Characteristics of the SARS-CoV-2 omicron HK.3 variant harbouring the FLip substitution

As of November, 2023, SARS-CoV-2 XBB variants, including EG.5.1 (XBB.1.9.2.5.1), the currently predominant lineage, have been circulating worldwide, according to Nextstrain datasets. The EG.5.1 strain has a characteristic amino acid substitution in the spike protein (S; S:F456L), which allows the strain to escape humoral immunity (appendix p 16).[1] EG.5.1 has further evolved, and its descendant lineage harbouring the S:L455F (ie, EG.5.1+S:L455F) variant has emerged and has been named HK.3 (XBB.1.9.2.5.1.1.3). HK.3 was initially discovered in east Asia and is rapidly spreading worldwide. Notably, the XBB subvariants bearing both S:L455F and S:F456L substitutions, including HK.3, are defined as FLip variants. These FLip variants, including JG.3 (XBB.1.9.2.5.1.3.3), JF.1 (XBB.1.16.6.1), and GK.3 (XBB.1.5.70.3) have emerged concurrently, suggesting that the acquisition of these two substitutions confers a growth advantage to XBB in the human population.[2,3] We investigated the virological properties of HK.3 as a representative of the FLip variants.

We estimated the relative effective reproduction number (R_e) of HK.3 on the basis of genome surveillance data obtained from 13 countries reporting the substantial presence of HK.3 with a Bayesian hierarchical multinomial logistic regression model (appendix pp 9–14, 16).[4] The global mean R_e for HK.3 was 1·29 times higher than that of XBB.1.5 and 1·12 higher than that of EG.5.1, suggesting that HK.3 might soon become the predominant lineage worldwide. As of Oct 15, 2023, the HK.3 variant has outcompeted EG.5.1 in countries such as Australia, China, South Korea, and Singapore (appendix p 16).

Next, to identify whether the enhanced infectivity of HK.3 contributes to its higher R_e, we constructed lentivirus-based pseudoviruses carrying the S proteins XBB.1.5, EG.5.1, HK.3, and an XBB.1.5 derivative, XBB.1.5+L455F. Although the S:L455F substitution significantly increased the infectivity of XBB.1.5, the infectivity of HK.3 (identical to EG.5.1+S:L455F) was similar to that of EG.5.1 (appendix p 16). The difference in the effect of S:L455F between XBB.1.5 and EG.5.1 might be attributed to the epistatic effects due to the S protein structures of XBB.1.5 and EG.5.1. These results suggest that the increased R_e of HK.3 is not owing to the increased infectivity caused by S:L455F.

We then performed a neutralisation assay using breakthrough infection serum samples (XBB.1.5 [n=20], XBB.1.9 [n=15], XBB.1.16 [n=20], or EG.5.1 [n=18]) to address whether HK.3 evades the antiviral response of humoral immunity induced by breakthrough infection of these variants. The 50% neutralisation titre (NT_{50}) for all breakthrough infection serum samples tested against XBB.1.5+S:L455F was significantly lower than that observed against the parental XBB.1.5 strain (appendix p 16). Notably, the NT_{50} for EG.5.1 breakthrough infection serum samples against HK.3 was significantly lower (1·6 times, p=0·0003) than that observed against EG.5.1 (appendix p 16). Thus, the increased R_e of HK.3 might be partly attributed to the enhanced immune evasion from humoral immunity elicited by breakthrough infection subvariants of XBB, including EG.5.1, its ancestor. S:L455F is a key mutation leading to this immune evasion.

JI has received consulting fees and honoraria for lectures from Takeda Pharmaceutical. KSat has received consulting fees from Moderna Japan and Takeda Pharmaceutical and has received honoraria for lectures from Gilead Sciences, Moderna Japan, and Shionogi & Co. All other authors declare no competing interests. YKo, AP, and OP contributed equally. This work was supported in part by the Japan Agency for Medical Research and Development (AMED) Strategic Center of Biomedical Advanced Vaccine Research and Development for Preparedness and Response (SCARDA) Japan Initiative for World-leading Vaccine Research and Development Centers UTOPIA (JP223fa627001, to KSat), AMED SCARDA Programme on R&D of New Generation Vaccine including New Modality Application (JP223fa727002, to KSat); AMED Research Programme on Emerging and Re-emerging Infectious Diseases (JP22fk0108146, to KSat; JP21fk0108494, to G2P-Japan Consortium and KSat; JP21fk0108425, to KSat; JP21fk0108432, to KSat; JP22fk0108511, to G2P-Japan Consortium and KSat; JP22fk0108516, to KSat; JP22fk0108506, to KSat); AMED Research Programme on HIV/AIDS (JP22fk0410039, to KSat); JST PRESTO (JPMJPR22R1, to JI); JST CREST (JPMJCR20H4, to KSat); JSPS KAKENHI Grant-in-Aid for Early-Career Scientists (23K14526, to JI); JSPS Core-to-Core Program (A. Advanced Research Networks) (JPJSCCA20190008, to KSat); JSPS Research Fellow DC2 (22J11578, to KU); JSPS Research Fellow DC1 (23KJ0710, to YKo); The Tokyo Biochemical Research Foundation (to KSat); and The Mitsubishi Foundation (to KSat). Members of the G2P-Japan Consortium are listed in the appendix (p 18).

*Yusuke Kosugi, Arnon Plianchaisuk, Olivia Putri, Keiya Uriu, Yu Kaku, Alfredo A Hinay Jr, Luo Chen, Jin Kuramochi, Kenji Sadamasu, Kazuhisa Yoshimura, Hiroyuki Asakura, Mami Nagashima, Jumpei Ito, The Genotype to Phenotype Japan (G2P-Japan) Consortium, *Kei Sato*

Division of Systems Virology, Department of Microbiology and Immunology, The Institute of Medical Science (YKo, AP, OP, KU, YKa, AAH, LC, JI, KSat), Graduate School of Medicine (YKo, KU, KSat), International Research Center for Infectious Diseases, The Institute of Medical Science (JI, KSat), International Vaccine Design Center, The Institute of Medical Science (KSat), The University of Tokyo, Tokyo, Japan; Department of Biomedicine, School of Life Sciences, Indonesia International Institute for Life Sciences (i3L), Jakarta, Indonesia (OP); Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Japan (LC); Interpark Kuramochi Clinic, Utsunomiya, Japan (JK); Department of Global Health Promotion, Tokyo Medical and Dental University, Tokyo, Japan (JK); Tokyo Metropolitan Institute of Public Health, Tokyo, Japan (KSad, KY, HA, MN, KSat); Collaboration Unit for Infection, Joint Research Center for Human Retrovirus infection, Kumamoto University, Kumamoto, Japan, (KSat); CREST, Japan Science and Technology Agency, Kawaguchi, Japan (KSat)

1 Kaku Y, Kosugi Y, Uriu K, et al. Antiviral efficacy of the SARS-CoV-2 XBB breakthrough infection sera against omicron subvariants including EG.5. *Lancet Infect Dis* 2023; **23:** e395–96.

2 Ito J, Suzuki R, Uriu K, et al. Convergent evolution of SARS-CoV-2 omicron subvariants leading to the emergence of BQ.1.1 variant. *Nat Commun* 2023; **14:** 2671.

3 Bloom JD, Neher RA. Fitness effects of mutations to SARS-CoV-2 proteins. *Virus Evol* 2023; **9:** vead055.

4 Yamasoba D, Kimura I, Nasser H, et al. Virological characteristics of the SARS-CoV-2 omicron BA.2 spike. *Cell* 2022; **185:** 2103–15.e19.

Lancet Microbe 2023

Published Online
https://doi.org/10.1016/S2666-5247(23)00373-7

For more on the **Nextstrain datasets** see https://nextstrain.org/ncov/gisaid/global/6m

See Online for appendix

For more on **EG.5.1** see https://github.com/sars-cov-2-variants/lineage-proposals/issues/414

For more on the **variants** see https://github.com/cov-lineages/pango-designation