

Genotype 4 Hepatitis E Virus in France: An Autochthonous Infection With a More Severe Presentation

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Among hepatitis E virus (HEV) infections diagnosed in 2011 by the French Reference Centre for HEV, 9 were due to genotype 4, which until recently was limited to Asia. Sequences from autochthonous cases formed a single cluster very similar to Belgian swine sequences. Clinical presentation differed from genotype 3 infections.

Keywords. HEV; HEV genotype 4; epidemiology; virulence.

Hepatitis E virus (HEV) is a single-stranded RNA virus grouped into 4 major genotypes. Genotypes 1 and 2 are restricted to humans and are hyperendemic in developing countries where HEV transmission occurs via the fecal-oral route, whereas genotype 3 (G3) and genotype 4 (G4) can infect both humans and other mammals; zoonotic and foodborne transmission is suggested for these genotypes [1, 2]. Until recently, human and animal infections due to G4 were limited to Asia [1]. In 2008, a G4 infection was reported in a German patient with no travel history [3]. A G4 strain was then detected in Belgian swine in 2011 [4], and the first autochthonous G4 infection was reported in France [5], followed by 2 other cases in southern France associated with the consumption of raw figatelli [6]. More than 300 cases of HEV infection are reported annually in France [7], mostly involving G3 strains. In 2011, 280 HEV RNA-positive infections were identified by the National Reference Centre for HAV and HEV, including 9 infections due to

HEV G4. The objective of the present study was to characterize the genetic diversity of G4 strains and the clinical characteristics of the cases identified in 2011.

METHODS

The following parameters were retrieved from the Reference Centre database: date of diagnosis, age, sex, alanine aminotransferase (ALT) levels, presence or absence of jaundice, risk factors for HEV infection, immunosuppression context, HEV serology, and HEV genotype. The routine diagnosis of HEV infection was implemented using anti-HEV immunoglobulin M (IgM) and immunoglobulin G testing (Adaltis, Milan, Italy), and HEV RNA detection targeting the open reading frame (ORF)-3 region (Ceeram, La Chapelle sur Erdre, France). The HEV genotype was determined by the phylogenetic analysis of 347 nucleotides (nt) within ORF-2 (nt 5996–6343) [8]. To further characterize the G4 isolates, additional analyses were performed on an 887 base-pair fragment encompassing nt 105–991 within ORF-1, as described elsewhere [4]. Phylogenetic analyses were conducted under MEGA5 software. For statistical analyses, the Mann-Whitney *U* test and Fisher exact test were performed using Statistica version 6 software (Stat Soft France, 2004).

RESULTS

Among the 280 infections with detectable HEV RNA, 260 strains were successfully genotyped. As expected, G3 was the most frequent genotype, identified in 94.5% of cases. G1 and G4 were identified in 5 (2%) and 9 cases (3.5%), respectively. Most G4 cases (7/9) were diagnosed during the fourth quarter of 2011, and most (6/9) were diagnosed in patients living in northern France.

From patients infected with HEV-G3, 239 had available clinical data. Of them, 31 were excluded because they had an ongoing chronic infection. It was then possible to compare the clinical features of 208 HEV G3-acutely infected patients to the 9 G4-infected patients (Table 1). Anti-HEV IgM were present in all G4-infected patients and in all but 3 G3-infected patients, of whom 2 were liver transplant patients and 1 was receiving immunosuppression for an autoimmune disease. No statistical difference was observed with respect to age (mean, 56 ± 13 years vs 48 ± 17 years; *P* = .10), but the usual male predominance of G3 infections was less pronounced relative to G4

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Table 1. Clinical Characteristics of Hepatitis E Virus Genotype 3- and 4-Infected Patients

Clinical Characteristics	G4 (n = 9)	G3 (n = 208)	G3 ID (n = 41)	G3 Non-ID (n = 167)	PValue (G4 vs G3)	PValue (G4 vs G3-ID)	PValue (G4 vs G3 Non-ID)
Age, y, mean ± SD	48 ± 17	56 ± 13	56 ± 14	57 ± 12	.10	.40	.04
Male	4/9 (44%)	152/208 (73%)	34/41 (83%)	118/167 (71%)	.07	.02	.13
ALT, IU/mL, mean ±	3835 ± 1913	1792 ± 1416	1297 ± 955	1909 ± 1484	.0003	<.0001	.003
Jaundice	7/8 (87%)	48/106 (45%)	9/20 (45%)	39/86 (45%)	.028	.088	.028

Abbreviations: ALT, alanine aminotransferase; G3, genotype 3; G4, genotype 4; ID, presence of immunodepression; SD, standard deviation.

infections (73% of G3-infected patients were male G3 compared to 44% for G4; $P = .07$), and significantly higher ALT levels were observed in G4 infections (mean, 3835 ± 1913 vs 1792 ± 1416 IU/mL; $P = .0003$). However, no G4-infected patient was immunosuppressed in this small series, whereas this was the case for 20% of G3-infected patients. As expected, immunosuppression was associated with lower ALT levels among G3-infected patients (mean, 1297 ± 955 vs 1909 ± 1484 IU/mL; $P = .0013$). Nevertheless, G4-infected patients still presented with higher ALT levels than did immunocompetent G3-infected patients ($P = .003$). Presence or absence of jaundice was reported for 114 patients. Jaundice was more frequent in G4-infected patients than in G3-infected patients (87% vs 45%; $P = .028$), including G3 immunocompetent patients (87% vs 45%; $P = .028$). However, the difference was not significant when compared to immunosuppressed patients, maybe because the presence of comorbidities in these latter. In addition, although data on factor V levels were not available for most G3 infections, thus hampering any comparison, a significant proportion of G4-infected patients (4/9) presented with liver failure, as defined by plasma prothrombin time <50%.

Just 1 patient reported traveling abroad (to China) within 6 weeks of the onset of symptoms (patient 1). Risk factors could be evaluated in 7 of the remaining 8 patients: the consumption of cured pork products was mentioned in 5 cases, and contact with farm animals in 2.

A phylogenetic analysis of ORF-2 sequences (Figure 1) identified a cluster that included the 8 autochthonous G4 strains isolated in 2011. The French G4 strain reported by Tessé et al in 2011 (GenBank accession number GU982294 [5]) clustered close to these 8 sequences. The G4 strain imported from China clustered separately from this cluster, together with sequences retrieved from GenBank and isolated in China.

A phylogenetic analysis of ORF-1 sequences (Figure 2) enabled comparisons with strains isolated from Belgian swine and those described in Marseille in 2011 (GenBank accession numbers HQ857384 and JN944587 [4, 6]). Autochthonous G4 strains formed a single cluster that included the Marseille

strain. Again, the G4 strain imported from China clustered separately from the autochthonous strains. The autochthonous G4 strains had 99.2%–100% of sequence homology within ORF-1; homology with the Belgian swine sequence was 94.8%–95.1%.

DISCUSSION

In France, autochthonous HEV infection is mainly due to genotype 3F [7]. However, the present study, as well as 2 other reports [5, 6] suggest the emergence of HEV G4 in France. As previously reported in studies from Asia [9], HEV G4 infection has certain clinical specificities. Indeed, patients tend to be younger, are less often men, and present with more severe disease involving higher aminotransferase levels and higher bilirubinemia, as reflected by the more frequent occurrence of jaundice. In the present study as well as in the report from Mizuo et al [9], the same limitations concerning the number of patients used for comparisons can apply. In fact, although exhaustive clinical data are presented for 25 G4-infected patients in this latter report, these data were only compared to 7 cases of G3-infected patients. However, similar observations from different populations may lend credence to a greater virulence of HEV G4.

Phylogenetic analyses showed that a distinct G4 lineage is circulating in Europe. Indeed, imported and autochthonous sequences belong to distinct clusters: the sequences from autochthonous cases formed a single cluster that included previously published G4 sequences from France [5, 6] and strong similarities with Belgian swine sequences [4].

It remains unclear how HEV G4 was first introduced into swine in Europe. Very few data are available, and HEV G4 has not so far been described in French swine. However, the genetic divergence from Asian strains strongly suggests that HEV G4 is circulating in Europe. The spread of HEV G4 should therefore be monitored closely, particularly as its clinical presentation may be more severe.

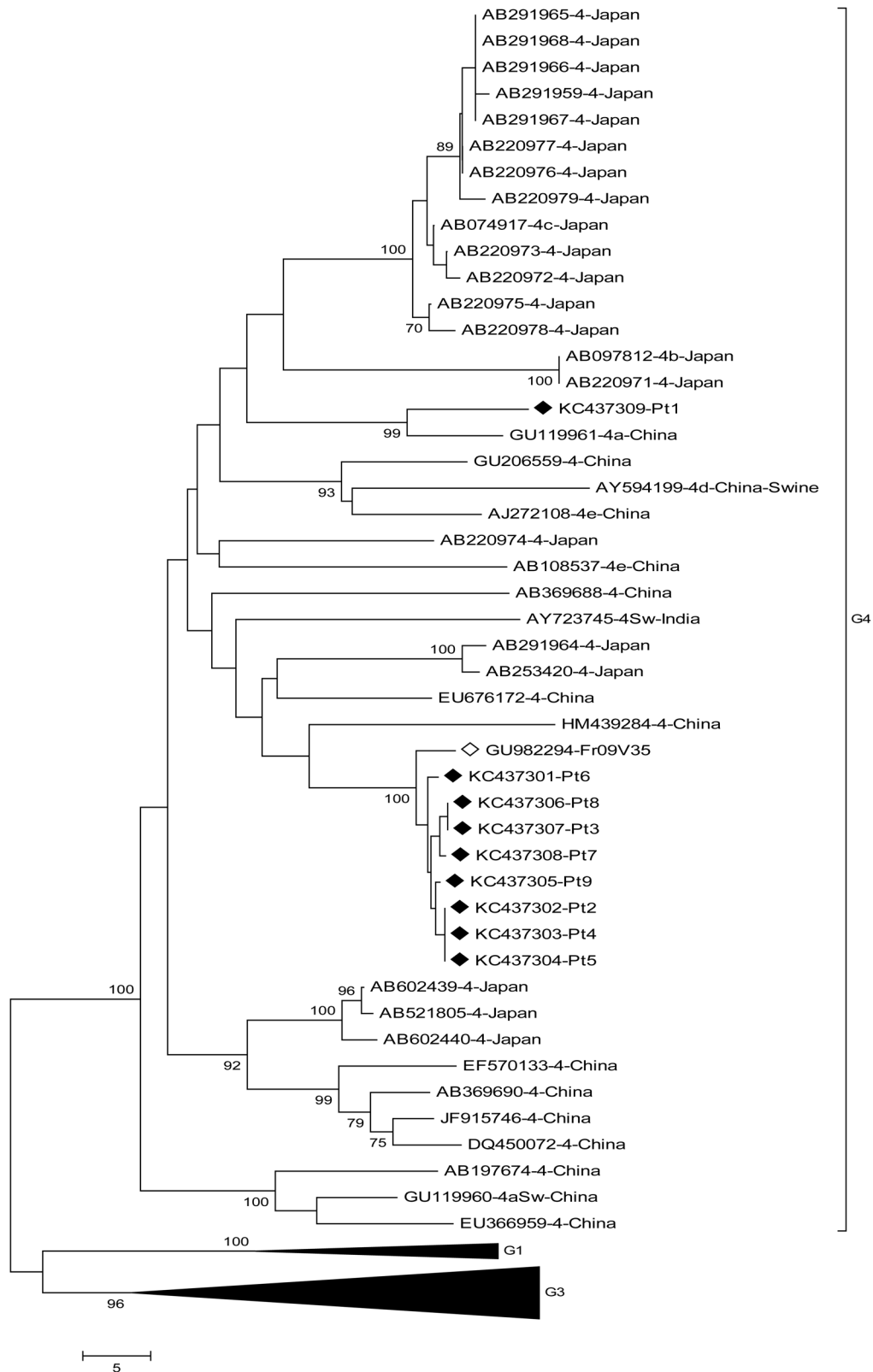


Figure 1. Phylogenetic analyses were performed in MEGA5 software using the neighbor-joining method from a Kimura 2-parameter distance matrix based on partial nucleotide sequences of the open reading frame (ORF)-2 (347 nucleotides). The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. Full-length GenBank reference sequences are indicated by their accession number. ◆ indicates the genotype 4 (G4) sequences described by the present study, with their GenBank accession number. ◇ indicates previously published European G4 sequences.

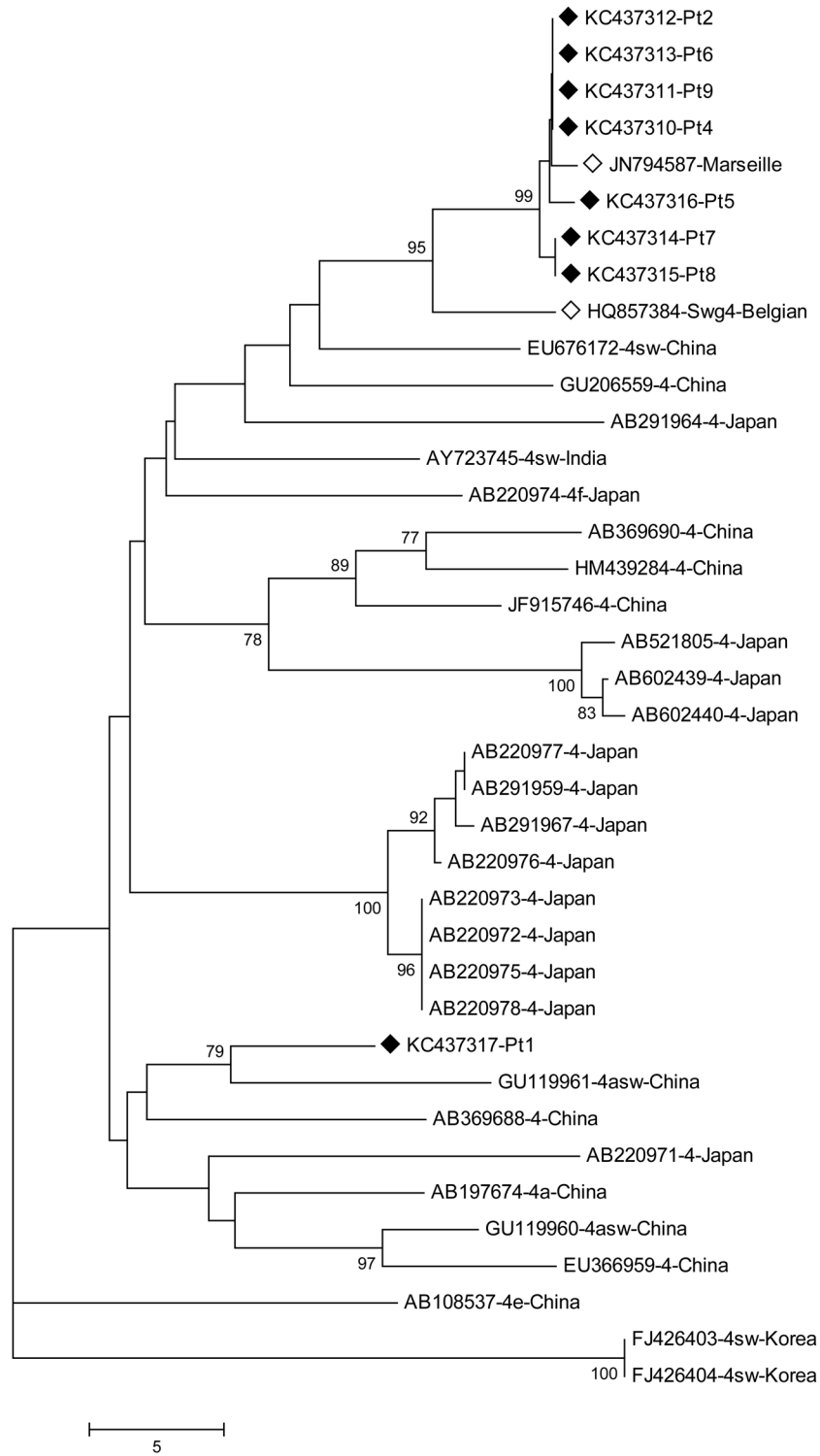


Figure 2. Phylogenetic analyses were performed in MEGA5 software using the neighbor-joining method from a Kimura 2-parameter distance matrix based on partial nucleotide sequences of the open reading frame ORF1 (887 nucleotides). The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. Full-length GenBank reference sequences are indicated by their accession number. ◆ indicates the genotype 4 (G4) sequences described by the present study, with their GenBank accession number. ◇ indicates previously published European G4 sequences.

Notes

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