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Letter

Genetic characteristics of H1N1 influenza virus outbreak in China in early 2023

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Dear Editor,

Influenza A virus (IAV) exhibits rapid mutability and antigenic mutations. Since the 20th century, IAV has caused at least four pandemics (Yang et al., 2022). Presently, seasonal H3N2 and H1N1 are the prevailing human-adaptive IAV subvariants responsible for human epidemics. The implementation of public health interventions since the commencement of the COVID-19 pandemic has effectively curtailed global influenza circulation (Olsen et al., 2021). Following the downregulation of the prevention and control strategies against COVID-19, global influenza activity is showing a recovery.

The 2009 pandemic H1N1 virus (A(H1N1)pdm09) possesses a strong capacity for human-to-human transmission and has been circulating worldwide for over a decade since its discovery in North America in April 2009 (Novel Swine-Origin Influenza A Virus Investigation Team, 2009; Naffakh and Van Der Werf, 2009). At the end of 2022, A(H1N1)pdm09 was mainly active in the Americas, Europe, and the Eastern Mediterranean region. In February 2023, it was beginning to circulate in China and the human cases were in explosive growth. However, the genetic characteristics of A(H1N1)pdm09 in the post-COVID-19 period have not been clarified. Here, we investigated the genetic characteristics of A(H1N1)pdm09 viruses from Hunan and Jiangsu provinces in China, aiming to establish a foundation for the prediction and prevention of influenza epidemics.

At the beginning of 2023, A(H1N1)pdm09 became the most prevalent type of influenza virus in the world, and cases increased rapidly in February, peaking at 47.13%. China contributed the vast majority of the A(H1N1)pdm09-related specimens (WHO, 2023a). In the first quarter of 2023, we identified 48 positive specimens with A(H1N1)pdm09 in Hunan (n = 24) and Jiangsu (n = 24) provinces in China. The methods of sample processing and sequencing analysis refer to our previous studies (Bi et al., 2016, 2020). The genomes of these strains have been deposited in China National Microbiology Data Center (NMDC, https://nmdc.cn/) (accession numbers in Supplementary Table S1) and the Global Initiative on Sharing Avian Influenza Data (GISAID, https://gisaid.org/) database (accession numbers: EPI2715310 to EPI2715313 and EPI2715315 to EPI2715692).

The hemagglutinin (*HA*) and neuraminidase (*NA*) genes of these viruses were fully sequenced and analyzed for homology. It was observed that the nucleotide sequence identity values for *HA* and *NA* were within the range of 98.1%–100% and 98.7%–100%, respectively. Similarly, their amino acid sequence identities were 98.1%–100% and 98.5%–100%. A comparative analysis between our strains and the vaccine strains, as recommended by the World Health Organization (WHO), was also performed. Compared to the recommended vaccine strain A/Victoria/2570/2019, which was used for the 2021–2023 influenza season, the *HA* and *NA* nucleotide sequences demonstrated a range of 98.1%–98.8% and 99.0%–99.1% identity, and their amino acid sequence identities were in a range of 97.2%–98.6% and 98.7%–99.6%, respectively. Compared to the A/Victoria/4897/2022 vaccine strain, newly recommended by WHO in February of 2023 and used for the 2023–2024 season, the *HA* and *NA* nucleotide sequences possessed a range of 98.3%–99.7% and 99.1%–99.4% identity, and their amino acid sequences displayed a range of 98.1%–99.3% and 98.7%–99.4% identity, respectively. These results indicate the obvious genetic divergence between the prevalent A(H1N1)pdm09 viruses and the vaccine strains.

To gain a comprehensive understanding of the evolutionary patterns exhibited by A(H1N1)pdm09 strains, a thorough phylogenetic analysis of complete genomes was conducted. In the *HA* phylogeny (Fig. 1A), the vaccine strain A/Victoria/2570/2019 belonged to 6B.1A.5a.2, while

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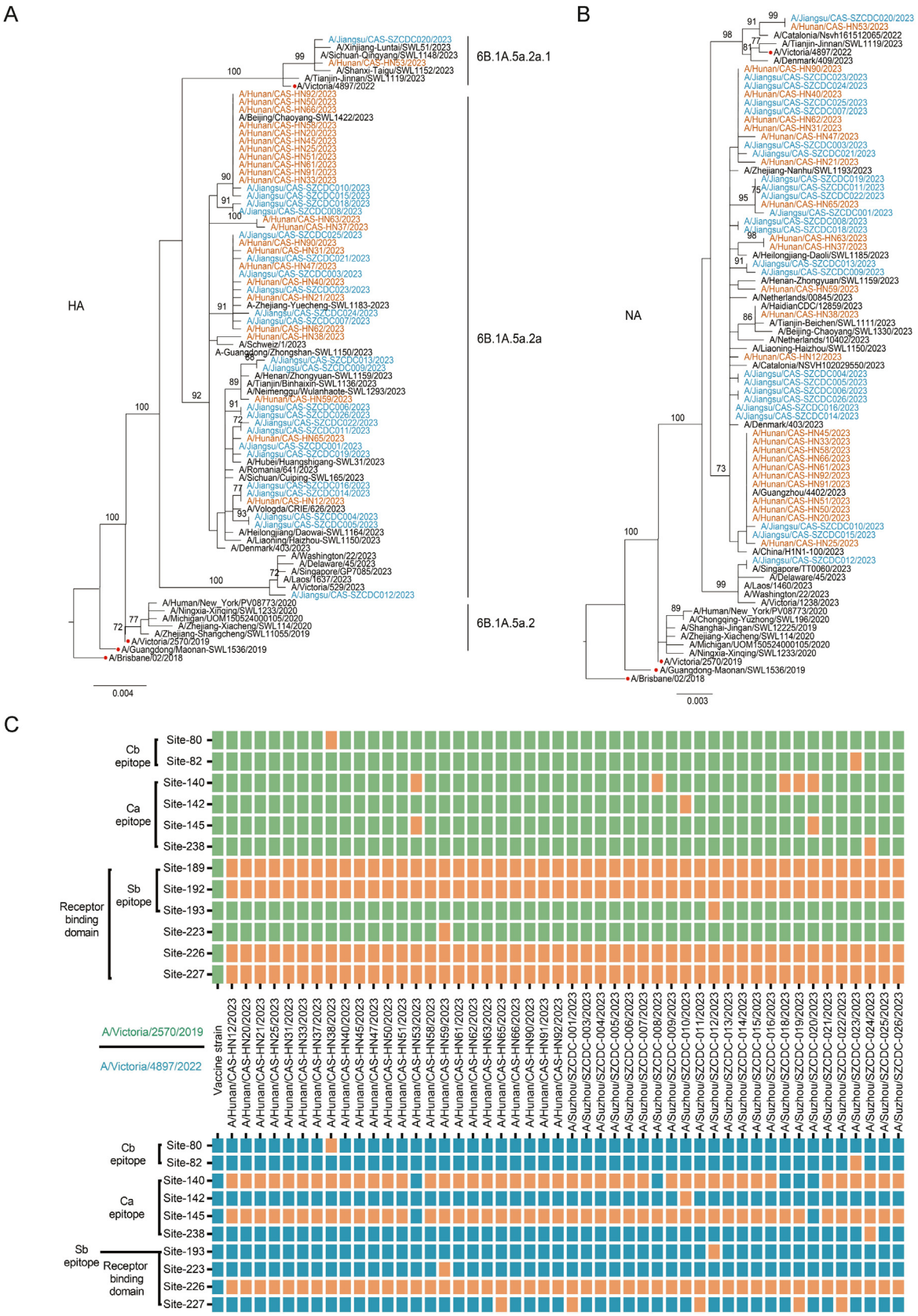

Fig. 1. Phylogenetic analysis and amino acid mutations of *HA* and *NA* genes of the viruses identified in this study. **A** Phylogenetic tree of the *HA* gene. **B** Phylogenetic tree of the *NA* gene. Orange and light blue indicate the strains isolated from Hunan and Jiangsu, respectively. The recommended vaccine strains are marked by red dots. **C** The amino acid substitutions in the *HA* of the identified viruses compared to the recommended vaccine strains. Green indicates that the amino acid residue is consistent with A/Victoria/2570/2019 vaccine strain, blue indicates that the amino acid residue is consistent with A/Victoria/4897/2022 vaccine strains, and orange indicates the different amino acid residue compared to the vaccine strains.

95.8% of our H1N1 strains (n = 46) fell within clade 6B.1A.5a.2a, and 4.2% (n = 2) of strains together with the latest vaccine strain A/Victoria/4897/2022 were in clade 6B.1A.5a.2a.1. These results indicate a gap between the outbreak A(H1N1)pdm09 viruses and the recommended vaccine strains in the two consecutive periods, and suggesting that there may be a mismatch between the epidemic viruses and vaccine strains. The *NA* phylogeny yielded consistent results (Fig. 1B). Next, we used K = r/2t (Kimura, 1980) to estimate the evolutionary rates of *HA* and *NA* substitutions during 2009 to late 2019 and late 2019 to late 2022. A/California/07/2009 and A/Victoria/2570/2019 were used as the reference strains during 2009 to late 2019. The evolutionary rates of *HA* and *NA* substitutions were 2.15×10^{-3} site/year and 1.74×10^{-3} site/year, respectively. A/Victoria/2570/2019 and A/Victoria/4897/2022 were used as the reference strains during late 2019 to late 2022. The evolutionary rates of *HA* and *NA* substitutions were 2.78×10^{-3} site/year and 1.90×10^{-3} site/year, respectively. The evolutionary rate of the A(H1N1)pdm09-like viruses during the COVID-19 pandemic seems to pose a quicker trend than that between 2009 and 2019.

Mutation in the *HA* gene is one of the primary causes of variation in the antigenicity and pathogenicity of the influenza virus, and the mutations on *NA* gene may induce resistance to the antiviral drugs of *NA* inhibitor (NAI) (Whitley et al., 2013; Mohan et al., 2021). So, we further analyzed the substitutions in *HA* and *NA* genes. The H3 and N2 numbering system was followed in this study. Compared with the ancestor strain A/California/07/2009, the antigenic sites on the *HA* of these viruses in this study exhibited 16 substitutions (Supplementary Table S2). Furthermore, as shown in Fig. 1C and Supplementary Table S3, there were nine mutations on *HA* antigenic sites of the circulating viruses compared to the vaccine strain A/Victoria/2570/2019. The *HA*-S80P and R82K substitutions are at the Cb epitope; P140S, A142D, K145R, and E238K are at the Ca epitope; and A189T, Q192E, and S193I are at the Sb epitope. Compared with the newly recommended vaccine strain A/Victoria/4897/2022, the prevalent viruses also possess seven mutations. The *HA*-S80P and R82K substitutions are at the Cb epitope; S140P, A142D, R145K, and E238K are at the Ca epitope; and S193I is at the Sb epitope (Fig. 1C and Supplementary Table S4). The mutation of amino acid sequences at antigenic sites is known as “Antigenic drift” (Wilson et al., 1981; Hay et al., 2001), which is one of the primary mechanisms through which IAVs evolve to evade the immune system (Palese, 2004). These mutations on *HA* antigenic sites, together with the genetic and phylogenetic divergence, indicate a mismatch between these newly identified viruses and the current and next vaccine strains. In addition, it is necessary to exercise vigilance regarding the potential risks associated with mutations occurring in the receptor-binding domain. Five of the 48 strains have *HA*-X227S substitution, which could increase the human receptor binding affinity (Xu et al., 2022) and may further impact the transmissibility of influenza viruses. In comparison with A/California/07/2009, A/Victoria/2570/2019, and A/Victoria/4897/2022, the NAI-resistance mutations in *NA* of these newly identified viruses were not observed in this study.

In the internal genes of the strains in this study, the key substitution affecting virus pathogenicity and transmissibility was not found. The nucleotide sequence identities of internal genes among our viruses exhibited the following ranges: *MP* (98.6%–100%), *NP* (97.5%–100%), *NS* (98.6%–100%), *PA* (98.2%–100%), *PB1* (98.5%–100%), *PB2* (97.9%–100%); and the amino acid sequence identities were *MP* (98.2%–100%), *NP* (98.6%–100%), *NS* (97.8%–100%), *PA* (98.5%–100%), *PB1* (98.9%–100%), *PB2* (98.9%–100%).

The implementation of prevention and control measures in response to the COVID-19 pandemic has also effectively controlled the spread of influenza viruses, resulting in a decline in influenza activity globally (Olsen et al., 2021). Consequently, there has been a decrease in public concern regarding the influenza virus, leading to a decline in both vaccination rates and natural immunity against influenza in the human populations (Razzaghi et al., 2022). Vaccination is widely recognized as the most effective measure for preventing influenza. In February 2023, the WHO updated the recommended vaccine strains for the 2023–2024 northern hemisphere influenza season (WHO, 2023b). However, our study revealed that a significant proportion of the strains prevalent in the regions of China in the first quarter of 2023 were in distinct clades compared to the recommended vaccine strains. Concurrently, our study showed that the evolutionary rates of the A(H1N1)pdm09-like strain during the COVID-19 pandemic exceeded those between 2009 and 2019. This suggests that there may be a recessive transmission of the strain during the pandemic and acceleration of evolutionary rates in response to the environmental pressures imposed by diverse prevention and control measures worldwide and the competition of co-circulation with COVID-19 (Sooryanarain et al., 2015; Yang et al., 2022; Liu et al., 2023). These findings imply that the influenza vaccine products based on the recommended vaccine strain may not provide significant protection against the forthcoming seasonal influenza epidemics in China. The variations in prevention and control strategies for the COVID-19 pandemic across different regions in the world have probably disrupted the prediction and recommendation system for vaccine strains against the global seasonal influenza. The movement of individuals and the diminished immunity of the population have heightened the potential of local outbreaks of seasonal influenza at any given time on Earth. Consequently, prevention and control measures in response to other epidemics may result in irregular influenza outbreaks over a certain period, affecting the immune efficacy of the influenza vaccine products based on conventional vaccine renewal programs. Certainly, more evidence on the antigenic relationships between the epidemic viruses and vaccine strains needs to be further investigated using hemagglutinin inhibition and microneutralization tests.

Overall, the findings of this study suggested the necessity for an expeditious and precise vaccine update program in the aftermath of the COVID-19 pandemic. Certainly, the enhancement of surveillance remains the key to a better understanding of and control of influenza in the future.

Footnotes

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