test to all the tested SNPs indicated that the genetic inflation factor lambda was 1.02 for the second stage (**Supplementary Fig. 1a** online), implying a low possibility of false positive associations due to population stratification. All 11 SNPs are located within or around the *HLA-DPA1* and *HLA-DPB1* locus (**Fig. 2**). We also conducted age- and sex-adjusted analysis using a logistic regression model, and confirmed similar association after adjustment (data not shown).

To validate the result of the discovery-phase analysis, we carried out replication analyses using three independent cohorts. We selected the most or second-most strongly associated SNPs from each *HLA-DP* locus (rs9277535 on *HLA-DPB1* and rs3077 on *HLA-DPA1*, respectively), as we failed to design a Taqman or Invader probe for rs2395309 on *HLA-DPA1*. We first examined two independent sets of Japanese case–control samples comprising 274 cases and 274 controls (age-, sex- and alcohol consumption–matched cohort from BioBank Japan) as well as 718 cases and 1,280 controls. We found significant associations at two SNP loci in both studies ($P = 1.06 \times 10^{-16} \sim 1.96 \times 10^{-6}$; **Table 1**). We also genotyped 308 individuals with chronic hepatitis B and 546 healthy controls in Thailand, and further confirmed the association at the two loci, rs3077 ($P = 6.53 \times 10^{-6}$) and rs9277535 ($P = 6.52 \times 10^{-8}$).

To combine these studies, we conducted a meta-analysis with a fixed-effects model using the Mantel-Haenszel method. As shown in **Table 1** and **Supplementary Figure 1b**, the odds ratios (OR) were quite similar across the four studies (the second stage of GWAS and three replication studies) and no heterogeneity was observed. Mantel-Haenszel P values for independence were 2.31×10^{-38} for rs3077 (OR = 0.56, 95% confidence interval (CI) = 0.51–0.61), and 6.34×10^{-39} for rs9277535 (OR = 0.57, 95% CI = 0.52–0.62).

The 11 SNPs showing significant associations are located within a 50-kb region including *HLA-DPA1* and *HLA-DPB1* (**Fig. 2**). Although the *HLA* region is known to show extensive linkage disequilibrium (LD) spanning over 7 Mb, the LD block including these 11 SNPs (surrounded by a bold line in **Fig. 2a**) was not in strong LD with the other *HLA* loci. In accordance with the extent of LD, only SNPs around the *HLA-DPA1* and *HLA-DPB1* genes showed very strong associations with chronic HBV (surrounded by a bold line in **Fig. 2b**), and SNPs outside of this particular LD block did not have significant association.

HLA-DPA1 and *HLA-DPB1* encode the HLA-DP α and β chains, respectively. HLA-DPs belong to the HLA class II molecules that form heterodimers on the cell surface and present antigens to CD4-positive T lymphocytes. HLA-DPs are highly polymorphic, especially in exon 2, which encodes antigen-binding sites. We thus considered that the association of these SNPs with chronic HBV might reflect variations in antigen-binding sites that might affect the immune response to HBV. We genotyped *HLA-DPA1* and *HLA-DPB1* alleles by direct sequencing of exon 2 (cases at second stage and controls at first stage) and found significant association of chronic hepatitis B with *HLA-DPA1**0103, *DPA1**0202, *DPB1**0402 and *DPB1**0501 ($P = 2.93 \times 10^{-11}$, 4.45×10^{-8}, 2.27×10^{-7} and 6.98×10^{-7}, respectively; **Supplementary Table 3** online). Because sequence variants in exon 2 of *HLA-DPA1* and *HLA-DPB1* could be linked to individual nucleotide variants, we inferred haplotypes using the 11 SNPs and variants in exon 2, and found very strong LD among them (**Supplementary Fig. 2**

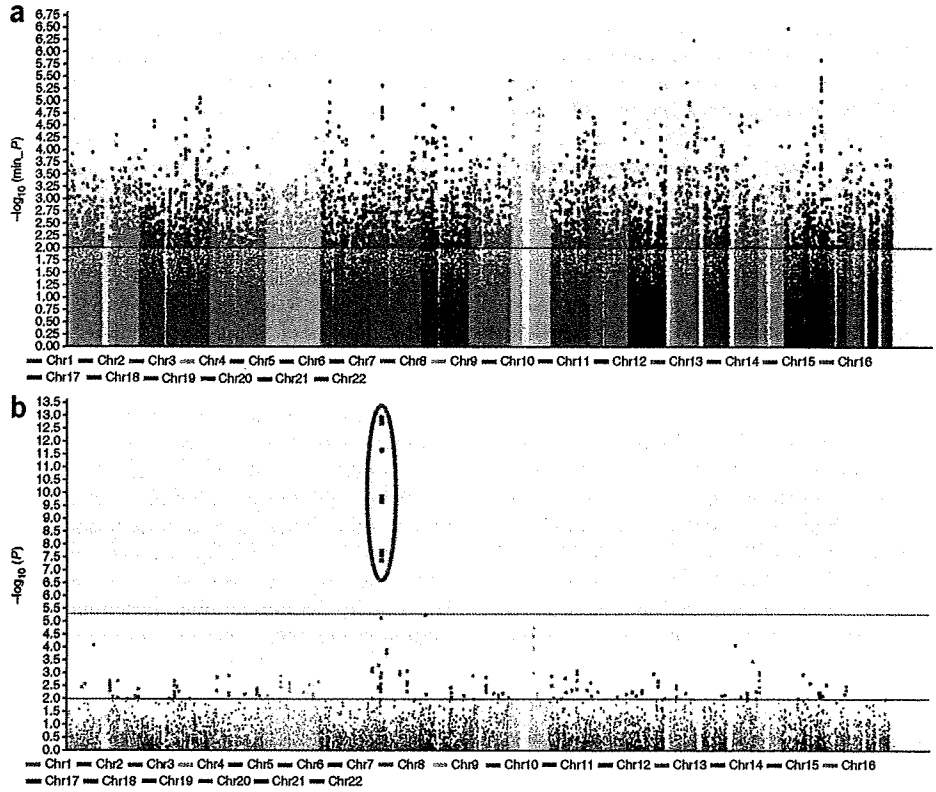

Figure 1 Results from a two-stage genome-wide association study. (**a**) $-\log_{10}$ P value plot at the first stage. Each P value is the minimum of Fisher's exact tests for three models: dominant, recessive and allele frequency model. (**b**) $-\log_{10}$ P value plot at the second stage. P values were calculated by 1-d.f. Cochrane-Armitage trend test. The large dots circled by red on the chromosome 6 showed significant associations ($P < 5.06 \times 10^{-6}$) with chronic hepatitis B.

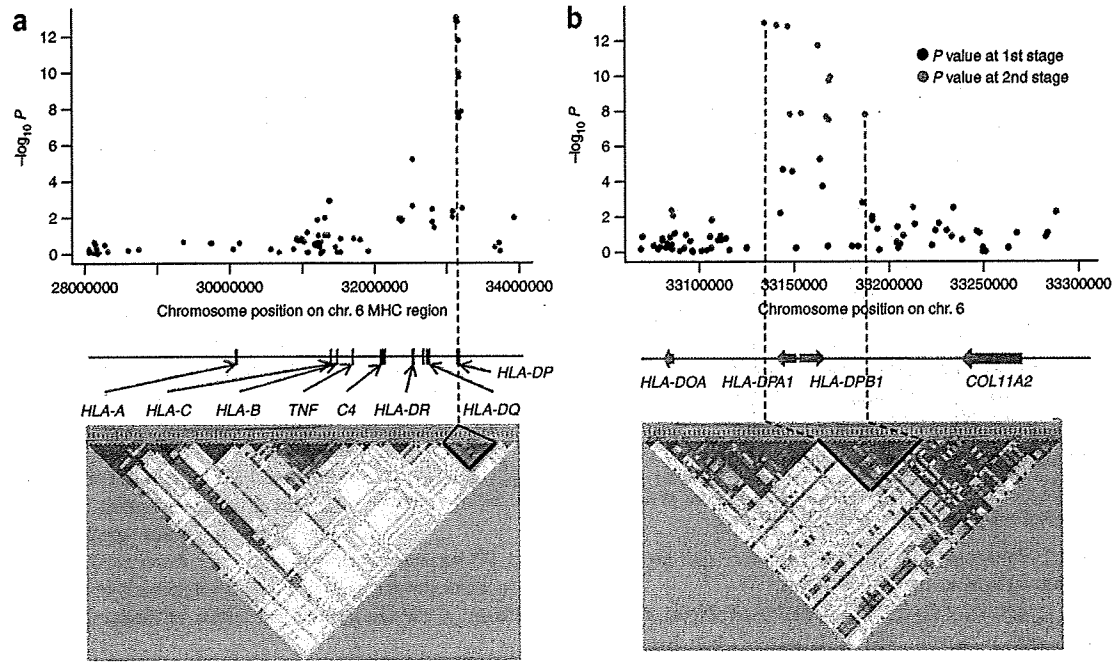

Figure 2 Case–control association results and linkage disequilibrium map of the MHC region. (**a**) *P*-value plot, genomic structure and LD map of the second stage within the extended MHC region of chromosome 6. The LD map based on *D′* was drawn using the genotype data of the cases and the controls in the second stage. (**b**) *P*-value plot, genomic structure and LD map around the *HLA-DPA1* and *HLA-DPB1* region. Black dots and red dots represent *P* values in the first and the second stage, respectively. The LD map based on *D′* was drawn using the genotype data of the cases and the controls in the first stage.

online). Case–control analyses revealed four associated haplotypes: *DPA1**0103-*DPB1**0402 and *DPA1**0103-*DPB1**0401 showed protective effects ($P = 6.00 \times 10^{-8}$, OR = 0.52, 95% CI = 0.35–0.75 and $P = 0.002$, OR = 0.57, 95% CI = 0.33–0.96, respectively), whereas *DPA1**0202-*DPB1**0501 and *DPA1**0202-*DPB1**0301 were associated with susceptibility to chronic hepatitis B ($P = 5.79 \times 10^{-6}$, OR = 1.45, 95% CI = 1.16–1.81 and $P = 0.002$, OR = 2.31, 95% CI = 1.39–3.84, respectively; **Table 2**). We also found various sets of SNPs (tagging SNPs) that could predict *HLA-DP* alleles (**Supplementary Table 4** online). Taken together, our findings strongly implicate an association of genetic variants in the *HLA-DPA1* and *HLA-DPB1* genes with chronic hepatitis B.

HLA-DR13 was reported to have a protective effect against persistent HBV infection in different populations[9,13,14]. Comparison of genotypes of *HLA-DRB1**1301 and *1302 alleles (both corresponding to HLA-DR13) and Illumina HumanHap550 SNPs in 333 of the first-stage control samples revealed that the A allele of rs11752643 was in strong LD with HLA-DR13 ($r^2 = 0.83$, $D' = 1$). However, the association between rs11752643 and chronic hepatitis B was not significant in our second stage GWAS, with an uncorrected *P* value of 1.04×10^{-4} (**Supplementary Table 5** online). In addition, the association of chronic hepatitis B with rs3077 and rs9277535 remained highly significant ($P = 2.11 \times 10^{-10}$ and 1.73×10^{-9}, respectively) after adjustment for rs11752643 using a logistic

Table 1 Results of replication studies and meta-analysis

SNP	Nearest gene	Allele (1/2)	Stage	Cases			Controls			OR (95%CI)[a]	P^b	P_{het}^c
				11	12	22	11	12	22			
rs3077	*HLA-DPA1*	A/G	GWAS second stage	42	240	324	197	598	472	0.57 (0.49–0.66)	1.26E–13	
			First replication	25	95	152	50	122	102	0.53 (0.41–0.69)	1.73E–06	
			Second replication	64	237	410	197	596	485	0.55 (0.47–0.63)	1.06E–16	
			Third replication	28	109	163	85	250	210	0.61 (0.49–0.75)	6.53E–06	
			Meta-analysis[d]							0.56 (0.51–0.61)	2.31E–38	0.84
rs9277535	*HLA-DPB1*	A/G	GWAS second stage	58	254	294	230	619	418	0.59 (0.51–0.69)	1.78E–12	
			First replication	26	102	144	49	132	91	0.54 (0.42–0.69)	1.96E–06	
			Second replication	68	264	376	227	604	445	0.56 (0.48–0.64)	1.81E–16	
			Third replication	29	136	139	107	273	155	0.56 (0.46–0.69)	6.52E–08	
			Meta-analysis[d]							0.57 (0.52–0.62)	6.34E–39	0.85

Odds ratio and *P* values for independence test were calculated by the Mantel-Haenszel method.
[a]Odds ratio of minor allele from two-by-two allele frequency table. [b]*P* values of Pearson's χ^2 test for allele model. [c]Result of Breslow-Day test. [d]Meta-analysis of all four studies.

Table 2 Haplotype analysis

No.	Haplotype[a]	Frequency (cases)	Frequency (controls)	P^b	OR[b] (95% CI)
1	GG-*DPA1*0202*-TCG-*DPB1*0501*-GAGATT	0.428	0.347	5.79E-06	1.45 (1.16–1.81)
2	AA-*DPA1*0103*-CCA-*DPB1*0201*-AGTGCC	0.165	0.192	0.052	Reference
3	GG-*DPA1*0201*-TCG-*DPB1*0901*-GGGGTC	0.129	0.124	0.642	1.21 (0.91–1.61)
4	AA-*DPA1*0103*-CTA-*DPB1*0402*-AGTGCC	0.042	0.096	6.00E-08	0.52 (0.35–0.75)
5	AA-*DPA1*0103*-CCA-*DPB1*0401*-AGTGCC	0.018	0.038	0.002	0.57 (0.33–0.96)
6	GG-*DPA1*0202*-TCG-*DPB1*0301*-GGGGTC	0.036	0.018	0.002	2.31 (1.39–3.84)
7	GG-*DPA1*0202*-TCG-*DPB1*0202*-AGTGCC	0.020	0.027	0.257	0.88 (0.51–1.52)
8	GG-*DPA1*0202*-TCG-*DPB1*0201*-AGTGCC	0.022	0.024	0.662	0.97 (0.57–1.65)
9	GG-*DPA1*0201*-TCG-*DPB1*0501*-GAGATT	0.029	0.018	0.057	1.81 (1.06–3.08)
10	GG-*DPA1*0201*-TCA-*DPB1*1301*-GGTGCC	0.022	0.016	0.172	1.69 (0.95–3.03)
11	AA-*DPA1*0103*-CTG-*DPB1*0301*-GGGGTC	0.011	0.016	0.246	0.74 (0.36–1.53)
12	GG-*DPA1*0201*-TCG-*DPB1*1401*-GGGGTC	0.012	0.012	0.877	1.25 (0.61–2.53)

Controls of the first stage and cases of the second stage were analyzed.
[a]Haplotypes consisting of rs2595309, rs3077, *HLA-DPA1*, rs2301220, rs9277341, rs3135021, *HLA-DPB1*, rs9277535, rs10484569, rs3128917, rs2281388, rs3117222 and rs9380343 are shown. [b]*P* values, odds ratios and its 95% confidence intervals of each haplotype were calculated as described in the Methods.

regression model. Thus, our findings clearly indicate that hepatitis B is associated with variants in the *HLA-DP* loci.

A number of reports have described association of several *HLA* and non-*HLA* genes with persistent HBV infection[12,15], but their results were not consistent among the studies, and none of them indicated a possible involvement of the *HLA-DP* locus. This study is the first GWAS to investigate host genetic factors associated with chronic hepatitis B. One genome-wide linkage analysis using 318 microsatellite markers in the Gambian population suggested that the chromosome 21q22 region contains a susceptibility locus for persistent HBV infection[16]. However, our GWAS analysis failed to support this result, possibly owing to ancestry differences or different modes of viral transmission (the vertical transmission in Japan versus the horizontal transmission in Gambia).

To investigate the correlation between the incidence of hepatitis B infection and these polymorphisms, we evaluated the frequencies of rs3077 and rs9277535 in 11 different HapMap3 populations (**Supplementary Table 6** online). Our association analysis indicated that A alleles at both rs3077 and rs9277535 were associated with protective effects for chronic hepatitis B. Notably, the frequencies of these two alleles were lower in Asian and African populations, especially in the Chinese population, compared with European and Central American populations. Although disease prevalence is not determined solely by genetic factors, the findings presented in our manuscript suggest that genetic factors might exert substantial influence on the prevalence of infectious disease.

Antigen presentations on HLA class II molecules to CD4-positive helper T cells and on class-I molecules to CD8-positive cytotoxic T cells are considered to be critical for the immune response against exposure to HBV. Although cytotoxic T cells are suspected to have major roles in viral clearance, helper T cells are also essential in the immune response to acute infections[17]. HLA-DPs have a structure similar to other classical HLA class II molecules, but their roles in the immune response have not been well characterized, except the association with berylliosis[18]. The 11 SNPs we found showing strong association with chronic HBV infection were in very strong LD with *HLA-DP* alleles. Because the subsequent haplotype analyses identified significant association of chronic hepatitis B with haplotypes containing the *HLA-DPA1* and *HLA-DPB1* genes, we suspected that variations in HLA-DP molecules would affect the ability for antigen presentation of HLA class II molecules on immune cells and result in weak (or no) immune response and persistent HBV infection. A previous report that implicated *HLA-DPA1**0103 and *DPB1**0402 to be candidate predictive factors for antibody production after HBV vaccination[19] supports this hypothesis. It should be noted that the lack of information regarding exposure to HBV for each control might underestimate the effect size obtained in this study but does not inflate the type 1 error rate.

In summary, we have demonstrated that genetic variants in the *HLA-DP* genes are strongly associated with chronic hepatitis B in the Asian population. Considering the function of HLA-DP molecules, our findings suggest that antigen presentation on HLA-DP molecules might be critical for virus elimination and have an important role in the pathogenesis of chronic hepatitis B. An understanding of the molecular mechanism by which *HLA-DP* variants confer risk of chronic hepatitis B should shed light on its pathogenesis and facilitate development of new therapies for treatment of the disease and prevention of disease progression.

METHODS

Samples. Characteristics of each cohort group are shown in **Supplementary Table 1**. Case and control samples used in this study for the Japanese population were obtained from the BioBank Japan at the Institute of Medical Science, the University of Tokyo[20], except case samples of the second replication and control samples of the first stage of the GWAS. From the registered samples in BioBank Japan, we selected individuals that were clinically diagnosed as having chronic hepatitis B. The diagnosis of chronic hepatitis B was conducted based on HBsAg-seropositivity and elevated serum aminotransferase levels for more than six months according to the guideline for diagnosis and treatment of chronic hepatitis (see URLs section below). The control groups consisted of 2,821 individuals that were registered in BioBank Japan as subjects with diseases other than chronic hepatitis B. Subjects who were positive for HBsAg were excluded from the controls. We obtained 934 Japanese control DNAs in the first stage from volunteers in the Osaka-Midosuji Rotary Club, Osaka, Japan. Case samples for the second replication cohort ($n = 718$, RIKEN) were collected at Toranomon Hospital as well as at hospitals participating in the Hiroshima Liver Study Group (for a list of doctors participating in this study group, see URLs section below). Cases and controls for the Thai replication study ($n = 308$ and 546, respectively) were collected at Ramathibodi Hospital, Mahidol University, Thailand. The diagnosis of chronic hepatitis B was based on HBsAg-seropositivity and elevated serum aminotransferase levels. All participants provided written informed consent. This research project was approved by the ethical committees at the Institute of Medical Science, the University of Tokyo, the Center for Genomic Medicine (formerly SNP Research Center), RIKEN and Ramathibodi Hospital, Mahidol University.

SNP genotyping. We applied the two-stage approach as described previously[21]. For the first stage, we genotyped 188 individuals with chronic hepatitis B and 934 controls using the Illumina HumanHap550v3 Genotyping BeadChip. After excluding nine cases with call rate of <0.98, we applied SNP quality control (call rate of ≥ 0.99 in both cases and controls and P value of Hardy-Weinberg equilibrium test of $\geq 1.0 \times 10^{-6}$ in controls): 499,544 SNPs on autosomal chromosomes passed the quality control filters and were further analyzed. Among the SNPs analyzed in the first stage, we selected the top 12,000 SNPs showing the smallest P values for the second stage. SNPs with minor allele frequency (MAF) of ≤ 0.1 in both case and control samples were excluded from the further analysis. In the second stage, we genotyped an additional panel of 616 cases using an

Affymetrix GeneChip Custom 10K array. After excluding nine cases with call rate of <0.95, all cluster plots were checked by visual inspection by trained staff, and SNPs with ambiguous calls were excluded. Ninety-four randomly selected case samples in the first stage were re-genotyped in the second stage, and SNPs with concordance rates of <98% between two assays (Illumina and Affymetrix) were excluded from the further analysis. We used genome-wide screening data of other diseases (uterine cervical cancer, esophageal cancer, hematological cancer, pulmonary tuberculosis, ovarian cancer, uterine body cancer and keroid) as controls for the second stage. All the samples were genotyped using the Illumina HumanHap550v3 Genotyping BeadChip, and the same quality-control filters as the first screening were applied. As a result, we analyzed 9,875 SNPs in 607 cases and 1,267 controls in the second stage and found 11 SNPs ($P < 5.06 \times 10^{-6}$) to be significantly associated with chronic hepatitis B after Bonferroni correction. These first and second stages are defined as the discovery phase of the research, and the following replication studies are defined as the replication phase. In the replication analyses, we used TaqMan genotyping system (Applied Biosystems) or the multiplex PCR-based Invader assay (Third Wave Technologies).

***HLA-DPA1* and *HLA-DPB1* genotyping.** We analyzed *HLA-DP* genotypes using 607 cases (in the second stage of GWAS) and 934 controls (in the first stage of GWAS). Exon 2 of the *HLA-DPA1* and *HLA-DPB1* genes were amplified and directly sequenced according to the protocol of International Histocompatibility Workshop Group[22]. *HLA-DPA1* and *DPB1* alleles were determined based on the alignment database of dbMHC.

Statistical analysis. In the first stage of the GWAS, Fisher's exact test was applied to a two-by-two contingency table in three genetic models: an allele frequency model, a dominant-effect model and a recessive-effect model. At the second stage of GWAS and replication analyses, statistical significance of the association with each SNP was assessed using a 1-degree-of-freedom Cochrane-Armitage trend test. Significance levels after Bonferroni correction for multiple testing were $P = 5.06 \times 10^{-6}$ (0.05/9,875) in the second stage and $P = 0.025$ (0.05/2) in replication analyses. Age- and sex-adjusted odds ratios were obtained by logistic regression analysis. Odds ratios and confidence intervals were calculated using the major allele as a reference. The meta-analysis was conducted using the Mantel-Haenszel method. Heterogeneity among studies was examined by using the Breslow-Day test. To assess the association of each *HLA* allele, we used Fisher's exact tests on two-by-two contingency tables with or without each *HLA* allele. To analyze the association of haplotypes, we used R package haplo.stats. *P* values for each haplotype were given by the results of a score test, and odds ratios and 95% confidence intervals were calculated from coefficients of GLM model. Odds ratios of each haplotype were calculated relative to the second major haplotype in **Table 2**, because the most common haplotype was the disease-associated haplotype. All of these statistical values were calculated by function haplo.cc. We used Haploview software to analyze linkage disequilibrium values between *HLA-DR13* and SNPs.

Software. For general statistical analysis, we used R statistical environment version 2.6.1 or PLINK1.03 (ref. 23). To draw the LD map, we used Haploview software[24]. Estimation of haplotype frequencies and analysis of haplotype association were performed by R package haplo.stats[25]. Sequence variants in exon2 of *HLA-DPA1* and *HLA-DPB1* were analyzed by Polyphred.

URLs. The Japan Society of Hepatology, http://www.jsh.or.jp/medical/gudelines/index.html; Hiroshima Liver Study Group, http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html; PLINK1.03, http://pngu.mgh.harvard.edu/~purcell/plink/; R package haplo.stats, http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm; Polyphred, http://droog.gs.washington.edu/polyphred/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

Y.N. conceived the study; Y.N., Y.K., Y.D., M.K. and K.M. designed the study; Y.K., S.W., H.O. and N.H. performed genotyping; Y.K., T.T., M.K., N.K., Y.N. and K.M. wrote the manuscript; T.K., A.T., T.T. and N.K. performed data analysis at the genome-wide phase; Y.N., K.M. and M.K. managed DNA samples belong to BioBankJapan; K.C. and H.K. managed second replication samples; W.C., A.P. and T.S. managed third replication samples in Thailand; Y.K. summarized the whole results; Y.N. obtained funding for the study.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Intra-arterial 5-fluorouracil/interferon combination therapy for advanced hepatocellular carcinoma with or without three-dimensional conformal radiotherapy for portal vein tumor thrombosis

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Abstract
Background The aim of this study was to elucidate the efficacy of intra-arterial 5-fluorouracil (5-FU) and interferon (IFN) α combined with three-dimensional conformal radiotherapy (3D-CRT) for portal vein tumor thrombosis (PVTT).
Methods The study groups were 16 HCC patients with PVTT treated with 5-FU/IFN combined with 3D-CRT (RT group) and 16 matched controls treated with 5-FU/IFN alone (non-RT group). We compared the survival rate, response, time to progression (TTP), portal hypertension-related events (PREs) and safety.
Result Complete response (CR) of PVTT, partial response (PR), stable disease (SD) and progressive disease (PR) were noted in three (19%), nine (56%), four (25%) and zero patients of the RT group, one (6%), three (19%), seven (44%) and five (31%) patients of the non-RT group, respectively. The objective response rate of PVTT was higher in the RT group ($P = 0.012$). The rate of PREs (variceal rupture, worsening of esophagogastric varices and emerging of uncontrollable ascites) was lower in the RT group than in the non-RT group ($P = 0.0195$). The median survival time of the RT group (7.5 months) was not significantly different from that of the non-RT group (7.9 months). RT-induced liver disease was not observed.
Conclusion 5-FU/IFN combination with 3D-CRT for PVTT improved the response rate of PVTT and reduced the incidence of portal hypertension-related events.

Keywords Hepatocellular carcinoma · Portal vein tumor thrombosis · Radiotherapy · 5-FU · IFN

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers and causes of cancer death worldwide [1–3]. Development of new diagnostic techniques, such as ultrasonography, computed tomography (CT), magnetic resonance imaging and angiography, and advancements in therapeutic modalities, such as surgical resection, radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), transcatheter arterial chemoembolization (TACE), radiotherapy (RT) and intra-arterial infusion via implantable drug delivery systems have gradually improved the prognosis of HCC patients [4–8]. Nevertheless, the prognosis of patients with advanced HCC and portal vein tumor thrombosis (PVTT) is still poor [9–13]. PVTT causes widespread intrahepatic and extrahepatic dissemination by spreading out of tumor cells through the portal tract. Furthermore, PVTT, especially PVTT in the first branch (Vp3)

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or in the main trunk (Vp4), causes portal hypertension resulting in variceal rupture, uncontrollable ascites, ischemic liver failure and worsening of performance status (PS) [14]. These pathological states lead to simultaneous worsening of quality of life (QOL); hence, any treatment for HCC is contraindicated under such conditions, especially for those patients with HCC and PVTT in Vp3 or Vp4.

Recent advances in implantable drug delivery systems have facilitated repeated arterial infusion of chemotherapy agents. Because hepatic arterial infusion chemotherapy (HAIC) increases local tissue drug concentrations and consequently reduces the side effects of anticancer agents, this therapeutic modality is suitable for HCC patients with PVTT and poor hepatic reserve. Among several anticancer agents, intra-arterial 5-fluorouracil (5-FU) and systemic interferon (IFN) α were reported as one of the most effective combination chemotherapies for HCC with PVTT [15–18]. On the other hand, advances in three-dimensional conformal radiotherapy (3D-CRT) have allowed the delivery of higher radiation doses to the tumor and at the same time minimized the radiation dose to normal tissue, resulting in improvement of the antitumor effect and minimization of damage to normal tissue. This modality makes possible local irradiation for PVTT in patients with poor hepatic reserves [19, 20]. Despite the development of new chemotherapies and radiotherapies, the prognosis of HCC patients with Vp3/4 is still less than 1 year, and the response rate is less than about 50%.

In various malignancies such as lung cancer and esophageal cancer, the synergistic effects of the combination of chemotherapy and radiotherapy have been reported [21–24]. Recently, Han et al. [25] reported that the use of combination therapy of HAIC containing 5-FU/cisplatin (CDDP) and 3D-CRT in HCC patients with Vp3/4 showed a response rate of 45%. To our knowledge, however, there is no report about the combination therapy of 5-FU/IFN and 3D-CRT. In addition, it is still unclear whether RT has any additional effects on 5-FU/IFN. Accordingly, we performed a retrospective case-control study of intra-arterial 5-FU/IFN combination therapy with or without 3D-CRT for advanced HCC with Vp3/4. The aim of the present study was to elucidate the efficacy of 5-FU/IFN combination therapy with 3D-CRT by comparing survival, response, time to progression (TTP), portal hypertension related-events (PREs) and safety in the two groups.

Patients and methods

Study design and eligibility

The enrollment criteria were as follows: HCC with Vp 3 or Vp 4, PS 0 or 1, Child-Pugh A or B, serum total bilirubin <3.0 mg/dl, leukocyte count >2,000/ml, platelet count >50,000/ml, serum creatinine <1.5 mg/dl, at least a 4-week rest period of no treatment since any previous treatment for HCC, no recent history of upper gastrointestinal bleeding, no history of heavy alcohol abuse, and no other serious medical condition that would interfere with participation in this study. Extrahepatic metastases were not exclusion criteria when we considered these were not prognostic factors of the patients. The exclusion criteria included HCC with inferior vena cava or hepatic vein tumor thrombosis. All patients were asked to provide a written informed consent for this study, which was approved by the Institutional Review Board of Hiroshima University.

From June 2003 to January 2008, 18 patients met the above criteria for 5-FU/IFN combined with 3D-CRT for Vp3/4. Two patients refused the therapy; thus, 16 patients were enrolled for the therapy. The 16 patients were defined as the RT group. To compare the clinical efficacy of the therapy, we retrospectively selected 16 patients treated with 5FU/IFN alone, matched 1:1 with patients of the RT group for sex, age, grade of portal vein invasion, grade of ascites and status of esophagogastric varices (form and red color sign) [26]. These patients were defined as the non-RT group. Patients of the non-RT group were selected from among 29 HCC patients with PVTT treated by 5-FU/IFN. The decision to use or not to use 3D-CRT was left to the attending physician. The baseline characteristics of patients of the two groups are shown in Table 1. There were no differences between the two groups with regard to the PS, etiology, HCC stage, main tumor size, tumor volume, extrahepatic metastases, α-fetoprotein (AFP), AFP-L3, des-γ-carboxy prothrombin (DCP), Child-Pugh grade, leukocyte count, hemoglobin, platelet count, total bilirubin, albumin and indocyanine green retention rate at 15 min (ICG-R (15)).

Treatment protocol

For the RT group, patients received 3D-CRT concomitantly with the first course of intra-arterial 5-FU/IFN. Patients received 3D-CRT in the Division of Radiation Oncology at our hospital. Patients of the RT group received high-energy photon beam irradiation using 18 or 6 MV, delivered by a three-dimensional conformal technique (CLINAC 2300 C/D linear accelerators, Varian Medical Systems Inc., Palo Alto, CA). The planning CT determined the gross tumor volume (GTV) as only the PVTT. The clinical target volume (CTV) was determined including GTV and intrahepatic tumor forming the basal part of PVTT. The planning target volume (PTV) represented the CTV plus a 10–20-mm margin in all directions for internal motion and set-up error. Four to five portal fields were

Table 1 Clinical profile of 32 patients with hepatocellular carcinoma and portal vein tumor thrombosis

	5FU/IFN combination with 3D-CRT	5FU/IFN alone	*P* value
Number	16	16	
Age (years)[a]	65.5 (35–79)	64 (53–76)	Matched
Sex (male/female)	15/1	15/1	Matched
PS (0/1)	14/2	12/4	NS
Etiology: HBV/HCV/other	4/9/3	5/8/3	NS
HCC stage (IVA/IVB)	11/5	13/3	NS
Grade of portal vein invasion (Vp 3/4)[b]	8/8	8/8	Matched
Main tumor size[a]	70 (36–130)	64 (22–115)	NS
Tumor volume (≤50/>50%)	11/5	13/3	NS
Extrahepatic metastases (yes/no)	6/10	4/12	NS
AFP (ng/ml)[a]	184.8 (<5–153,200)	586.7 (6–165,500)	NS
AFP-L3 (%)[a]	48.1 (<0.5–88.8)	27.1 (<0.5–87.6)	NS
DCP (mAU/ml)[a]	8,992 (36–392,790)	9,513 (61–722,140)	NS
Child-Pugh grade (A/B)	12/4	13/3	NS
Platelet count ($\times 10^3/\mu l$)[a]	11.3 (5.7–32.8)	14.4 (6.7–54.4)	NS
Total bilirubin (mg/dl)[a]	0.9 (0.4–1.9)	0.9 (0.6–1.9)	NS
Albumin (g/dl)[a]	3.8 (2.6–5)	3.7 (3.1–4.3)	NS
Ascites (absent/present)	12/4	12/4	Matched
ICG-R(15) (%)[a]	17.6 (3.2–32)	25.4 (6.8–47)	NS
Previous treatment (performed/not performed)	5/11	5/11	NS
Radiation dose (Gy)[a]	39 (30–45)	–	
Form of EV (F0/F1/F2)[c]	4/10/2	9/5/2	Matched
Red color sigh of EV (RC0/RC1/RC2)[c]	12/3/1	13/2/1	Matched
Form of GV (F0/F1/F2)[c]	10/6/0	11/3/2	Matched
Red color sign of GV (RC0/RC1/RC2)[c]	16/0/0	15/1/0	Matched

PS Eastern Cooperative Oncology Group performance status, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma, *AFP* α-fetoprotein, *AFP-L3* lens culinaris agglutininreactive fraction of α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *ICG-R (15)* indocyanine green retention rate at 15 min, *EV* esophageal varices, *GV* gastric varices

[a] Data are median values (range)

[b] PVTT grade: *Vp3* tumor thrombus in the first branch of the portal vein, *Vp4* tumor thrombus in the trunk of the portal vein

[c] Endoscopic findings of esophagogastric varices: *F* form, *F0* absence of varices, *F1* small straight, *F2* enlarged tortuous, *RC* red color sign, *RC0* no RC, *RC1* localized RC, *RC2* between localized and entire circumference RC

used. Outlined target volumes, total liver tissue and organ at risk structures, including the spinal cord, bilateral kidneys and intestinal tract nearby targets, were transferred to the treatment planning system (Pinnacle3, Philips Medical Systems, Eindhoven, The Netherlands) with reference to the diagnostic enhanced CT images. The prescribed dose was 30, 39 or 45 Gy in accordance with the dose-volume constraint of normal tissue and liver function. Ninety-five percent of the PTV should receive at least 95% of the prescribed dose. 50% of the liver tissue should not receive more than 25 Gy, 50% of each kidney not more than 20 Gy and maximum dose to the spinal cord, intestinal tract and esophagus not more than 40 Gy. Five, eight and three patients received a dose of 30, 39 and 45 Gy, in daily doses of 3 Gy per fraction, respectively.

In both groups, patients received repeated arterial infusions of anticancer agents via an injection port. One course of chemotherapy represented 2 weeks. 5-FU (500 mg/body weight/day; Kyowa Hakko, Tokyo) was administered within 5 h using a mechanical infusion pump on days 1–5 of the first and second weeks (5 g in one course). Recombinant IFN α-2b (Intron A, Schering-Plough Pharmaceuticals, Osaka, Japan), 3×10^6 U (3 MU), or natural IFN α (OIF, Otsuka Pharmaceuticals, Tokyo), 5×10^6 U (5 MU), was administered intramuscularly on days 1, 3 and 5 of each week (total dose, 18 and 30 MU, respectively). In principle, treatment was repeated several times unless PS changed to 3 or 4 during the treatment. A 2- to 4-week rest period of no treatment was allowed after each treatment course. As for the two types of IFN, we reported previously

similar effects of recombinant IFN α-2b and natural IFN α when combined with intraarterial 5-FU for the treatment of advanced HCC [15]. The arterial catheter was implanted as described previously [27].

Evaluation

The maximum response to therapy was assessed with contrast-enhanced CT at 1–2 months after completion of the first course of the treatment, and then every 2–3 months. The response was defined according to the response evaluation criteria in solid tumors (RECIST) [28]. A complete response (CR) was defined as disappearance of all target/non-target lesions, no appearance of any other lesion, and normalization of AFP and DCP. CR was confirmed at 4 weeks after the first evaluation of CR. A partial response (PR) was defined as a decrease of at least 30% in the sum of the longest diameter of target lesions with the baseline sum of the longest diameter of target lesions as the reference. Progressive disease (PD) was defined as an increase of at least 20% in the sum of the longest diameter of target lesions. Stable disease (SD) was defined as meeting neither the PR nor PD criteria. We evaluated the response to the therapies of PVTT and intrahepatic tumor as well as overall response. We also evaluated changes in ascites, esophagogastric varices and PS during the clinical course. We defined variceal rupture, worsening of esophagogastric varices and emerging of uncontrollable ascites as portal hypertension-related events (PREs).

Esophagogastric varices were assessed by endoscopic examination at 2–3 months after completion of the first course of treatment and then every 3–6 months. The varices-related endoscopic findings were evaluated according to the general rules proposed by the Japanese Research Society for Portal Hypertension [26]. In brief, the form of the varices (F factor) was classified as small straight (F1), enlarged tortuous (F2) or large coil-shaped (F3). The red color (RC) sign of the mucosal area covering the varices (red colored blood visualized underneath a very thin vascular wall) was classified according to the criteria of the Japanese Research Society for Portal Hypertension as negative (RC0), localized (RC1), between localized and entire circumference (RC2), and entire circumference (RC3). Worsening of esophagogastric varices was defined as deterioration in the *F* or RC factor of esophagogastric varices.

Adverse reactions were assessed every week during the treatment using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) (version 3.0) [29].

Radiotherapy-induced liver disease (RILD) manifested by the development of anicteric elevation of the alkaline phosphatase level of at least twofold and nonmalignant ascites in the absence of documented progressive disease, or elevated transaminases of at least fivefold the upper limit of normal or of pretreatment level, as proposed by Lawrence et al. [30].

Statistical analysis

Data were analyzed statistically on 1 July 2008. Differences between groups were examined for statistical significance using the Mann–Whitney test (*U* test), logistic regression test and χ^2 test where appropriate. The cumulative survival rate, TTP and PREs were calculated from the initial date of the therapy and assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log rank test. Univariate analyses of predictors of survival and TTP were assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log rank test. Variables that achieved statistical significance ($P < 0.05$) or those with *P* value of less than 0.10 on univariate analysis were entered into the multivariate analysis. Multivariate analyses of predictors of survival and TTP were assessed by Cox proportional hazard model. All aforementioned analyses were performed using the SPSS program (version 11, SPSS Inc., Chicago, IL). In this study, we assessed the survival benefits, PREs and safety of 5FU/IFN combined with 3D-CRT for HCC patients with PVTT.

Results

Response

Response of intrahepatic HCC

All patients received at least one course of the therapy. In the RT group, three patients were treated with 1 course, six with 2 courses, 5 with three courses and two patients with >4 courses. In the non-RT group, four patients were treated with 1 course, six with 2 courses, two with 3 courses and four with >4 courses. With regard to the maximum response of the intrahepatic tumor, of the RT group, one (6%), two (13%), eight (50%) and five (31%) patients and of the non-RT group, one (6%), three (19%), eight (50%) and four (25%) patients showed CR, PR, SD and PD, respectively. There were no statistically significant differences with regard to objective response (CR and PR) rates of intrahepatic HCC between the two groups ($P = 1.0$, Table 2).

Response of portal vein tumor thrombosis

With regard to the maximum response of PVTT, of the RT group, three (19%), nine (56%), four (25%) and zero (0%) patients and of the non-RT group, one (6%), three

Table 2 Response of intrahepatic HCC to treatment

	CR	PR	SD	PD	Response rate[a] (%)	*P* value
5-FU/IFN combination with 3D-CRT	1	2	8	5	19	1.0
5-FU/IFN alone	1	3	8	4	25	

HCC hepatocellular carcinoma, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *5-FU* 5-fluorouracil, *IFN* interferon, *3D-CRT* three-dimensional conformal radiotherapy

[a] Response rate = CR + PR/CR + PR + SD + PD

Table 3 Response of portal vein tumor thrombosis to treatment

	CR	PR	SD	PD	Response ratea[a] (%)	*P* value
5-FU/IFN combination with 3D-CRT	3	9	4	0	75	0.012
5-FU/IFN alone	1	3	7	5	25	

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *5-FU* 5-fluorouracil, *IFN* interferon, *3D-CRT* three-dimensional conformal radiotherapy

[a] Response rate = CR + PR/CR + PR + SD + PD

(19%), seven (44%) and five (31%) patients showed CR, PR, SD and PD, respectively. The objective response rates were 75 and 25% in the RT group and the non-RT group, respectively. The objective response rate of PVTT was significantly higher in the RT group than the non-RT group ($P = 0.012$, Table 3).

Overall response

With regard to the maximum overall response, one (6%), two (13%), eight (50%) and five (31%) patients of the RT group and one (6%), three (19%), eight (50%) and four (25%) patients of the non-RT group showed CR, PR, SD and PD, respectively. There were no statistically significant differences with regard to overall response rates between the two groups.

Three (19%) patients of the RT group and four (25%) patients of the non-RT group showed objective response of both intrahepatic HCC and PVTT. Nine (56%) patients of the RT group and none of the non-RT group showed objective response of PVTT alone. None of each group showed objective response of intrahepatic HCC only (Table 4).

Time to progression

Overall time to progression

In the RT group, the cumulative overall TTP rates at 3, 6 and 12 months were 37.5, 62.5 and 85.0%, respectively. In the non-RT group, the cumulative overall TTP rates at 3, 6 and 12 months were 38.1, 58.7 and 72.5%, respectively. There were no significant differences with regard to overall TTP between the two groups ($P = 0.792$). The median TTP was 3.6 months (95% CI, 3.1–4.1 months) for the RT group and 3.8 months (95% CI, 1.2–6.4 months) for the non-RT group.

Table 4 Objective response of intrahepatic HCC and portal vein tumor thrombosis

	5-FU/IFN combination with 3D-CRT (%)	5-FU/IFN alone (%)
Both intrahepatic HCC and PVTT	3/16 (19)	4/16 (25)
PVTT only	9/16 (56)	0/16 (0)
Intrahepatic HCC only	0/16 (0)	0/16 (0)

HCC hepatocellular carcinoma, *5-FU* 5-fluorouracil, *IFN* interferon, *3D-CRT* three-dimensional conformal radiotherapy, *PVTT* portal vein tumor thrombosis

Time to progression of portal vein tumor thrombosis

In the RT group, the cumulative TTP of PVTT rates at 3, 6 and 12 months were 6.7, 27.4 and 27.4%, respectively. In the non-RT group, the cumulative TTP of PVTT rates at 3, 6 and 12 months were 38.1, 51.9 and 51.9%, respectively (Fig. 1). Univariate analysis identified positivity of HCV antibody ($P = 0.0134$), PS = 0 ($P = 0.0001$) and absence of extrahepatic metastases ($P = 0.0044$) as significant factors of TTP of PVTT. Multivariate analysis identified PS = 0 ($P = 0.012$), absence of extrahepatic metastases ($P = 0.005$) and 5-FU/IFN combined with RT ($P = 0.020$)

as significant and independent factors of TTP of PVTT (Table 5).

Incidence of portal hypertension-related events

During the observation period, rupture of esophageal varices occurred in zero patients and five (31.3%) patients of the RT and non-RT groups, respectively. Rupture of gastric varices did not occur in this cohort. The cumulative rupture rate of the esophageal varices at 6 and 12 months in the non-RT group were 21 and 37%, respectively. Worsening of esophagogastric varices was observed in two and ten patients of the RT group and non-RT group, respectively. The cumulative worsening of the esophagogastric varices rate at 6, 12 and 24 months were 14.3, 14.3 and 57.1% in the RT group, respectively, and 57.6, 68.2 and 84.0% in the non-RT group, respectively. The cumulative rupture rate of esophageal varices was significantly higher in the non-RT group than in the RT group ($P = 0.040$). The worsening of the esophagogastric varices rate was significantly higher in the non-RT group than in the RT group ($P = 0.0244$, Fig. 2). In addition, none of the RT group and three patients of the non-RT group required preventive therapy for varices, such as Hassab's operation, endoscopic injection sclerotherapy (EIS) and endoscopic variceal ligation (EVL) due to pending variceal rupture during the follow-up period.

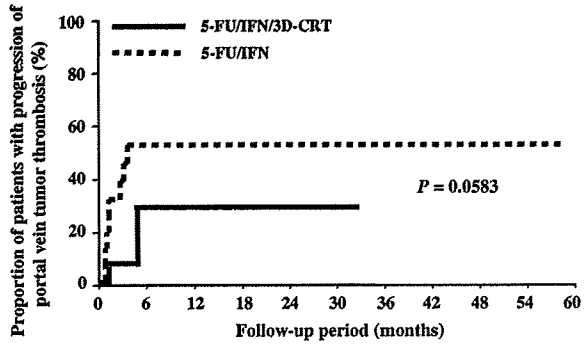

Fig. 1 Proportion of patients who showed progression of portal vein tumor thrombosis. Comparison between patients treated with 5-FU/IFN/three-dimensional conformal radiotherapy and 5-FU/IFN alone (log-rank test)

At the commencement of this study, four (25%) patients of the RT group and four (25%) of the non-RT group developed controllable ascites. None showed uncontrollable ascites. During the follow-up period, uncontrollable ascites was noted in four (25%) patients of the RT group and ten (62.5%) of the non-RT group. In the RT group, the cumulative uncontrollable ascites rates at 6, 12, 24 months were 13, 13 and 57%, respectively, and in the non-RT group were 52, 60 and 74%, respectively. Although there was no statistical difference between the RT and non-RT group, the cumulative uncontrollable ascites rate of the RT group tended to be lower than that of the non-RT group ($P = 0.064$).

We defined variceal rupture, worsening of esophagogastric varices and development of uncontrollable ascites as portal hypertension-related events (PREs). In the RT group, the cumulative PREs-free rates at 3, 6, 12 and 24 months were 93.8, 79.3, 79.3 and 19.8%, respectively. In the non-RT group, the cumulative PREs-free rate at 3, 6, 12 and 24 months were 37.5, 30, 30 and 15%, respectively. The

Table 5 Univariate and multivariate analyses of predictors of time to progression of portal vein tumor thrombosis

Variable	Univariate analysis	Multivariate analysis		
	P value	Hazard ratio	95% CI	*P* value
Age (≤65 vs. >65)	0.1738			
Sex (M vs. F)	0.4026			
HCV antibody (positive vs. negative)	0.0134	–	–	0.187
Child Pugh stage (A vs. B, C)	0.0716	–	–	0.140
PS (0 vs. 1)	0.0001	6.726	1.532–29.527	0.012
Intrahepatic tumor volume (≤50 vs. >50%)	0.5577			
Extrahepatic metastases (absence vs. presence)	0.0044	9.988	1.992–50.077	0.005
Vp (3 vs. 4)	0.8389			
AFP (≤1,000 vs. >1,000)	0.7778			
AFP-L3 (≤40 vs. >40)	0.5893			
DCP (≤10,000 vs. >10,000)	0.2378			
3D-CRT (combination with vs. without)	0.0583	6.287	1.340–29.505	0.020

HCV hepatitis C virus, *PS* Eastern Cooperative Oncology Group performance status, *Vp3* tumor thrombus in the first branch of the portal vein, *Vp4* tumor thrombus in the trunk of the portal vein, *AFP* α-fetoprotein, *AFP-L3* lens culinaris agglutininreactive fraction of α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *3D-CRT* three-dimensional conformal radiotherapy

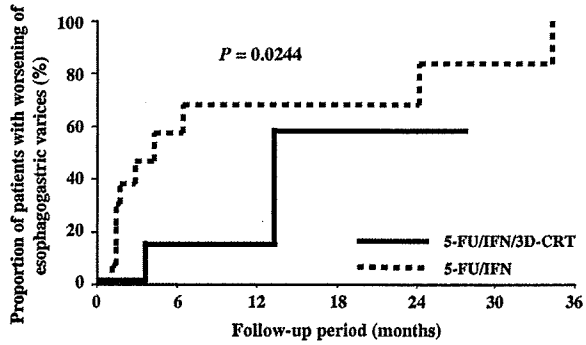

Fig. 2 Proportion of patients with worsening of esophagogastric varices. Comparison between patients treated with 5-FU/IFN/three-dimensional conformal radiotherapy and 5-FU/IFN alone (log-rank test)

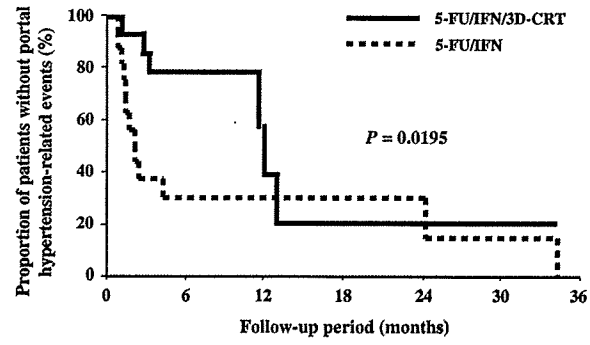

Fig. 3 Proportion of patients without portal hypertension-related events (variceal rupture, worsening of esophagogastric and emerging of uncontrollable ascites). Comparison between patients treated with 5-FU/IFN/three-dimensional conformal radiotherapy and 5-FU/IFN alone (log-rank test)

difference in the cumulative PREs-free rate between the RT group and the non-RT group was significant ($P = 0.0195$, Fig. 3).

The median PS worsening-free periods were 6.6 (range 1.2–35.2) and 2.8 months (range 1.0–59.0) for the RT group and non-RT group, respectively. The median PS worsening-free period was longer in the RT group than in the non-RT group.

Survival

Data of the 32 patients showed that the median survival time (MST) was 7.9 months (95% CI, 4.6–11.2 months), and the cumulative survival rates at 6, 12 and 24 months were 61.3, 30.1 and 21.5%, respectively. The MST of the RT group [7.5 months (95% CI, 0.0–15.0 months)] was not significantly different from that of the non-RT group [7.9 months (95% CI, 6.1–9.7 months)] ($P = 0.871$, Fig. 4).

Univariate analysis identified positivity of HCV antibody ($P = 0.0009$), PS = 0 ($P = 0.0003$), absence of extrahepatic metastases ($P = 0.0002$) and objective response of both intrahepatic HCC and PVTT ($P = 0.0020$) as significant factors of overall survival. Multivariate analysis identified PS = 0 ($P = 0.020$), absence of extrahepatic metastases ($P = 0.001$) and objective response of intrahepatic HCC and PVTT ($P = 0.005$) as significant and independent factors of overall survival (Table 6).

Adverse reactions and complications

Table 7 lists the toxicity data for all patients during and after the treatment. Fever, fatigue, nausea and anorexia were the most common adverse events, but these were mostly NCI-CTC grade 1 or 2. NCI-CTC grade 3 or 4 adverse reactions relative to the RT group and the non-RT

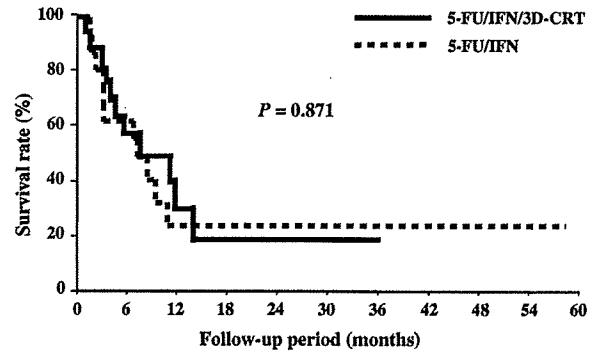

Fig. 4 Overall survival rate of patients treated with 5-FU/IFN/three-dimensional conformal radiotherapy and 5-FU/IFN alone (log-rank test)

group were as follows: leukopenia was observed in nine (56.3%) and three (18.8%) patients, thrombocytopenia in six (37.5%) and five (31.3%) patients and anorexia in one (6.3%) and one (6.3%) patient, respectively. The proportion of patients with NCI-CTC grade 3 or 4 leukopenia tended to be higher in the RT group than in the non-RT group ($P = 0.066$). Two patients of the RT group developed NCI-CTC grade 4 leukopenia and required administration of granulocyte colony-stimulating factor. None required platelet transfusion. None developed upper gastrointestinal ulcers associated with 3D-CRT. Furthermore, none developed RILD, and hepatic failure related to 5-FU/IFN with 3D-CRT was not observed during the follow-up period.

After 3D-CRT and one course of 5-FU/IFN, Child-Pugh classification did not change in the RT group: 13 patients, from A to B: 2 patients and from A to C: 1 patient. After one course of 5-FU/IFN, Child-Pugh classification did not change in the non-RT group: 13 patients, from A to B: 2 patients and from B to A: 1 patient. In the RT group, one

Table 6 Univariate and multivariate analyses of predictors of overall survival

Variable	Univariate analysis	Multivariate analysis		
	P value	Hazard ratio	95% CI	*P* value
Age (≤65 vs. >65)	0.2643			
Sex (M vs. F)	0.6535			
HCV antibody (positive vs. negative)	0.0009	–	–	0.136
Child Pugh stage (A vs. B, C)	0.4592			
PS (0 vs. 1)	0.0003	3.643	1.223–10.850	0.020
Intrahepatic tumor volume (≤50 vs. >50%)	0.4963			
Extrahepatic metastases (absence vs. presence)	0.0002	5.870	2.164–15.923	0.001
Vp (3 vs. 4)	0.3170			
AFP (≤1,000 vs. >1,000)	0.9743			
AFP-L3 (≤40 vs. >40)	0.7701			
DCP (≤10,000 vs. >10,000)	0.4399			
3D-CRT (combination with vs. without)	0.8710			
Objective response of PVTT only	0.4586			
Objective response of intrahepatic HCC + PVTT	0.0020	8.064	1.890–34.412	0.005
Variceal rupture	0.7345			

HCV hepatitis C virus, *PS* Eastern Cooperative Oncology Group performance status, *Vp3* tumor thrombus in the first branch of the portal vein, *Vp4* tumor thrombus in the trunk of the portal vein, *AFP* α-fetoprotein, *AFP-L3* lens culinaris agglutininreactive fraction of α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *3D-CRT* three-dimensional conformal radiotherapy, *PVTT* portal vein tumor thrombosis, *HCC* hepatocellular carcinoma

Table 7 Adverse reactions (NCI-CTC v3.0 grade 3/4) during and after the treatment

	5-FU/IFN combined with 3D-CRT		5-FU/IFN		*P* value
Grade:	3	4	3	4	
Leukopenia	7 (43.8%)	2 (12.5%)	3 (18.8%)	0	0.066
Anemia	0	0	0	0	NS
Thrombocytopenia	6 (37.5%)	0	5 (31.3%)	0	NS
Anorexia	1 (6.3%)	0	1 (6.3%)	0	NS

5-FU 5-fluorouracil, *IFN* interferon, *3D-CRT* three-dimensional conformal radiotherapy

patient with the Child-Pugh classification worsened from A to C. Hepatic failure in this patient was due to rapid progression of intrahepatic HCC and was not associated with 3D-CRT.

Causes of death

At the time of analysis, 10 patients were still alive, and 22 patients had died of disease. All patients died of cancer-related disease. Nineteen patients died of hepatic failure because of progression of intrahepatic HCC. Among the 19 patients, 4 died of HCC rupture. Three patients died of bleeding esophageal varices; all were in the non-RT group. In the RT group, none died of bleeding of esophageal varices. None of the 22 patients died of extrahepatic metastases.

Discussion

Advanced HCC is often accompanied by PVTT. However, there is no established standard therapeutic modality for HCC with PVTT, especially Vp3 and Vp4. The prognosis is often poor; the reported MST associated with symptomatic treatment of patients with HCC and PVTT is shorter than 90 days [9, 10], and the degree of portal vein tumor thrombosis is a fairly reliable factor in predicting survival [31]. The poor prognosis depends on (1) rapid progression of HCC by spread of tumor cells through the portal tract and (2) portal hypertension by PVTT causing various complications, such as variceal rupture, ascites and ischemic liver failure. Especially, Vp3/4 results in deterioration of PS of these patients, and consequently any treatment for HCC is considered contraindicated.

It was reported that surgical resection of HCC with Vp3/4 is limited to highly selected group of patients such as those with good hepatic reserve and relatively small primary tumors. The reason for the limitation is related to the potential intraoperative death or the high recurrence rate postoperatively [32, 33]. Although TACE was widely applied to unresectable HCC, the reported outcome was poor, especially for HCC with Vp3/4 [34, 35]. Previous studies also reported the efficacy of HAIC for PVTT. Several groups reported the combination therapy of intraarterial 5-FU and CDDP for HCC with Vp3/4 [36, 37]. Recent reports described the efficacy and survival benefits of combination therapy of intraarterial 5-FU and systemic IFN-α [15–18]. IFN-α acts as a modulator by increasing the level of thymidine phosphorylase, which is an enzyme responsible for biochemical activation of 5-FU [38, 39]. In addition, IFN-α suppresses cancer cells directly and/or indirectly via several pathways, such as inhibition of the cell cycle, boosting p53 activation and activation of immunocytes [40–45]. The response rate to 5-FU/IFN in HCC patients with PVTT seems superior to 5-FU/CDDP [15–18, 36, 37, 46, 47]. The combination therapy of 5-FU/IFN for Vp3/4 has been reported with MST, with the response rate ranging from 6.9 to 11.8 months and 44–52%, respectively [17, 18].

Other studies also reported the safety and efficacy of local radiotherapy for PVTT. 3D-CRT monotherapy for Vp3/4 has been reported with MST, with the response rate in Vp3/4 ranging from 9.6 to 10.7 months and 45–46%, respectively [19, 20].

Despite the development of chemotherapies and radiotherapies, the prognosis of HCC patients with Vp3/4 is still less than 1 year, and the response rate is less than about 50% [17–20].

Recent studies reported the synergistic effects of chemotherapy combined with radiotherapy in several malignancies, such as lung cancer and esophageal cancer [21–24]. The combination of chemotherapy and radiotherapy interacts in several ways. For example, the combination of RT, 5-FU and carboplatin raises the concentration of 5-FU in head and neck tumors [48]. Furthermore, the combination of RT, 5-FU and doxorubicin or CDDP improved the radiosensitivity of HepG2 cell lines [49]. With regard to HCC, radiotherapy was first used in combination with TACE [50, 51]. Recently, Han et al. [25] reported the use of 3D-CRT with HAIC consisting of low-dose cisplatin and 5-FU, with a 13-month MST and 45% response rate. However, to our knowledge, there are no reports about the combination of 5-FU/IFN and 3D-CRT. Accordingly, we applied this combination therapy for advanced HCC with Vp3/4.

In this study, the response rate of PVTT was significantly higher ($P = 0.012$) in the RT group than in the non-RT group (75 vs. 25%). The response rate to 5-FU/IFN/3D-CRT was higher than that reported in a previous study using 3D-CRT alone (45%), which also applied RECIST for evaluation of the response rate, similar to the present study [20]. In addition, in the same study, the reported response rate of PVTT was 22% for <44 Gy and 80% for ≥44 Gy [20]. In our study, a positive response of PVTT was observed in 4/5 (80%), 6/9 (67%) and 2/2 (100%) patients who received RT at a dose of 30, 39 and 45 Gy. Comparison with the above study indicates a higher response rate with lower RT dose. These results suggest that RT acts synergistically with 5-FU/IFN and improves the outcome.

In the present study, the high response rate (75%) of PVTT in the RT group was associated with excellent secondary benefits. Shrinkage of PVTT improved portal hypertension and avoided PREs. In other words, it significantly reduced the rate of variceal rupture and worsening of varices, compared with the non-RT group ($P = 0.040$ and 0.0244, respectively). It is noteworthy that none of the patients of the RT group and three patients of the non-RT group required preventive therapy for varices, such as Hassab's operation, EIS and EVL, during the follow-up period. In addition, uncontrollable ascites was less likely to develop in patients of the RT group than non-RT group.

The response rates of intrahepatic tumor in the RT group and the non-RT group were similar. We believe this is due to the limitation of the irradiation area to PVTT.

There is almost no information regarding the toxicity of combination therapy of 3D-CRT and 5-FU/IFN. Previous studies reported the safety of 3D-CRT alone for PVTT. The reported rate of 0–1.7% RILD was observed by 3D-CRT alone for PVTT with a dose range of 18–54 Gy [19, 20]. In our study, RILD was not observed, and changes in the hepatic reserve before and after treatments were similar in the two groups. NCI-CTC grade 3 or 4 leukopenia tended to be more frequently observed in the RT group than in the non-RT group ($P = 0.066$), but there was no statistical difference between the two groups. Two patients with grade 4 leukopenia required treatment with granulocyte colony-stimulating factor, but no discontinuation of the chemotherapy based on well-preserved hepatic reserve.

In this study, three patients in the non-RT group died of bleeding from esophageal varices. When variceal ruptures occurred, these three patients received the best supportive care because of deterioration of HCC. We estimated their prognosis at that stage to be only a few months, even if ruptures did not occur. Accordingly, we speculate that death related to esophageal varices has little influence on the overall survival of the non-RT group.

In this study, the objective response rate of PVTT was higher in the RT group than in the non-RT group. In addition, 5FU/IFN with 3D-CRT did not worsen the hepatic reserve. However, the overall survival was similar in the two groups. In this study, we analyzed the objective response of both intrahepatic HCC and PVTT, not the objective response of PVTT alone, as contributing to overall survival. The objective response rate of both intrahepatic HCC and PVTT was similar between the two groups. We regarded this point as the reason for the similar survival rate in the two groups.

In conclusion, 5-FU/IFN combined with 3D-CRT attained a high response rate of PVTT by low RT dose. The combination therapy improved the response rate of PVTT and halted any deterioration of portal hypertension, resulting in reduced incidence of portal hypertension-related events, such as variceal rupture and uncontrollable ascites, and resulted in maintenance of PS and QOL compared with 5-FU/IFN alone. The results also showed that 5-FU/IFN combination therapy with 3D-CRT is well tolerated. However, any generalized statement on the results of this study should be guarded due to the small sample size. Further prospective studies are needed to investigate the factors involved in the survival benefits of 5-FU/IFN combination therapy with 3D-CRT.

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Case Report

The first Japanese case of COACH syndrome

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COACH syndrome is a disorder characterized by hypoplasia of cerebellar vermis, oligophrenia, congenital ataxia, coloboma and hepatic fibrosis, and 21 cases have been reported to date. Here we describe the first Japanese case of COACH syndrome, who was diagnosed at the age of 37 years and never progressed to liver failure. The patient was found to have delayed developmental milestones at the age of 5 months and mental retardation at the age of 7 years. She had been treated for hepatopathy of unknown origin from the age of 22 years. She was admitted to Hiroshima University Hospital at the age of 37 years after the identification of esophageal varices on a routine upper endoscopy. Computed tomography of the abdomen revealed portal hypertension and splenomegaly, and liver biopsy showed liver fibrosis. In addition, she had coordination disorder and dysarthria. Brain magnetic resonance images revealed hypoplasia of cerebellar vermis. The final diagnosis was COACH syndrome. She underwent endoscopic injection sclerotherapy for esophageal varices. From that point until her death from ovarian cancer at the age of 41 years, the liver function tests were stable without an episode of hematemesis. Physicians should be aware of COACH syndrome when they examine young patients who present with hepatopathy, portal hypertension of unknown origin and cerebellar ataxia.

INTRODUCTION

COACH SYNDROME IS one of the oculo-encephalo-hepato-renal syndromes and is a rare disorder with hypoplasia (or aplasia) of the cerebellar vermis, oligophrenia, congenital ataxia, coloboma and hepatic fibrosis. Verloes and Lambotte[1] were the first to describe this syndrome, and only 21 cases of COACH syndrome have been reported to date (Table 1). The term "congenital hepatic fibrosis" (CHF) was introduced by Kerr *et al.*[14], and a special subgroup of CHF is COACH syndrome. CHF is probably the most common cause of non-icteric hepatosplenomegaly and is encountered mainly in children and young adults.[8] Patients with CHF are rarely discovered after the age of 30 years.[15] In fact, only two of the 21 previously reported cases of COACH syndrome were diagnosed over the age of 30. Summerfield *et al.*[16] and Desmet[17] indicated that CHF resulted in hepatosplenomegaly and portal hypertension with normal liver function. However, five of the 21 previously reported cases progressed to liver failure and three of these five underwent liver transplantation. Here we describe the first Japanese case diagnosed as COACH syndrome at the age of 37 years who never progressed to liver failure until her death from ovarian cancer.

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CASE REPORT

OUR PATIENT WAS a 37-year-old Japanese female. She was born in 1966 as the youngest child of healthy unrelated parents after an uneventful pregnancy. Her older brother and sister were healthy. The family history was unremarkable. Her birth weight was 3 kg. She presented with delayed developmental milestones and was able to control her head at the age of 5 months. When she entered elementary school at the age of 7 years, she was found to have developmental delays. At the age of 22 years, she developed spontaneous pneumothorax and was found to have elevated liver transaminase levels. Since then, she had been treated with ursodeoxycholic acid for hepatopathy of unknown

Table 1 Comparison of the present patient with the previously reported cases of COACH syndrome

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Cerebellar vermis hypo/aplasia	+	+	NS	NS	+	+	NS	NS	+	NS	+	+	+	+	+	NS	NS	+	+	NS	+	+
Mental retardation	+	+	NS	+	+	NS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	NS	+
Ataxia	+	+	+	+	+	+	NS	+	+	+	+	+	+	+	+	+	+	+	+	+	NS	+
Chorioretinal coloboma	+	+	NS	+	+	+	+	NS	+	+	+	+	+	+	–	+	+	+	+	+	+	–
Hepatic fibrosis	+	+	+	+	NS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Portal hypertension	–	NS	+	+	–	+	+	NS	NS	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal abnormalities	–	–	NS	+	NS	+	NS	+	+	+	+	NS	+	–	+	+	+	–	NS	+	–	–
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hypotonia	+	NS	NS	+	NS	NS	NS	–	+	NS	+	NS	+	+	+	NS	+	+	+	+	NS	–
Ptosis	+	NS	NS	NS	+	+	+	+	NS	NS	+	–	+	–	+	–	+	NS	NS	–	NS	–
Nystagmus	–	NS	NS	+	+	+	+	+	+	NS	+	–	+	+	+	–	+	NS	NS	–	NS	–
Dysmorphic signs	+	+	NS	+	+	NS	+	+	NS	NS	+	+	+	+	+	+	+	+	+	+	+	+
Esophageal varices	–	NS	NS	+	NS	+	+	NS	NS	+	+	–	+	+	NS	NS	+	+	+	+	+	+
History of hematemesis	–	NS	NS	+	NS	–	+	NS	NS	+	+	+	–	+	NS	NS	+	+	–	+	+	+
Therapy	–	NS	NS	TIPS	NS	–	NS	NS	NS	NS	NS	–	–	NS	NS	NS	NS	NS	–	TIPS	NS	EIS
Gender	M	M	F	F	F	F	F	F	F	F	M	F	M	M	M	F	M	F	F	F	M	F
Age (years)†‡	1.4†	34†	46‡	23†	6†	7†	14†	1.6‡	6‡	6‡	7†	11†	7†	15‡	2.6†	4†	1.2†	8†	11†	18†	7†	37†

1, Foell *et al.*,[2] 2, 3, Wiesner *et al.*,[3] 4, 5, Kumar and Rankin;[4] 6, Barzilai *et al.*,[5] 7, 8, Thompson and Baraitser;[6] 9, 10, Dietrich and Straub;[7] 11–13, Verloes and Lambotte;[1] 14, 15, Gentile *et al.*,[8] 16, 17, Hunter *et al.*,[9] 18, Herzog *et al.*,[10] 19 Kirchner *et al.*,[11] 20, Uemura *et al.*,[12] 21, Gleeson *et al.*,[13] 22, our patient.
†Age at liver biopsy or when diagnosed as COACH syndrome. ‡Age at death. + present. – absent.
EIS, endoscopic injection sclerotherapy; F, female; M, male; NS, not specified; TIPS, transjugular intrahepatic portosystemic shunt.

Table 2 Laboratory data on admission

Complete blood count			LDH	172	IU/L	Tumor marker		
WBC	4690	/μL	ALP	466	IU/L	AFP	<5.0	ng/mL
RBC	4.12×10^6	/μL	γGTP	83	IU/L			
Hb	12.7	g/dL	TP	7.5	g/dL	Virus markers		
Ht	37.8	%	Alb	4.2	g/dL	HBsAg	(−)	
Plt	134×10^3	/μL	TC	161	mg/dL	HCVAb	(−)	
			TTT	5	U			
Blood coagulation test			ZTT	12	U	Autoantibodies		
PT	90	%	BUN	15	mg/dL	ANA	(−)	
			Cr	0.48	mg/dL	AMA(M2)	(−)	
Blood chemistry			CRP	<0.3	mg/dL			
TBil	0.9	mg/dL	FBS	79	mg/dL			
AST	35	IU/L	HbA_{1c}	3.9	%			
ALT	38	IU/L	NH_3	47	μg/mL			

AFP, α-fetoprotein; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibody; ANA, antinuclear antibody; AST, aspartate aminotransferase; Cr, creatinine; CRP, c-reactive protein; FBS, fasting blood sugar level; Hb, hemoglobin; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C antibody; LDH, lactate dehydrogenase; Plt, platelets; PT, prothrombin time; RBC, red blood cells; TBil, total bilirubin; TC, total cholesterol; TTT, thymol turbidity test; WBC, white blood cells; ZTT, zinc sulfate turbidity test.

origin at a local clinic. At the age of 37 years, an upper endoscopy was performed as part of a routine examination and found esophageal varices (Lm, F2, Cb, RC1, Te).[18] She was referred to our hospital for admission and treatment of esophageal varices.

Physical examination showed micrognathia, saccadic eye movement, hepatosplenomegaly and varicose veins on both lower legs. No coloboma or hypotonia were present. Neurological examination showed mental delayed development and cerebellar ataxia. Based on the Wechsler Adult Intelligence Scale – Revised (WAIS-R), her intelligence quotient (IQ) was evaluated as verbal IQ 69, performance IQ 61 and full-scale IQ 61. She presented with scanning speech. She was unable to walk with tandem gait or to stand on one leg. She was poor at the nose–finger–nose test and had an intention tremor.

Laboratory tests (Table 2) showed aminotransferases within the normal range, but the cholestatic parameters were increased (alkaline phosphatase 466 IU/L, γ-glutaryltransferase 83 IU/L). Platelet count was low (134×10^3/μL). Viral hepatitis and autoimmune hepatitis were excluded. Renal function tests were normal and banded karyotyping at the 400–550 band level of resolution was normal (46, XX).

Ultrasound examination and computed tomography (CT) (Fig. 1a,b) of the abdomen showed hepatomegaly, large collateral vessels and splenomegaly associated with portal hypertension. Both kidneys were normal. Ultrasound-guided liver biopsy (Fig. 2a,b) was performed and showed hepatic fibrosis, containing bile ductular proliferation and mild inflammatory infiltrate of lymphocytes and neutrophils. This pathology was diagnostic for congenital hepatic fibrosis.

Magnetic resonance imaging (MRI) of the brain (Fig. 3) showed hypoplasia of the inferior part of the cerebellar vermis, so-called the Molar Tooth Sign. The electroencephalogram was normal. Based on the

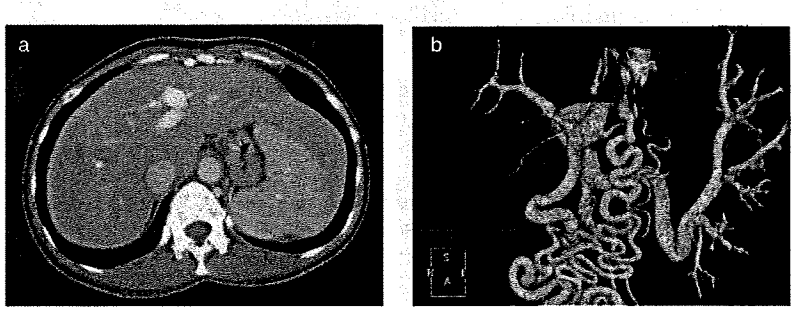

Figure 1 Computed tomography of the abdomen. (a) Axial image shows hepatosplenomegaly. (b) Multiplanner reconstruction image of the portal vein shows portosystemic collaterals associated with portal hypertension.

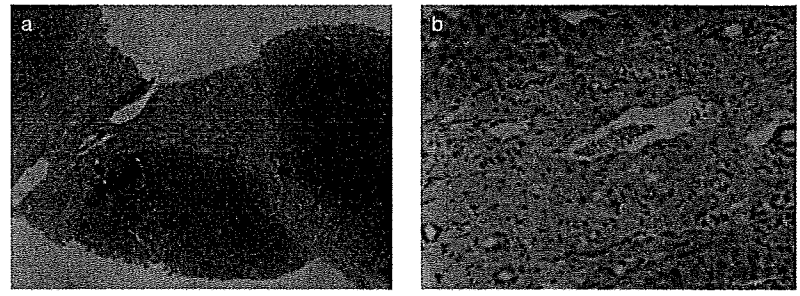

Figure 2 Liver biopsy. Hepatic fibrosis with bile duct proliferation and with mild inflammation including lymphocytes and neutrophils. There are no findings of narrowing or occlusion of the portal vein. (a) Azan, magnification × 40. (b) Hematoxylin and eosin, magnification × 400.

previously reported cases, physical, laboratory and imaging findings, these histopathological findings established the diagnosis of COACH syndrome.

The patient underwent endoscopic injection sclerotherapy (EIS) for esophageal varices in order to prevent potential rupture. Esophageal varices improved to Lm, F0, Cw, RC0, UI[18] two weeks after the treatment. Subsequently, she was treated with protirelin. Ataxia did not improve, but liver function tests stabilized. At the age of 40 years, she presented with lumbago. Detailed examination showed an ovarian cancer. At the age of 41 years, she died from ovarian cancer. Until her death, hematemesis and/or liver failure never occurred.

DISCUSSION

WE OFTEN EXPERIENCE patients with liver disorders of unknown origin. This case was followed as hepatopathy of unknown origin from the age of 22 years. A routine medical check up identified esophageal varices requiring treatment. CT, ultrasound, MRI, liver biopsy and physical examination revealed that she suffered from COACH syndrome. COACH syndrome is a very rare disorder consisting of cerebellar vermis hypoplasia (or aplasia), oligophrenia, congenital ataxia, coloboma and hepatic fibrosis. An autosomal recessive mode of inheritance was suggested,[16] but the primary cause of this disease remains unknown.

Liver dysfunction and portal hypertension-related COACH syndrome is due to CHF. Kerr *et al.*[14] were the first to describe CHF as a distinct entity from cirrhosis. CHF is an inherited malformation, defined pathologically by bands of fibrous tissue within the liver, linking portal tracts and containing multiple bile ductules.[14,17] The main characteristics of CHF are normal liver parenchyma, fibrosis of the portal spaces and ductal changes.[19] The lack of inflammatory infiltrates in the connective tissue differentiates the congenital form of fibrosis from the acquired form.[8]

The clinical picture of CHF includes hepatosplenomegaly and portal hypertension with normal liver function.[16] Complications of portal hypertension, including hematemesis due to esophageal varices, gastrointestinal hemorrhage and hypersplenism, are frequently observed.[20] Our case had esophageal varices due to

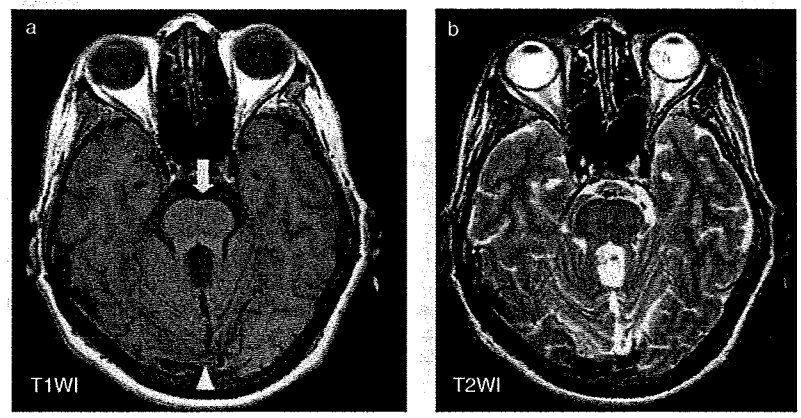

Figure 3 Axial magnetic resonance images show the molar tooth sign (arrow) and cerebellar vermis hypoplasia (arrowhead). (a) T1-weighted image. (b) T2-weighted image.

portal hypertension and underwent EIS. Since then, she did not develop hematemesis. Twelve of the previously reported 21 cases had esophageal varices and two cases underwent transjugular intrahepatic portosystemic shunt (TIPS) (Table 1).

Many cases underwent close investigation to diagnose the presence of oligophrenia and were diagnosed as COACH syndrome. Although the past history in our case also indicated delayed developmental milestones in early childhood, like other cases, close examination was not made. Therefore, the diagnosis of COACH syndrome was made at a relatively advanced age. The late appearance of symptoms and their clinical evolution suggest that CHF is a dynamic and progressive condition. Some studies indicate that there is a progressive build-up of liver fibrosis over the years.[21,22] Five of the previously reported 21 cases progressed to liver failure and three of these five underwent liver transplantation. In comparison, our case never progressed to liver failure in her lifetime, although she had esophageal varices due to portal hypertension.

Five of the previously reported 21 cases with COACH syndrome died. One patient died from liver failure without liver transplantation, one died from acute hepatitis C after liver transplantation, one died from hematemesis from esophageal varices, one died from renal failure and one died from aspiration pneumonia. Complications of hepatopathy are major contributing factors to morbidity and mortality during the course of the disease. Most reported cases died from COACH syndrome-related disorders. However, our patient died from ovarian cancer after a clinical course free of liver failure or hematemesis. Whether COACH syndrome is related to ovarian cancer or not is not clear at this stage. To date, 10 CHF cases with liver tumor have been reported,[11] five patients with cholangiocellular carcinoma, two patients with hepatocellular carcinoma and three patients with benign liver tumor. However, none of the 21 patients with COACH syndrome was reported to have malignant tumors beyond the hepatobiliary system.

In summary, we experienced the first Japanese case of COACH syndrome. Physicians should be aware of COACH syndrome when they examine young patients who present with hepatopathy, portal hypertension of unknown origin and cerebellar ataxia.

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