Tohoku J. Exp. Med., 2015, **236**, 263-271

Characteristics of 20 Patients with Autochthonous Acute Hepatitis E in Hokkaido, Japan: First Report of Bilateral Facial Palsy Following the Infection with Genotype 4 Hepatitis E Virus

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Autochthonous hepatitis E is increasingly being recognized in industrialized countries, including Japan. Although neurological abnormalities have been sporadically reported as an extrahepatic manifestation of hepatitis E virus (HEV) infection, it is rare and has not been reported in Japan. The present study aimed to characterize a total of 20 patients consecutively diagnosed with sporadic acute hepatitis E at a city hospital in Hokkaido, Japan, during 2001-2014, focusing on a patient complicated with neuropathy. Seventeen patients were infected with genotype 4 HEV, while the remaining three patients were with genotype 3 HEV. Although a 67-year-old male with severe hepatitis did not have predisposing factors associated with the development of neurological disorders, such as diabetes mellitus and the use of immunosuppressive agents, he developed bilateral peripheral facial palsy six days after admission. A neurological examination revealed the inability to smile, frown, close his eyes completely or puff out his cheeks. MRI brain scans were considered to be normal. Although it took 83 days after admission for the total bilirubin levels to normalize, his neurological symptoms resolved gradually within three weeks without any sequelae following conservative therapy. A full-length genomic analysis of the HEV strain (HE-JA30) isolated from the patient belonged to genotype 4 and was closest to that currently circulating in Hokkaido, Japan. This is the first report of HEV-associated neuropathy in Japan. While all of previous reports on HEV-related neuropathy involve genotype 3 HEV, the present report is unique in that genotype 4 HEV is responsible for the neuropathy.

Keywords: bilateral facial palsy; extrahepatic manifestation; full genome; genotype 4; hepatitis E virus
Tohoku J. Exp. Med., 2015 August, **236** (4), 263-271.

Introduction

Hepatitis E virus (HEV), *family Hepeviridae, genus Orthohepevirus* (Smith et al. 2014), is now recognized to be an important pathogen of acute hepatitis, and both HEV and hepatitis caused by HEV appear to exist virtually ubiquitously worldwide (Emerson and Purcell 2013). In Japan, autochthonous HEV strains were first recovered in 2001 from a Japanese patient with sporadic acute hepatitis E who had no history of traveling abroad in addition to domesticated pigs independently (Okamoto et al. 2001; Takahashi et al. 2001). Zoonotic food-borne transmission of HEV via the ingestion of the meat or viscera of infected animals, including pigs, wild boar and deer, is the main route of HEV transmission in Japan (Takahashi and Okamoto 2014).

There are currently four known genotypes of HEV that infect humans. Genotypes 1 and 2 have all been isolated during human epidemic outbreaks in developing countries, while genotypes 3 and 4 have been isolated in humans as well as animals in both developing and industrialized countries and exhibit the characteristics of zoonosis (Takahashi and Okamoto 2014). Pigs are the most frequent HEV reservoir among animals in Japan (Nishizawa et al. 2003; Yazaki et al. 2003), and autochthonous HEV strains obtained from humans and animals in Japan belong to genotype 3 or 4 (Takahashi and Okamoto 2014; Inagaki et al. 2015; Takeuchi et al. 2015).

A range of extrahepatic manifestations have been described in association with HEV infection in European and Asian countries. These complications include neurological disorders (Kamar et al. 2011; Cheung et al. 2012; Santos et al. 2013; van den Berg et al. 2014; Woolson et al.

Received May 29, 2015; revised and accepted July 2, 2015. Published online July 28, 2015; doi: 10.1620/tjem.236.263.
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2014), myasthenia gravis (Belbezier et al. 2014), thrombocytopenia (Fourquet et al. 2010), aplastic anemia (Shah et al. 2012) glomerulonephritis (Kamar et al. 2012) and acute pancreatitis (Deniel et al. 2011). Among them, neurological complications are more frequently reported, including Guillain-Barré syndrome, brachial neuritis, transverse myelitis, cranial nerve palsy and peripheral neuropathy (Kamar et al. 2011; Cheung et al. 2012; van den Berg et al. 2014; Woolson et al. 2014). However, such neurological complications as extrahepatic manifestations of clinical or subclinical HEV infection have not yet been reported in the literature in Japan, although cases of autochthonous acute or fulminant hepatitis E are increasingly being seen in this country.

The present study was conducted to characterize a total of 20 patients consecutively diagnosed with sporadic acute hepatitis E at a city hospital in Hokkaido, Japan, during 2001-2014, focusing on a patient complicated with bilateral peripheral facial palsy. This is the first report of HEV-associated neuropathy in Japan. While all genotyped patients with HEV-related neuropathy in the literature involve genotype 3 HEV, the present report is unique in that genotype 4 HEV is responsible for the neuropathy.

Materials and Methods

Serum samples from sporadic cases of acute hepatitis E

Serum samples were obtained at the first visit from 20 consecutive patients seen at the Center for Gastroenterology, Kobayashi Hospital in Kitami, Hokkaido, Japan with a diagnosis of acute hepatitis E during the period of 2001-2014. All subjects were negative for immunoglobulin (Ig) M class antibodies against hepatitis A virus (HAV), hepatitis B virus (HBV) markers (anti-HBV core IgM and hepatitis B surface antigen (HBsAg)), anti-hepatitis C virus (anti-HCV) and IgM class antibodies against cytomegalovirus (CMV) and Epstein-Barr virus (EBV). The presence of anti-HAV IgM, anti-HBV core IgM, HBsAg and anti-HCV was examined using commercially available kits (Abbott Japan, Tokyo, Japan). The presence of antibodies against HEV was tested as described previously (Takahashi et al. 2005), and qualitative and quantitative detection of HEV RNA was performed as described below. Other viral markers were determined at a commercial laboratory (SRL, Tokyo, Japan) using enzyme-linked immunosorbent assays (ELISAs). Among the 20 patients, nine (Patients 1-9) were described in our previous studies, focusing on the consumption of pig liver and/or intestine as well as the disease severity (Yazaki et al. 2003; Mizuo et al. 2005). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committee of Jichi Medical University School of Medicine. All patients participating in this study gave informed consent, and Patient 5 approved the use of the photos (Fig. 1).

Qualitative and quantitative detection of HEV RNA

Total RNA extracted from 100 μl of serum using TRIZOL-LS (Life Technologies, Carlsbad, CA) was reverse-transcribed, and subsequent nested PCR (ORF2-457 PCR) was performed with primers derived from areas of the ORF2 region that were well conserved across all four genotypes (1-4), using the previously described method (Mizuo et al. 2002). When HEV RNA was undetectable with the ORF2-457 PCR, the samples were subjected to ORF2/3-137 PCR (Inoue et al. 2006b). The HEV genotype was determined using a phylogenetic analysis of the amplicons (412 nucleotides (nt): primer sequences at both ends excluded), as described below.

HEV RNA was quantitated using real-time detection via reverse transcription (RT)-PCR according to a previously described method (Takahashi et al. 2008) with a QuantiTect Probe RT-PCR Kit (QIAGEN, Tokyo, Japan).

Amplification of the full-length HEV genome

Total RNA was extracted from 900 μl of the serum sample obtained from a patient (Patient 5) with autochthonous severe acute hepatitis E and bilateral facial palsy and subjected to cDNA synthesis followed by nested PCR of 10 overlapping regions, including the extreme 5′- and 3′-terminal regions; the amplified regions excluding the primer sequences were nt 1-50 (50 nt), nt 37-1199 (1,163 nt), nt 991-2134 (1,144 nt), nt 2056-3142 (1,087 nt), nt 3029-3899 (871 nt), nt 3622-4603 (982 nt), nt 4401-5175 (775 nt), nt 4663-5439 (777 nt), nt 5343-6396 (1,054 nt) and nt 6357-7252 (896 nt). The extreme 5′-end 50-nt sequence was determined according to a modified rapid amplification of cDNA ends (RACE) technique with the FirstChoice RLM-RACE kit (Ambion, Austin, TX), as previously described (Okamoto et al. 2001). Amplification of the 3′-end sequence (nt 6357-7239: poly (A) tail excluded) was performed using the RACE method as previously described (Okamoto et al. 2001).

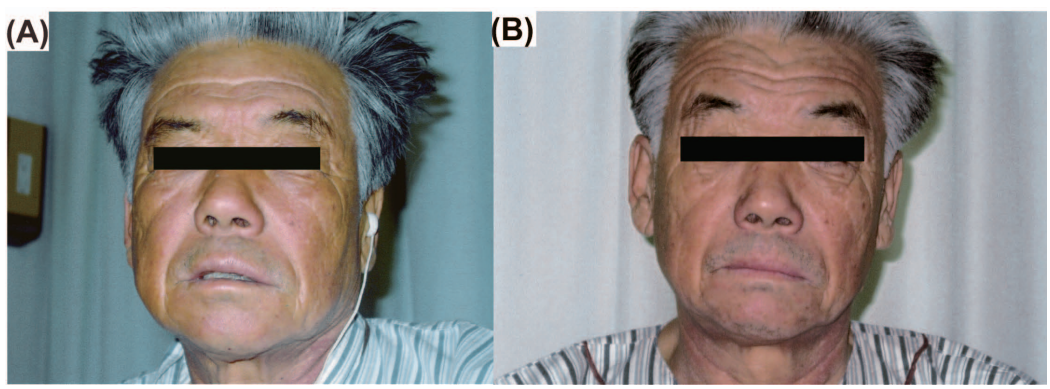

Fig. 1. Face of Patient 5 with bilateral peripheral facial palsy. On the sixth hospital day (A), he could not smile, laugh, frown, whistle, talk properly, close his eyes completely, puff out his cheeks and wrinkle forehead skin. On day 20 (B), his neurological symptoms were almost gone, although there was still the slight deviation of the left angle of his mouth.

Determination of the nucleotide sequences and phylogenetic tree analysis

The amplification products were sequenced directly on both strands using a BigDye Terminator v3.1 Cycle Sequencing kit (Life Technologies) on an Applied Biosystems 3130xl Genetic Analyzer (Life Technologies). The sequence analysis was performed using the Genetyx software program (version 12.0.6; Genetyx, Tokyo, Japan), and multiple alignments were generated with the CLUSTAL Omega software program (version 1.2.0) (Goujon et al. 2010). A phylogenetic tree was constructed according to the neighbor-joining method with the Kimura two-parameter model and 1,000 replicates of bootstrap resampling tests, as implemented in the MEGA6 software program (version 6.0.6) (Tamura et al. 2013).

Results

Characteristics of 20 patients with sporadic acute hepatitis E

The demographic and virological features as well as the associated diseases of 20 consecutive patients with autochthonous, sporadic acute hepatitis E are shown in Table 1. The majority of the patients (18/20, 90%) were male. The age of the patients ranged from 38 to 86 years, with a mean age of 60.6 years, and patients 50 years of age or older accounted for 95% of the total. Of note, one (Patient 5) of the 20 patients had a lowest prothrombin activity of less than 40%, unaccompanied by hepatic encephalopathy, and was diagnosed with severe acute hepatitis. None of the patients, including Patient 5, progressed to fulminant hepatitis. Of note, Patient 15 contracted acute renal failure following the development of acute hepatitis E, but his renal function was normalized after a hemodialysis treatment for one and a half months. Although 10 patients had suffered from gastric cancer, diabetes mellitus, fatty liver, hyperlipidemia, ulcerative colitis, cerebral infarction or chronic hepatitis C, before the development of acute hepatitis E (Table 1), the remaining 10 patients, including Patients 5 and 15, had no such underlying diseases.

Although IgG anti-HEV and IgA anti-HEV antibodies

Table 1. Characteristics of patients with sporadic acute hepatitis E in Hokkaido, Japan.

Patient No.[a]	Age (yrs)	Sex	Year of onset	Peak ALT (IU/L)	Peak AST (IU/L)	Peak T-Bil (mg/dl)	Lowest PT%	IgM anti-HEV (OD_{450})[b]	IgA anti-HEV (OD_{450})[b]	HEV RNA (copies/ml)[b]	HEV genotype	Associated diseases
1	57	M	2001	3,261	2,219	14.1	75	1.738 (+)	2.486 (+)	7.5×10^3	4	Gastric cancer
2	51	M	2002	2,773	1,296	7.4	113	2.499 (+)	2.547 (+)	2.0×10^3	3	Diabetes mellitus, fatty liver and hyperlipidemia
3	61	M	2002	3,132	1,930	5.6	74	> 3.000 (+)	> 3.000 (+)	8.6×10^1	4	Ulcerative colitis
4	86	M	2002	1,305	1,619	26.0	66	1.039 (+)	> 3.000 (+)	9.8×10^4	4	Cerebral infarction
5[c,d]	**67**	**M**	**2003**	**3,866**	**3,321**	**26.2**	**36**	**2.592 (+)**	**> 3.000 (+)**	**5.7×10^4**	**4**	**Bilateral facial palsy**[e]
6	75	M	2003	1,667	1,493	5.6	99	0.461 (+)	> 3.000 (+)	2.1×10^4	4	–[f]
7	38	M	2004	2,916	1,569	3.3	100	> 3.000 (+)	> 3.000 (+)	4.0×10^5	4	–
8	61	M	2004	4,471	3,670	4.3	59	0.732 (+)	2.035 (+)	3.4×10^6	4	Hyperlipidemia
9	52	M	2004	152	59	34.2	93	2.591 (+)	2.394 (+)	3.2×10^2	4	–
10	56	M	2005	4,348	3,339	3.6	100	2.234 (+)	> 3.000 (+)	3.3×10^5	4	–
11	53	M	2006	2,425	365	3.5	99	2.785 (+)	> 3.000 (+)	1.0×10^3	4	Chronic hepatitis C
12	78	F	2006	1,763	1,558	22.5	52	2.082 (+)	2.707 (+)	7.6×10^6	4	Liver cirrhosis and diabetes mellitus
13	55	F	2006	318	485	11.6	100	2.834 (+)	1.151 (+)	(+) < 10	4	After resection for gastric cancer
14	59	M	2007	2,491	2,277	2.0	101	2.842 (+)	> 3.000 (+)	5.7×10^4	4	–
15	51	M	2007	2,415	222	12.0	98	> 3.000 (+)	2.886 (+)	1.1×10^5	4	Acute renal failure[e]
16	71	M	2010	1,651	1,140	3.4	102	1.754 (+)	> 3.000 (+)	4.3×10^3	3	Hyperlipidemia
17	71	M	2010	3,625	2,940	7.6	111	2.963 (+)	1.817 (+)	5.8×10^5	4	–
18[d]	66	M	2012	2,119	2,056	6.4	99	0.120 (−)	1.155 (+)	3.9×10^6	4	–
19[d]	53	M	2013	451	87	8.6	123	> 3.000 (+)	2.984 (+)	6.3×10^3	4	–
20[d]	51	M	2014	794	119	3.5	115	2.541 (+)	1.671 (+)	5.5×10^3	3	Fatty liver

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HEV, hepatitis E virus; PT, prothrombin activity; T-Bil, total bilirubin.

[a]Patients 1-4 correspond to Patients 3, 4, 7 and 9 in the previous report by Yazaki et al. (2003) and Patients 1-9 correspond to Cases 9, 11, 15, 17, 21, 27, 28, 30 and 31 in the previous report by Mizuo et al. (2005).

[b]Detected in serum samples obtained on the first visit.

[c]Patient 5 with severe hepatitis is highlighted in bold-type.

[d]Patient 19 is a heavy drinker, while Patients 5, 18 and 20 are habitual drinkers.

[e]Patients 5 and 15 suffered from bilateral facial palsy and acute renal failure, respectively, following the development of acute hepatitis E.

[f]None.

were detectable in all 20 patients, IgM anti-HEV was undetectable in one patient (Patient 18). The HEV load on the first visit was variable, ranging from < 10 copies/ml (Patient 13) to 7.6 × 10^6 copies/ml (Patient 12): HEV RNA was detectable in all but one patient (Patient 13) using ORF2-457 PCR, whereas HEV RNA was detectable in Patient 13 using ORF2/3-137 PCR. Among the 20 patients, 17 patients (85%), including Patient 5 with severe acute hepatitis and Patient 15 complicated with acute renal failure, had genotype 4 HEV, all of which were classified into a cluster of the Hokkaido strain, while the remaining three patients (Patients 2, 16 and 20) were infected with genotype 3 HEV. The partial nucleotide sequences of the HEV strains determined in the present study were deposited into the DDBJ/GenBank/EMBL databases under accession numbers LC022734-LC022744.

The source of HEV infection was unknown in three patients (Patients 13, 14 and 16), while the remaining 17 patients had consumed raw or undercooked pig liver and/or intestine within two months before the onset of acute hepatitis.

Bilateral facial palsy in Patient 5

In May 2003, a 67-year-old male was referred to Kobayashi Hospital with a clinical diagnosis of acute liver injury 12 days after the appearance of general fatigue and dark urine. He exhibited a deteriorated general condition and jaundice on the referral visit, and laboratory data revealed elevation of the serum liver enzyme levels (aspartate aminotransferase, 3,321 IU/L; alanine aminotransferase, 3,866 IU/L; lactate dehydrogenase, 717 IU/L; alkaline phosphatase, 331 IU/L; and γ-glutamyl transferase, 133 IU/L). The prothrombin activity was low (36%), while the total bilirubin level was highly elevated (26.2 mg/dl) (Table 2). The patient had no history of traveling abroad, receiving blood or blood-related products or using injection drugs. Although viral markers of acute hepatitis A, B and C were negative in the serum, the IgM, IgA and IgG classes of anti-HEV antibodies and HEV RNA were detected, which led to a diagnosis of acute hepatitis E. After hospitalization, the patient's liver function and general malaise improved with conservative therapy.

However, on the sixth hospital day, he developed bilateral facial palsy (Fig. 1A). A neurological examination revealed the inability to smile, laugh, frown, whistle, talk properly, close his eyes completely, puff out his cheeks and wrinkle forehead skin. However, power in all limbs was normal; deep tendon reflexes were normal. Sensory examination was unremarkable and there were no cerebellar signs. In addition, he did not experience impaired hearing or dizziness, suggesting that Guillain-Barré syndrome was unlikely. Cerebral magnetic resonance imaging (MRI) scans were considered to be normal. The cerebrospinal fluid was clear, although the protein and glucose levels were slightly elevated at 185 mg/dl (reference: 8-48) and 78 mg/dl (reference: 50-75), respectively, and the white blood cell count was 5 cells/μL (Table 2). Based on the findings of MRI brain scans and neurological examinations, the patient was diagnosed with the complication of bilateral peripheral facial palsy. Following conservative therapy without the administration of steroids or immunoglobulin, the facial palsy resolved on day 20 (Fig. 1B), although the slight deviation of the left angle of mouth was present, which disappeared within the next few days.

A liver biopsy performed on day 27 showed the findings compatible with acute viral hepatitis. Despite a spontaneous recovery of the neurological symptoms within three weeks, without any sequelae, it took 47 and 83 days after admission for the serum liver enzyme and total bilirubin levels to normalize.

Nucleotide sequence analysis of the HE-JA30 strain obtained from Patient 5

The HE-JA30 strain recovered from the serum of Patient 5 with bilateral facial palsy had a genomic length of 7,239 nt, excluding the poly(A) tract at the 3′ terminus. The HE-JA30 genome possessed three major ORFs: ORF1, -2 and -3 had coding capacities of 1,707 amino acids (aa) (nt 26-5146), 660 aa (nt 5188-7167) and 114 aa (nt 5174-5515), respectively. The 5′-UTR comprised 25 nt, while the 3′-UTR consisted of 72 nt (excluding the poly(A) tail). The entire genomic sequence of the HE-JA30 strain was deposited into the DDBJ/GenBank/EMBL databases under accession number LC022745.

A full-genome analysis confirmed that the HE-JA30 strain belonged to genotype 4 and was clustered with the Hokkaido genotype 4 strains, with the highest identity of 99.8% to the HE-JA28 strain (AB220976) (Inoue et al. 2006a), whose entire genomic sequence is known (Fig. 2). HE-JA30 had six nucleotide positions (2378, 2782, 2842, 5274, 6309 and 7032) with a mixture of two nucleotides (T/C or A/G), while HE-JA28 had four such nucleotide positions (2322, 2869, 2959 and 5750). When a pyrimidine base (T or C) was considered to be identical to a mixture of T/C and when a purine base (A or G) was considered to be identical to a mixture of A/G, HE-JA30 and HE-JA28 differed from each other by only two nucleotides (nt 3598 and 4657) over the entire genome, unaccompanied by amino acid differences. No amino acid alterations were observed even in the hypervariable region within ORF1 (amino acids 707-791), indicating that both isolates are derived from the same strain, although the HE-JA28 isolate was recovered from a patient who lived in a different area in Hokkaido and developed sporadic acute hepatitis E at a different point in time (December, 2002).

Discussion

The present study shows the characteristics of 20 patients with autochthonous acute hepatitis E seen in Hokkaido, Japan during 2001-2014 and the findings of a case of autochthonous severe acute hepatitis E in Japan, in which the patient demonstrated neurological symptoms as

Table 2. Laboratory data on admission.

Hematology		Autoantibodies	
WBC	7,700/μl	ANA	(−)
RBC	466 × 10^4/μl	AMA	(−)
Hemoglobin	14.2 g/dl		
Hematocrit	43.2%	Cancer-associated markers	
Platelets	11.4 × 10^4/μl	AFP	17 ng/ml
		CEA	2.9 ng/ml
Blood Chemistry		CA19-9	11 U/ml
Total protein	6.0 g/dl		
Albumin	3.2 g/dl	Viral markers	
T-Bil	26.2 mg/dl	IgM anti-HAV	(−)
D-Bil	17.5 mg/dl	HBsAg	(−)
AST	3,321 IU/L	IgM anti-HBc	(−)
ALT	3,866 IU/L	Anti-HBc	(+)
LDH	717 IU/L	HBV DNA	(−)
ALP	331 IU/L	Anti-HCV	(−)
γ-GT	133 IU/L	HCV RNA	(−)
ChE	124 IU/L	IgM anti-CMV	(−)
T. chol	120 mg/dl	IgG anti-CMV	(+)
Triglycerides	153 mg/dl	IgM anti-EBV VCA	(−)
CRP	1.6 mg/dl	IgG anti-EBV VCA	(+)
Ammonia	114 μg/dl	IgG anti-EBNA	(+)
		IgM anti-HSV	(−)
Electrolytes and renal function		IgG anti-HSV	(+)
Na	140 mEq/L	Anti-HIV-1/2	(−)
K	3.2 mEq/L	IgG anti-HEV	(+)
Cl	103 mEq/L	IgM anti-HEV	(+)
BUN	6 mg/dl	IgA anti-HEV	(+)
Creatinine	0.64 mg/dl	HEV RNA	(+)
Uric acid	4.3 mg/dl	HEV genotype	4
Blood coagulation		Cerebrospinal fluid	
Prothrombin activity (%)	36%	Protein	185 mg/dl
Hepaplastin test	21%	Glucose	78 mg/dl
APTT	54.3 sec	WBC	5 cells/μl
Fibrinogen	182 mg/dl	RBC	0 cells/μl
Antithrombin III	32%		

γ-GT, γ-glutamyl transferase; AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANA, antinuclear antibodies; AMA, antimitochondrial antibodies; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CEA, carcinoembryonic antigen; ChE, cholinesterase; CMV, cytomegalovirus; CRP, C-reactive protein; D-Bil, direct bilirubin; EBV, Epstein-Barr virus; EBNA, EBV nuclear antigen; HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; HBc, hepatitis B core; HCV, hepatitis C virus; HEV, hepatitis E virus; HSV, herpes simplex virus; LDH, lactate dehydrogenase; RBC, red blood cell count; T-Bil, total bilirubin; T. chol, total cholesterol; VCA, viral capsid antigen; WBC, white blood cell count.

an extrahepatic manifestation of HEV infection. The present case was unique in that the patient had bilateral peripheral facial palsy as a neurological manifestation of HEV infection and was infected with genotype 4 HEV. A full-length sequence analysis of the HEV genome suggests that viral factors may have contributed minimally to the development of neurological complications of HEV infection in this patient.

In general, simultaneous bilateral facial palsy is relatively uncommon and occurs in 0.3-2.0% of cases of facial palsy (Stahl and Ferit 1989). The present case may be the first reported case to involve bilateral facial palsy as an extrahepatic manifestation of HEV infection. Of note, however, the presentation of bilateral facial palsy has been

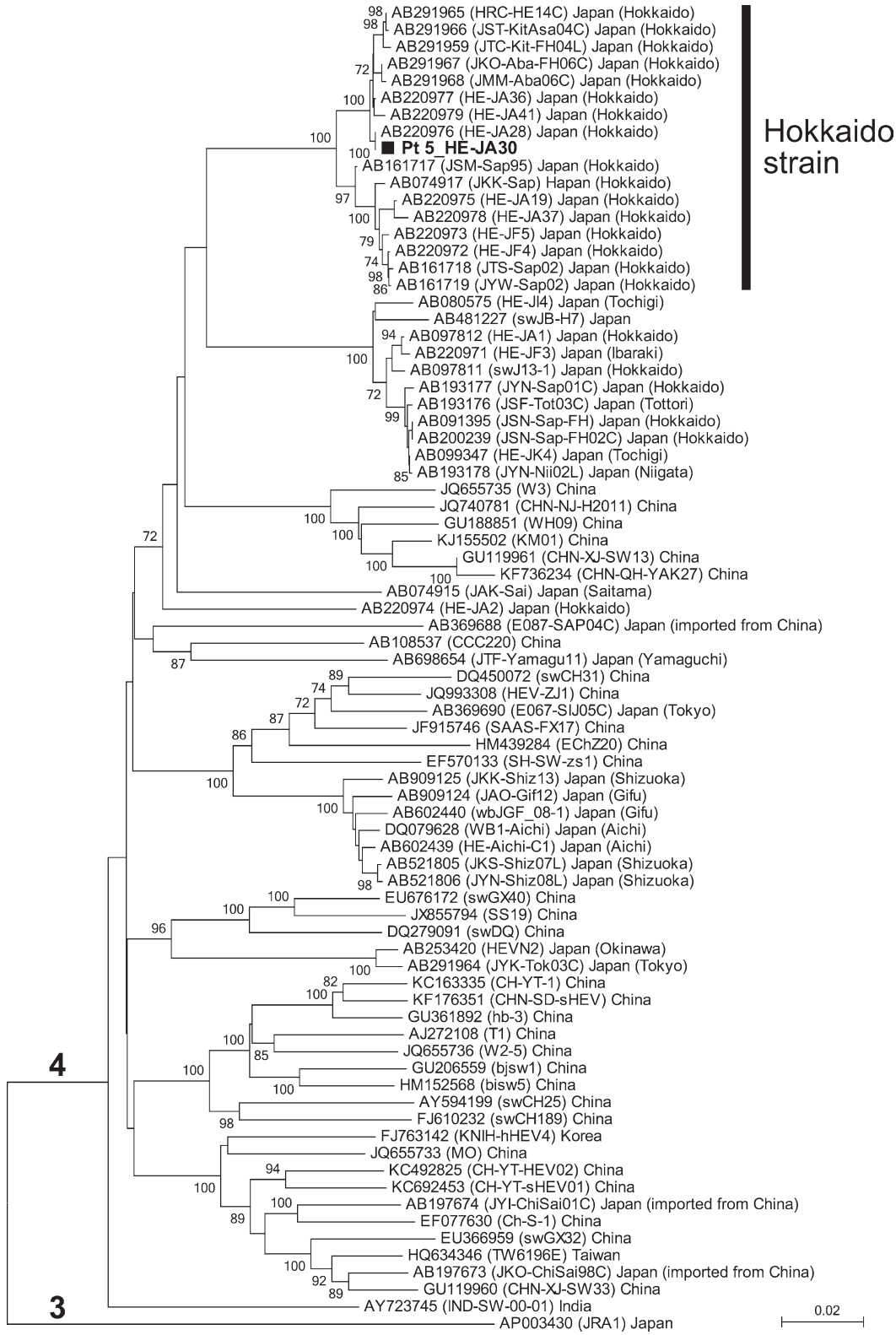

Fig. 2. Phylogenetic tree of full-length genotype 4 HEV strains. Phylogenetic tree was constructed according to the neighbor-joining method based on the entire or near-entire genomic sequences of the HE-JA30 isolate obtained in this study with 76 reference sequences of genotype 4 and the outgroup isolate of genotype 3 (AP003430). The reference sequences are shown with accession nos., followed by the isolate name in parenthesis and the name of the country and/or prefecture in parenthesis where it was isolated. The genotype 4 Hokkaido-indigenous strains are highlighted with a vertical bar. The HE-JA30 isolate obtained from Patient 5 in this study is shown in bold and marked with a closed box. The bootstrap values (> 70%) are indicated for the nodes as a percentage of the data obtained from 1,000 resampling tests. The scale bar is in units of nucleotide substitutions per site.

reported in patients with infections of other viruses, such as human immunodeficiency virus, Japanese encephalitis virus or EBV; similar but unilateral changes have also been reported in EBV-infected patients with Bell's palsy (Coddington et al. 2010; Ruiz and Kirmani 2012; Verma and Praharaj 2012). Therefore, it seems likely that bilateral facial palsy occurs as an extrahepatic manifestation of HEV infection.

Although extrahepatic manifestations of HEV infection have scarcely been presented at academic meetings [https://www.med.kyushu-u.ac.jp/neuro/chihoukai/past/202program.pdf (in Japanese)] and there is no previous literature regarding such manifestations in Japan, several neurological manifestations have been described in patients with HEV infection in European countries, including Belgium, France, Germany, the Netherlands, Portugal and the United Kingdom (Loly et al. 2009; Kamar et al. 2011; Cheung et al. 2012; Santos et al. 2013; Scharn et al. 2014; van den Berg et al. 2014; van Eijk et al. 2014; Woolson et al. 2014), as well as Asian countries, including Bangladesh, China and India (Dixit et al. 2006; Jha et al. 2012; Geurtsvankessel et al. 2013; Chen et al. 2014; Wu et al. 2015). A recent case series from Southwest England and Toulouse, France found a 5.6% prevalence (seven out of 126) of neurological complications in cases of locally acquired HEV infection (Kamar et al. 2011). A systematic, retrospective review of data for 106 cases of autochthonous hepatitis E in the United Kingdom identified eight (7.5%) patients who presented with neurological syndromes, including brachial neuritis, Guillain-Barré syndrome, peripheral neuropathy, neuromyopathy and vesicular neuritis (Woolson et al. 2014). In the Netherlands, it has been reported that 5% of patients with Guillain-Barré syndrome have an associated acute HEV infection (van den Berg et al. 2014). In addition, five cases (10.6%) of acute HEV infection were identified in a total group of 47 patients with neuralgic amyotrophy in the United Kingdom and the Netherlands (van Eijk et al. 2014). In the present study, one of the 20 consecutive patients with autochthonous acute hepatitis E had neurological symptoms, accounting for 5% of the total, this prevalence being similar to that observed in previous reports from European countries. However, further studies with larger numbers of hepatitis E patients are required to clarify the precise prevalence of extrahepatic (particularly, neurological) manifestations of HEV infection in Japan.

Several mechanisms of HEV causing neurological disease have been proposed. In cases of Guillain-Barré syndrome after infection with Campylobacter, influenza virus and cytomegalovirus, anti-ganglioside antibodies are thought to play a pathogenic role in the onset of neurological symptoms via molecular mimicry (Kusunoki and Kaida 2011), and the production of these antibodies may be triggered by HEV infection (Cheung et al. 2012). Brachial neuritis is also thought to be autoimmune in origin in genetically susceptible individuals (Cheung et al. 2012). The pathogenesis of HEV causing peripheral neuropathy and other neurological disorders may involve multiple mechanisms, including predisposing and host immune factors, thus accounting for the variety of manifestations (Kamar et al. 2011; Cheung et al. 2012; Woolson et al. 2014). Diabetes mellitus, alcohol intake and the use of immunosuppressive agents are known to be associated with the development of neurological disorders (Peltier and Russell 2002; Mahmood et al. 2009; Chopra and Tiwari 2012). Although Patient 5 was not diabetic nor hypertensive, and had no history of trauma, blood transfusion or the use of immunosuppressive drugs in the three months preceding onset of facial palsy, he was a habitual drinker and had consumed alcohol of approximately 60-80 g per day for more than 40 years. Continued excessive alcohol intake in Patient 5 may have been a non-viral trigger for the development of neuropathy. Patients 18-20 were also heavy or habitual drinkers (Table 1). However, they did not develop neurological disorders following clinical HEV infection, suggesting that alcohol intake is not a significant predisposing factor for HEV-associated neuropathy.

In the present study, a full-genome analysis excluded the possibility that viral factor(s) may be associated with the development of neurological complications of HEV infection, as a previously reported patient with autochthonous acute hepatitis E, unaccompanied by neurological symptoms, harbored exactly the same HEV strain (HE-JA28) (Inoue et al. 2006a). The isolation of different viral sequences within the serum and cerebrospinal fluid of the same patient suggests the possible emergence of HEV with neurotropic quasispecies that can directly affect the nervous system (Kamar et al. 2010, 2011). Unfortunately, however, stored cerebrospinal fluid was not available for detection of HEV RNA in the present study. Risk factors for the development of neurological complications of HEV infection should be clarified in future studies.

In conclusion, the present study reported the onset of bilateral peripheral facial palsy as an extrahepatic manifestation of acute HEV infection in a Japanese patient infected with genotype 4 HEV. HEV infection should be considered in the differential diagnosis of neurological disorders associated with abnormal liver function tests in Japan.

Acknowledgments

The authors thank Professor Tohru Matsuura, Division of Neurology, Department of Medicine, Jichi Medical University for his helpful discussion during preparation of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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