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1 **Phylogenetic investigation of HCV-4d epidemic in Paris MSM HIV**
2 **population reveals a still active outbreak and a strong link to the**
3 **Netherlands**

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22 **Objectives.** HCV epidemic is evolving quickly despite new treatments and due to behaviour
23 changes increasing at-risk situations. We investigated potential origins and evolution of the
24 HCV-4d French emergence among HIV-infected MSM, in Paris in 2003.

25 **Methods.** We analyzed all HCV sequences from Paris initial outbreak with all newly available
26 sequences publicly available, including sampling date and geographical location, resulting in
27 184, 68, 156, 107, 13 and 2 sequences from France, Netherlands, other European countries,
28 Africa, Middle-East or Turkey, Americas and Asia, respectively. Phylogenetic reconstruction
29 was performed by maximum likelihood and Bayesian approaches.

30 **Results.** HCV-4d sequences from Europe were strongly separated from non-European
31 sequences. Sequences from the initial Paris outbreak were all included into two well
32 separated and supported clusters with branch support at 100%, mean genetic distance <2.8
33 subst./100 nucleotides and >3.4 subst./100 nucleotides between their common ancestor
34 and the previous node. The largest cluster interleaved French (n=98) and Dutch (n=28)
35 sequences, suggesting several translocations between these countries. This cluster included
36 41 French sequences from Lyon sampled after 2014, highlighting its continuous spread
37 within France since the initial outbreak. The smallest cluster included one Paris sequence
38 with United-Kingdom sequences (n=9).

39 **Conclusion.** A few previous works have shown HCV-4d transmissions occurring between a
40 few countries. In our work, we suggest a new and large connection between France and
41 Netherlands MSM communities and highlight a well separated pan-European transmission
42 network. Large collaborative networks are needed to investigate ongoing transmissions
43 across countries and help specific prevention measures.

44

45

46 **Introduction**

47 The hepatitis C virus (HCV) epidemic has displayed several epidemiologic changes in the last
48 decade in Europe with an increasing prevalence among men who have sex with men (MSM)
49 infected with human immunodeficiency virus (HIV), from 0.42/100 person-years in 1991 to
50 1.34/100 in 2010 [1], but also among HIV-negative MSM, from 0.15/100 person-years to
51 1.2/100 person-years [2,3]. The decreasing condoms use and ChemSex emergence including
52 intravenous injections (“slam”) and new biomedical prevention methods such as pre-
53 exposure prophylaxis (PrEP) against HIV raise concerns about other sexually transmitted
54 infections (STIs) in high-risk populations, including HCV [4]. HCV transmissions between HIV-
55 negative and positive populations are increasingly reported [5]. The use of new HCV directly
56 acting agents (DAA), raising hope of an HCV eradication, is also associated with an increasing
57 number reinfections in the absence of targeted interventions for at-risk behaviour
58 populations [6].

59 The HCV global epidemiology has also been evolving since the last decades. HCV subtype 4,
60 previously restricted to Central Africa and the Middle East, is now found in Western Europe
61 at a low but increasing prevalence, [estimated at 6% of HCV strains in early 2010’s](#) [7]. An
62 important outbreak of HCV subtype 4d, primarily associated with people who inject drugs,
63 started in Paris in 2003 and illustrates these changes. This outbreak occurred between 2003
64 and 2007 among HIV-infected MSM without any traditional risk factor for HCV infection. The
65 only factor associated with HCV acquisition at the time was highly at-risk sexual behaviour,
66 including unprotected receptive anal intercourse with multiple casual partners, concomitant
67 erosive STI (*i.e.* primary syphilis), group sex, fist-fucking, shared sex-toys, and use of
68 recreational drugs [8].

69 In this work, we performed a phylogenetic analysis of HCV strains from the French 2003-
70 2007 outbreak to better understand the origin of this epidemic, its evolution and potential
71 links with other countries.

72

73 **Methods**

74 *Patients and sequences database*

75 All partial NS5B gene (328 nucleotides) sequences from HIV-infected MSM diagnosed with
76 HCV subtype 4d infection in Paris between 2003 and 2007 were included in this study (n=26)
77 [8]. All HCV-4d NS5B sequences available by the 30th June of 2019 were retrieved from
78 GenBank database (<http://www.ncbi.nlm.nih.gov/genbank>). We annotated all sequences
79 with collection date and sampling location using data available in GenBank or related
80 publications. Poor quality, clonal or iterative sequences were discarded resulting in a dataset
81 of 530 sequences (184 from France, 68 from the Netherlands, 156 from other European
82 countries, 107 from Africa, Middle-East or Turkey, 13 from Americas and 2 from Asia and
83 India, cf. Supplementary table).

84 *Phylogenetic analyses*

85 Sequences were aligned using Clustal omega 1.2.4 and manually inspected in AliView 1.18.
86 The best fitting nucleotide substitution model was identified as GTR-G using ModelFinder
87 implemented in IQ-Tree 1.6.1. A phylogenetic tree was inferred by maximum of likelihood
88 reconstruction using IQ-Tree 1.6.1 with GTR-G model and ultrafast bootstrapping (1000
89 replicates). To check the potential clusters observed with French sequences, a lighter
90 phylogenetic tree, only including sequences from the Northern countries, has also been
91 inferred by Bayesian methods using MrBayes 3.2.7 with GTR-G model.

92

93 **Results**

94 The maximum likelihood phylogenetic reconstruction is shown in Figure 1. All but one of the
95 26 viruses from Paris during the 2003-2007 HCV outbreak formed a single monophyletic
96 cluster, herein called cluster A. This cluster was strongly supported by maximum likelihood
97 (branch support of 100%) and Bayesian (posterior probabilities of 100%) phylogenetic
98 frameworks. It was also well separated from other HCV 4d strains with a genetic distance of
99 0.034 substitutions per nucleotide between the most recent common ancestor of cluster A
100 and the previous node. Mean and maximal genetic distances within cluster A were 0.028 and
101 0.084 substitutions per nucleotide, respectively. Within cluster A, 73 other French sequences
102 were also included. These sequences were sampled in Lyon from 2014 to 2017 (n=41) and

103 Paris in 2016 (n=32), supporting the idea of a national outbreak rather than a localized one.
104 The cluster also included 28 sequences from the Netherlands sampled from 2001 to 2017
105 and interleaved in the whole cluster. Among all the French HCV 4d sequences, 54% are
106 included in cluster A (98/184) and 41% of all Dutch sequences (28/68). Sequences from
107 other countries were rare in cluster A: three sequences from United Kingdom, sampled
108 between 2004 and 2007 (3 out of 93 English sequences), 3 from Italy sampled in 2014 and
109 2015 (3/14), and one from Germany (1/3) sampled in 2014.

110 One sequence from the 2003 Paris outbreak was found in a second cluster, herein called
111 cluster B. It is formed of 11 sequences, is strongly supported at 100% [by both phylogenetic](#)
112 [frameworks](#) and well separated from other HCV 4d with a genetic distance of 0.036
113 substitutions per nucleotide between the ancestor of cluster B and the previous tree node.
114 Mean and maximal genetic distances within cluster B were 0.026 and 0.049 substitutions per
115 nucleotide, respectively. The Paris outbreak sequence, sampled in 2006, was the only French
116 sequence in this cluster. Most of cluster B sequences were sampled in the United Kingdom
117 from 2004 to 2014 (n=9). The last sequence of this cluster was sampled in Belgium in 2014.

118

119 **Discussion**

120 The face of HCV infection amongst MSM is evolving quickly due to behaviour changes and
121 new therapeutic strategies.

122 [A few previous works have been conducted about the recent emergence of HCV-4d. A strong](#)
123 [connection between Netherlands and Germany has already been observed as well as](#)
124 [between Quebec and Europe](#) [9,10]. [Our work highlights](#) close links between the French and
125 Netherlands MSM communities as emphasized by HCV transmission with several possible
126 translocation events between these two countries within cluster A. The large representation
127 of HCV 4d among French and Dutch sequences can be explained by local epidemics of this
128 subtype but potentially also by sampling bias due the lack of systematic sequencing. As all
129 HCV-4d sequences from Lyon have been sampled during the acute phase of the infection [5],
130 we also evidence that this outbreak is still spreading a decade later and has reached Lyon,
131 the second largest French city. [Charre et al. also previously evidenced that it should have](#)
132 [been transmitted to Lyon through HIV-negative MSM that have, later on, infected HIV-](#)
133 [positive MSM, highlighting the new inter-relation between these two previously distinct](#)
134 [population also observed in Netherlands](#) [11,12]. [This is in line with the recent French survey](#)

135 [PREVAGAY](#), conducted in 2015 among MSM attending gay venues in 5 French metropolitan
136 cities, were most HCV-positive patients with ongoing infections were infected with HCV-4d
137 [13]. The few sequences from United Kingdom, Italy and Germany included in cluster A
138 suggest a low diffusion of this strain outside Netherlands and France. In our work, we also
139 observed a tidy interconnection of all European sequences, [strongly separated from Central](#)
140 [Africa or Middle-East sequences](#), suggesting a pan-European network better explained by
141 holiday resort of high-risk populations rather than global immigration.

142 As acute HCV infection is mostly asymptomatic, repeated screening, new screening
143 approaches and behavioural interventions should be implemented in at-risk populations
144 [14]. More systematic and standardized sequencing approaches, along with phylogenetic
145 frameworks and epidemiological investigations, could allow contact tracing for targeted
146 prevention and a deeper understanding of epidemiological shifts, as increasingly done
147 worldwide for HIV surveillance. This is of peculiar importance as the globally decreasing HCV
148 epidemic is increasingly driven by regular outbreaks in specific populations with
149 international connections.

150 **Transparency declaration**

151 Pr Descamps and Dr Ghosn report personal fees from ViiV Healthcare, personal fees from
152 Gilead Science, personal fees from Janssen Cilag, personal fees from MSD, outside the
153 submitted work. All the other authors have no conflict of interest to disclose.

154

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158

159 **Authors' contributions**

160 JG, MLC, DD and CD conceived the study, provided expertise and contributed towards the
161 review of the manuscript. BV and SH carried out data collection, analysis and initial drafting
162 of the manuscript. QLH, SL and MS carried out data curation, assisted the analysis and
163 critically reviewed the manuscript.

164

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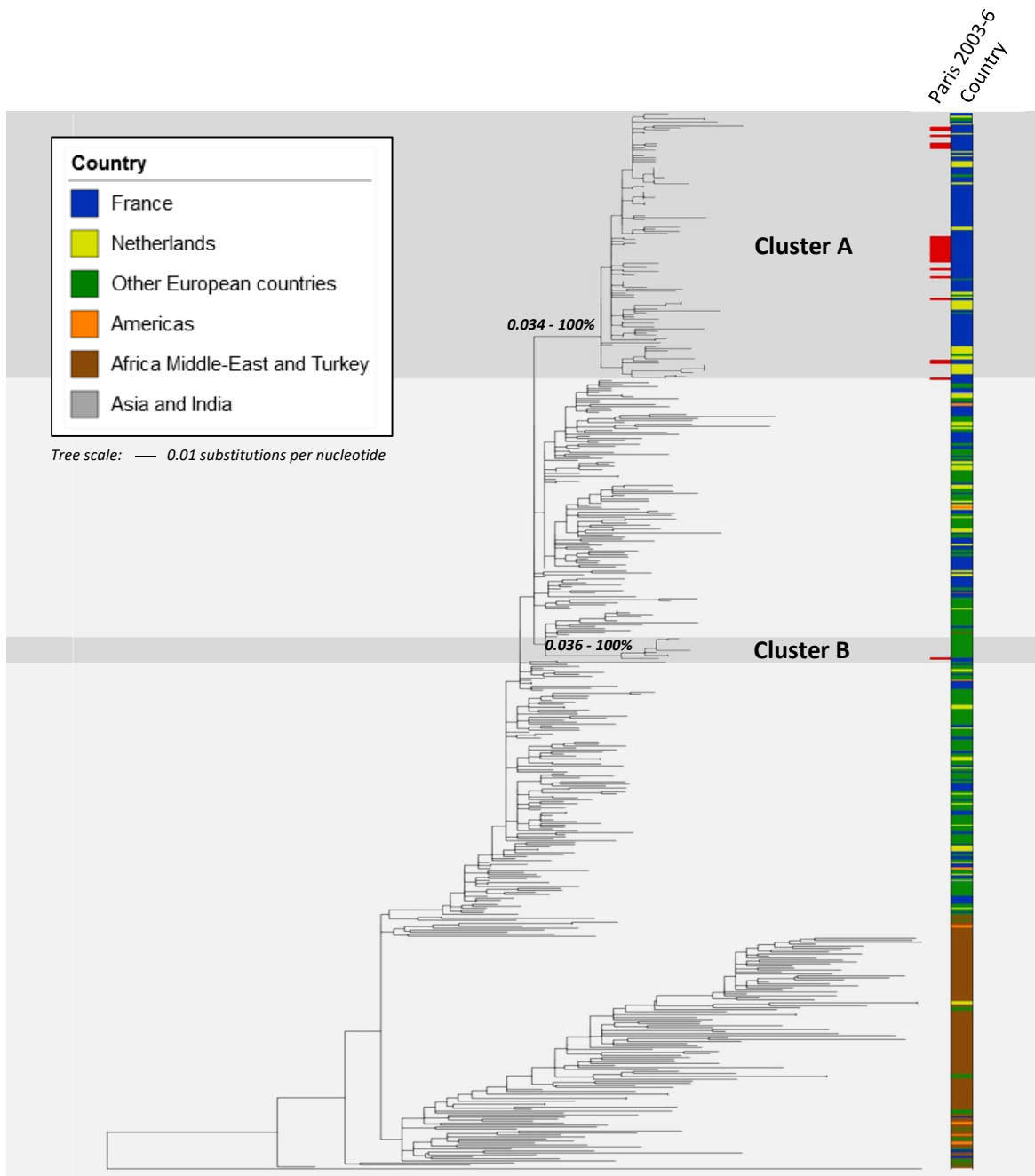


Figure. Phylogenetic tree of all included HCV 4d sequences. A total of 530 HCV-4d sequences are included (184 from France, 68 from the Netherlands, 156 from other European countries, 107 from Africa, Middle-East or Turkey, 13 from Americas and 2 from Asia and India, cf. Supplementary table). Sequences for the Paris outbreak from 2003 to 2007 are indicated by a red mark and the country of sampling is indicated by a specific color on the right side of the tree. Clusters A and B are highlighted in dark grey, branch support values obtained by ultrafast bootstrap (expressed in percentage) and genetic distance (expressed in substitutions per nucleotide) of their most common recent ancestors with the previous node are indicated.