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Short communication

Comparison of SARS-CoV-2 variant lethality in human angiotensin-converting enzyme 2 transgenic mice

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ABSTRACT

This study compared the lethality of severe acute respiratory syndrome coronavirus 2 variants belonging to the S, V, L, G, GH, and GR clades using K18-human angiotensin-converting enzyme 2 heterozygous mice. To estimate the 50% lethal dose (LD_{50}) of each variant, increasing viral loads (10^0–10^4 plaque-forming units [PFU]) were administered intranasally. Mouse weight and survival were monitored for 14 days. The LD_{50} of the GH and GR clades was significantly lower than that of other clades at 50 PFU. These findings suggest that the GH and GR clades, which are prevalent worldwide, are more virulent than the other clades.

Abbreviations

SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
hACE2	human angiotensin-converting enzyme 2
KDCA	Korea Disease Control and Prevention Agency
PFU	plaque-forming unit
LD50	50% lethal dose

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belongs to the genus Coronavirus, the family Coronaviridae and the order Nidovirales, is first identified in Wuhan at the end of 2019 and has rapidly spread worldwide since then. At the beginning of the pandemic caused by SARS-CoV-2, the Global Initiative for Sharing All Influenza Data (https://www.gisaid.org) announced that SARS-CoV-2 can be subdivided into seven clades, namely S, V, L, G, GH, GR, and GV, according to whole-genome sequencing data. The SARS-CoV-2 genome continuously undergoes mutations, among which the D614G mutation (observed in G, GH, GR, and GV variants) in the spike protein-coding gene rapidly increased in frequency and is now the dominant variant worldwide (Ibarrondo et al., 2020; Mercatelli and Giorgi, 2020). Currently, G groups including GR, GH, and GV have spread across most countries. However, the virulence and transmissibility of the different SARS-CoV-2 variants are unclear. Herein, K18-human angiotensin-converting enzyme 2 (hACE2) transgenic mice were infected with various SARS-CoV-2 variants to compare their pathogenicity.

SARS-CoV-2 variants were obtained from the National Culture Collection for Pathogens of the Korea Disease Control and Prevention Agency (KDCA). Their genetic information was collected from the GenBank database: MW466791.1 (S clade: A), MW466795.1 (L clade: B), MW466797.1 (V clade: B), MW466798.1 (GR clade: B.1.1), MW466799.1 (G clade: B.1), and MW466800.1 (GH clade: B.1.497). Each virus was propagated in Vero-E6 cells, and the aliquots were stored at −80°C until use. The viruses used in this experiment were collected at the third passage.

The growth properties and plaque morphologies of SARS-CoV-2 variants in Vero-E6 cells were investigated. No significant difference was observed between the growth kinetics of the six clades (Fig. 1A). The G clade formed larger plaques than the other clades in Vero-E6 cells (Fig. 1B). As the loss of furin-cleavage site (PRRAR) of spike protein has been reported in Vero-E6 cultured SARS-CoV-2, which results in attenuated viral pathogenesis (Johnson et al., 2021; Lau et al., 2020), the spike gene sequences of the viruses were analyzed, and there were no deletion of furin-cleavage site on the spike protein of each virus (Fig. 1C).

All animal experiments were approved by the Institutional Animal Care and Use Committee of KDCA (Approval No. KCDC-088-20-2A) and performed under the Guidelines for the Care and Use of Laboratory Animals of Korea National Institute of Health. Six-week-old male transgenic K18-hACE2 mice were purchased from The Jackson

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Laboratory (Bar Harbor, ME, USA). For intranasal infection, K18-hACE2 mice were anesthetized with tiletamine/zolazepam and xylazine combination injected intraperitoneally. Subsequently, each virus was administered intranasally in decreasing 10-fold concentrations (from 10^4 to 10^0 plaque-forming units [PFU] in a volume of 30 μL) to groups of four or five mice, and a back titration was performed to check the virus titers. The infected mice were monitored daily for 14 days for weight loss and mortality. When the body weight decreased to 75% of the initial body weight, the mice were anesthetized and humanely euthanized. The 50% lethal dose (LD_{50}) was determined by assessing the number of dead and live mice on day 14, as previously described (Ramakrishnan, 2016).

Mice infected with L, G, V, and S clades at 10^4 PFU started losing weight four days after inoculation until they exhibited a reduction of 25% of body weight or died (Fig. 2A). However, the weight reduction in GR and GH groups at the same PFU was observed one day earlier than in the other clade groups. All the mice infected with 10^4 PFU of SARS-CoV-2 died within eight or nine days (Fig. 2B). The titers of LD_{50} were calculated as 501, 316, 200, 125, 50, and 50 PFU for the L, G, V, S, GR, and GH clades, respectively (Table 1). In conclusion, the GR and GH clades were at least 2–10 times more lethal than the other clades.

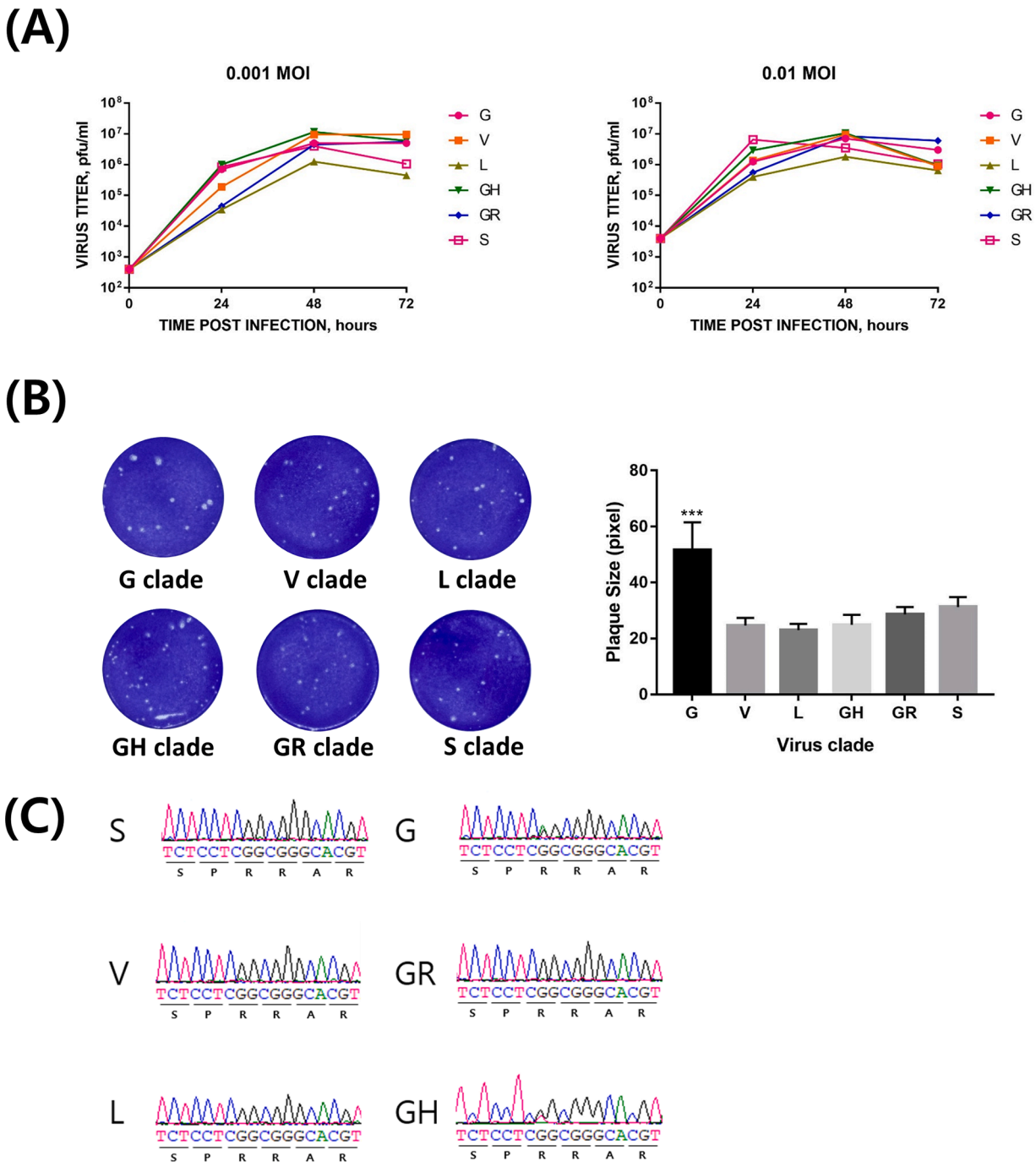

Fig. 1. Growth kinetics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants in Vero-E6 cells. **(A)** Confluent monolayers of Vero-E6 cells infected with the virus at a multiplicity of infection of 0.01−0.001 PFU/cells. Cell supernatants were collected at 24, 48, and 72 h, and virus titers were determined using plaque-forming units (PFU). **(B)** Representative photographs of viral plaques and their corresponding sizes. Plaque sizes were measured in pixels using ImageJ (NIH). Data are mean ± SEM for 3 replicates. (*** $p < 0.001$ analyzed by one-way analysis of variance-Dunnett's multiple comparison test) **(C)** Representative nucleotide and amino acid sequence of the furin cleavage sites of SARS-CoV-2 variants.

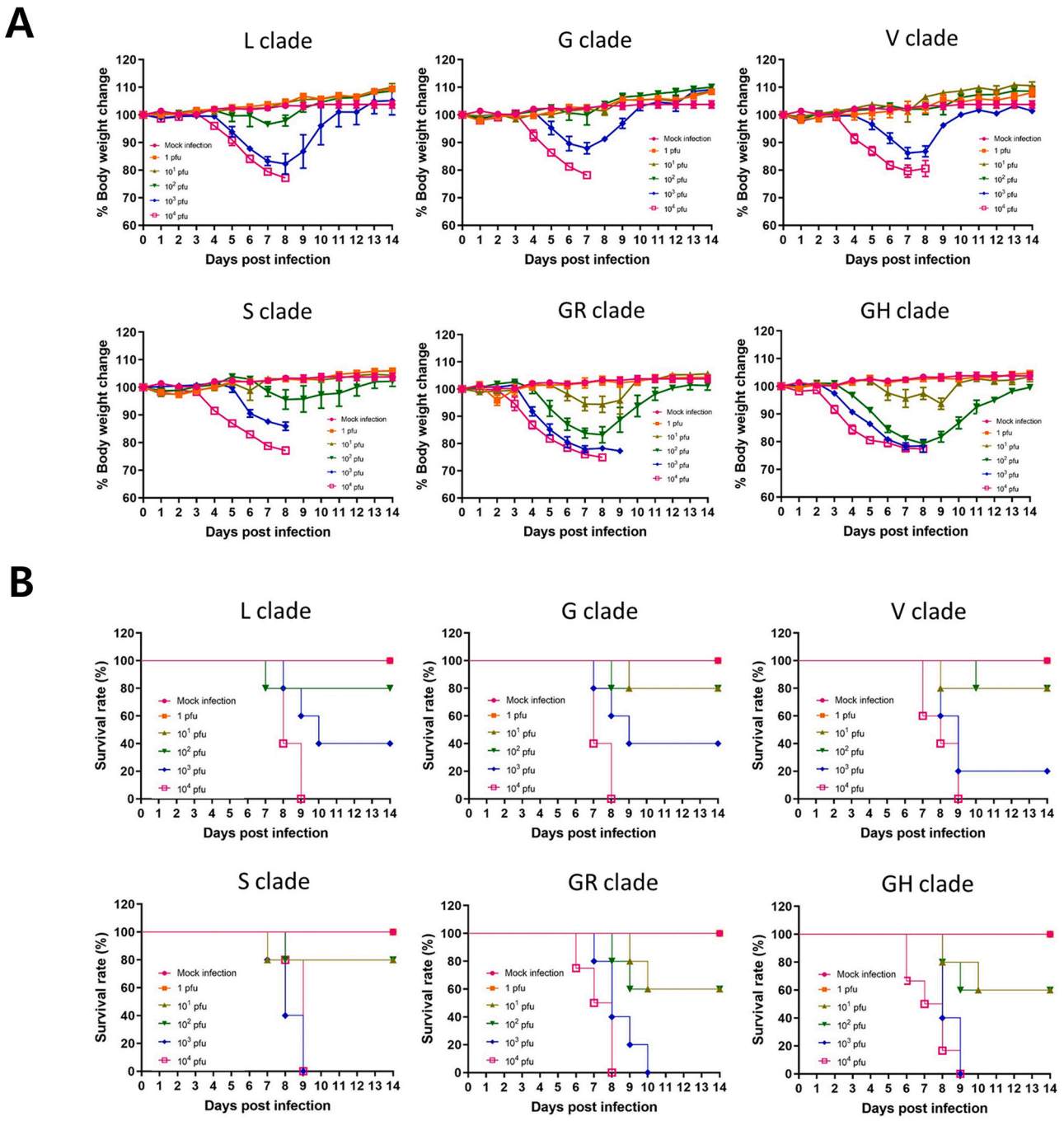

Fig. 2. Body weight change and survival in K18-human angiotensin-converting enzyme 2 (hACE2) transgenic mice. Mice ($n = 4$ or 5) were infected intranasally with 10-fold serial dilutions of different severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) clades. Body weight **(A)** and survival **(B)** were monitored daily for 14 days.

Previous studies exploring SARS-CoV-2 infection in transgenic K-18-hACE2 mice only used high or low doses of the D614 type virus, and thus, the exact LD_{50} was not measured (Yinda et al., 2021; Golden et al., 2020). Herein, the viral replication fitness and lethality of six SARS-CoV-2 clades were evaluated in Vero-E6 cells and transgenic K-18-hACE2 male mice, respectively. No differences in virus growth were observed in Vero-E6 cells. Plaque morphology of G clade was larger than other five clades. This finding is consistent with recent findings suggesting that compared with the original D614 virus, the SARS-CoV-2 spike protein substitution D614G variant enhances viral replication and infectivity in the human lung epithelial cell line Calu-3 or in primary human upper airway tissues but not in Vero-E6 cells (Plante et al., 2020). However, the D614G variant did not result in more severe pathogenicity in hamsters and patients (Lorenzo-Redondo et al., 2020). Hence, further studies using infectious cDNA clones with specific amino acid changes are required to evaluate whether the GR (N-G204R substitution) or GH clade (NS3-Q57H substitution) may result in increased infectiousness and transmissibility, as well as to determine the virus variant-associated disease severity using *in vivo* models (Plante et al., 2020).

This is the first study that compares the pathogenicity of the different SARS-CoV-2 clades identified in the ongoing pandemic. However, the use of only male heterozygous hACE2 mice was a limiting factor, as there is a possibility of differences in SARS-CoV-2 pathogenicity and

Table 1
SARS-CoV-2 variants lethal dose in K18-hACE2 transgenic mice.

Virus clades	Challenge dose (PFU/mouse)	No. of deaths/No. of challenged mice(%)	LD_{50}(PFU)[a]
L	10^4 10^3 10^2 10^1 10^0	5/5 (100) 3/5 (60) 1/5 (20) 0/5 (0) 0/5 (0)	501
G	10^4 10^3 10^2 10^1 10^0	5/5 (100) 3/5 (60) 1/5 (20) 1/5 (20) 0/4 (0)	316
V	10^4 10^3 10^2 10^1 10^0	5/5 (100) 4/5 (80) 1/5 (20) 1/5 (20) 0/4 (0)	200
S	10^4 10^3 10^2 10^1 10^0	4/4 (100) 5/5 (100) 1/5 (20) 1/5 (20) 0/4 (0)	125
GR	10^4 10^3 10^2 10^1 10^0	4/4 (100) 5/5 (100) 2/5 (40) 2/5 (40) 0/4 (0)	50
GH	10^4 10^3 10^2 10^1 10^0	4/4 (100) 5/5 (100) 2/5 (40) 2/5 (40) 0/4 (0)	50

[a] LD_{50} values were calculated from the data shown in Fig. 2 and using the method described in Ramakrishnan (2016).

lethality with sex. Therefore, future animal studies should include both male and female mice. Nevertheless, the current findings will be valuable for basic research on the pathogenicity of emerging SARS-CoV-2 variants, including the mink variant, B.1.1.7, and B.1.351, and will be useful in preclinical studies for the efficacy evaluation of SARS-CoV-2 vaccine candidates and therapeutic agents.

CRediT authorship contribution statement

Tae-Young Lee: Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Hansaem Lee:** . **Nayoung Kim:** . **Pyeonghwa Jeon:** . **Jun-Won Kim:** . **Hee-Young Lim:** Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Jeong-Sun Yang:** Visualization, Writing – review & editing. **Kyung-Chang Kim:** . **Joo-Yeon Lee:** Visualization, Writing – review & editing, Conceptualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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