and 22.9% (82/358), respectively. The SNP genotype distribution was in Hardy–Weinberg equilibrium (*P*-value was non-significant). The median age at onset of the HCC patients was 69.76 years, and approximately 55% were male.

Primary end-point

Table 2 shows the age at onset of patients with HCC and the associations among rs738409 genotypes, sex, BMI, alcohol consumption, HCV genotype and HCV viral load. The median ages (1st–3rd quartile) at onset in patients with HCC for the rs738409 GG and non-GG (CC/CG) genotypes were 67.8 years (range, 60.6–74.0) and 69.9 years (range, 65.2–75.6), respectively. The median age was significantly younger in patients with the rs738409 GG genotype than in those with non-GG genotype ($P = 0.004$). In multivariate analysis, early age at onset of HCC was independently associated with rs738409 GG genotype ($P < 0.001$), male sex ($P = 0.004$) and higher BMI ($P = 0.03$). The median ages at onset of patients with HCC for the CC and CG genotypes were 70.3 and 69.7 years, respectively. The Jonckheere–Terpstra trend test showed a significant trend across the GG, CG and CC alleles ($P = 0.005$; Fig. 1). One hundred and sixty-six patients had histories of blood transfusion. The median (1st–3rd quartile) intervals between blood transfusion and the onset of HCC in patients with rs738409 GG and non-GG (CC/CG) genotypes were 39.96 (range, 33.43–45.84) and 40.85 years (range, 33.52–46.76), respectively. In multivariate analysis, the median interval between blood transfusion and the onset of HCC was significantly shorter in patients with rs738409 GG genotype ($P = 0.008$) and male sex ($P < 0.001$) (Table 3).

Secondary end-point

Table 4 shows the clinical findings and associations between the rs738409 genotypes at the time of HCC onset. The rs738409 GG genotype was significantly associated with a higher aspartate aminotransferase (AST) level (69.5 vs 59.0 IU/L, $P = 0.02$), a lower prothrombin time (72.95% vs 78.00%, $P = 0.008$) and a higher prevalence of histological steatosis (40.00% *vs.* 22.16%, $P = 0.01$) compared to the non-GG genotype after adjustment for sex, BMI and alcohol consumption. There were no significant associations between rs738409 genotype and histological stage of fibrosis or histological grade of disease activity. Figure 2 shows the

Table 2 Factors associated with the age at onset of HCC ($n = 358$)

Variable	Median	1st–3rd quartile	*P*-value	
			Univariate	Multivariate†
PNPLA3 genotype			0.004	<0.001
GG	67.81	60.58–73.97		
CC/CG	69.87	65.20–75.62		
Sex			<0.001	0.004
Male	68.59	62.09–74.20		
Female	71.81	65.98–76.26		
BMI			0.07	0.03
>25	68.95	63.05–73.50		
≤25	70.49	64.32–75.57		
Alcohol consumption			0.02	0.11
>50 g/day	68.25	59.75–73.35		
≤50 g/day	70.12	64.80–75.47		
HCV genotype			0.2	
Genotype 1	69.87	64.35–75.53		
Genotype 2	68.65	63.50–74.17		
Viral load			0.09	0.06
High‡	70.57	65.08–75.82		
Low§	68.89	63.75–74.59		

†Stepwise regression analysis for the age at onset of hepatocellular carcinoma (HCC; the dependent variable) using *PNPLA3* genotype, sex, body mass index (BMI), alcohol consumption, hepatitis C virus (HCV) genotype and HCV viral load as independent variables.
‡At or above the median value.
§Below the median value.

Table 3 Factors associated with the time between HCV infection and the development of HCC ($n = 166$)

Variable	Median	1st–3rd Quartile	*P*-value	
			Univariate	Multivariate†
PNPLA3 genotype			0.47	0.008
GG ($n = 40$)	39.96	33.43–45.84		
CC/CG ($n = 126$)	40.85	33.52–46.76		
Sex			0.04	<0.001
Male	38.54	31.95–44.93		
Female	42.45	35.67–47.25		
BMI			0.75	–
>25 kg/m^2	37.94	32.91–45.60		
≤25 kg/m^2	40.85	33.70–46.87		
Alcohol consumption			0.26	–
>50 g/day	40.13	28.55–45.33		
≤50 g/day	40.87	33.79–46.76		
HCV genotype			0.09	–
Genotype 1	41.46	34.20–46.92		
Genotype 2	37.80	28.70–45.44		
Viral load			0.008	0.11
High‡	41.81	35.18–48.28		
Low§	38.53	30.79–45.12		

†Stepwise regression analysis of age at onset of hepatocellular carcinoma (HCC; the dependent variable) using *PNPLA3* genotype, sex, body mass index (BMI), alcohol consumption, hepatitis C virus (HCV) genotype, HCV viral load and the age at blood transfusion as independent variables.
‡At or above the median value.
§Below the median value.

histological findings for CC, CG and GG genotypes. The increment in the G allele was significantly associated with a higher prevalence of steatosis, as demonstrated by the Cochran–Armitage trend test (CC 13.11% vs CG 28.45% vs GG 40.00%, respectively; $P = 0.004$).

DISCUSSION

IN THIS STUDY, we found that the risk allele of *PNPLA3*, which was strongly correlated with significant liver steatosis, also may be a risk factor for hepatocarcinogenesis in CHC patients. Median age at onset of HCC was significantly younger ($P < 0.001$), and the median interval between blood transfusion and the onset of HCC was significantly shorter ($P = 0.008$) in patients with the rs738409 GG genotype than in those with non-GG genotypes after adjustment for sex, BMI, alcohol consumption, HCV genotype and HCV viral load.

Earlier age at HCC onset or shorter time between HCV infection and the development of HCC in the GG genotype was thought to be caused by the acceleration of liver fibrosis. The patients with the rs738409 GG genotype may reach the stage of advanced cirrhosis and develop HCC in their early age or shorter time after HCV infection. Previous studies reported hepatic steatosis as a risk factor for progressed fibrosis and HCC in CHC patients.[4,42] The *PNPLA3* polymorphism was originally reported as a determinant of liver fat content,[23] and a significant association between rs738409 SNP and histological evidence of steatosis (≥5%) was identified in the present study. The *PNPLA3* polymorphism was thought to affect the susceptibility to HCC in CHC patients via alteration of lipid accumulation in the liver.

Although this was not confirmed histologically, the *PNPLA3* GG genotype was also significantly associated with higher AST level and tended to be associated with a higher prevalence of progressed histological fibrosis compared to the non-GG genotypes (74.0% vs 60.5%, $P = 0.11$) at the time of HCC onset. Moreover, the GG genotype was associated with a lower prothrombin time, which suggests depressed liver function. Increased lipid accumulation in the *PNPLA3* GG genotype may enhance the risks of hepatic inflammation, fibrosis and impairment of liver function in CHC patients.

Table 4 Associations between *PNPLA3* genotype and clinical findings at the time of HCC onset ($n = 358$)

Variable	Median/number (1st–3rd quartile)		*P*-values	
	GG	Non-GG	*P*-value	Adjusted *P*-value†
Platelet count ($\times 10^4/\mu L$)	10.05 (7.73–12.78)	10.30 (7.68–13.35)	0.53	–
AST (IU/L)	69.5 (49.0–88.5)	59.0 (43.0–83.5)	0.048	0.02§
ALT (IU/L)	59.0 (42.0–93.3)	55.0 (37.0–86.3)	0.29	–
TB (mg/dL)	0.8 (0.6–1.1)	0.8 (0.6–1.1)	0.85	–
Albumin (g/dL)	3.7 (3.3–3.9)	3.7 (3.4–3.9)	0.41	–
PT (%)	73.0 (67.3–79.0)	78.0 (69.0–90.0)	0.004	0.008§
Viral load (log IU/mL)	4.73 (4.51–4.94)	4.75 (4.35–5.20)	0.90	–
LDL cholesterol (mg/dL)	77.2 (63.1–90.3)	74.7 (57.6–93.6)	0.77	–
Triglyceride (mg/dL)	82.0 (59.0–108.0)	87.0 (66.0–114.0)	0.32	–
Fasting plasma glucose (mg/dL)	100.0 (88.5–116.0)	103.0 (91.3–121.8)	0.20	–
Plasma insulin (μg/mL)	12.0 (8.0–18.0)	12.0 (9.0–19.0)	0.67	–
Histological findings ($n = 235$)				
Fibrosis				
F0–3	13	73	0.11	–
F4	37	112		
Activity				
A0–1	30	112	0.93	–
A2–3	20	73		
Steatosis‡				
<5%	30	144	0.02	0.01¶
≥5%	20	41		

†Adjusted for sex, BMI and alcohol consumption (independent variables). The dependent variables of each *P*-value are the items in the leftmost fields of the corresponding row (e.g. platelet count, AST, ALT).
‡Odds ratio (95% CI) for the GG allele was 2.43 (1.24–4.77), and the 95% CI of each proportion is shown in parentheses for this outcome.
§*P*-value by stepwise regression analysis.
¶*P*-value by stepwise logistic regression analysis.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; HCC, hepatocellular carcinoma; LDL, low-density lipoprotein; PT, prothrombin time; TB, total bilirubin.

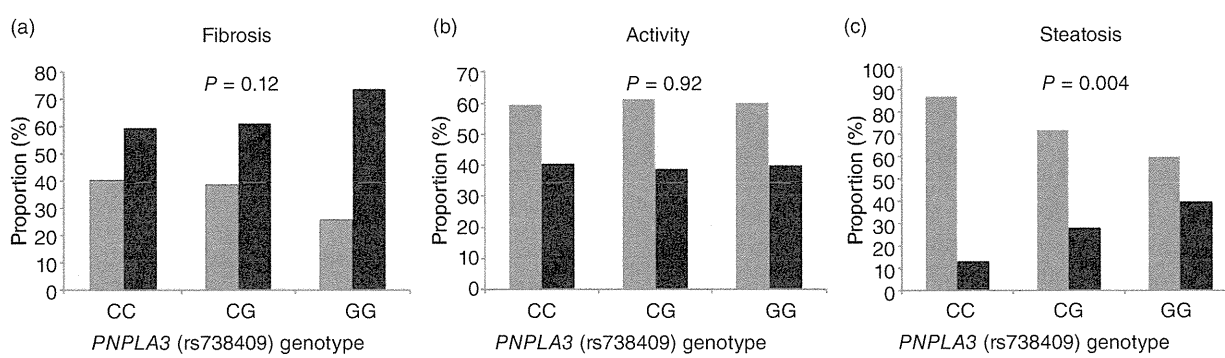

Figure 2 Bar plot: prevalence of fibrosis (F1–3 vs F4, a), necroinflammation (A1 vs A2–3, b) and steatosis (<5% vs ≥5%, c) in 235 patients with chronic hepatitis C. The proportions are shown on the Y axis. *P*-values of the frequency distributions are shown (Cochran–Armitage trend test). ■, F1–3; ■, F4; ■, A1; ■, A2–3; ■, <5%; ■, ≥5%.

One study investigated the impact of the *PNPLA3* polymorphism on liver steatosis and fibrosis in CHC patients.[36] In this study, the cumulative incidence of HCC during the follow-up period was significantly higher in patients with the GG genotype.[36] The *PNPLA3* polymorphism is also associated with susceptibility to HCC in patients with other causes of hepatitis.[34,43] Our data suggest that the *PNPLA3* rs738409 polymorphism may provide important information that will assist identification of patients at particular risk for HCC.

In the present study, early age at onset of HCC was also independently associated with male sex and higher BMI, and the median interval between blood transfusion and the onset of HCC was significantly associated with male sex. These results are consistent with previous reports of male sex and higher BMI as independent risk factors for HCC development in CHC patients.[9,44,45]

A limitation of the present study is its retrospective design. The histology samples at the time of initial treatment were obtained via ultrasound-guided aspiration at the time of percutaneous tumor ablation or surgical resection. To minimize the risk of bleeding, ultrasound-guided aspiration was not performed for patients with a platelet count of less than 6 ($\times 10^4/\mu L$). Therefore, the histological samples were collected from a biased group of patients. Another limitation is the cross-sectional study design and the lack of controls without HCC. We are unable to confirm whether the age at onset of HCC (primary outcome of the present study) is an adequate indicator of susceptibility to HCC from the current study alone. Further prospective study is needed to validate the current results.

In conclusion, the *PNPLA3* rs738409 C>G polymorphism may play a significant role in hepatocarcinogenesis in CHC patients. Thus, this genetic factor should be taken into consideration when determining a treatment strategy intended to prevent the future development of HCC in CHC patients.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

IL28B minor allele is associated with a younger age of onset of hepatocellular carcinoma in patients with chronic hepatitis C virus infection

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Abstract
Background IL28B polymorphisms were shown to be associated with a response to peg-interferon-based treatment in chronic hepatitis C (CHC) and spontaneous clearance. However, little is known about how this polymorphism affects the course of CHC, including the development of hepatocellular carcinoma (HCC). We evaluated the influence of IL28B polymorphisms on hepatocarcinogenesis in CHC patients.
Methods We genotyped the rs8099917 single-nucleotide polymorphism in 351 hepatitis C-associated HCC patients without history of IFN-based treatment, and correlated the age at onset of HCC in patients with each genotype.
Results Frequencies of TT, TG, and GG genotypes were 74.3 % (261/351), 24.8 % (87/351), and 0.9 % (3/351), respectively. The mean ages at onset of HCC for TT, TG, and GG genotypes were 69.9, 67.5 and 66.8, respectively. In multivariate analysis, IL28B minor allele (TG and GG genotypes) was an independent risk factor for younger age at onset of HCC ($P = 0.02$) in males ($P < 0.001$) with higher body mass index (BMI; $P = 0.009$). The IL28B minor allele was also associated with a lower probability of having aspartate aminotransferase-to-platelet ratio index (APRI) >1.5 (minor vs. major, 46.7 vs. 58.6 %; $P = 0.01$), lower AST (69.1 vs. 77.7 IU/L, $P = 0.02$), lower ALT (67.8 vs. 80.9 IU/L, $P = 0.002$), higher platelet count (12.8 vs. 11.2 $\times$ 10^4/μL, $P = 0.002$), and higher prothrombin time (79.3 vs. 75.4 %, $P = 0.002$).
Conclusions The IL28B minor allele was associated with lower inflammatory activity and less progressed fibrosis of the liver; however, it constituted a risk factor for younger-age onset of HCC in CHC patients.

Keywords rs8099917 · Hepatocarcinogenesis · Interferon-λ · Risk allele · Fibrosis

Abbreviations

AFP	α-Fetoprotein
APRI	Aminotransferase platelet ratio index
CHC	Chronic hepatitis C
GWAS	Genome-wide association study
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
IL28B	Interleukin 28B
PCR	Polymerase chain reaction
peg-IFN	peg-Interferon
RIG- I	Retinoic acid-inducible gene-I
SNP	Single-nucleotide polymorphism
SVR	Sustained viral response
TLR3	Toll-like receptor 3

Introduction

Hepatitis C virus (HCV) infection is one of the major causes of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1]. Currently, patients with chronic

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hepatitis C (CHC) are treated with a combination of peg-interferon (peg-IFN) and ribavirin [2, 3]. Recently, HCV nonstructural 3/4A serine protease inhibitors combined with PEG-IFN and RBV were reported to achieve higher sustained viral response (SVR) rates in genotype 1 patients compared to conventional PEG-IFN/RBV. These triple therapies are considered to be the next standard of care for patients with CHC virus infection [4, 5].

Genetic variations near the interleukin 28B (IL28B) gene, encoding the type III IFN-λ3, were shown to be strongly associated with the response to peg-IFN and ribavirin treatment in patients with CHC [6–8] and also spontaneous clearance of HCV [9]. Host immune cells produce IFN and other cytokines in response to viral infection. In response to HCV, cellular sensors detect the double-stranded RNA via the retinoic acid-inducible gene-I (RIG-I) and toll like receptor 3 (TLR3) and activate a pathway to produce antiviral cytokines, including alpha and beta IFNs that trigger an antiviral response to eradicate the virus [10, 11].

Genetic polymorphisms of genes involved in innate immunities are likely to influence the strength and nature of this defense system [12]. Besides its antiviral properties, IFN-λ exhibits antitumor activity; in fact, several experimental studies in cell lines and in animal models demonstrated that the activation of type III IFN induces apoptosis [13] and antitumor activities [14–16]. Thus, this genetic factor is thought to influence the natural course of HCV infection, including the development of HCC. However, little is known about the influence of IL28B polymorphisms on hepatocarcinogenesis in patients with CHC.

In the present study, we examined the association between the rs8099917 single-nucleotide polymorphism (SNP) at the IL28B locus with the age at onset of HCC and other clinical findings in patients with CHC who had no history of receiving IFN-based treatment.

Materials and methods

Patients

The patients analyzed in the present study were derived from an HCV study cohort of the University of Tokyo Hospital. In this cohort, we enrolled the patients who visited the liver clinic at our institute between August 1997 and April 2009, and agreed to provide blood samples for human genome studies along with written informed consent according with the Declaration of Helsinki. All patients underwent laboratory blood tests at the time of enrollment in our cohort. The result of the blood tests were recorded with the information on alcohol consumption and BMI of each patient. The patients who were positive for hepatitis B surface antigen and had a history of biliary disease were excluded. All subjects in our cohort were Japanese, and this research project was approved by the ethics committees of the University of Tokyo (No. 400).

From this cohort, we examined the patients who had developed new-onset HCC and received initial therapy in our institute by January 31, 2010, and with available sample for genotyping. We excluded the patients with a history of receiving IFN-based treatment. Finally, 351 patients were enrolled for this study, and the association between the age at onset of HCC and the IL28B genotype was analyzed. Patient follow-up and Diagnosis of HCC was performed as previously described [17, 18].

IL28B genotyping

Human genomic DNA was extracted from the whole blood of each patient. Genotyping for the IL28B rs8099917 T/G polymorphism was performed by polymerase chain reaction (PCR) using the TaqMan predesigned SNP Genotyping Assay (Applied Biosystems, Foster City, CA) as recommended by the manufacturer. Allele-specific primers were labeled with fluorescent dye (FAM or HEX) and used in the PCR reaction. Aliquots of the PCR products were genotyped using an allele-specific probe of the SNP on a real-time PCR thermocycler (MX3000P, Stratagene, La Jolla, CA). Samples were subjected to 50 cycles of denaturation for 15 s at 92 °C, annealing of primers for 30 s at 60 °C, and elongation for 30 s at 60 °C.

Study endpoint

We analyzed the relationship between the age at onset of HCC (the primary endpoint of this study) and host factors, including the IL28B genotypes, sex, BMI, alcoholic consumption, and HCV genotype. We also examined the relationship between IL28B genotypes and the clinical findings at the time of enrollment in our cohort (the secondary endpoint), such as the biochemical markers and presence of liver fibrosis. Liver biopsies were only available in a small number of patients (48); liver fibrosis was assessed using the aspartate aminotransferase platelet ratio index (APRI), and an APRI of >1.5 was classified as bridging fibrosis or cirrhosis (F stage 3–4) [19].

Statistical analysis

Continuous variables were presented as the mean ± standard deviation (SD) while categorical variables were expressed as frequencies (%). Categorical data were analyzed using the Chi square test, and stepwise logistic regression analyses were used to adjust the influence of IL28B genotype by other covariates such as sex, BMI (<25

or not), and alcoholic consumption (<50 g/day or not). For continuous data, the univariate associations were evaluated using the Student's t test or nonparametric Wilcoxon rank-sum test as appropriate. Since the age at onset of HCC (the primary endpoint of this study) satisfied the assumption of normal distribution (Kolmogorov–Smirnov test, $P > 0.05$), we used stepwise regression analysis to adjust the influence of IL28B genotype by sex, BMI (<25 or not), and alcoholic consumption (<50 g/day or not). All statistical analyses were two-sided, and the threshold of the reported P values for significance was accepted as <0.05. All statistical analyses were performed using R 2.13.1 software (http://www.r-project.org).

Results

Patient characteristics

Patient characteristics are shown in Table 1. Frequencies of the rs8099917 TT, TG, and GG genotype were 74.3 % (261/351), 24.8 % (87/351), and 0.9 % (3/351), respectively. The SNP genotype distribution was in Hardy–Weinberg equilibrium (P value was not significant). We defined the IL28 major genotype as homozygous for the major sequence (TT) and the IL28B minor genotype as homozygous (GG) or heterozygous (TG) for the minor sequence. The mean age at onset of the HCC patients was 69.3 years, and approximately 60 % were male. The mean age at the time of enrollment was 67.2 years and the follow-up period was 27.9 months in average.

Table 1 Clinical characteristics and genotype distributions in the study cohort ($n = 351$)

Parameter	Values
Mean age at onset of HCC, in years	69.26 ± 8.07
Mean age at the time of enrollment, in years	67.16 ± 8.32
Male sex	200 (57.0 %)
BMI >25	70 (20.0 %)
Alcohol consumption (>50 g/day)	75 (21.4 %)
IL28B genotype	
TT	261 (74.3 %)
TG	87 (24.8 %)
GG	3 (0.9 %)
T allele frequency	0.87
HCV genotype	
Genotype 1	240 (68.4 %)
Genotype 2	91 (25.9 %)
Not tested	20 (5.7 %)

Continuous variables were represented as the mean ± standard deviation (SD) and categorical variables were as number and frequencies (%)

Primary endpoint

Table 2 shows the age at onset of patients with HCC and the associations among IL28B genotypes, sex, BMI, alcohol consumption, and HCV genotype. The mean age at onset in patients with HCC for the IL28B major and minor genotypes were 69.88 ± 7.97 and 67.48 ± 8.17, respectively, and significantly higher in patients with the IL28B major genotype than in those with the minor genotype ($P = 0.02$). In multivariate analysis, the age at onset of HCC was significantly younger in patients with the IL28B minor genotype ($P = 0.02$, Fig. 1), independently of male sex ($P < 0.001$) and higher BMI ($P = 0.009$). The characters of HCC, such as sizes (2.56 vs. 2.40 cm, $P = 0.41$) or the numbers (1.94 vs. 2.23, $P = 0.54$) at diagnosis were not significantly different between IL28B major and minor genotypes. We also analyzed the interval between blood transfusion and the onset of HCC in 161 patients who have histories of blood transfusion which had been the major cause of HCV infection in Japan [20]. The mean interval between blood transfusion and the onset of HCC for the IL28B major and minor genotypes were 39.09 ± 9.99 and 38.86 ± 9.27 years, respectively ($P = 0.9$; data not shown).

Secondary endpoint

Table 3 shows the clinical findings and associations between the IL28B genotypes at the time of enrollment in our cohort. The IL28B major genotype was significantly associated with a higher probability of having an APRI >1.5 (58.62 vs. 46.67 %, $P = 0.01$; Fig. 2), a lower platelet count (11.15 vs. 12.80 × 10^4/μL, $P = 0.002$), a higher AST level (77.69 vs. 69.12 IU/L, $P = 0.02$), a higher ALT level (80.92 vs. 67.79 IU/L, $P = 0.002$), and a lower prothrombin time (75.40 vs. 79.27 %, $P = 0.002$) compared to the IL28B minor genotype after adjustment for sex, BMI, alcoholic consumption, and the age at enrollment of our cohort. A lower γ-GTP level was significantly associated with the IL28B major genotype in univariate analysis, and alcoholic consumption, sex, and age were stronger factors associated with the γ-GTP level. Thus, after adjustment for these factors, the IL28B genotype was not extracted as a significant factor associated with the γ-GTP level. Histological assessments of liver fibrosis were performed in 248 patients at the time of initial therapy. The prevalence of histologically proved liver cirrhosis (F4) was 65.6 % (118/180) in patients with major genotype and 51.5 % (35/68) in those with minor genotype. The prevalence of liver cirrhosis was significantly higher in patients with major genotype after adjustment for sex, BMI, alcoholic consumption, and the age at the time of initial therapy for HCC ($P = 0.045$, data not shown).

Table 2 Factors associated with the age at onset of HCC

Variable	Mean	Standard deviation (SD)	*P* value	
			Univariate	Multivariate[a]
IL28B genotype			0.02	0.02
Major (TT)	69.88	7.97		
Minor (TG/GG)	67.48	8.17		
Sex			<0.001	<0.001
Male	67.94	8.48		
Female	71.02	7.16		
BMI			0.01	0.009
>25	66.87	9.11		
≤25	69.86	7.70		
Alcohol consumption			0.11	–
>50 (g/day)	67.78	9.37		
≤50 (g/day)	69.67	7.65		
HCV genotype			0.29	–
Genotype 1	69.65	7.59		
Genotype 2	68.22	8.79		

[a] Stepwise regression analysis for the age at onset of HCC (the dependent variable) using IL28B genotype, sex, BMI, alcohol consumption, and HCV genotype as independent variables

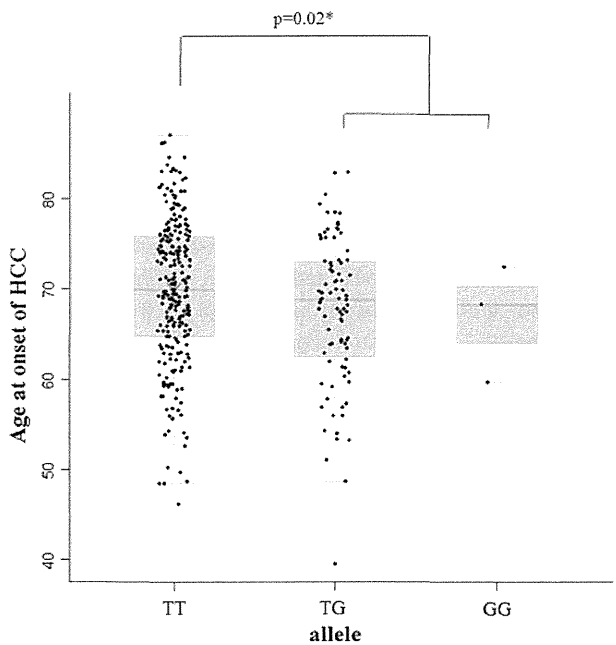

Fig. 1 *Box and whisker and dot plot* distributions of the age at onset of HCC in each genotype. The mean age at onset of HCC for the IL28B major and minor genotypes were 69.88 ± 7.97 and 67.48 ± 8.17, respectively, and was significantly higher in patients with the IL28B major genotype than in those with the minor genotype ($P = 0.02$). *P values after adjustment for sex, BMI, and alcoholic consumption

Discussion

In the present study, we evaluated the association between the IL28B polymorphism and the age at onset of HCC in patients with CHC. The IL28B minor genotype was significantly associated with younger age at onset of HCC with well known risk factors for the development of HCC such as male gender and higher BMI [21] without prior IFN-based treatment. Our previous study analyzing a susceptibility locus for HCV-induced HCC using a genome-wide association study (GWAS) could not detect the significant association between IL28B genotypes and the development of HCC in a cross-sectional distribution analysis between patients with and without HCC in more than 3,000 samples [22]. Also, IL28B alleles were not identified as a susceptibility locus for HCV-induced HCC in another GWAS study [23]. The cross-sectional distribution analyses may have underestimated the susceptibility to HCC because it could not take into consideration the future development of HCC and the duration after the past onset of HCC. Moreover, although GWAS would provide an effective and unbiased approach for revealing risk alleles for genetically complex non-Mendelian disorders, the risk of multiple comparisons made in a GWAS have resulted in reports of false positive results (Type 1 errors), and if the correction is overly conservative or the power is inadequate, false negative results (Type 2 errors) [24–26]. The relation between IL28B polymorphism and the susceptibility to HCC is still controversial. A previous study from Japan reported that the rs8099917 TT genotype was associated with a lower incidence of HCC even in non-responders to IFN based treatment [27] that was in agreement with the present study. Another study from Italy evaluating the association between genome frequency and the presence of cirrhosis due to hepatitis C, hepatitis B, alcohol use, and other factors also showed a higher prevalence of the IL28B minor allele in patients with HCC

Table 3 Associations between the IL28B genotype and clinical findings at the time of enrollment in our cohort

Variable	Mean/proportion (standard deviation; SD)		*P* values	
	Major (TT)	Minor (TG/GG)	*P* value	Adjusted *P* value[¶]
APRI >1.5[a]	58.62 % (52.38–64.66)	46.67 % (36.07–57.69)	0.07	0.01[‖]
Platelet count ($\times 10^4/\mu L$)	11.15 (5.00)	12.80 (5.43)	0.01	0.002**
AST (IU/L)	77.69 (45.14)	69.12 (38.16)	0.12	0.02**
ALT (IU/L)	80.92 (60.45)	67.79 (41.78)	0.17	0.002**
T.B (mg/dL)	0.90 (0.40)	0.83 (0.39)	0.02	–
Alb (g/dL)	3.69 (0.46)	3.71 (0.46)	0.9	–
ALP (IU/L)[b]	236.4 (81.75)	216.4 (58.96)	0.08	0.11**
γGTP (IU/L)[c]	76.83 (65.34)	87.23 (42.92)	0.005	–
PT (%)[d]	75.40 (13.36)	79.27 (13.13)	0.02	0.002**

[¶] Adjusted for sex, BMI, alcoholic consumption, and the age at enrollment (independent variables). The dependent variables of each *P* values are the items in the leftmost fields of corresponding rows (the proportion of having APRI >1.5, platelet count, AST, ALT and so on)

[‖] *P* value by stepwise logistic regression analysis

** *P* value by stepwise regression analysis

[a] Odds ratio (95 % CI) for major allele was 1.88 (1.13–3.11), and 95 % confidence interval (CI) of each proportion is parenthesized for this outcome

[b] Missing in 115 patients

[c] Missing in 112 patients

[d] Missing in 4 patients

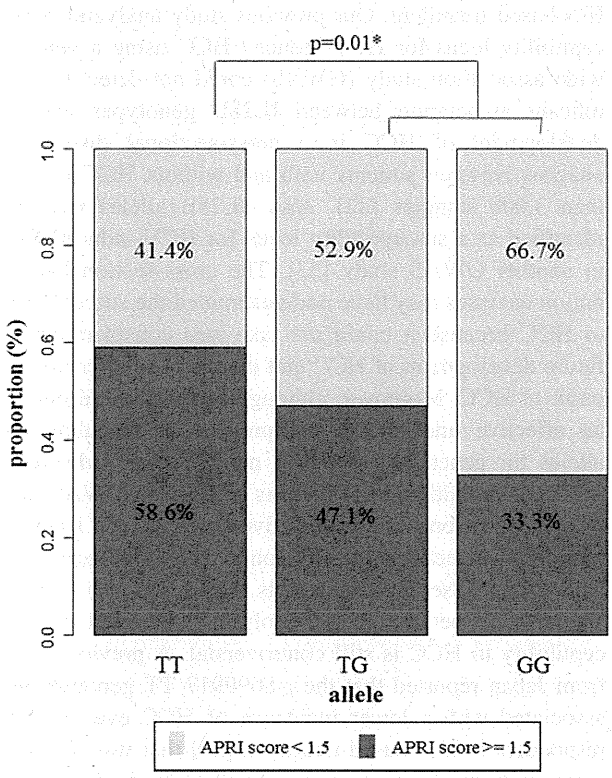

Fig. 2 *Bar plot* the proportion of having an AST-to-platelet ratio (APRI) score >1.5 in each allele. **P* values after adjustment for sex, BMI, alcoholic consumption, and the age at enrollment

compared to those without HCC [28]. However, other studies showed no relation between IL28B polymorphism and the susceptibility to HCC [29–32]. Some studies have reported the HCV genotype 1 as a risk factor associated with HCC in patients who had CHC [33–35]; however, we could not find a significant association between the HCV genotype and hepatocarcinogenesis in the present study. Our data showed no relationship between the duration of HCV infection in the patients with a history of blood transfusion. The mean age of blood transfusion was not significantly different between patients with major and minor genotypes (28.99 in major genotype vs. 27.60 in minor genotype, $P = 0.18$). Moreover, older age at HCV infection was reported to be associated with more rapid disease progression [36]. Thus, the difference in the duration of HCV infection may have little effect on the result of the present study. The IL28B genotype may have a critical role in the onset of HCC. Moreover, only about 45 % of all patients in the present study have the history of blood transfusion; hence, further analysis with larger samples may be indicated.

Previous studies evaluating patients with chronic HCV infection showed severer histological inflammatory activity and fibrosis, as well as higher ALT levels and APRI scores in patients homozygous for the IL28B major alleles [29, 32, 37, 38]. Similarly, in the present study, the IL28B

major genotype was significantly associated with a higher probability of having an APRI >1.5 and a higher ALT level; and the prevalence of histologically proved liver cirrhosis (F4) was significantly higher in patients with major genotype at the age at the time of initial therapy for HCC. Given the association between the IL28B major allele and the severe inflammatory activity or progressed fibrosis, the IL28B allele is thought to be associated with the susceptibility to HCC via a mechanism that is independent of controlling an activity of HCV infection.

Recent experimental studies have suggested that IFN-λ has an antitumor activity. In esophageal cancer cell lines expressing IFN-λ receptor complexes, IFN-λ1 suppressed growth via the induction of the G1 phase arrest or apoptosis [39]. An antitumor activity of IFN-λ was also shown in the B16 melanoma, BNL hepatoma, Colon 26, and neuroendocrine BON1 tumor cells [40–43]. One probable explanation for the paradoxical result of the present study is that the more aggressive inflammatory activity of patients with IL28B major genotype may reflect a stronger immune response to the virus, which may also have anti-tumor effects. However, the innate immune responses and antitumor activity via IFN-λ, as well as the mechanism underlying the association of the IL28B genotype, have not been elucidated. Further studies are needed to determine the functional role of the IL28B gene in relation to the course of chronic HCV infection, including hepatocarcinogenesis.

Because of the retrospective design, this study is limited by the absence of some important clinical details such as information about the histological findings of fibrosis and inflammation. Although the APRI is a useful index for the prediction of fibrosis, the limitation of this score has been reported in previous studies [44, 45]. Prospectively designed studies are needed to confirm our findings. However, observing chronic HCV-infected patients without antiviral treatment would be nearly impossible in the future. In this regard, the present study may have important implications.

In conclusion, the IL28B minor genotype was associated with a younger age of onset of HCC in patients with CHC, and this association was completely independent of the response to IFN-based treatment. Hepatocarcinogenesis appeared to be suppressed in patients who had CHC with the IL28B major genotype, despite higher inflammatory activity and progressed fibrosis of liver. The current findings may provide a clinically important information in the follow-up or HCC screening of cirrhotic patients.

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Conflict of interest None of the authors have any conflicts of interest.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Acute liver disease in Japan: a nationwide analysis of the Japanese Diagnosis Procedure Combination database

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Abstract
Background Accurate data on the incidence of acute liver disease (ALD) is lacking in most countries. We investigated the incidence of ALD-related admission in Japan using a large sample in a nationwide Japanese database.
Methods Data from the Diagnosis Procedure Combination database were analyzed for 1 July to 31 December 2007–2010. Patient characteristics, in-hospital mortality, and clinical practices, including drugs and procedures during hospitalization, were analyzed.
Results We identified 10509 patients with ALD from a total of 11.61 million inpatients in the database. The median age was 53 years and 54.7 % were male. The annual incidence of ALD-related hospital admission was estimated to be 131.1 cases/1 million people. The overall mortality rate was 5.9 % (622 cases). The infant (0–3 years), child (4–18 years), and adult in-hospital mortality rates were 2.7 % (7/261), 1.0 % (5/494), and 6.3 % (610/9754), respectively. The infant and child mortality rates were significantly lower than the adult mortality rate (Chi square test: $P = 0.03$ and $P < 0.001$, respectively). Hepatitis A virus- and hepatitis C virus-induced ALD had favorable outcomes, with in-hospital mortality rates of approximately 2 %. Plasma exchange and continuous hemodiafiltration were performed in 5.3 % (556 cases) and 3.4 % (360 cases) of all ALD cases, respectively.
Conclusions In-hospital mortality of ALD in Japan was acceptably low, and was affected by the etiology and patient background characteristics. The present study adds important information on the incidence and prognosis of ALD in Japan. Improvement of public health surveillance systems is necessary for population-based patient monitoring.

Keywords Acute hepatitis · Diagnosis Procedure Combination · Nationwide database · In-hospital mortality · Clinical practices

Abbreviations

AIH	Autoimmune hepatitis
ALD	Acute liver disease
ALF	Acute liver failure
A/AoCLF	Acute or acute-on-chronic liver failure
CHDF	Continuous hemodiafiltration
DPC	Diagnosis Procedure Combination
FH	Fulminant hepatitis
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEV	Hepatitis E virus
ICD-10	International classification of diseases and related health problems, tenth revision

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Introduction

Acute liver disease (ALD) is characterized by acute inflammation with varying degrees of necrosis and collapse of the hepatic architectural framework. ALD is generally a transient self-limiting disease regardless of the etiology. However, the severity of the disease is variable, and some patients progress to a fatal form, acute liver failure (ALF). ALF is a serious but rare clinical syndrome marked by sudden loss of hepatic function in a person with no prior history of liver disease. Clinically, the syndrome manifests itself as a severe impairment of liver function with hepatocellular necrosis, leading to hepatic encephalopathy, systemic inflammation, and multiorgan failure [1, 2].

In Japan, the definition and classification of fulminant hepatitis (FH), which was the representative disease entity associated with ALF, were originally established at the Inuyama Symposium in 1981 [3]. However, because of the differences in the demographic and clinical features of ALF between Japan and Europe or the United States, the diagnostic criteria for FH in Japan differed from those for ALF in Europe and the United States [4, 5]. Therefore, the diagnostic criteria for FH in Japan needed to be revised to correspond to those for ALF in Europe and the United States, and the Intractable Hepato-Biliary Disease Study Group of Japan recently determined the diagnostic criteria for ALF [6, 7].

Since the wide variety of symptoms in ALD makes it challenging to establish surveillance systems, accurate data on the incidence of ALD is lacking in most countries. Even in countries with a reporting system for infectious diseases, which are the major causes of ALD, there are few reliable data on the incidence of viral infection because reporting is not always mandatory and many cases are left unreported.

The Diagnosis Procedure Combination (DPC) database is a database containing discharge abstract and administrative claims of inpatients who are admitted to secondary or tertiary care hospitals in Japan [8–10], and represents approximately 40 % of inpatient admissions to such hospitals. The database contains a large number of samples, and can, thus, be used to investigate the incidence of ALD on an objective basis. The present study analyzed the incidence of admission related to ALD in Japan using the DPC database. In the database, clinical data to define the presence of ALF (i.e., prothrombin time, degree of encephalopathy, or length of illness) were not accessible. Therefore, we analyzed patients with ALD, who may include the entire cases of ALF, in a comprehensive manner. The aim of the present study was to collect detailed information on the clinical consequences for hospitalized ALD patients and estimate the public health burden of ALD in Japan.

Materials and methods

Data source

The DPC database contains the following information: hospital location; patient demographics; diagnosis, comorbidities at admission, and complications after admission recorded with Japanese text and International Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) codes; therapeutic procedures encoded by Japanese original codes; length of stay; discharge status, including in-hospital death; and total costs. A survey of the DPC hospitals is conducted by the DPC Study Group between 1 July and 31 December each year, and is funded by the Ministry of Health, Labour and Welfare, Japan. All 82 university teaching hospitals in Japan are obliged to adopt the DPC system, whereas adoption by community hospitals is voluntary. The survey started in 2003 with 82 teaching hospitals, and the numbers of participating hospitals and registered patients have since increased. The numbers of cases in the database were 2.99, 2.86, 2.57, and 3.19 million in 2007, 2008, 2009, and 2010, respectively, and represented approximately 40 % of all inpatient admissions to secondary and tertiary care hospitals in Japan.

The requirement for informed consent was waived in this study, because of the anonymous nature of the data. Study approval was obtained from the institutional review board of The University of Tokyo.

Samples

We obtained impatient data for 2007–2010. First, we identified patients with ICD-10 code-based diagnoses of hepatitis by any causes (ICD-10 codes, K70–K77), and those with viral, bacterial, or parasitic infections that may cause ALD through infectious diseases (A00–B99), from the 11.61 million inpatients included in the DPC database for 2007–2010. We then identified patients with Wilson's disease (E830), Budd–Chiari syndrome (I820), and acetaminophen overdose (T391), which are independent from the items of hepatitis (K70–K77) in the DPC database. Second, we manually checked the registered diagnoses in the Japanese texts for all of the screened cases to confirm the diagnosis of ALD. We excluded cases with a "suspected" diagnosis. We then excluded cases with diagnoses of chronic hepatitis (B170, B180–B189, K713–K715, and K721–K739) and liver cirrhosis (K702, K703, K717, K740–K742, K745, and K746) as well as cases with gastric and esophageal varices (I850, I859, and I864), which may imply the presence of chronic hepatitis. We also excluded cases with malignancy and those with a past history of liver transplantation.

Data description

The ALD patients were categorized according to their etiologies including viral hepatitis [hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), or hepatitis E virus (HEV)], alcohol-induced hepatitis, drug-induced hepatitis, and autoimmune hepatitis (AIH). The patients' age, sex, length of stay, in-hospital mortality, and total costs for hospitalization were summarized for each subgroup.

The DPC database includes the records of clinical practices performed on each patient. The clinical procedures examined during hospitalization were use of prostaglandin E1, corticosteroid injection, albumin preparation, platelet transfusion, fresh-frozen plasma transfusion, continuous hemodiafiltration (CHDF), plasma apheresis, plasma exchange, and liver transplantation.

Estimation of incidence of hospitalization for ALD

We estimated the incidence of hospitalization for ALD based on the number of beds in all acute care hospitals in Japan, and hospitals that had joined the DPC database. We assumed that there was no seasonality in the hospitalizations for ALD. To adjust for the influence of bed volume imbalance, we stratified the hospitals based on bed volume categories. The estimated annual number of ALD cases (Y_i) and the 95 % confidence interval (CI) were calculated with the following equation using Wald confidence intervals for the population proportion [11]:

$$Y_i/N_i = p_i \pm Z\sqrt{p_i(1-p_i)/(n_i \times 2)},$$

where N_i is the total number of beds in all acute care hospitals in Japan, n_i is the number of beds in the DPC hospitals, $p_i = X_i/(n_i \times 2)$ (X_i is the observed number of ALD cases in DPC hospitals between July and December, 2007–2010), and $Z = 1.96$.

Estimation of incidence of hospitalization for acute or acute-on-chronic liver failure

In the present study, we included the patients with alcoholic hepatitis which were usually excluded from the disease entity of ALF [6, 7, 12]. We defined the fatal form of ALD in the present study as acute or acute-on-chronic liver failure (A/AoCLF). A/AoCLF (or ALF) is not covered by a distinctive ICD code, and the clinical data that define the presence of A/AoCLF (or ALF) were not accessible. Therefore, we assumed that those who underwent plasma exchange were A/AoCLF cases for estimation of the mortality from A/AoCLF in the DPC database. This can be an acceptable approximation of the number of A/AoCLF cases with a minimal possibility of underreporting [13], because more than 90 % of ALF patients in Japan undergo plasma exchange [4]. We estimated the incidence of hospitalization for A/AoCLF in the same way used for ALD.

Statistical analysis

The examined variables were expressed as the median with the 1st and 3rd percentiles (continuous variables) and frequencies (categorical variables). The significance of differences among groups was assessed by the Chi square test. The threshold for significance was a value of $P < 0.05$. All statistical analyses were conducted using IBM SPSS version 19.0 (IBM SPSS, Armonk, NY, USA).

Results

Etiologies and clinical characteristics of ALD in Japan

A total of 10509 ALD cases were identified between 1 July and 31 December in 2007–2010. Overall, 54.7 % of cases (5748) were male, and the median age was 53 years. The peak age for male patients was in their 60s, while the peak age for female patients was in their 50s (Fig. 1). The most frequent cause of ALD was indeterminate (35.1 %), followed by drugs (16.1 %) and alcohol (15.6 %) (Fig. 2a). When restricted to A/AoCLF cases, HBV-induced A/AoCLF accounted for 20.3 % of all cases of A/AoCLF

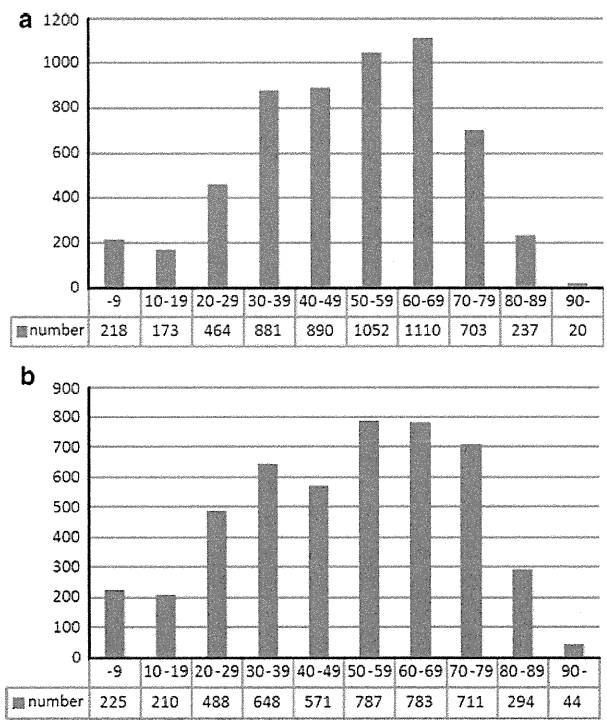

Fig. 1 Age distributions of the 10509 patients with ALD

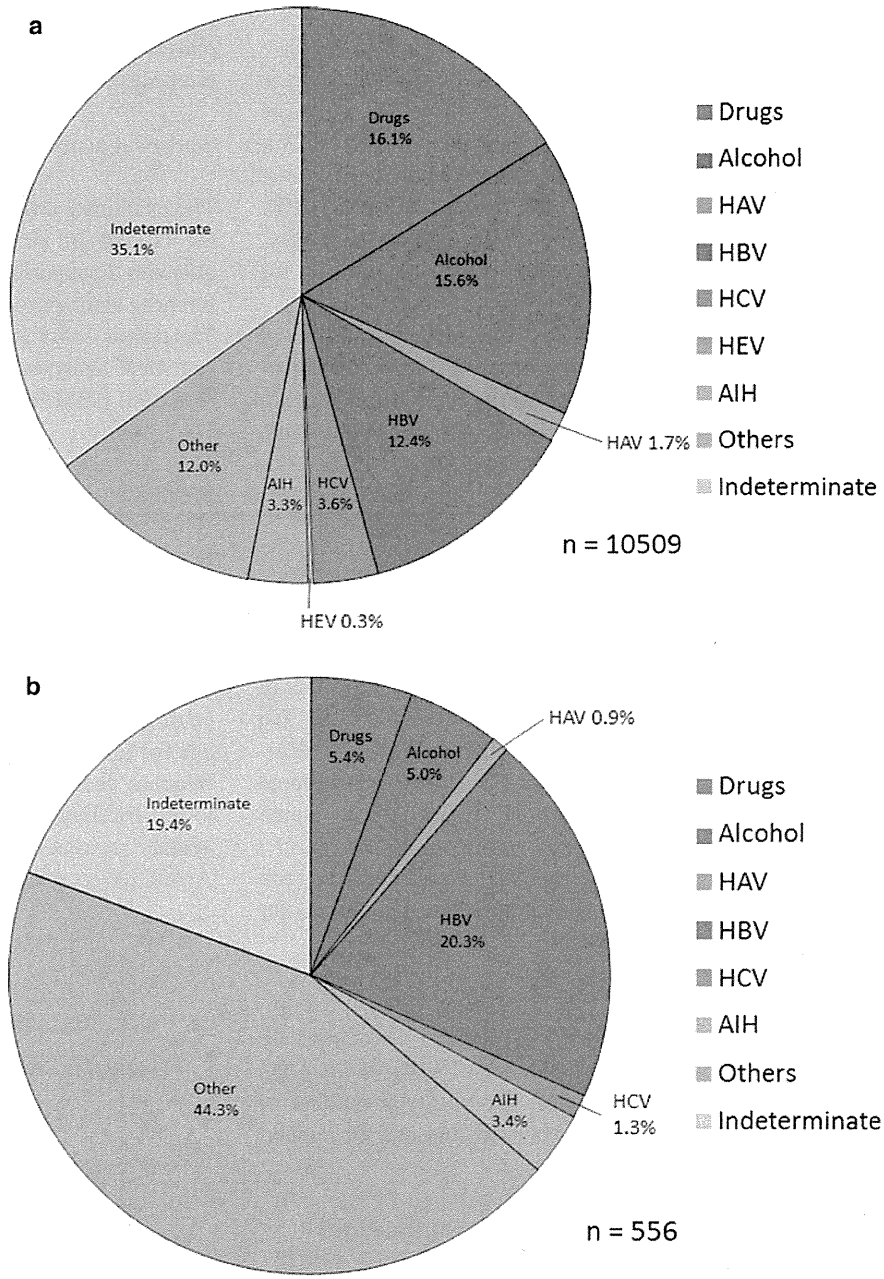

Fig. 2 **a** Etiologies of ALD in the total of 10509 patients in the DPC database between 2007 and 2010. **b** Etiologies of A/AoCLF in the total of 556 patients in the DPC database between 2007 and 2010

(Fig. 2b). Table 1 shows the etiologies and clinical characteristics of ALD cases in Japan. The proportion of male patients was highest in alcoholic hepatitis (83.7 %) and lowest in AIH (21.6 %). The overall mortality rate was 5.9 % (622 cases), and the etiologies of ALD affected the clinical outcomes. ALD induced by HAV or HCV had favorable outcomes with in-hospital mortality rates of approximately 2 %, compared with ALD induced by HBV, AIH, or HEV that had in-hospital mortality rates of 6.1–7.1 %. The hospitalization related to AIH was associated with the longest hospital stay and highest cost (median: 30 days and US$17183, respectively), followed by HEV-induced hepatitis (median: 27 days and US$15822, respectively).

Clinical practices

Table 2 summarizes the clinical procedures performed for the ALD cases. Overall, only 55 patients (0.5 %) underwent liver transplantation. Various treatments other than liver transplantation were provided to the ALD patients. Plasma exchange and CHDF were performed in 556

Table 1 Etiologies and clinical characteristics of 10509 cases of ALD

Features	Drugs ($n = 1690$)	Alcohol ($n = 1642$)	HAV ($n = 177$)	HBV ($n = 1303$)	HCV ($n = 383$)	HEV ($n = 28$)	AIH ($n = 342$)	Others[c] ($n = 1260$)	Indeterminate ($n = 3684$)
Age (years)[a]	63 (49–74)	55 (45–65)	47 (32–60)	41 (31–56)	57 (42–67)	57 (48–65)	60 (49–62)	32 (18–54)	53 (34–68)
Male sex, *n* (%)	679 (40.2)	1374 (83.7)	101 (57.1)	917 (70.4)	209 (54.6)	22 (78.6)	74 (21.6)	615 (48.8)	1757 (47.7)
Length of stay (days)[a]	16 (10–25)	14 (9–23)	16 (11–25)	18 (11–26)	13 (8–21)	27 (19–51)	30 (18–47)	13 (8–22)	14 (9–23)
In-hospital mortality, *n* (%)	48 (2.8)	57 (3.5)	3 (1.7)	80 (6.1)	9 (2.3)	2 (7.1)	23 (6.7)	144 (11.4)	256 (6.9)
Hospitalization costs (US$)[a, b]	9227 (5856–14322)	8278 (5225–13029)	9211 (6764–15075)	10801 (6862–16905)	7609 (3962–12572)	15822 (11720–25552)	17183 (11146–27143)	8393 (5312–20354)	8629 (5515–13888)

[a] Median (1st quartile–3rd quartile)

[b] The exchange rate was assumed to be 80 Japanese yen for US$1

[c] Epstein–Barr virus infection, 479 (38.1 %) cases; cytomegalovirus infection, 178 (14.1 %) cases; echinococcus infection, 31 (2.5 %) cases; herpes simplex virus infection, 7 (0.6 %) cases; Wilson disease, 6 (0.5 %) cases; leptospiral infection, 1 (0.1 %) case; adenovirus infection, 1 (0.1 %) case; other or unspecified viruses or parasites, 554 (44.0 %) cases

(5.3 %) and 360 (3.4 %) cases of all ALD cases, respectively. Plasma exchange was performed in 133, 141, 122, and 160 cases of all ALD cases in 2007, 2008, 2009, and 2010, respectively. Fresh-frozen plasma, platelet, and albumin preparations were used in 961 (9.1 %), 350 (3.3 %), and 748 (7.1 %) cases, respectively. Corticosteroids injection was most commonly used in AIH. The overall mortality in patients who underwent plasma exchange was 44.2 % (246/556 cases).

ALD in children

Of all the ALD cases, infant (0–3 years) and child (4–18 years) cases accounted for 261 (2.5 %) and 494 (4.7 %) cases, respectively. The etiologies of pediatric ALD were HBV in 5.8 % of cases, HAV in 1.6 %, AIH in 0.9 %, HCV in 0.8 %, and other causes in 45.1 %. The remaining 41.6 % of cases were considered indeterminate. The distribution of the etiologies differed from that in adults (Fig. 3). Overall, the in-hospital mortality rates of infant, child, and adult cases were 2.7 % (7/261), 1.0 % (5/494), and 6.3 % (610/9754), respectively. The mortality was significantly lower in infants and children than in adults (Chi square test; $P = 0.03$ and $P < 0.001$, respectively).

Estimated incidences of hospital admission for ALD and A/AoCLF in Japan

The estimated annual incidence of ALD in Japan, calculated by the equation using Wald confidence intervals, was 16645 cases (95 % CI: 15877–17413) (Table 3). According to the Population Census Data, the population of Japan in 2008 was approximately 127 million, indicating that the estimated annual incidence of hospitalization for ALD was 131.1 cases/1 million people. The annual numbers of A/AoCLF cases were estimated to be 598, 662, 643, and 698 cases in 2007, 2008, 2009, and 2010, respectively.

Discussion

In the present study, we used a large nationwide administrative claims database to evaluate the incidence of hospitalization related to ALD in Japan, which was estimated to be 131.1 cases/1 million people/year. Although Japan has a regulation requiring reports for several infectious diseases, our previous study analyzing the incidence of acute hepatitis B [13] using the DPC database revealed underreporting of acute hepatitis B in the National Epidemiological Surveillance for Infectious Disease, which is based on the Infectious Control Law [14]. Non-mandatory reporting systems will inevitably result in underestimation

Table 2 Clinical practices

Treatments	Drugs (n = 1690)	Alcohol (n = 1642)	HAV (n = 177)	HBV (n = 1303)	HCV (n = 383)	HEV (n = 28)	AIH (n = 342)	Others (n = 1260)	Indeterminate (n = 3684)
Transplantation	0 (0)	0 (0)	0 (0)	10 (0.8)	1 (0.3)	0 (0)	3 (0.9)	34 (2.7)	7 (0.2)
Plasma exchange	30 (1.8)	28 (1.7)	5 (2.8)	113 (8.7)	7 (1.8)	0 (0)	19 (5.6)	246 (19.5)	108 (2.9)
CHDF	11 (0.7)	19 (1.2)	2 (1.1)	61 (4.7)	3 (0.8)	0 (0)	13 (3.8)	160 (12.7)	91 (2.5)
Plasmapheresis	0 (0)	5 (0.3)	0 (0)	0 (0)	0 (0)	1 (3.6)	0 (0)	2 (0.2)	3 (0.1)
Prostaglandin E1	23 (1.4)	25 (1.5)	2 (1.1)	15 (1.2)	3 (0.8)	0 (0)	3 (0.9)	38 (3.0)	34 (0.9)
Cyclosporin A	5 (0.3)	0 (0)	0 (0)	15 (1.2)	3 (0.8)	0 (0)	3 (0.9)	11 (0.9)	12 (0.3)
Corticosteroids injection	215 (12.7)	103 (6.3)	25 (14.1)	167 (12.8)	9 (2.3)	3 (10.7)	105 (30.7)	329 (26.1)	499 (13.5)
Fresh-frozen plasma	51 (3.0)	74 (4.5)	20 (11.3)	158 (12.1)	9 (2.3)	8 (28.6)	45 (13.2)	327 (26.0)	269 (7.3)
Platelet transfusion	17 (1.0)	16 (1.0)	4 (2.3)	43 (3.3)	9 (2.3)	2 (7.1)	15 (4.4)	150 (11.9)	94 (2.6)
Albumin preparation	47 (2.8)	107 (6.5)	8 (4.5)	80 (6.1)	11 (2.9)	3 (10.7)	46 (13.5)	212 (16.8)	234 (6.4)

Data are shown as n (%)

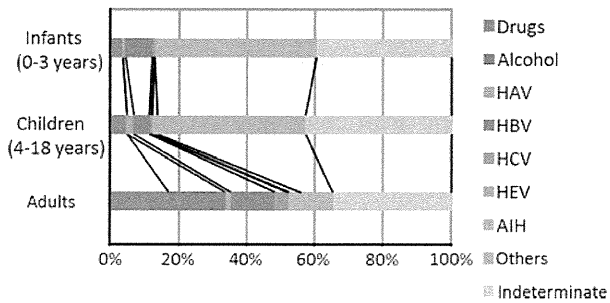

Fig. 3 Etiology of acute hepatitis in each generation

of the occurrence of disease, and thus impair health policy evaluation and decision making. In the DPC database, the diagnosis upon hospitalization is a required item, and is thought to be completely free from recall bias.

The frequency distribution of etiologies differs geographically worldwide [15, 16]. In a previous ALF study from the United States, drugs (including acetaminophen)-induced ALF were shown to be responsible for more than 50 % of ALF cases [17]. In the present study, drug-induced A/AoCLF accounted for only 5.4 % of all cases of A/AoCLF, which is far lower than the value reported in the previous ALF study from the United States. Consistent with the present study, drug-induced ALF was reported to account for about 5–15 % of cases in previous nationwide analyses in Japan [4, 6, 18]. On the other hand, HBV-induced ALF accounted for the largest proportion (about 30–40 %) of ALF cases in Japan [4, 6, 18], which is much higher than the value reported in the United States [17]. In the present study, HBV-induced A/AoCLF constituted 20.3 % of A/AoCLF cases, which is lower than the values in previous reports in Japan. ICD-10 code-based diagnosis of chronic hepatitis may have been attached to more than a few inactive HBV carriers. We excluded such patients from the present study, although acute exacerbation of hepatitis in asymptomatic HBV carriers is considered as acute hepatitis in Japan. This may explain the discrepancy.

The DPC database includes the records of clinical practices performed on each patient. Thus, we can track the use of medications and procedures including plasma exchange and liver transplantation. Artificial liver support with plasma exchange plays a central role in the treatment of ALF in Japan. The results showed that the annual estimated numbers of A/AoCLF cases were 598–698 from 2007 to 2010. These figures may be acceptable in light of an epidemiological survey of nationally-designated intractable diseases, which recently estimated the number of ALF patients in Japan to be 429 cases/year [19]. The overall fatality of A/AoCLF was estimated to be 44.2 % in the present study. In Japanese nationwide studies, the survival probabilities of patients with ALF were reported to be 47.8 % in the survey from 1998 to 2003 [4], and 47.4 % in the survey from 2004 to 2009 [18]. Similarly, the probability of spontaneous survival was reported to be approximately 45 % in the United States [17].

In the present study, ALD induced by HAV or HCV had favorable outcomes with regard to mortality, compared with that induced by HBV, HEV, or AIH, which is compatible with previous studies that reported favorable outcomes of ALF induced by HAV and unfavorable outcomes of ALF induced by HBV or AIH [17]. Moreover, the mortality of infants and children hospitalized for ALD was significantly lower than that of adults. We cannot know the severity of ALD from the DPC database, which may leave room for the possibility that children with ALD were more prone to be admitted to hospitals with less severe conditions. Favorable outcomes of ALF in children were also reported [20].

Table 3 Estimated number of annual ALD patients in Japan

Bed volume	Number of acute care beds in Japan (N_i)	Number of acute care Beds in DPC hospitals (n_i)	Number of ALD patients in DPC hospitals for 2 years (X_i)[a]	Estimated number of all ALD patients in Japan (Y_i) (95 % confidence interval)
≤399	566658	119853	4843	11449 (10997–11900)
400–599	175715	89627	3008	2949 (2801–3096)
600–799	88870	49740	1477	1319 (1225–1414)
≥800	78995	50245	1181	928 (854–1003)
Total	910238	309465	10509	16645 (15877–17413)

[a] Data were collected from 6 months (July–December) of each 4 years (2007–2010)

Table 4 Estimated number of annual ALD patients and annual incidence (per 1 million people) of acute hepatitis in Japan

Features	Drugs	Alcohol	HAV	HBV	HCV	HEV	AIH	Others	Indeterminate
Estimated number	2788 (2476–3100)	3021 (2709–3334)	267 (169–366)	1815 (1552–2076)	574 (428–720)	40 (1–79)	474 (340–609)	1918 (1653–2183)	5748 (5293–6203)
Annual incidence/1 million people	22.0 (19.5–24.4)	23.8 (21.3–26.3)	2.1 (1.3–2.9)	14.3 (12.2–16.4)	4.5 (3.4–5.7)	0.32 (0.01–0.62)	3.7 (2.7–4.8)	15.1 (13.0–17.2)	45.3 (41.7–48.9)

Data are shown as n (95 % confidence interval)

In the present study, ALD induced by indeterminate etiology accounted for the greatest proportion (35.1 %) of all cases of ALD. A previous study showed the possibility that patients with ALF induced by HBV, AIH, or drugs may be included in ALF with indeterminate etiology, using a data-mining approach [21]. However, data on a total of 104 items, including information inaccessible in the DPC database such as past history or laboratory data, are required to categorize patients by this approach. Future improvements to the DPC database are encouraged to enable access to more information, which will allow us to undertake further useful approaches.

Alcoholic hepatitis often develops in patients with chronic liver disease caused by habitual alcohol consumption. Thus, in the recently determined criteria for ALF (as well as the previous criteria for FH) in Japan, patients with alcoholic hepatitis are usually excluded from the disease entity of ALF (or FH) [4, 6, 7, 12]. However, alcoholic hepatitis may develop in patients with minimal liver injury, and is still included as an etiological factor for the disease entity of ALF in Europe and the United States [5]. In addition, alcoholic hepatitis is associated with a high fatality rate [22]. Therefore, alcohol-induced ALD is thought to constitute a major health burden. For these reasons, in the present study, we did not exclude patients with alcoholic hepatitis unless accompanied by another diagnosis such as chronic hepatitis or cirrhosis.

This study has several limitations. First, the sample collection in the DPC database is not based on a random sampling method, and thus the hospital distribution tends to be biased. Although the DPC database represents approximately 40 % of all admissions to secondary and tertiary care hospitals in Japan, participating hospitals tend to be medium-to-large-sized institutions with beds for more severe ALD patients. The mortality could,, therefore be overestimated by excluding less severe patients in small-sized hospitals. However, ALD is relatively common and hospitalization related to ALD in small-to-medium-sized hospitals is thought to account for a considerable portion. Indeed, 4843 (46.1 %) cases were derived from hospitals with less than 400 beds, as shown in Table 3. Hence, this limitation may be not too serious. Second, the DPC database leaves room for the possibility of inaccurate reporting of diagnoses. Although we excluded cases with diagnoses of chronic liver diseases, some patients with acute exacerbation of chronic liver disease might have been registered as ALD, resulting in an overestimation of the ALD incidence in Japan. Third, as noted above, important clinical data such as prothrombin time or degree of encephalopathy were unavailable in the DPC database. Consequently, we could not learn from the database the etiology or clinical characteristics of ALF cases, which should be characterized by the presence of encephalopathy and prothrombin international normalized ratio of >1.5. Fourth, the DPC survey is only conducted between July and December each year, and therefore data between January and June were not available. The sample may therefore be biased, especially in ALDs related to seasonal causes such as HAV or HEV.

Fifth, patients may have been referred from the first hospital to another hospital for specialized treatment, such as liver transplantation. In this case, the two admissions would be recorded separately in the DPC database, leaving the possibility that such patients were enrolled in the analyses in a duplicated manner. Finally, the DPC database only includes inpatient data, and; therefore, we cannot know the incidence of ALD cases treated in outpatient settings from the database. However, the most severe cases are likely to be included in the inpatient database (Table 4).

In conclusion, the present study has demonstrated the incidence of ALD and the clinical practices performed on ALD patients in Japan using the nationwide DPC database. The overall in-hospital mortality of ALD in Japan was 5.9 % in the DPC database, which was affected by the etiologies as well as the patients' background characteristics. Since the DPC database does not cover the whole admission in Japan and also does not cover patients in outpatient settings, the overall burden of the disease may still remain to be evaluated. Improvement of public health surveillance systems is necessary for population-based patient monitoring.

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Conflict of interest None of the authors have any conflicts of interest.

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