

1 **Virological characteristics of the SARS-CoV-2 JN.1 variant**

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29 Word count: 430/500 words, 7/8 references

30 **Abstract**

31 The SARS-CoV-2 BA.2.86 lineage, first identified in August 2023, is  
32 phylogenetically distinct from the currently circulating SARS-CoV-2 Omicron  
33 XBB lineages, including EG.5.1 and HK.3. Comparing to XBB and BA.2, BA.2.86  
34 carries more than 30 mutations in the spike (S) protein, indicating a high  
35 potential for immune evasion. BA.2.86 has evolved and its descendant, JN.1  
36 (BA.2.86.1.1), emerged in late 2023. JN.1 harbors S:L455S and three mutations  
37 in non-S proteins. S:L455S is a hallmark mutation of JN.1: we have recently  
38 shown that HK.3 and other "FLip" variants carry S:L455F, which contributes to  
39 increased transmissibility and immune escape ability compared to the parental  
40 EG.5.1 variant. Here, we investigated the virological properties of JN.1.

41 **Text**

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45 carries more than 30 mutations in the spike (S) protein, indicating a high  
46 potential for immune evasion.<sup>1-4</sup> BA.2.86 has evolved and its descendant, JN.1  
47 (BA.2.86.1.1), emerged in late 2023. JN.1 harbors S:L455S and three mutations  
48 in non-S proteins (**Figure 1A**). S:L455S is a hallmark mutation of JN.1: we have  
49 recently shown that HK.3 and other "FLip" variants carry S:L455F, which  
50 contributes to increased transmissibility and immune escape ability compared to  
51 the parental EG.5.1 variant.<sup>5</sup> Here, we investigated the virological properties of  
52 JN.1. We estimated the relative effective reproductive number ( $R_e$ ) of JN.1 using  
53 genomic surveillance data from France, the United Kingdom and Spain, where  
54 >25 sequences of JN.1 have been reported, using a Bayesian multinomial  
55 logistic model (**Figures 1B, 1C, Table S3**).<sup>6</sup> The  $R_e$  of JN.1 in these three  
56 countries was higher than that of BA.2.86.1 and HK.3, one of the XBB lineages  
57 with the highest growth advantage at the end of November 2023 (**Figure 1B**).<sup>5</sup>  
58 These results suggest that JN.1 may soon become the dominant lineage  
59 worldwide. Indeed, by the end of November 2023, JN.1 has already overtaken  
60 HK.3 in France and Spain (**Figure 1C**).

61 The *in vitro* ACE2 binding assay<sup>7</sup> showed that the dissociation constant  
62 ( $K_D$ ) value of the JN.1 receptor-binding domain (RBD) is significantly higher than  
63 that of the BA.2.86 RBD (**Figure 1D**), suggesting that S:L455S decreases the  
64 binding affinity to the human ACE2 receptor. In contrast, the pseudovirus assay  
65 showed that the infectivity of JN.1 is significantly higher than that of BA.2.86  
66 (**Figure 1E**). This discrepancy (**Figures 1D, 1E**) would be due to the difference  
67 between monomeric RBD and trimerized whole S protein (see also  
68 **Supplementary Discussion**). We then performed a neutralization assay using  
69 rodent sera infected with BA.2.86 or immunized with BA.2.86 S protein. In both  
70 cases, the 50% neutralization titer ( $NT_{50}$ ) against JN.1 was comparable to that  
71 against BA.2.86 (**Figures 1F, 1G**), suggesting that S:L455S does not affect the  
72 antigenicity of BA.2.86. On the other hand, the  $NT_{50}$  of breakthrough infection  
73 (BTI) sera with XBB.1.5 and EG.5.1 against JN.1 was significantly lower than  
74 that of HK.3 (2.6- to 3.1-fold) and BA.2.86 (3.8-fold) (**Figures 1H, 1I**).  
75 Furthermore, JN.1 shows robust resistance to monovalent XBB.1.5 vaccine sera  
76 compared to BA.2.86 (**Figure 1J**). Taken together, these results suggest that  
77 JN.1 is one of the most immune-evading variants to date. Our results suggest  
78 that S:L455S contributes to increased immune evasion, which partly explains the  
79 increased  $R_e$  of JN.1.

80 **Grants**

81 Supported in part by AMED SCARDA Japan Initiative for World-leading Vaccine  
82 Research and Development Centers "UTOPIA" (JP223fa627001, to Ken J Ishii  
83 and Kei Sato), AMED SCARDA Program on R&D of new generation vaccine  
84 including new modality application (JP223fa727002, to Ken J Ishii and Kei Sato);  
85 AMED Research Program on Emerging and Re-emerging Infectious Diseases  
86 (JP22fk0108146, to Kei Sato; JP21fk0108494 to G2P-Japan Consortium and  
87 Kei Sato; JP21fk0108425, to Kei Sato; JP21fk0108432, to Kei Sato;  
88 JP22fk0108511, to G2P-Japan Consortium and Kei Sato; JP22fk0108516, to  
89 Kei Sato; JP22fk0108506, to Kei Sato); AMED Research Program on HIV/AIDS  
90 (JP22fk0410039, to Kei Sato); JST PRESTO (JPMJPR22R1, to Jumpei Ito); JST  
91 CREST (JPMJCR20H4, to Kei Sato); JSPS KAKENHI Fund for the Promotion of  
92 Joint International Research (International Leading Research) (JP23K20041, to  
93 G2P-Japan and Kei Sato); JSPS KAKENHI Grant-in-Aid for Early-Career  
94 Scientists (23K14526, to Jumpei Ito); JSPS Core-to-Core Program (A. Advanced  
95 Research Networks) (JPJSCCA20190008, Kei Sato); JSPS Research Fellow  
96 DC2 (22J11578, to Keiya Uriu); JSPS Research Fellow DC1 (23KJ0710, to  
97 Yusuke Kosugi); The Tokyo Biochemical Research Foundation (to Kei Sato);  
98 The Mitsubishi Foundation (to Kei Sato); International Joint Research Project of  
99 the Institute of Medical Science, the University of Tokyo (to Jiri Zahradnik); and  
100 the project of National Institute of Virology and Bacteriology, Programme  
101 EXCELES, funded by the European Union, Next Generation EU (LX22NPO5103,  
102 to Jiri Zahradnik). We wish to express our gratitude to CEU Universities and  
103 Santander Bank (Ayudas a la movilidad internacional de los investigadores en  
104 formación de la CEINDO) as well as to the Federation of European Biochemical  
105 Societies (FEBS; Short-Term Fellowship), for their financial support to Miguel  
106 Padilla-Blanco during the first and second part of his internship period at  
107 BIOCEV, respectively.

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109 **Declaration of interest**

110 J.I. has consulting fees and honoraria for lectures from Takeda Pharmaceutical  
111 Co. Ltd. K.S. has consulting fees from Moderna Japan Co., Ltd. and Takeda  
112 Pharmaceutical Co. Ltd. and honoraria for lectures from Gilead Sciences, Inc.,  
113 Moderna Japan Co., Ltd., and Shionogi & Co., Ltd. The other authors declare no  
114 competing interests. All authors have submitted the ICMJE Form for Disclosure  
115 of Potential Conflicts of Interest. Conflicts that the editors consider relevant to  
116 the content of the manuscript have been disclosed.

117 **Figure 1. Virological features of JN.1**

118 **(A)** Frequency of mutations in JN.1 and other lineages of interest. Only  
119 mutations with a frequency  $>0.5$  in at least one but not all the representative  
120 lineages are shown.

121 **(B)** Estimated relative  $R_e$  of the variants of interest in France, United Kingdom,  
122 and Spain. The relative  $R_e$  of EG.5.1 is set to 1 (horizontal dashed line). Violin,  
123 posterior distribution; dot, posterior mean; line, 95% Bayesian confidence  
124 interval.

125 **(C)** Estimated epidemic dynamics of the variants of interest in France, United  
126 Kingdom, and Spain from April 1, 2023 to November 16, 2023. Countries are  
127 ordered according to the number of detected sequences of JN.1 from high to low.  
128 Line, posterior mean, ribbon, 95% Bayesian confidence interval.

129 **(D)** Yeast surface display affinity between the RBD of the BA.2.86 SARS-CoV-2  
130 variant or BA.2.86 that contained the L455S mutation and mACE2 was  
131 measured by yeast surface display. The dissociation constant ( $K_D$ ) value  
132 indicates the binding affinity of the RBD of the SARS-CoV-2 S protein to soluble  
133 ACE2 when expressed on yeast. Statistically significant differences versus  
134 BA.2.86 is determined by two-sided Student's  $t$  tests.

135 **(E)** Lentivirus-based pseudovirus assay. HOS-ACE2/TMPRSS2 cells were  
136 infected with pseudoviruses bearing each S protein of B.1.1 or BA.2 sublineages.  
137 The amount of input virus was normalized to the amount of HIV-1 p24 capsid  
138 protein. The percentage infectivity of B.1.1, BA.2 and JN.1 are compared to that  
139 of BA.2.86. The horizontal dash line indicates the mean value of the percentage  
140 infectivity of BA.2.86. Assays were performed in quadruplicate, and a  
141 representative result of four independent assays is shown. The presented data  
142 are expressed as the average  $\pm$  SD. Each dot indicates the result of an individual  
143 replicate. Statistically significant differences versus BA.2.86 is determined by  
144 two-sided Student's  $t$  tests.

145 **(F-J)** Neutralization assay. Assays were performed with pseudoviruses  
146 harboring the S proteins of B.1.1, BA.2, BA.2.86, JN.1 and HK.3. The following  
147 sera were used: sera from six hamsters infected with BA.2.86 **(F)**; sera from ten  
148 mice immunized with SARS-CoV-2 BA.2.86 S **(G)**; convalescent sera from fully  
149 vaccinated individuals who had been infected with XBB.1.5 (eight 3-dose  
150 vaccinated donors, six 4-dose vaccinated donors, four 5-dose vaccinated donors  
151 and one 6-dose vaccinated donor. 19 donors in total) **(H)**; and EG.5.1 (one  
152 2-dose vaccinated donor, four 3-dose vaccinated donors, five 4-dose vaccinated  
153 donors, four 5-dose vaccinated donors and four 6-dose vaccinated donors. 18  
154 donors in total) **(I)**. Assays were also performed with pseudoviruses harboring  
155 the S proteins of BA.2.86 and JN.1. The following two sera were used:  
156 vaccinated sera from fully vaccinated individuals who had not been infected (8  
157 donors) and vaccinated sera from fully vaccinated individuals who had been  
158 infected with XBB subvariants (after June, 2023) (10 donors). Sera were  
159 collected before vaccination ('Pre') and 3-4 weeks after XBB.1.5 monovalent  
160 vaccination ('Post') **(J)**. Assays for each serum sample were performed in  
161 triplicate to determine the 50% neutralization titer ( $NT_{50}$ ).

162 Each dot represents one NT<sub>50</sub> value, and the geometric mean and 95%  
163 confidence interval are shown. The number in parenthesis indicates the  
164 geometric mean of NT<sub>50</sub> values. The horizontal dash line indicates the detection  
165 limit (40-fold) and the number of samples with neutralization titer under the limit  
166 are shown below the dash line. In **F-J**, statistically significant differences versus  
167 JN.1 were determined by two-sided Wilcoxon signed-rank tests, and p values  
168 are indicated in parentheses. The fold changes of NT<sub>50</sub> from that of JN.1 are  
169 indicated with “X”. In **F** and **G**, \*, p<0.05; \*\*, p<0.01 versus JN.1.

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