Genetic and Pathogenic-Diversity of Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) in South Korea

Seok-Min Yun, …, Joo-Yeon Lee, Young Ki Choi

*JCI Insight.* 2019. [https://doi.org/10.1172/jci.insight.129531](https://doi.org/10.1172/jci.insight.129531).

**Research In-Press Preview** Infectious disease Virology

**Graphical abstract**

Find the latest version:

https://jci.me/129531/pdf
Genetic and Pathogenic Diversity of Severe Fever with Thrombocytopenia Syndrome virus (SFTSV) in South Korea

Seok-Min Yun1,2,†, Su-Jin Park1,3,†, Young-II Kim1,3,†, Sun-Whan Park4, Min-Ah Yu1,3, Hyeok-II Kwon1,3, Eun-Ha Kim1,3, Kwang-Min Yu1,3, Hye Won Jeong1,5, Jungsang Ryou2, Won-Ja Lee2, Youngmee Jee6, Joo-Yeon Lee2,* and Young Ki Choi1,3,*

1Department of Microbiology, College of Medicine and Medical Research Institute, Chungbuk National University, Cheongju, Chungcheongbuk-do, Republic of Korea
2Division of Emerging Infectious Disease and Vector Research, Center for Infectious Diseases Research, National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongju, Chungcheongbuk-do, Republic of Korea
3Zoonotic Infectious Diseases Research Center, Chungbuk National University, Cheongju, Chungcheongbuk-do, Republic of Korea
4Jeju National Quarantine Station, Centers for Disease Control and Prevention, Jeju, Jeju-do Republic of Korea
5Department of Internal Medicine, Chungbuk National University College of Medicine, Cheongju, Chungcheongbuk-do, Republic of Korea
6Center for Infectious Diseases Research, National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongju, Chungcheongbuk-do, Republic of Korea

Running title: Genotype-dependent pathogenicity of SFTSV

†These authors contributed equally to this article.
*These authors were co-principal investigators.

Address for correspondence:
Conflict of interest statement:

The authors have no potential conflicts to disclose.
Abstract

To investigate the nationwide severe fever with thrombocytopenia syndrome virus (SFTSV) infection status, we isolated SFTSVs from severe fever with thrombocytopenia syndrome (SFTS)-suspected patients in 207 hospitals throughout South Korea between 2013 and April of 2017. A total of 116 SFTSVs were isolated from 3,137 SFTS-suspected patients with an overall 21.6% case fatality rate. Genetic characterization revealed that at least six genotypes of SFTSVs are co-circulating in South Korea with multiple reassortments among them. Of these, the genotype B-2 strains were the most prevalent (n=48, 36.1%) followed by the A and F genotypes. Clinical and epidemiologic investigations revealed that genotype B strains were associated with the highest case-fatality rate (34.8%, 32/92), while genotype A caused only one fatality out of ten patients. Further, ferret infection studies demonstrated varied clinical manifestations and case mortality rates of different strains of SFTSV, which suggests this virus could exhibit genotype-dependent pathogenicity.

Keywords: severe fever with thrombocytopenia syndrome virus (SFTSV), clinical manifestations, genotypes, pathogenesis
Introduction

Severe fever with thrombocytopenia syndrome (SFTS), an emerging tick-borne infectious disease with a high fatality rate and symptoms including severe fever and thrombocytopenia, was first reported in China in 2011 (1, 2). The causative agent, the severe fever with thrombocytopenia syndrome virus (SFTSV), was subsequently identified in South Korea and Japan in 2013 following lethal infections in humans (3, 4). SFTSV (now renamed Huaiyangshan banyangvirus) belongs to the genus Banyangvirus in the family Phenuiviridae (5, 6) along with other novel tick-borne Banyangviruses including Heartland virus and Guertu virus (5, 7, 8). The genome of SFTSV has negative-stranded RNA segments including the L segment, which encodes the RNA-dependent RNA polymerase (RdRp), M segment, which encodes the surface glycoproteins (Gn and Gc), and the S segment harbors a nucleoprotein (NP), and a non-structural S segment (NS) protein, which is encoded via an ambisense strategy (2, 9). Since the first report of SFTSV in humans in 2011 (2), the number of human cases has rapidly increased each year in China, South Korea, and Japan (10-12). Although the average case-fatality rate varies between regions and years (13), the mean mortality rate of SFTS cases has remained relatively high in Japan (27%), South Korea (23.3%), and China (5.3 to 16.2%) (10-12). Further, SFTSV infection has clinical features including high fever, thrombocytopenia, and leukocytopenia and can cause central nervous system manifestations including headache, confusion, and seizure (14-16). The reason for the difference in mortality rates between countries and the mechanisms underlying the varied clinical manifestations caused by this infection are largely unknown; however, underlying disease conditions are suspected (17, 18). Recently, several studies reported the identification of at least six different genotypes of SFTSV in East Asian countries and that the prevalence of each genotype varied by country (19-21).

To understand the differing clinical manifestations of SFTSV infections, several studies were analyzed to compare host factors such as age, cytokine profile in sera, proportions of peripheral blood mononuclear cells (PBMCs), and viral titers in the central
nervous system (22-25). However, whether the pathogenic potential of different SFTSV strains vary remains unclear. To this end, we characterized a total of 116 Korean SFTSVs isolates and investigated the epidemiological and clinical records of patients including age and clinical outcomes. Further, we evaluated pathogenic characteristics of each SFTSV genotype using an animal model.
**Results**

**Characteristics of SFTS in human patients**

A total of 3,137 serum samples were collected from hospitalized SFTS-suspected patients between 2013 and April 2017. These patients experienced symptoms of SFTS, such as high fever (≥ 38°C), vomiting, diarrhea, fatigue, thrombocytopenia, leukocytopenia, and spots resulting from tick bites, and were treated at 207 hospitals throughout South Korea. Of these, 342 specimens were found to be positive for SFTSV by real-time RT-PCR, and there were 74 (21.6%) confirmed fatalities (Figure 1A). Of the 342 SFTSV-positive cases, a total of 116 human-derived SFTSVs were isolated in Vero E6 cells. Of these, 38 (32.8%) viruses were obtained from fatal cases and 26 were isolated from specimens taken from farmers (26/116, 22.4%). The detailed regional distribution of SFTS cases analyzed in this study is summarized in Supplemental Figure 1.

The most frequent symptoms of SFTS were high fever (≥ 38°C), gastrointestinal symptoms (vomiting, diarrhea, and nausea), and thrombocytopenia and/or leukocytopenia (Supplemental Table 1). Patient age ranged from 19 to 89 years with a mean of 66.6, and the majority of virus-isolated cases were patients aged ≥ 50 years (Figure 1B and C). Moreover, those ≥70 years (46/74, 62.2%) showed the highest mortality rate (p < 0.05), followed by patients 60-69 years (22/74, 29.7%) and 50-59 years (6/74, 8.1%), suggesting age is an important factor for SFTSV-induced fatality (Figure 1C). There were no gender differences in infection or case-fatality rates (Figure 1D).

**Genetic and phylogenetic analyses**

To investigate the genetic characteristics of SFTSVs, full genomic sequences of all 116 Korean SFTSVs were analyzed using the NGS deep sequencing method. These sequences, as well as all known SFTSV full-length sequences available in GenBank, were subjected to phylogenetic analysis (Figure 2 and Supplemental Figure 2). The results show that Korean SFTSVs can be roughly classified into A-F genotypes based on the previous
classification method used in China and Korea (19, 26). The majority of Korean SFTSV isolates (n=92/133, 69.2%) are classified as the B genotype. However, it is noteworthy that repeated analysis of genotype B strains resulted in their subdivision into three different genotypes, B-1, B-2 and B-3, with strong bootstrap values (>70) (Figure 2). In addition, the L and M segments of the 16MS373 strain, as well as the L segments of the 16KS77 and KADGH4 strains, do not cluster with known A to F genotype groups although their internal M and/or S segments cluster with genotype B-1, B-2, or B-3 (Figure 2). Further, the L segment of the ZJ2013-06 Chinese isolate and three strains of Japanese origin (SPL057A, SPL097A, and SPL100A) also align with unclassified clades (Figure 2A).

Genotypes of Korean SFTSVs

Based on the phylogenetic analyses, the Korean SFTSVs were differentiated into six genotypes, which belonged to one of pure A to F genotype groups and their reassortant groups (Figure 3). In the A to F pure genotype groups, the most prevalent genotype was B-2 (n=48, 36.1%) followed by B-3 (n=28, 21.1%) and B-1 (n=16, 12.0%) (Figure 3A). Although the first Korean SFTSV strain identified was genotype F in 2012, its prevalence was relatively lower than B (B-1, B-2, and B-3) and A genotypes (Figure 4A). Further, while genotype D viruses (n=5, 3.8%) were detected in 2013 and 2014, in recent years this genotype has rarely been detected in Korea. In contrast, genotype C and E SFTSV strains were not detected during this study (Figure 3A and 4A). The most prevalent genotype in Japan was B-2 and the overall genotype distribution was similar to that seen in South Korea, although genotypes A and F were not reported (Supplemental Table 2). In contrast, the Chinese SFTSV strains were diverse with 14 genotypes reported, the most prevalent being genotype F.

In reassortant groups, R-3 (n=6), which results from recombination between the B-1 and B-3 genotypes, was the most common followed by R-2, which results from recombination between B-1 and B-2 genotypes (n=4) (Figure 3B). Further, even in a single
strain detected various reassortants were present, such as R-1 (B-1 and B-2), R-4 (B-3 and F), R-5 (C and D), and R-6 (B-1 and D) (Figure 3B). The R-7 virus has an unclassified L gene and M and S genes from B-2 and B-1 genotypes, respectively. In addition, the R-8 virus also has the unclassified L gene, while the M and S segments of this strain originated from B-3 and B-1 genotypes, respectively. Further, the R-9 virus also contains unclassified L and M segments, while the S segment is originated from of genotype B-1 (Figure 3B). These results suggest that Korean SFTSV strains are actively undergoing evolution through dynamic reassortments resulting in the creation of various novel genotypes.

**Association between the case fatality rate and SFTSV genotype**

To investigate whether different genetic phenotypes induce different rates of case-fatalities, we first analyzed the human mortality rate across genotypes (Figure 4). Most genotypes of SFTSV were detected annually starting in 2013, but the reassortants (R-1, R-2, R-5, R-8, and R-9) were first detected in 2016 with low incidence (Figure 4A). Although most genotypes of SFTSVs were associated with fatalities, the B-2 genotype showed the highest incidence (48 of 133 cases) and a significantly higher mortality rate (21 of 48 patients, 43.8%) than the other genotypes ($p < 0.05$) (Figure 4B). The F genotype also showed a high mortality rate (4 of 9 cases, 44.4%), although the incidence rate was relatively lower than that of B-3 (8 of 28 cases) and B-1 (3 of 16 cases) genotypes. In addition, the A genotype showed the lowest mortality rate (1 of 10 cases) compared with the other genotypes (Figure 4B). Of the reassortant genotypes, R-3 showed the strongest correlation with high mortality (4 of 6 cases, 67% mortality rate) followed by the R-2 genotype (2 of 4 cases, 50% mortality).

Although only single cases of each of the other novel R genotypes were detected in this study, it should be noted that genotype R-6, R-7, and R-9 viruses were isolated from fatal cases of SFTSV infection. Further, there was a clear association between the case mortality rate and age of infected patients as revealed by analysis of clinical records of confirmed
SFTSV cases (Figure 4C). Most fatalities were in patients older than 60 (42/47 cases, 89.4%), although 5 fatalities (10.6%) occurred in patients aged 50 to 59 ($p < 0.05$).

**Genotype-specific pathogenicity of SFTSVs**

To confirm that different genotypes of SFTSVs exhibit different clinical manifestations, each randomly selected SFTSV strain (A[CB2], B-1[CB3], B-2[CB7], B-3[CB6], D[CB8], F[16KS89], R-1[16MS299], R-2[16MS310], R-3[CB1], R-4[KACNH2], R-5[16MS322], R-6[16KS55], R-7[KADGH4], R-8[16KS77], R-9[16MS373]) was inoculated into young adult and aged ferrets, a proven SFTSV infection model (27-29), and clinical symptoms, hematology, and mortality were monitored for 14 days. To optimize the infection dose in ferrets, we analyzed the growth property of each selected virus in Vero E6 cells (Supplemental Table 3). The B-1 virus showed the most efficient replication compared with the other viruses ($10^{7.6}$ FFU/ml) and the B-2, B-3, R1, R2, R3, R4 and R6 exhibited peak titers of approximately $10^{6.6-7.3}$ FFU/ml. The genotype A, D, F, R5, R7, R8, and R9 strains demonstrated attenuated viral titers in cells ($10^{5.3-5.9}$ FFU/ml) compared with the other genotypes (Supplementary Table 3). However, all viruses tested in this study grew up to at least $10^{5.0}$ FFU/ml at peak titers, hence we determined the $10^{5.0}$ FFU/ml dose to be the optimal ferret infection dose.

In this study, none of the young adult ferrets infected with any of the genotypes succumbed or experienced weight loss (Supplemental Figure 3 and 4). Further, most young adult ferrets showed only mildly elevated body temperatures from 4 to 6 dpi and short periods of viremia (within 10 dpi) compared with aged ferrets (Figure 5 and 6, and Supplemental Figure 3, 4 and 5). In contrast, in aged ferrets, pure genotypes B (B-1, B-2, and B-3) and D caused 100% mortality within 12 dpi with high fever, significant body weight loss (more than 20%), and high virus RNA copy numbers in collected blood samples (higher than $4 \log_{10}$/ml at 8 to 10 dpi) (Figure 5B-C, Figure 6A-B and Supplemental Figure 3B-E). Genotype A and F-infected aged ferrets showed 40% (2/5) and 60% (3/5) mortality,
respectively (Figure 5A and Figure 6C). Further, viral RNAs were detected until 12 dpi in surviving ferrets infected with genotype A and F virus, although their body weight and temperature recovered to the normal range (Supplemental Figure 3A and F). In reassortant genotype groups, R-2, R-3 and R-5 caused 100% mortality with high fever and body weight loss (more than 20%) (Supplementary Figure 4B, C and E, and Supplementary Figure 5B, C and E). Further, these groups showed much higher peak viral RNA copy numbers (>4.0 log10/ml) in sera compared with the other reassortant groups (<4.0 log10/ml) (Supplementary Figure 5A-I). In aged ferrets the R-1, R-4, R-6, and R-7 viruses caused 80% (4/5), 60% (3/5), 40% (2/5), and 40% (2/5) mortality (Supplementary Figure 5A, D, F and G), while R-8 and R-9 caused only 20% (1/5) mortality (Supplementary Figure 5H and I) with mildly elevated body temperatures and viral RNA copy numbers (below 4.0 log10/ml) (Supplemental Figure 4H and I, and Supplementary Figure 5H and I).

Hematological analysis revealed that most pure- and reassortant genotype-infected young adult ferrets maintained normal platelet numbers (more than 300 x 10^3/µl), although a single B-1 and a single D genotype-infected ferret showed decreased platelet numbers at 4 and 8 dpi, respectively (Figure 5B and Figure 6B). Interestingly, all fatal cases exhibited persistent severe thrombocytopenia (more than 4 days below the 100 x 10^3/µl), while surviving animals recovered their platelet numbers within 12 days even in aged ferrets (Figure 5 and 6, and Supplemental Figure 5). In young adult ferrets, white blood cell (WBC) counts slightly decreased at 4 to 6 dpi compared with the initial day of infection, but remained in the normal range (2.5–16.7 x 10^6/µl). Further the ALT and AST levels were slightly increased until 8 dpi, but returned to the normal range (ALT normal range: 49–242.8 UI⁻¹ or AST normal range: 40.1–142.7 UI⁻¹) after 10 dpi. (Supplemental Figure 6 and 7). However, in aged ferrets, regardless of the genotype, most SFTSV-infected ferrets exhibited rapid thrombocytopenia, decreased WBC counts, and increased AST/ALT levels (from 4 to 10 dpi), although surviving ferrets had recovered to the normal range of each parameter at 14 dpi. (Supplemental Figure 6 and 7). Taken together, these results demonstrate that both
the age of the infected animal and the SFTSV genotype are associated with varied clinical outputs, suggesting the genotype-specific pathogenic potential of this virus.
Due to the increasing incidence of SFTSV human infections there is an elevated level of public concern. Therefore, in this study we investigated the epidemiological and genetic diversity of SFTSV strains in South Korea. In addition, we evaluated the pathogenic characteristics of each genotype of SFTSV using an experimental, age-dependent ferret model.

During the study period, a total of 3,137 suspected SFTSV specimens were collected from 207 hospitals throughout South Korea, and 342 cases, mainly elderly, were confirmed as positive for SFTSV infection. Epidemiological investigation of SFTS cases in which virus was isolated showed that 38 (32.8%) cases were fatal and of these a majority of the patients were ≥ 50 years of age. The high prevalence and mortality rate in the elderly is in agreement with corresponding data from other studies (30). The phylogenetic tree of 335 SFTSV strains, including 116 strains identified in this study, revealed that most Korean SFTSVs can be classified into the six previously identified genotypes (A-F) (19, 26), and the B genotype can be subdivided into at least three different genotypes (B-1, B-2, and B-3). Further, the results of the inconsistent phylogenetic clustering of each segment demonstrate that at least 6 genotypes were co-circulating in South Korea with 9 reassortments among them. The segmented nature of the Banyangvirus lends itself to genetic reassortment, which is an important molecular mechanism contributing to the genetic diversity necessary for viral evolution (31). Although there have been a few reports of genetic reassortment of SFTSV in China and Japan (21, 31, 32), this is the first evidence of large and complex reassortment events, as at least nine different reassortant genotypes were present in South Korea (Figure 3B). This suggests that SFTSV strains undergo active evolution in nature through reassortment resulting in the creation of novel genotypes.

Genetic and phylogenetic analyses revealed that the B-2 (n=48, 36.1%) genotype was the most common in South Korea followed by B-3 (n=28, 21.1%) and B-1 (n=16, 12.0%) (Figure 3A), while the F (n=9) and D (n=5) genotypes were infrequently found. The highest
mortality and incidence rates were observed in patients infected with genotype B-2 (43.8%, 21 of 48 cases). In contrast, out of ten patients with genotype A only a single case was fatal (10%). The reported average case-fatality rate caused by SFTSV infections varies greatly in East Asia, with China at 5.3%-16.2%, Japan at 20%, and South Korea at 23.3% (11, 33, 34). Therefore, comparison of the virus genotype and mortality rates suggests that the differences in reported case-mortality rates might be associated with the differential distribution of SFTSV genotypes across countries. Interestingly, genetic analysis of reported full-length sequences revealed that the most prevalent genotype in Japan was B-2, which exhibited a high case mortality rate, while genotypes A and F were not reported. In contrast, the most prevalent genotype in China was genotype F (44.3%) followed by genotype A (21.5%), both of which exhibited relatively low case mortality rates in Korea (35). These results suggest that, in addition to the age of the patients, prevalence of different genotypes in each country might also be a factor in the varied mortality rates between countries.

Previous studies demonstrated that aged ferrets mimic clinical signs (fever, hematological changes) of human SFTSV infection (27-29). Further, severe illness was exhibited only in aged ferrets, which is similar to that seen in humans over 60 years old. Thus, these results demonstrate that aged ferrets are a suitable SFTSV animal model. Therefore, we utilized aged ferrets, which reflect the clinical symptoms of SFTSV infection, to investigate genotype-dependent differences in the pathogenesis of SFTSV infection. Aged ferrets infected with genotypes B (B-1, B-2, and B-3), D, R-2, R-3 and R-5 showed 100% mortality, while genotypes A, F, R-1, R-4, R-6 and R-7 induced attenuated virulence with attenuated viral titers and delayed death, with 40% to 60% mortality, respectively (Figure 5, Figure 6 and Supplemental Figure 5). Further, only one of five aged ferrets infected with genotypes R-8 and R-9 succumbed to infection and these groups of ferrets exhibited only moderate body weight loss (Supplemental Figure 4H and I). These results are similar to what is reported for genotypic variants of Rift Valley fever viruses, which exhibited different levels of virulence in a mouse model (36). However, the low number of clinical cases of
infection with certain SFTSV genotypes, especially of the reassortant genotypes, impedes
the determination of the association between genotype and case mortality. Further, recently,
Song et al demonstrate that fatality induced by SFTSV infection is associated with the
absence of specific IgGs to viral nucleocapsid and glycoprotein due to a failure in B-cell
class switching (37). Similarly, in our preliminary ELISA study with ferret sera, we found that
SFTSV-infected young healthy ferrets exhibited a strong IgG response, while aged ferrets
showed very limited IgG responses against the nucleoprotein of the B-1/2014 SFTSV strain
([Su-Jin Park] unpublished observations). However, in contrast to human cases, most
fatalities occurred in 10 to 12 days after infection of ferrets, which is a relatively short time to
induce full antibody responses against antigens. Therefore, it is hard to determine if there is
an association between the absence of virus-specific B cell immunity and mortality in this
study. In this regard, further continuous surveillance of SFTS patients with genomic- and
pathogenic analyses of detailed clinical manifestations are needed to better understand the
association between fatality and specific genotypes.

Overall, the evidence from this study greatly increases our understanding of the
genetic and pathogenic diversity of SFTSVs in East Asian countries. Virological and clinical
manifestation analyses revealed that there are close associations among the clinical
manifestations (case-fatality), age of patients, and genotypes of SFTSVs. In addition,
continuous and efficient surveillance remains vital and beneficial for the prevention of a
severe outbreak of SFTSV.

Methods

Specimen collection of SFTS suspected patients and clinical evaluation

A total of 3,137 specimens (sera or cerebrospinal fluid) were collected from SFTS-suspected
patients who experienced symptoms of SFTS, such as high fever (≥ 38°C), vomiting,
diarrhea, fatigue, thrombocytopenia, and leukocytopenia in 207 hospitals throughout South
Korea between 2013 and April 2017. SFTSV infection was confirmed in collected specimens by one step reverse-transcription (RT-PCR) using an *M* segment-based SFTSV-specific primer set [MF3 (5′-GATGAGATGGTCCATGCTGATTCT-3′) and MR2 (5′-CTCATGGGGTGGAATGTCCTCAC-3′)] (38). To compare demographic characteristics of SFTS cases, information regarding age, gender, occupation, residential address, and presence or absence of death were also collected by an epidemiologic investigation of SFTS patients.

**SFTSV isolation from patient specimens**

For virus isolation, Vero E6 cells (derived from an African green monkey kidney epithelial cell (CRL-1586; ATCC, Manassas, VA, USA)) were inoculated with the patient specimens, and cultured in Dulbecco’s Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) containing 2% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) with penicillin and streptomycin (P/S, Gibco, Grand Island, NY, USA) placed in 37°C incubator supplemented with 5% CO₂. After 14 days of incubation, isolated viruses were identified with RT-PCR. To make the stock viruses but minimize the unnecessary mutation of SFTSV during the virus isolation, one or two additional cell cultures were conducted and stored at -80°C until further uses.

**Genetic characterization and phylogenetic analysis**

For whole genome sequencing, PCR fragments covering whole SFTSV genome were amplified by RT-PCR. Whole SFTSV genome cDNA libraries for use in deep sequencing using the next-generation sequencing (NGS) method were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) with an average insert size of 300 bp. The sequencing run was performed on a Miniseq system (Illumina, San Diego, CA, USA) using a MiniSeq mid-output kit resulting in 2 × 150 nucleotides paired-end reads (39). Primer set information for each segment will be provided upon request. The
nucleotide sequences obtained from this study were assembled using the QIAGEN Bioinformatics CLC workbench program (version 10.1.1; CLC bio, Aarhus, Denmark).

Genetic and phylogenetic analyses were conducted by aligning published full-length sequences of SFTSV obtained from China, Japan, and South Korea (GenBank). A total of 335 full-length sequences of the L, M, and S segments (158 from China, 43 from Japan, and 134 from South Korea including this study) were included in the analyses (Supplemental Table 4). Multiple sequence alignments were performed using the Clustal W algorithm in MEGA version 6.0 (40). Phylogenetic analyses were performed based on the complete open reading frame (ORF) sequences of L, M, and S segments of SFTSVs using the Maximum Likelihood (ML) method based on the Kimura 2-parameter model. The reliability of the ML tree was evaluated by the bootstrap test with 1,000 replications.

**In vitro growth properties of each virus genotype in cells**

Vero E6 cells were cultured in six-well plates and then infected with each virus genotype at a multiplicity of infection (MOI) of 0.001 for 1 hour, washed and incubated at 37°C in a 5% CO₂ atmosphere. Cell culture supernatants were collected at 1 to 6 dpi and virus titers were determined by FFU/ml.

**Ferret infection studies with different SFTSV genotypes**

Of the isolated SFTSV strains, a total of fifteen different genotypes of SFTSVs (based on the number of prevalence) were selected and inoculated into each group of young adult (<2 years old, *Mustela putorius furo*) or aged female and male ferrets (>4 years old, *Mustela putorius furo*) (n=5) (ID Bio, Cheongju, Korea), respectively, with 10⁵.0 FFU/ml strain as previously described (27). For the analysis of hematological parameters and viral load, blood was collected every other day for 14 days in EDTA tubes and analyzed for hematological parameters using a Celltac hematology analyzer (MEK-6550J/K, Nihon
Kohden, Tokyo, Japan). For virus titration in sera from infected animals, the number of viral RNA copies was measured as previously described (27).

**Study approval**

Viruses were isolated from serum samples collected from the Chungbuk National University Hospital, in accordance with the approved procedures by the Institutional Review Board of Chungbuk National University Hospital (IRB #. 2017-05-002-001). All animal experiments were approved by the Medical Research Institute, a member of Laboratory Animal Research Center of Chungbuk National University (LARC) (approval number: CBNUA-1804-18-01), and were conducted in strict accordance and adherence to relevant policies as mandated under the Guidelines for Animal Use and Care of the Korea Centers for Disease Control (K-CDC) in an enhanced biosafety level 3 (BSL3) containment laboratory.

**Statistics**

Asterisks indicate statistically significant differences in mortality rates between each genotype infected patients, and weight loss, temperature change, blood analysis, viral load and titer between groups and across time points as determined by two tailed Mantel Cox method, Mann-Whitney U test, two way ANOVA with Sidak comparison, or two way ANOVA with Tukey’s test. Statistical analyses were performed using GraphPad Prism version 8.2.0 for Windows (GraphPad Software, La Jolla, CA, USA). P-values ($p$) less than 0.05 were considered statistically significant.
Author Contributions

SMY, SJP, YIK, and YKC conceived and designed the study. SMY, SJP, YIK, SWP, MAY, HIK, EHK, KMY, HWJ, JR, WJL, YJ, and JYL conducted sample collection, and virus isolation. SMY, SJP, YIK, and HWJ analyzed sample sequence and data. SMY, SJP, YIK, JYL, and YKC wrote a manuscript. SMY, SJP, and YIK contributed equally to this article as co-first authors.
Acknowledgements

We thank all involved staffs in the diagnosis of SFTS and epidemiological investigation of SFTS patients during 2013-2017. This work was supported by the Ministry of Health & Welfare (government–wide R&D fund project for infectious disease research (HG18C0029)) and supported by intramural fund (2017-NI53002-00) of the Research of National Institute of Health, Korea Centers for Disease Control and Prevention.


Figure 1. Distribution of severe fever with thrombocytopenia syndrome (SFTS) cases between 2013 and 2016. (A) Suspected, confirmed, and isolated human cases of SFTS in South Korea from 2013 to 2016. (B) Age distribution of confirmed SFTS cases. (C) The number of SFTS fatalities in confirmed cases. (D) The ratio of non-fatal and fatal cases in each gender by year. M, male; F, female. Asterisks indicate statistically significant differences in mortality rates between each age group of infected patients as determined by two tailed Mantel Cox method or Mann-Whitney U test (* indicates $p < 0.05$).
Figure 2. Phylogenetic analysis based on the complete ORF sequences of (A) L, (B) M, and (C) S segments of Korean SFTSVs compared with those from China and Japan. Compressed versions of the Maximum Likelihood (ML) trees based on the Kimura 2-parameter model were constructed and tested by bootstrap analysis with 1,000 replications. The scale bar indicates the number of nucleotide substitutions per site, and the phylogenetic branches were supported with greater than 70% bootstrap values. The SFTSV strains from China, Japan, and South Korea are marked with black, red, and blue closed circles, respectively. The full versions of the ML trees in this study are shown in Supplemental Figure 2.
Figure 3. Genotypes of Korean SFTSVs isolated from human sera. The SFTSVs were assigned to different genotypes based on the genetic origin of each segment as determined by phylogenic analysis. The SFTSVs were differentiated into at least 15 genotypes including genetic reassortant viruses. Gene segments from top to bottom are L, M, and S segments. The red, yellow, black, light blue, purple, blue, pink, and green bars indicate pure genotype groups of (A) A, B-1, B-2, B-3, C, D, E, F, and reassortant groups (B) R-1 to R-9 including the unique clustering genotype (grey bar), respectively.
Figure 4. The prevalence and fatality rates of each genotype by year. (A) Incidence of each genotype of SFTSV from 2012 to 2017. (B) The ratio of non-fatal to fatal cases for each genotype. The case fatality percentage (%) is shown at the top of each graph. (C) The cases of non-fatal to fatal cases by age group. Asterisks indicate statistically significant differences in mortality rates between each genotype of infected patients determined by Fisher’s exact test (* indicates $p < 0.05$).
Figure 5. Pathogenicity of each randomly selected SFTSV pure genotype (A, B-1, B-2) in ferrets. Young adult (≤ 2 years, n=5) or aged female and male ferrets (> 4 years, n=5) were inoculated with 10^5.0 FFU/ml of each randomly selected SFTSV strain (A) A, (B) B-1 and (C) B-2. Survival (first lane), viral titers in serum (second lane), and platelet concentration (third lane) were assessed. The normal range of platelets (171.7–1,280.6 × 10^3 per µl) is marked by dashed lines in each panel. Comparison between results in young adult and aged ferrets is represented in blue and red, respectively. Both viral titers and platelet concentrations are presented as mean ± SEM. Asterisks (*) indicate statistical significance between infected young adult and infected aged ferrets per day post-infection as determined by Mantel-Cox method (survival), two way ANOVA with Sidak comparison (viral copy number and platelet). The significance (platelet) between non infected aged adult and infected aged ferret were calculated by two way ANOVA with Tukey’s test which are indicated with dagger (†) (*, † indicate p < 0.05, and †† indicate p < 0.001, *** indicate p < 0.0001).
Figure 6. Pathogenicity of each randomly selected SFTSV pure genotype (B-3, D, F) in ferrets. Young adult (< 2 years, n=5) or aged female and male ferrets (> 4 years, n=5) were inoculated with $10^{5.0}$ FFU/ml of each randomly selected SFTSV strain (A) B-3, (B) D and (C) F. Survival (first lane), viral titers in serum (second lane), and platelet concentration (third lane) were assessed. The normal range of platelets (171.7–1,280.6 × 10^3 per µl) is marked by dashed lines in each panel. Comparison between results in young adult and aged ferrets is represented in blue and red, respectively. Both viral titers and platelet concentrations are presented as mean ± SEM. Asterisks (*) indicate statistical significance between infected young adult and aged infected ferrets per day post-infection as determined by Mantel-Cox method (survival), two way ANOVA with Sidak comparison (viral copy number and platelet). The significance (platelet) between non infected aged adult and infected aged ferret were calculated by two way ANOVA with Tukey’s test which are indicated with dagger (†) (*, † indicate $p < 0.05$, and **, †† indicate $p < 0.001$, *** indicate $p < 0.0001$).