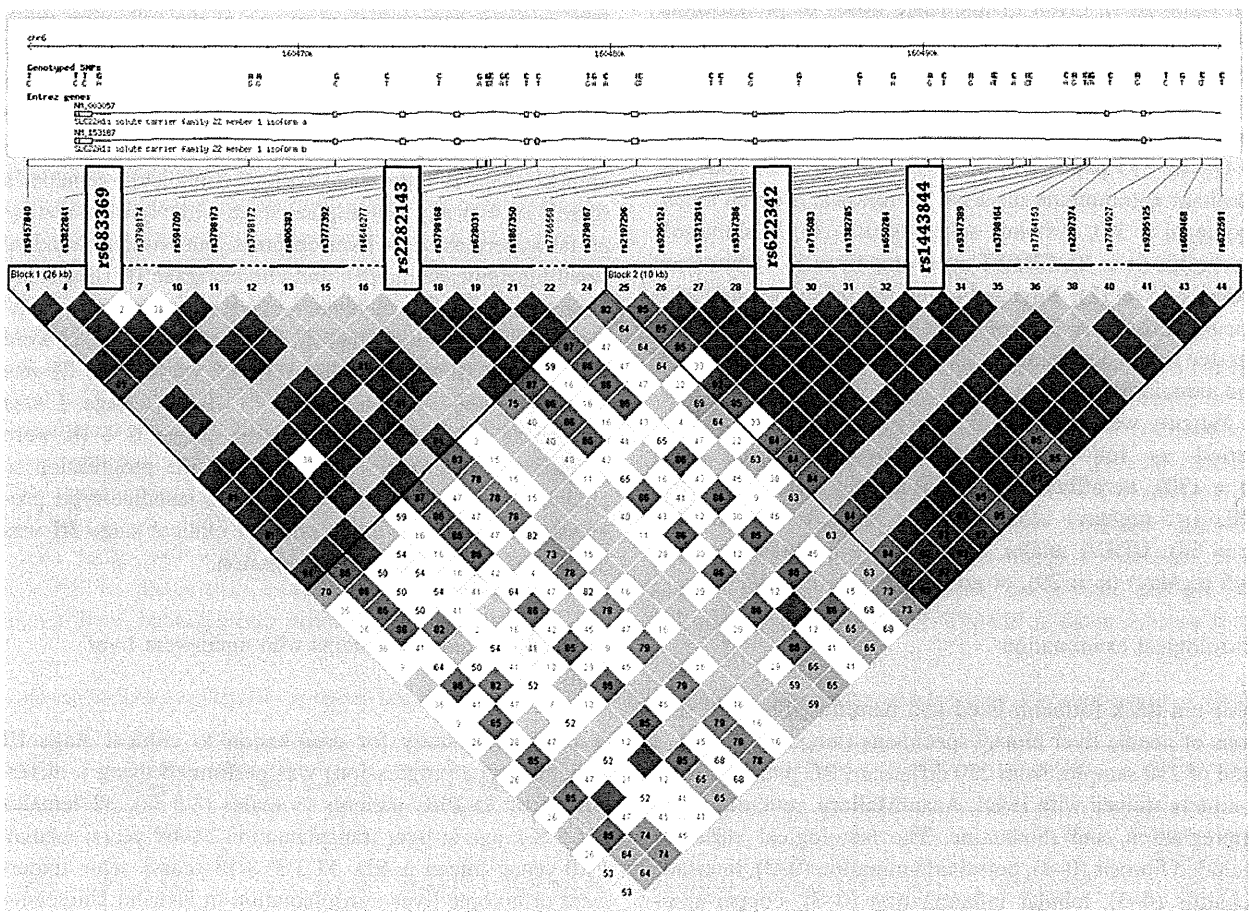

Fig. 1 Linkage disequilibrium (LD) plots for tag SNPs in *OCT1* using Haploview software version 4.2. The significant tag SNPs associated with the progression of PBC in the present study is highlighted in the *blue boxes*. Each *diamond* represents pairwise LD strength with color (D′/LOD method) and 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	*P* value	Early stage (stage I)	Late stage (stages II + III)	*P* value	Non-jaundice stage (stages I + II)	Jaundice stage (stage III)	*P* 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				
OCT-1/SLC22a1	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				
OCT-1/SLC22a1	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				
OCT-1/SLC22a1	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				
OCT-1/SLC22a1	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				
OCT-1/SLC22a1	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.

Table 8 Multivariate analysis for the progression to jaundice stage

Factor	*P* 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)

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

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.

Acknowledgments This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (#20590800, #23591006) to Minoru Nakamura; by a Grant-in Aid for Clinical Research from the National Hospital Organization to Minoru Nakamura; and by the Research Program of Intractable Disease provided by the Ministry of Health, Labor, and Welfare of Japan to Hiromi Ishibashi. We thank Drs. Seigo Abiru, Shinya Nagaoka (NHO Nagasaki Medical Center), Hajime Ota (NHO Kanazawa Medical Center), Tatsuji Komatsu (NHO Yokohama Medical Center), Jinya Ishida (NHO Nishisaitama Hospital), Hirotsugu Kouno (NHO Kure Medical Center), Michiyasu Yagura (NHO Tokyo Hospital), Masakazu Kobayashi (NHO Matsumoto Medical Center), Toyokichi Muro (NHO Oita Medical Center), Naohiko Masaki (National Center for Global Health and Medicine), Keiichi Hirata (NHO National Disaster Medical Center), Yukio Watanabe (NHO Sagamihara Hospital), Masaaki Shimada (NHO Nagoya Medical Center), Toshiki Komeda (NHO Kyoto Medical Center), Kazuhiro Sugi (NHO Kumamoto Medical Center), Eiichi Takesaki (NHO Higashi-Hiroshima Medical Center), Yukio Ohara (NHO Hokkaido Medical Center), Hiroshi Mano (NHO Sendai Medical Center), Haruhiro Yamashita (NHO Okayama Medical Center), Michiaki Koga (NHO Ureshino Medical Center), Masahiko Takahashi (NHO Tokyo Medical Center), Tetsuo Yamamoto (NHO Yonago Medical Center), Fujio Makita (NHO Nishigunma Hospital), Hideo Nishimura (NHO Asahikawa Medical Center), Hitoshi Takagi (NHO Takasaki General Medical Center), Noboru Hirashima (NHO Higashinagoya Hospital), and Kaname Yoshizawa (NHO Shinshu Ueda Medical Center) for obtaining informed consent and collecting serum and DNA samples from PBC patients.

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

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胆　道　27巻2号　176～187（2013）

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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，胆汁うっ滞を来た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」班班

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受付日；平成24年12月19日
採用日；平成25年2月13日

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
Symptoms at presentation			
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
*Laboratory data*1			
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
*Detection of autoantibodies*2			
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