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Divergent Gemycircularvirus in HIV-Positive Blood, France

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To the Editor: Gemycircularviruses are a group of recently discovered single-stranded DNA viruses, found initially in fungi in 2010 (*1*). These "myco-like" viruses have a genome ranging from 2.1 to 2.3 kb, containing 2 opposite open reading frames that probably code for a capsid protein (CP) and a spliced replication-associated protein (Rep). Related viruses have been subsequently identified in animal blood and fecal matter, raw and treated sewage, and insects and plant material, suggesting that gemycircularviruses may represent a large group of viruses exhibiting considerable genetic diversity (*2–8*). The presence of these viruses was recently extended to humans after gemycircularvirus sequences were identified in human blood and brain tissue (multiple sclerosis patient), cerebrospinal fluid, and fecal matter (*8,9*).

While investigating the virome content of an HIV-positive blood donation, we identified several gemycircularvirus-related sequences. The initial metagenomic approach involved an HIV-1–positive plasma sample (B genotype, ≈530 copies/mL) obtained from the French blood agency national plasma bank in Tours, France. A 4-mL aliquot was prepared for metagenomic analysis after filtration, concentration, and nucleases treatment. Next, particle-protected nucleic acids were recovered and used for the preparation of a next-generation sequencing library and its subsequent analysis (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/11/15-0486-Techapp1.pdf). Gemycircularvirus sequences identified among reads (1,680 vs. 82,560 reads total; ≈2%) were assembled into a resulting full-length sequence (GemyC1c) by using CodonCode Aligner version 5.1 (CodonCode Corporation, Centerville, MA, USA). This sequence was verified by using back-to-back specific primers, and the amplicon was cloned and sequenced according to the Sanger method.

The analysis of the GemyC1c sequence (2,109 nt, GenBank accession no. KP987887) revealed a genome divergent from those already available in databases, despite a similar genomic organization (Figure, panel A) and assignment to gemycircularviruses after BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis of putative CP and Rep proteins. This divergence was demonstrated by the phylogenetic analysis of the deduced CP (Figure, panel B), which exhibited ≈72% and ≈44% aa pairwise identity with the 2 closest gemycircularvirus CP sequences available in GenBank (gemycircularvirus c from mongoose feces [Conceicao-Neto N., unpub. data] and HCBI8.215 from cattle blood, respectively). Moreover, GemyC1c CP exhibited ≈30% pairwise identity with viral sequences identified previously in humans (BZ1 from feces, SL1 from cerebrospinal fluid, MSSI2.225 from blood). The deduced spliced Rep (major Rep1 and minor Rep2), seen in such viruses, contained putative rolling circle motifs I (LFTYS), II (HLHAFVD), and III (YATKD) retrieved from gemycircularviruses (*4*).

We subsequently investigated the presence of GemyC1c DNA in 128 HIV-positive plasma samples (French blood agency national plasma bank) along with 256 HIV-negative plasma samples (healthy blood donors, southeastern France; mean donor age 38 years; 136 men; 1 man:1.13 women). Plasma samples were prepared as described previously (*10*), and extracted nucleic acids were tested for GemyC1c DNA by using a specific PCR that included negative, positive, and extraction controls (online Technical Appendix).

Application of the above GemyC1c DNA detection system did not generate any positive signal in the 384 plasma samples in this study, suggesting that the presence

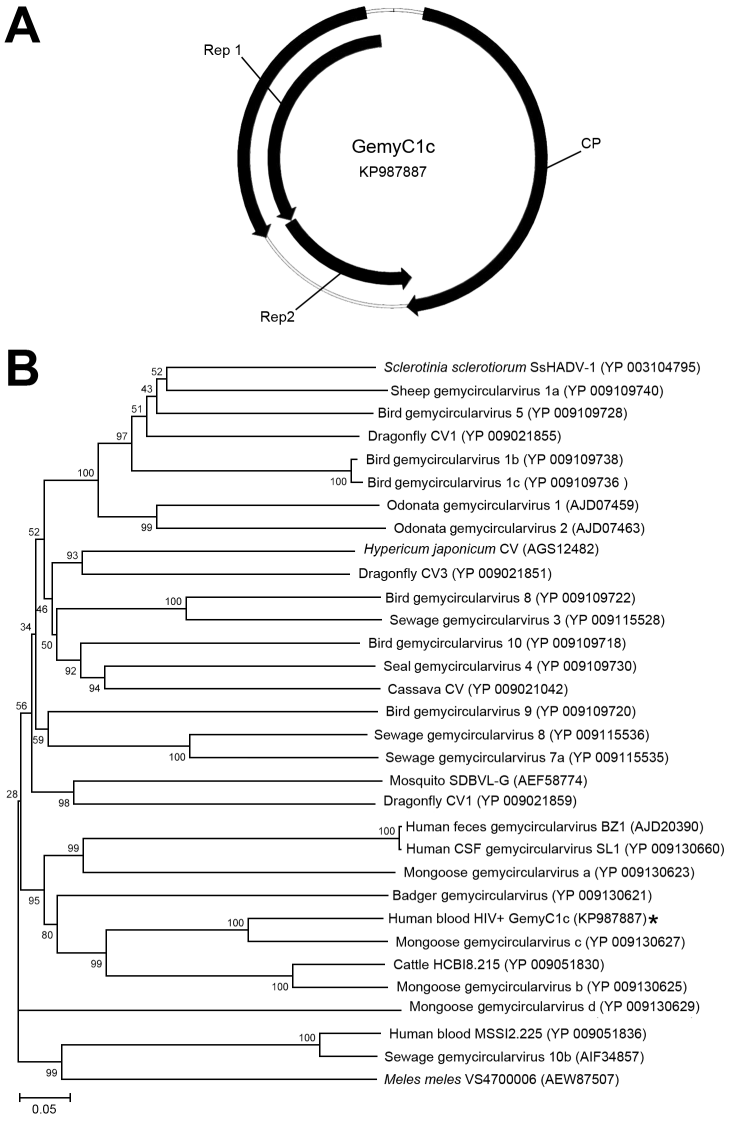

Figure. A) Predicted genomic structure of divergent gemycircularvirus (GemyC1c) isolated from HIV-positive blood, France. Arrows represent major open reading frames. Deduced capsid protein (CP) and spliced replication-associated proteins (Rep1/Rep2) are composed of 318 and 226/126 aa, respectively. B) Neighbor-joining phylogenetic tree constructed by using CP amino acid sequences of GemyC1c (asterisk) and genetically related gemycircularviruses. Bootstrap values were based on 1,000 replicates. Scale bar indicates amino acid substitutions per position.

of this virus in the blood of the populations tested was a rare occurrence. However, it is possible that other, divergent, GemyC1c-related sequences could be present in human blood but remain undetectable by the molecular assay used; the development of universal gemycircularvirus PCR systems is now expected.

Gemycircularviruses are potentially very stable in the environment. Because an unknown part of this group is able to infect fungi, possible contamination from the laboratory environment or nucleic acid extraction methods must be considered. The fact that the same genome was never identified in other libraries generated in our laboratory supports the absence of local contamination; of note, we were also able to detect GemyC1c DNA with PCR by using another plasma aliquot extracted by an alternative method (NucliSENS magnetic extraction; bioMérieux, Marcy l'Étoile, France) In addition, the systematic elimination of the first 35 mL of each blood donation, associated with filtration procedures and control of the temperature of stored plasma (–25°C), contributes to the reduction of bacterial/fungal contamination during blood collection. However, it is not possible to state that the GemyC1c sequence would belong to a human-tropic virus because an association of the virus with an unknown fungus is plausible. Thus, the presence of fungi in the gut, with fungi/virions having traversed the gut lining, or circulating in blood should be considered. Such aspects should prompt future investigations of the effective replication of gemycircularviruses in human or other mammalian cells.

Our discovery of the GemyC1c by a sequence-independent molecular approach was informative for several reasons: 1) this viral sequence would have been undetectable by PCR according to the high genetic divergence existing between GemyC1c and other gemycircularviruses

identified; 2) this finding adds clues to the identification of potential new co-infections occurring in HIV-infected persons; and 3) this finding underlines the need to investigate the virome content of blood samples in a research context of new microbes as potential threats for transfusion. Further studies aimed at exploring genetic diversity and natural history of gemycircularviruses in human hosts are needed.

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Co-infection with Drug-Susceptible and Reactivated Latent Multidrug-Resistant *Mycobacterium tuberculosis*

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To the Editor: Genotyping of *Mycobacterium tuberculosis* (MTB) has identified mixed infections involving >1 MTB strain (*1–4*), which are clinically relevant when different susceptibility patterns are involved (*4–7*). We describe a tuberculosis (TB) case-patient with mixed infection in an area of moderate incidence. In a low-resistance setting (monoresistance 4.6%; multiresistance 1.7%), 1 of the strains was drug susceptible and the other was multidrug-resistant (MDR). Molecular fingerprinting and epidemiologic research revealed that the infection corresponded to a recent infection by a susceptible strain and reactivation of an MDR TB strain. The patient was an HIV-negative woman, 47 years of age, who had immigrated to Spain from Romania and had been living in Almería for >3 years. TB was diagnosed in May 2014; she had experienced symptoms for 2 months. Her diagnosis was confirmed 3 years after being studied in Almería as a close contact of her husband, also from Romania, who had tested positive for MDR TB (resistant to rifampin and isoniazid). When she was observed in the contact trace, she tested positive for purified protein derivative, had been vaccinated against the *M. bovis* bacillus Calmette-Guérin strain, and had no radiologic findings or clinical symptoms. Based on the susceptibility profile of her husband, prophylaxis was not prescribed. Her husband adhered to anti-TB treatment for 20 months; all microbiological control test results had been negative since 2 months after starting therapy.

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Technical Appendix

Plasma sample preparation and NGS analysis

A 4-ml aliquot of the plasma sample was clarified by centrifugation, filtered (0.22 µm), and viral particles were concentrated by adding sterile polyethylene glycol (MW 8,000, Invitrogen, Cergy Pontoise, France) to a 4% final concentration before centrifugation (10,000 × g for 1 h at 4°C). The pellet was suspended in 600 µl of sterile water, treated with 550 U of Benzonase (Sigma, Saint-Quentin Fallavier, France), 300 U of DNase and 1.2 mg of RNase (both from Roche Diagnostics, Meylan, France), and incubated at 37°C for 45 min. Particle-protected nucleic acids were recovered using the High pure viral nucleic acids kit (Roche) and subsequently used for the preparation of a NGS library according to manufacturer's instructions (Nextera XT DNA sample preparation kit; Illumina, Paris, France); the final analysis was performed on a MiSeq sequencer (Illumina).

GemyC1c DNA specific detection and controls

Nucleic acids were tested for GemyC1c DNA by using a nested-PCR assay targeting the CP gene with primers C1cS1 (5′-GTTCCGATTTCCACCGATTTCCA-3′) / C1cR1 (5′-TGCAACTCAACAGTTTCTTGGAG-3′) for PCR-1 (206 bp), and primers C1cS2 (5′-GATTTCCAACAACAGAATCCTGG-3′) / C1cR2 (5′- CTTGGAGGCCTTTGACGAAACAC-3′) for PCR-2 (175 bp). Amplification conditions for the two consecutive PCR assays were 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 58°C for 40 s and 72°C for 1 min. Dilutions of a cloned partial sequence of GemyC1c permitted to estimate the sensitivity of the amplification assay to be <5 copies of target sequence by limiting-dilution assay. Negative (sterile water) and positive controls (cloned GemyC1c partial sequence) were used systematically. The quality of

the nucleic acids extraction procedure was also checked by a control PCR targeting members of the family *Anelloviridae*, a highly prevalent group of DNA viruses in humans (*1*).

GemyC1c sequence (2,109 nt, GenBank accession no KP987887)

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   1 taatattacc tctggacctt ggacccggac ccaggccggg tataaatagg tcccttcccc
  61 cgcggagatg ttttttttg ttgaggggta tgtcgcattt tacgtcatgg cttacgcaca
 121 ccgtcgcttt cggaggcgca ggttcgcccg ccgccggcca acattcgccc gtcgccgtgt
 181 cccccgtcgc tcttatggtc gccgtcgtcg gtcatccggt cgttccgttc ggtccattcg
 241 gaacatagcg gctcgcaagt gtcgcgacaa catgcttggc gttccgattt ccaccgattt
 301 ccaacaacag aatcctggcc ctctcaccat gtttggtgat accagccatg gatatttgtt
 361 ttctcccact gctcgtcgcg tcggttctct tgatgggtca ttacctcgcg gtattcacgc
 421 caattccgct gagcgttgga agacccggtg tttcgtcaaa ggcctccaag aaactgttga
 481 gttgcagcct aataatggag caggctggct gtggagacgc gtcgtatttt cctgcattgg
 541 cctattggag gaattcccta ccaatagtgt ggctgcgccc gactccactc gtgggtatgg
 601 tcgcgccatt tggaacatgc gggatggtag cgctgctgcc acccccctttt acaacgaaat
 661 ggccaattat cttttcgagg gtactcgatt gcgtgattgg atcaatcctt ttactgcgaa
 721 gttggatcgc agtattgtta cggtacattc ggacacgaca cgtaccattc agagtaacaa
 781 cccgaacggt acctacaaga ttttcaagcg gtattacccc gtgaacaggg gtattgtcta
 841 tgcggatgac gagtctggaa gtgggtcgaa gaatgacgcc cactacgctg ctggtacgac
 901 caaagggaat tcgggcgatc tgtttgtttt ggacctcttc aatgctatca atgatagttc
 961 ggagaatact atgaatttcg gagttcacgc caggtattac tggcatgagg gctctggaga
1021 gtaaagcctc tcccctatat aaactataat acaatttttt tccaaccaat catggtcgac
1081 gtcctcgctc attgggtcat tgttggatag ccaaatgcat ggtttacccc atgcgatgtg
1141 cttcttcttc ctgtacctgt cagtacagta gaactctgtc tgttgaccca accagctctt
1201 gtatgctggg aagaatttga aacctccttg gatgtcgtcg aatactgcat acgaacagtc
1261 tggatcctgt tcatactctt ccaaagagaa taatccacca aagtaggcat gggtgccaaa
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1381 acgcacctct gtcactcaga ggttagccca attagggtta gggttagggt tagggttagg
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1501 ggatagatgt tccctaaccc attcccgaat ctcaccatat tgtgtaagat cgaacgagta
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1621 gaatgaagtg aatgatttga cgagatggct gaaaagatgc gctgacgcaa gttcaaaaaa
1681 ctcctctcga gttgatgcat ctctgagttg gtcccatcca gcattctgtc gttcagcatt
1741 agaaccctgg ctaggttctg gtctgcgtag gctcccgcca catatgtcgc catctttcgt
1801 tgcgtaatcc caactgccac ttggacttcg gattgtagaa atgatatttg gatggaaacc
1861 ctcaacatcg aatttgcgca cgtctcgagt tctgtacttt ctgcgaaaat cgacgaaagc
1921 atgtagatgc gtcccaccat cgccgtgcgc ttctctgccg acgatacaat ctgctgagag
1981 agccgagaat aactccacga ctgctgatgg tcggagttgg ccgcattgcg agtaggtgaa
2041 taatccatag cgggcttgga atcgaaatga tgacataagc tttttcggaa gggtctggtc
2101 cagagtttt
```

Location:

CP: 68..1024; Rep1/Rep2: complement 997...2076; Overprinted ORF: complement 1392..2048.

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