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Human pegivirus isolates characterized by deep sequencing from hepatitis C virus-RNA and human immunodeficiency virus-RNA-positive blood donations, France

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Human pegivirus (HPgV, formerly GBV-C) is a member of the genus *Pegivirus*, family Flaviviridae. Despite its identification more than 20 years ago, both natural history and distribution of this viral group in human hosts remain under exploration. Analysis of HPgV genomes characterized up to now points out the scarcity of French pegivirus sequences in databases. To bring new data regarding HPgV genomic diversity, we investigated 16 French isolates obtained from hepatitis C virus-RNA and human immunodeficiency virus-RNA-positive blood donations following deep sequencing and coupled molecular protocols. Initial phylogenetic analysis of 5'-untranslated region (5'-UTR)/E2 partial sequences permitted to assign HPgV isolates to genotypes 2 ($n = 15$) and 1 ($n = 1$), with up to 16% genetic diversity observed for both regions considered. Seven nearly full-length representative genomes were characterized subsequently, with complete polyprotein coding sequences exhibiting up to 13% genetic diversity; closest nucleotide (nt) divergence with available HPgV sequences was in the range 7% to 11%. A 36 nts deletion located on the NS4B coding region (N-terminal part, 12 amino acids) of the genotype 1 HPgV genome characterized was identified, along with single nucleotide deletions in two genotype 2, 5'-UTR sequences.

KEYWORDS

blood, deep sequencing, France, GBV-C, HPgV, human pegivirus

1 | INTRODUCTION

Human pegivirus (HPgV), formerly known as GBV-C, is a member of the genus *Pegivirus*, family Flaviviridae, composed of isolates identified in various mammalian hosts.¹⁻³ Recent taxonomic evolutions and proposals related to these single-stranded RNA viruses allow the current description of 11 species within the genus *Pegivirus* (*Pegivirus A-K*), the human and nonhuman primate pegivirus isolates being assigned to species *Pegivirus C*.⁴ Despite a nonnegligible blood prevalence in healthy persons, that is 1% to 5% in developed countries and up to 20% in developing countries,

and higher values in immunocompromised patients, both natural history and potential implication of pegiviruses in host's health are largely unknown despite their identification more than 20 years ago.⁵⁻¹⁴ Of note, however, human immunodeficiency (HIV)-HPgV association studies were extensively explored, due to a possible beneficial effect in HIV infection motivating investigations about HIV-HPgV interactions at the molecular level.¹⁵⁻²² Mechanisms of viral persistence and host-immune modulation remain also poorly characterized.²³⁻²⁵ Previous studies allowed the identification of the viral genome in liver, spleen, bone marrow, and peripheral blood mononuclear cells, including T and

B lymphocytes, NK cells, and monocytes; the infection of progenitor hematopoietic stem cells as possible primary targets of the virus had been also suggested.^{26,27}

Exploration of diversity, distribution, and potential hosts of pegiviruses is in perpetual re-evaluation.^{28,29} In the case of human infections, at least six genotypes of the virus have been described, typically classified by phylogenetic comparison of 5'-untranslated region (5'-UTR), E2, or complete genomic sequences: genotype 1 is retrieved in West Africa, genotypes 1 and 2 in Europe and North America, genotype 3 in parts of Asia, genotype 4 in South East Asia, genotype 5 in South Africa, and genotype 6 in Indonesia; recombination events have also been described.³⁰⁻³⁵

Despite those advances, precise analysis of the literature reveals very poor information regarding HPgV nucleotide (nt) sequences characterized in France up to now. Thus, genomic information relative to previous epidemiological studies is mostly reduced to a set of short-viral sequences⁷; this lack of genetic data is also exemplified by the availability in the GenBank database of a unique full-length coding sequence of a French genotype 2 isolate of HPgV characterized in 1998 (GenBank accession no. AF104403).

To gain new insights about the diversity of these viruses, molecular and phylogenetic analyze of 16 French isolates of HPgV, obtained from hepatitis C virus (HCV)- and HIV-RNA-positive blood donations following deep sequencing and coupled molecular protocols, were investigated.

2 | MATERIALS AND METHODS

2.1 | Blood samples

HPgV-positive plasmas considered in this study were part of a sample collection ($n = 150$) entering a metagenomic research project investigating virome content of blood donations excluded from transfusion protocol (HBV-, HCV-, and HIV-positive samples stored at the French Blood Agency National Plasma Bank, Tours, France).

2.2 | Ethical statements

Blood donation is anonymous and voluntary. Informed consent forms were obtained systematically from blood donors for blood collection, processing, and testing for blood-borne pathogens. Viral strains described were identified in plasma units that were deemed unsuitable for transfusion due to the presence of an HBV, HCV, or HIV-positive biological marker. This study was approved by the French Blood Agency Scientific Committee.

2.3 | Nucleic acids preparation

One milliliter of plasma aliquots were clarified by centrifugation (13 000g for 10 minutes at 20°C), filtered (0.22 μ M), and subsequently treated with 915 U of Benzonase (Sigma, Saint-Quentin-Fallavier, France), 500 U of DNase and 2 μ g of RNase (both from

Roche Diagnostics, Meylan, France) for 45 minutes at 37°C. Particle-protected nucleic acids were recovered using the High Pure Viral Nucleic Acid Large Volume Kit (Roche Diagnostics) and resuspended in 30 μ L ultrapure water. Extracted DNA/RNA templates were then converted into double-stranded DNA using combined reverse transcription and 2-hour Phi-29 DNA polymerase treatments.^{36,37}

2.4 | Deep sequencing protocol and metadata analysis

Next-generation sequencing (NGS) libraries were prepared using the Nextera XT DNA sample preparation kit (Illumina, Paris, France), including DNA fragmentation/indexing, polymerase chain reaction (PCR) amplification/clean-up, and library normalization/pooling steps according to manufacturer's instructions; subsequent analysis was performed on a MiSeq sequencer (~65-hours running time) using the MiSeq Reagent Kit v3 600 cycles (both from Illumina).

Resulting paired-end reads (2 \times 300 nts) were analysed using CLC Genomics Workbench v.9.5.3 software (Qiagen, Courtaboeuf, France) implemented on dedicated workstations running on Linux and Windows environments. Briefly, a bioinformatics workflow was designed including sequence quality analysis, trimming and filtering steps, human and bacterial sequence removal (NCBI database for *Homo sapiens* GRCh37/hg19 and genomes of representative bacteria, respectively), de novo assembly, and final taxonomic assignments following local BLAST N/X analysis against nt and nr NCBI RefSeq genomes (July 2017).

Additional NCBI taxonomic assignment and exploration of viral species/families distributions were performed using programs MEGAN v.5.11.3 (<http://www-ab.informatik.uni-tuebingen.de/software/megan/>), KRONA v.2.7 (<https://github.com/marbl/Krona/>), and local Galaxy instances (<https://galaxyproject.org/>).

2.5 | HPgV isolates characterization and analysis

Deduced contigs of HPgV origin were first used as templates for sequence confirmation, and subsequent genotype assignment, using PCR systems targeting 5'-UTR³⁸ and envelope E2 (this study) (UTR-F: 5'-GGTCAYCYTGGTAGCCACTATAGG-3'; UTR-R: 5'-AAGAGAGACA TTGWAGGGCGACGT-3' and E2-F: 5'-GCGGGHGTGATGAGCCTTACHCC-3'; E2-R: 5'-CTGRACATGTATRAACCCRTCAGG-3', respectively). Amplification products were purified from the PCR reaction mixtures (MinElute Reaction Cleanup Kit; Qiagen), cloned into pGEM-T vector (Promega, Charbonnières-les-Bains, France) and transfected into *Escherichia coli* TOP10 (Invitrogen, Cergy-Pontoise, France); five clones from each PCR product were sequenced using universal M13 primers.

Following initial phylogenetic analysis, partial sequences considered as representative of the different HPgV isolates identified in terms of genetic diversity served as templates for sequences

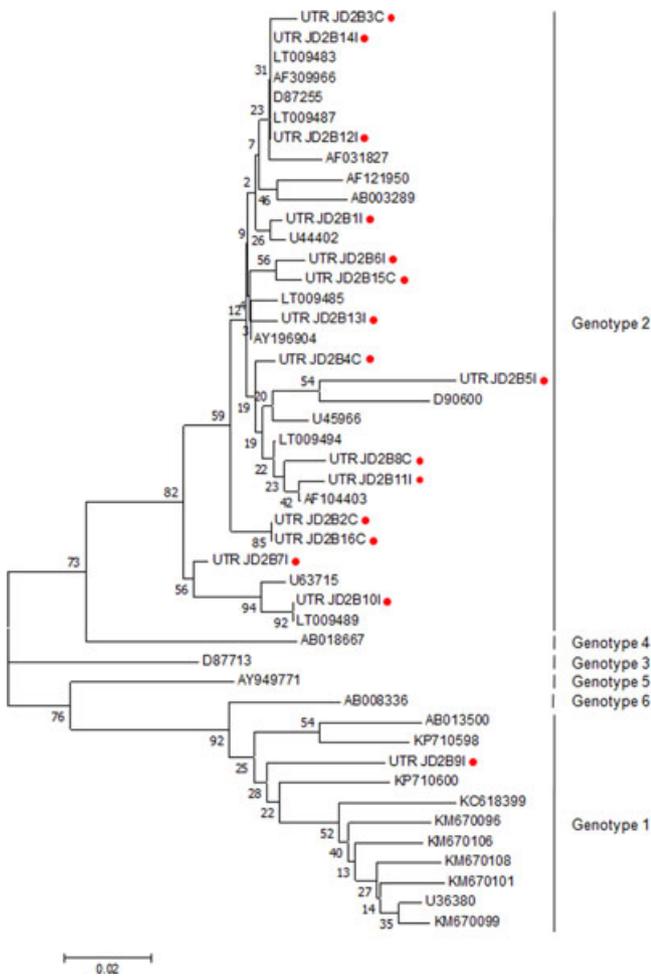


FIGURE 1 UTR phylogenetic tree constructed using HPgV representative isolates and French sequences characterized in this study (red circle). Analysis was done using a p-distance/neighbor-joining method; bootstrap values obtained from 1000 replicas are shown. Scale bar indicates the number of substitutions per site

extensions, in combination with deduced NGS contigs, and subsequent characterization of nearly full-length genomes.

Sequence alignments, mapping, and recombination analyses were subsequently performed using CLUSTALW2 v.2.1, CodonCode Aligner v.5.1.5 (CodonCode Corporation, Centerville, OH), and RDP4 v.4.94 programs, respectively.^{39,40} For the latter, default settings were used for all algorithms; breakpoints were considered if detected with three or more recombination detection methods at a $P < 0.05$. Phylogenetic analyses were performed using MEGA6 software.⁴¹ The GenBank reference sequences ($n = 30$) used to confirm HPgV genotypes, included AB013500, KP710598, U36380, KM670099, KM670101, KM670108, KM670106, KM670096, KC618399, and KP710600 (genotype 1); AF104403, LT009494, D90600, U45966, AY196904, LT009485, LT009487, LT009489, AF309966, D87255, LT009483, AF031827, U44402, AF121950, AB003289, and U63715 (genotype 2); D87713 (genotype 3); AB018667 (genotype 4); AY949771 (genotype 5); and AB008336 (genotype 6).

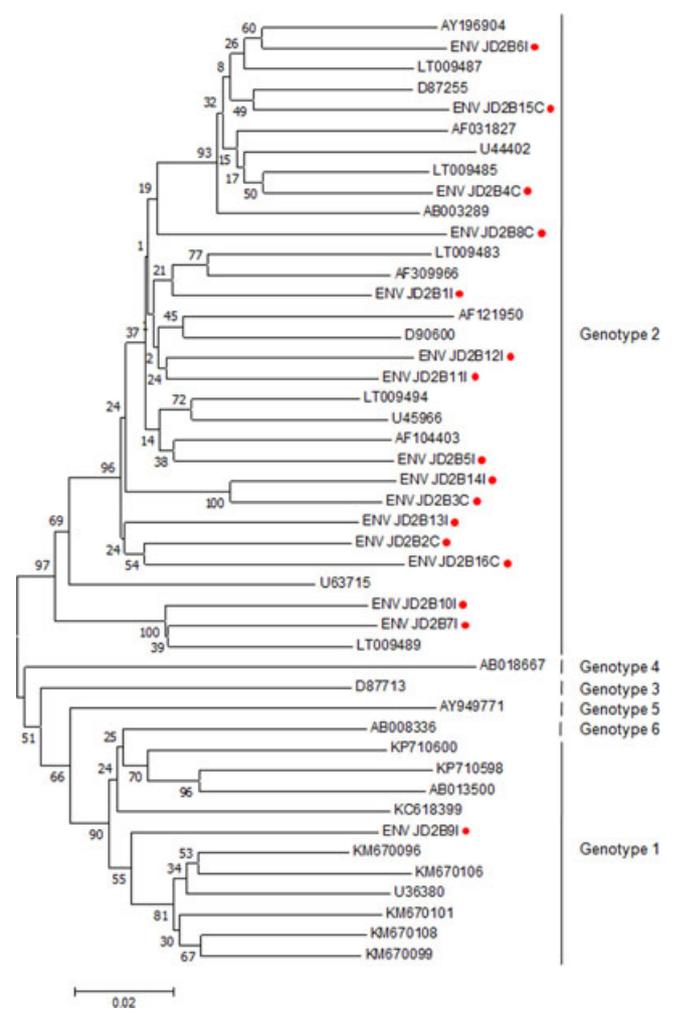


FIGURE 2 ENV phylogenetic tree constructed using HPgV representative isolates and French sequences characterized in this study (red circle). Analysis was done using a p-distance/neighbor-joining method; bootstrap values obtained from 1000 replicas are shown. Scale bar indicates the number of substitutions per site

Hydropathy profiles and transmembrane helix predictions were investigated online at ExpASY (<http://web.expasy.org/protscale/>) and CBS (<http://www.cbs.dtu.dk/services/TMHMM/>) servers, respectively.

3 | RESULTS

3.1 | Distribution of HPgV partial sequences identified

Bioinformatics treatment of Illumina reads (range, 3.33×10^4 to 2.44×10^6), and subsequent analysis of contigs taxonomic distribution, led to the identification of 16 NGS libraries (originating from six HCV-RNA and 10 HIV-RNA-positive plasma samples) exhibiting HPgV reads in the range 2.32×10^3 to 5.11×10^5 (~0.5% to ~75% of total viral sequences identified). HCV or HIV sequences were also retrieved systematically in the corresponding libraries, along with

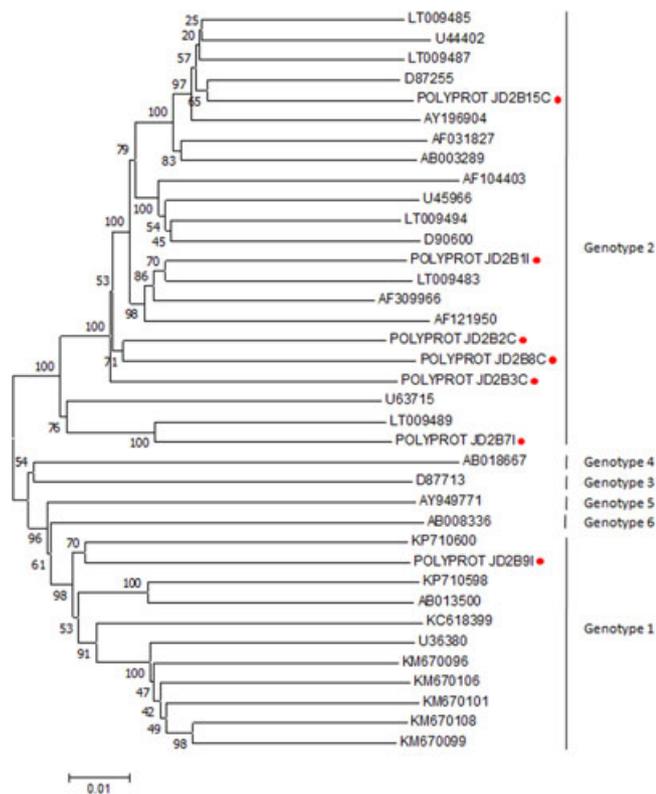


FIGURE 3 Polyprotein phylogenetic tree constructed using HPgV representative isolates and French sequences characterized in this study (red circle). Analysis was done using a p-distance/neighbor-joining method; bootstrap values obtained from 1000 replicas are shown. Scale bar indicates the number of substitutions per site

those of the highly prevalent Anelloviridae family (human viruses TTV, TTMV, and TTMDV).⁴²

Phylogenetic analysis of the 16 HPgV isolates performed using 5'-UTR and E2 sequences (159/160 and 1033 nts, respectively, primers excluded) (GenBank accession no. MH053106-30) permitted the assignment of 15 isolates to genotype 2 (HCV+ or HIV+ plasma), the remaining isolate being assigned to genotype 1 (HIV+ plasma) (Figures 1 and 2).

The observed genetic diversity between the 15 genotype 2 isolates was in the range 0% to 9% and 6% to 15% for the 5'-UTR and E2 regions, respectively; up to 16% genetic diversity was observed for both regions tested when the genotype 1 isolate was included; at the amino acid (aa) level, observed divergence was in the range 0% to 8% for the E2 region considered.

3.2 | Analysis of nearly full-length HPgV genomes

Partial HPgV sequences, originating from seven samples identified as representative of the 16 isolates in terms of genetic diversity, served as templates for sequence extension and subsequent characterization of nearly full-length genomes (range, 8937 to 9316 nts; GenBank accession no. MH053115-21) including complete polyprotein-coding

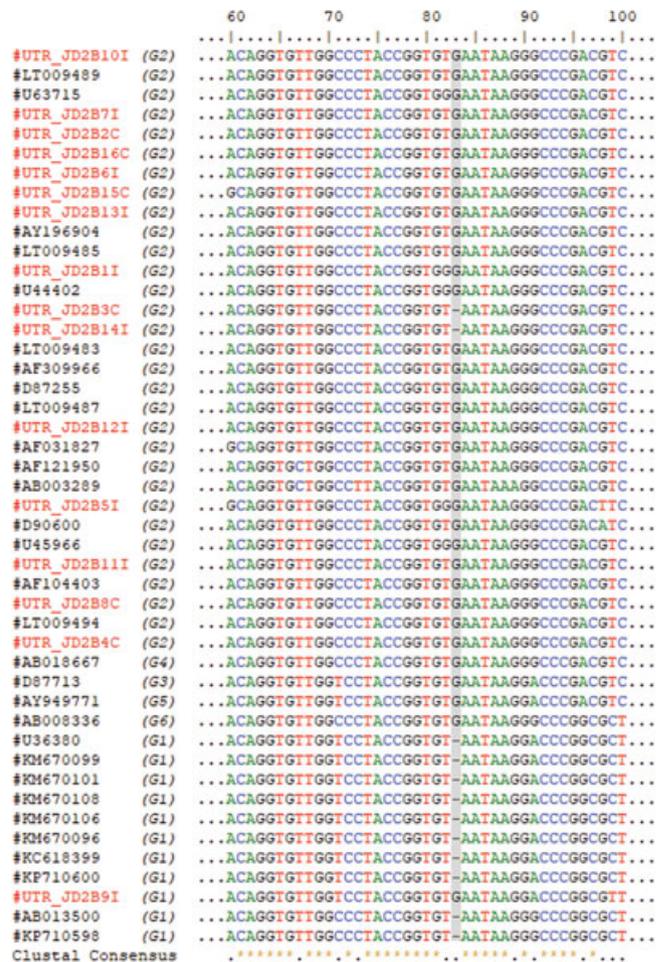


FIGURE 4 Partial UTR nucleotide alignment of HPgV representative isolates and French sequences characterized in this study (red). Position 83 showing single deletion is shaded

sequences (CDS). Sequence analysis confirmed genomic arrangements in accordance with putative cleavage site patterns identified previously for HPgV polyproteins.⁴³

Phylogenetic analysis of corresponding complete polyprotein CDS (8529 nts, genotype 2 isolates/8493 nts, genotype 1 isolate) validated both 5'-UTR and E2 initial genotype assignments (Figure 3).

Nucleotide divergence with closest HPgV polyprotein CDS available in GenBank database were in the 7% to 10% range for the six genotype 2 isolates, and 11% for the genotype 1 isolate; of note, the French genotype 2 isolate (AF104403) exhibited 10% to 12% divergence with genotype 2 sequences characterized here.

The observed genetic diversity between the six genotype 2 polyprotein CDS characterized reached 11%, and up to 13% when the genotype 1 polyprotein CDS was included. When the whole set of representative sequences ($n = 30$) was added to this analysis, observed nucleotide divergence values were in the 7% to 14% range (7% to 12% when considering genotype 1, or genotype 2 sequences only), with a 12% overall genetic distance.

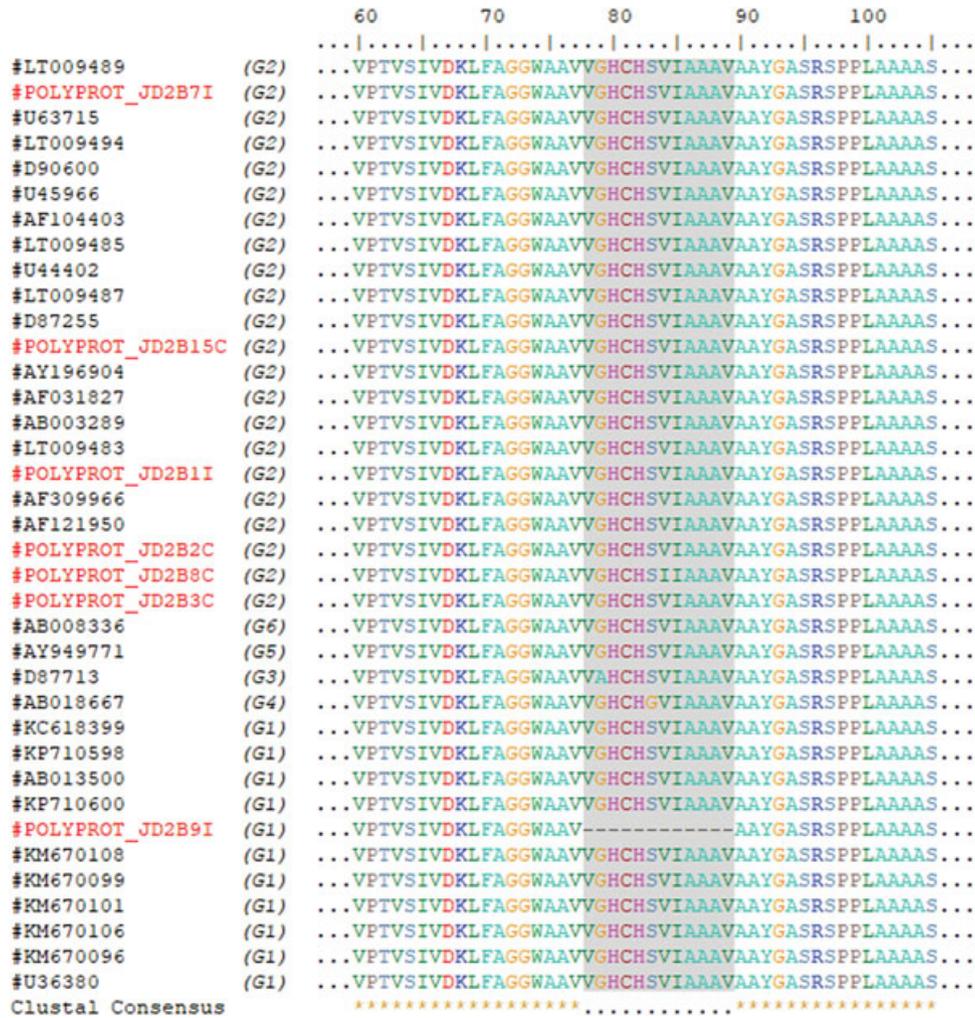


FIGURE 5 Partial NS4B amino acid alignment of HPgV representative isolates and French sequences characterized in this study (red). Positions 78-89 showing the 12 aa-deletion are shaded

3.3 | Analysis of insertions/deletions

Sequence alignments permitted to identify deletions in some sequences characterized:

- (1) A single nt deletion was observed in the UTR of isolates JD2B3C and JD2B14I (genotype 2) at nt position 83 of the alignment (Figure 4). Interestingly, such a deletion was also retrieved in genotype 1 reference sequences, except for isolate JD2B9I (genotype 1) characterized in this study.
- (2) A 36-nt deletion was identified in the polyprotein CDS of isolate JD2B9I (genotype 1); this deletion was confirmed by a control PCR using primers encompassing the corresponding region and the subsequent analysis of cloned amplicons. Investigation of putative cleavage sites within the polyprotein permitted to locate the resulting 12 aa deletion (VGHCHSVIAAAV) in the N-terminal portion of the deduced NS4B protein (269 aa), corresponding to aa positions 78 to 89 of the alignment (Figure 5). In silico analysis suggested a nontransmembrane location of this N-terminal part.

No insertion was observed within sequences characterized in this study.

3.4 | Analysis of recombination events

The RDP4 analysis of the seven full-length polyprotein CDS characterized did not permit to identify significant probability of recombination events according to selected parameters.

4 | DISCUSSION

Despite the discovery of first pegiviruses more than 20 years ago, the natural history of this growing viral group infecting human and nonhuman hosts remains only partially understood. As for many other viruses, advances related to their genetic diversity were linked to the evolution of laboratory protocols, initially with the help of sequence-specific, PCR-based approaches, and more recently using sequence-independent, massive sequencing.

By using the above mentioned approaches, we were able to identify and explore the genetic diversity of 16 strains identified in French human-blood samples excluded from transfusion protocol, and to characterize partial and/or nearly complete HPgV genomes. Our study was motivated by GenBank database analysis results pointing out the scarcity of French HPgV sequences available. Thus, we first analysed 5'-UTR and E2 HPgV sequences, and identified 15 isolates classified as genotype 2 and 1 isolate as genotype 1. The identification of such genotypes in France was in agreement with previous findings highlighting the predominance of HPgV genotype 2 isolates in several human cohorts (blood donors and polytransfused children), along with the occasional detection of other HPgV genotypes (ie, 1 and 3).^{7,8} This predominance was also observed in studies originating from other European countries, such findings being suggested, however, by the analysis of partial sequences, except for the recent characterization of UK full-length genomes.³⁴ We subsequently characterized seven nearly complete HPgV sequences, and confirmed their phylogenetic assignation to genotypes 2 ($n = 6$) and 1 ($n = 1$) by analysis of the complete polyprotein CDS. We observed a moderate genetic divergence among the seven French isolates (up to 13%) and by comparison with the closest sequences available in databases (up to 11%). Such moderate divergence values, also observed (up to 14% genetic diversity) when including 30 HPgV genotypes 1 to 6 reference sequences, were considered as typical for members of this viral group.³⁴ Thus, the observed genetic diversity among French genotype 2 isolates is consistent with well known aspects of pegiviruses natural history, that is, a very probable long-term presence and coevolution of the virus with humans, combined with multiple routes of transmission, persistent asymptomatic infections, and high rates of mutation/replication.^{2,11,25,27,34}

Genomic sequences characterized here did not exhibit evidence of recombination, such events being potentially retrieved among pegivirus members as demonstrated for several HPgV isolates originating from China, Germany, or USA.^{31,35,44-46} We identified, however, a 36 nts/12aa deletion located on the NS4B coding region (N-terminal part) of the genotype 1 isolate characterized in this study. By analogy with previous studies focusing on HCV and other Flaviviridae members, it is probable that pegivirus NS4B would play an important role on viral replication complex formation, associated with multiple interactions with cellular proteins as well.^{23,47,48} Investigation of biological impact of such deletion on viral cycle would require future studies involving, ideally, a cell line model able to support viral replication.

Deep sequencing permitted recent advances in deciphering HPgV diversity, not only by the characterization of new HPgV variants in diverse biologic samples but also by the discovery of the distantly-related hepegivirus 1 (HHpgV-1), sharing features with HCV and HPgV, in human blood.^{28,34,43} Even if HHpgV-1 sequences were not identified in our study, probably due to its very low frequency in human populations, application of our NGS protocol permitted to collect new data about HPgV genomic diversity, notably at the intragenotype level, by characterizing French isolates of the virus.

Improving knowledge of the natural history of this intriguing, still pathologically orphan, class of viruses is a motivating challenge, along with the investigation of associated genetic diversity among related hosts. Such perspectives join the dynamic of the fascinating exploration of the microbial content of human, and nonhuman, biological samples by deep sequencing strategies.

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REFERENCES

1. Simons JN, Leary TP, Dawson GJ, et al. Isolation of novel virus-like sequences associated with human hepatitis. *Nat Med.* 1995;1:564-569.
2. Hartlage AS, Cullen JM, Kapoor A. The strange, expanding world of animal hepaciviruses. *Annu Rev Virol.* 2016;3(1):53-75.
3. Stapleton JT, Fong S, Muerhoff AS, Bukh J, Simmonds P. The GB viruses: a review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus *Pegivirus* within the family Flaviviridae. *J Gen Virol.* 2011;92:233-246.
4. Smith DB, Becher P, Bukh J, et al. Proposed update to the taxonomy of the genera Hepacivirus and Pegivirus within the Flaviviridae family. *J Gen Virol.* 2016;97:2894-2907.
5. Anastassopoulou CG, Paraskevis D, Tassopoulos NC, et al. Molecular epidemiology of GB virus C/hepatitis G virus in Athens, Greece. *J Med Virol.* 2000;61(3):319-326.
6. Branco C, Esteves A, Piedade J, Parreira R. A new genotype 2 subcluster identified among GBV-C strains circulating in the Lisbon metropolitan area of Portugal. *J Med Virol.* 2010;82(3):452-459.
7. Castelain S, Francois C, Bonte D, et al. Epidemiological and quantitative study of GBV-C infection in french polytransfused children. *J Med Virol.* 2004;73:596-600.
8. Cantaloube JF, Gallian P, Biagini P, et al. Prevalence of GB virus type C/hepatitis G virus RNA and anti-E2 among blood donors in Southeastern France. *Transfusion.* 1999;39:95-102.
9. Jøgedal EL, Huik K, Pauskar M, et al. Prevalence and genotypes of GBV-C and its associations with HIV infection among persons who inject drugs in Eastern Europe. *J Med Virol.* 2017;89(4):632-638.
10. Lee CK, Tang JWT, Chiu L, et al. Epidemiology of GB virus type C among patients infected with HIV in Singapore. *J Med Virol.* 2014;86:737-744.
11. Marano G, Franchini M, Farina B, et al. The human pegivirus: a new name for an "ancient" virus. Can transfusion medicine come up with something new? *Acta Virol.* 2017;61(4):401-412.
12. Sahni H, Kirkwood K, Kyriakides TC, Stapleton J, Brown ST, Holodniy M, OPTIMA Study Team. GBV-C viremia and clinical events in advanced HIV infection. *J Med Virol.* 2014;86:426-432.
13. Singh S, Blackard JT. Human pegivirus (HPgV) infection in sub-Saharan Africa-A call for a renewed research agenda. *Rev Med Virol.* 2017;27(6):e1951.
14. Trinks J, Maestri M, Oliveto F, et al. Human pegivirus molecular epidemiology in Argentina: potential contribution of Latin

- American migration to genotype 3 circulation. *J Med Virol*. 2014; 86:2076-2083.
15. Bhattarai N, Stapleton JT. GB virus C: the good boy virus? *Trends Microbiol*. 2012;20:124-130.
 16. Ernst D, Greer M, Akmatova R, et al. Impact of GB virus C viraemia on clinical outcome in HIV-1-infected patients: a 20-year follow-up study. *HIV Med*. 2014;15:245-250.
 17. Gómara MJ, Sánchez-Merino V, Paús A, et al. Definition of an 18-mer synthetic peptide derived from the GB virus C E1 protein as a new HIV-1 entry inhibitor. *Biochim Biophys Acta*. 2016;1860:1139-1148.
 18. Hattori J, Okumura N, Yamazaki Y, et al. Beneficial effect of GB virus C co-infection in human immunodeficiency virus type 1-infected individuals. *Microbiol Immunol*. 2007;51:193-200.
 19. Lanteri MC, Vahidnia F, Tan S, et al. NHLBI REDS III Study. Downregulation of cytokines and chemokines by GB Virus C after transmission via blood transfusion in HIV-positive blood recipients. *J Infect Dis*. 2015;211:1585-1596.
 20. Mohr EL, Stapleton JT. GB virus type C interactions with HIV: the role of envelope glycoproteins. *J Viral Hepat*. 2009;16:757-768.
 21. Schwarze-Zander C, Blackard JT, Rockstroh JK. Role of GB virus C in modulating HIV disease. *Expert Rev Anti Infect Ther*. 2012;10:563-572.
 22. Wang C, Timmons CL, Shao Q, et al. GB virus type C E2 protein inhibits human immunodeficiency virus type 1 Gag assembly by downregulating human ADP-ribosylation factor 1. *Oncotarget*. 2015; 6:43293-43309.
 23. Chen S, Wu Z, Wang M, Cheng A. Innate immune evasion mediated by flaviviridae non-structural proteins. *Viruses*. 2017;9(10):291. <https://doi.org/10.3390/v9100291>
 24. Chivero ET, Bhattarai N, McLinden JH, Xiang J, Stapleton JT. Human Pegivirus (HPgV; formerly known as GBV-C) inhibits IL-12 dependent natural killer cell function. *Virology*. 2015;485:116-127.
 25. Chivero ET, Stapleton JT. Tropism of human pegivirus (formerly known as GB virus C/hepatitis G virus) and host immunomodulation: insights into a highly successful viral infection. *J Gen Virol*. 2015; 96:1521-1532.
 26. Bailey AL, Lauck M, Mohns M, et al. Durable sequence stability and bone marrow tropism in a macaque model of human pegivirus infection. *Sci Transl Med*. 2015;7:305ra144-305ra144.
 27. Chivero ET, Bhattarai N, Rydze RT, Winters MA, Holodniy M, Stapleton JT. Human pegivirus RNA is found in multiple blood mononuclear cells in vivo and serum-derived viral RNA-containing particles are infectious in vitro. *J Gen Virol*. 2014;95:1307-1319.
 28. Kapoor A, Kumar A, Simmonds P, et al. Virome analysis of transfusion recipients reveals a novel human virus that shares genomic features with hepaciviruses and pegviruses. *MBio*. 2015;6:e01466-15.
 29. Kapoor A, Simmonds P, Scheel TKH, et al. Identification of rodent homologs of hepatitis C virus and pegviruses. *MBio*. 2013;4:e00216-13.
 30. Ghai RR, Sibley SD, Lauck M, et al. Deep sequencing identifies two genotypes and high viral genetic diversity of human pegivirus (GB virus C) in rural Ugandan patients. *J Gen Virol*. 2013;94:2670-2678.
 31. Neibecker M, Schwarze-Zander C, Rockstroh JK, Spengler U, Blackard JT. Evidence for extensive genotypic diversity and recombination of GB virus C (GBV-C) in Germany. *J Med Virol*. 2011;83(4):685-694.
 32. Wu H, Padhi A, Xu J, Gong X, Tien P. Intra-host diversity and emergence of unique GBV-C viral lineages in HIV infected subjects in central China. *PLOS One*. 2012;7(11):e48417.
 33. Luk KC, Berg MG, Naccache SN, et al. Utility of metagenomic next-generation sequencing for characterization of HIV and human pegivirus diversity. *PLOS One*. 2015;10:e0141723.
 34. Bonsall D, Gregory WF, Ip CLC, et al. Evaluation of viremia frequencies of a novel human pegivirus by using bioinformatic screening and PCR. *Emerg Infect Dis*. 2016;22:671-678.
 35. Blackard JT, Ma G, Polen C, et al. Recombination among GB virus C (GBV-C) isolates in the United States. *J Gen Virol*. 2016;97: 1537-1544.
 36. Biagini P, Uch R, Belhouchet M, et al. Circular genomes related to anelloviruses identified in human and animal samples by using a combined rolling-circle amplification/sequence-independent single primer amplification approach. *J Gen Virol*. 2007;88:2696-2701.
 37. Uch R, Fournier PE, Robert C, et al. Divergent gemycircularvirus in HIV-positive blood, France. *Emerg Infect Dis*. 2015;21(11):2096-2098.
 38. Andonov A, Sauder C, Jacobsen H, Chaudhary R. Comparison of six sets of PCR primers from two different genomic regions for amplification of GB virus C/hepatitis G virus RNA. *J Clin Microbiol*. 1998;36(1):286-289.
 39. Larkin MA, Blackshields G, Brown NP, et al. Clustal W and clustal X version 2.0. *Bioinformatics*. 2017;23:2947-2948.
 40. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. RDP4: detection and analysis of recombination patterns in virus genomes. *Virus Evol*. 2015;1:vev003.
 41. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*. 2013;30:2725-2729.
 42. Biagini P. Classification of TTV and related viruses (anelloviruses). *Curr Top Microbiol Immunol*. 2009;331:21-33.
 43. Berg MG, Lee D, Coller K, et al. Discovery of a novel human pegivirus in blood associated with hepatitis C virus co-infection. *PLOS Pathog*. 2015;11:e1005325.
 44. Thézé J, Lowes S, Parker J, Pybus OG. Evolutionary and phylogenetic analysis of the hepaciviruses and pegviruses. *Genome Biol Evol*. 2015;7:2996-3008.
 45. Worobey M, Holmes EC. Homologous recombination in GB virus C/hepatitis G virus. *Mol Biol Evol*. 2001;18(2):254-261.
 46. Wu H, Padhi A, Xu J, Gong X, Tien P. Evidence for within-host genetic recombination among the human pegiviral strains in HIV infected subjects. *PLOS One*. 2016;11:e0161880.
 47. Li S, Yu X, Guo Y, Kong L. Interaction networks of hepatitis C virus NS4B: implications for antiviral therapy. *Cell Microbiol*. 2012; 14(7):994-1002.
 48. Zmurko J, Neyts J, Dallmeier K. Flaviviral NS4b, chameleon and jack-in-the-box roles in viral replication and pathogenesis, and a molecular target for antiviral intervention. *Rev Med Virol*. 2015;25(4):205-223.