Molecular genomic characterization of severe fever with thrombocytopenia syndrome virus isolates from South Korea

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Severe fever with thrombocytopenia syndrome (SFTS) is a tick-borne emerging infectious disease caused by the SFTS virus (SFTSV) and is a threat to public health due to its high fatality rate. However, details on tick-to-human transmission of SFTSV are limited. In this study, we determined the whole-genome sequence of a South Korean SFTSV strain (CUK-JJ01), compared it to those of other recent human SFTSV isolates, and identified the genetic variations and relationships among the SFTSV strains. The genome of CUK-JJ01 was consistent with the genome of other members of the genus *Phlebovirus*, including the large (L), medium (M), and small (S) segments of 6368, 3378, and 1744 nucleotides, respectively. Based on amino acid sequences of the M and S segments, which are used to distinguish the six SFTSV genotypes, CUK-JJ01 was classified as genotype B. Segment analysis revealed that the L, M, and S segments were 97.49%, 97.18%, and 97.94% similar to those of KAJNH2/2013/Korea, ZJZSH-FDE/2012/China, and KADGH/2013/Korea, respectively. Currently, only few studies on SFTSV have been conducted in Korean population and most were limited to serological analysis. Although the present study has limitations in terms of number of sample analyzed, the findings may serve as basis to understand the transmission and spread of SFTSV, as well as for the development of diagnostic and detection methods for viral recombinants by comparing the whole genome sequence of SFTSV isolates from South Korea and that of foreign isolates.

Keywords: SFTS, SFTSV, whole-genome sequencing, *Phlebovirus*, South Korea, phylogeny

Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne viral disease characterized by fever, gastrointestinal symptoms, leukopenia, and thrombocytopenia.

It was first reported in China in 2009, and subsequently in South Korea and western Japan in 2010 and 2013, respectively (Yu *et al.*, 2011; Kim *et al.*, 2013; Park *et al.*, 2014; Takahashi *et al.*, 2014). The etiological agent, SFTS virus (SFTSV), which belongs to the genus *Phlebovirus* of the family *Phenuiviridae*, is suspected to be a tick-borne virus based on evidence of its presence in four species of ticks, *Haemaphysalis longicornis*, *Amblyomma testudinarium*, *Ixodes nipponensis*, and *Rhipicephalus microplus* (Yu *et al.*, 2011; Zhang *et al.*, 2012; Yun *et al.*, 2015). Furthermore, this virus has also been isolated from or detected in domesticated animals (e.g., sheep, cattle, rodents, and dogs), small mammals such as rodents and shrews, and reptiles (Zhao *et al.*, 2012; Niu *et al.*, 2013; Liu *et al.*, 2014b).

SFTS is an acute infectious disease with a high mortality rate (approximately 6–30%) that can affect people at any age (Yu *et al.*, 2011; Ding *et al.*, 2013; Takahashi *et al.*, 2014; Yoshikawa *et al.*, 2015). Hospitalized patients that presented with clinical symptoms consistent with SFTS have been frequently reported in central and northeast China since March 2010. In 2009, the SFTSV was identified as the causative agent of SFTS (Xu *et al.*, 2011; Yu *et al.*, 2011; Liu *et al.*, 2014a). Importantly, human-to-human transmission of SFTSV was documented in a family cluster in China in 2011 (Bao *et al.*, 2011). The evidence demonstrates that SFTS has become an increasing threat to public health.

Like other phleboviruses, SFTSV contains a tripartite RNA genome consisting of three single-stranded RNA segments of negative polarity, designated as the large (L), medium (M), and small (S) segments (Yu *et al.*, 2011; Liu *et al.*, 2014a). The genome encodes an RNA-dependent RNA polymerase (RdRp) from the L segment, viral envelope glycoproteins Gn and Gc from the M segment, and a nucleoprotein (NP) and nonstructural protein (NS) in an ambisense orientation from the S segment (Yu *et al.*, 2011; Liu *et al.*, 2014a). The nomenclature for SFTSV genotypes is based on the country of origin, such as C1 to C5 for the Chinese lineage and J1 to J3 for the Japanese lineage. The phylogenetic analysis of a viral genome is indispensable for understanding the features of genotypic variation and the relationship of strains with their geographic distribution. For instance, it has been shown that eight strains of SFTSV recovered from patients in Japan clustered to an independent lineage separate from Chinese strains (Takahashi *et al.*, 2014). Recently, based on full-length sequencing of

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multiple SFTSV strains ($n = 159$) from China, South Korea, and Japan, Fu *et al.* (2016) have proposed a new nomenclature for SFTSV strains, which divides the viruses into six clades referred to as genotypes A to F.

Since the first reported fatal case in 2012 in Gangwon Province, South Korea (Kim *et al.*, 2013), concern regarding SFTSV has grown as the number of SFTS patients has increased annually with 36 cases reported in 2013, 55 cases in

2014, 79 cases in 2015, 165 cases in 2016, and 272 cases in 2017 (Korea Center for Disease Control, 2018). The mean mortality rate of these cases was approximately 20.9%.

According to a recent study in Vietnam (Tran *et al.*, 2019), virus-bearing *Amblyomma testudinarium* ticks and migratory birds played a role in dispersing SFTSV to Vietnam. In the current study, we analyzed the whole-genome sequence of a human SFTSV strain isolated from a patient who died

Table 1. Newly designed primers used in this study

Primer	Sequence (5'-3')	Location ^a	Size (bp)
L-GSP1	GCT GTC CAG TCA GGA TCA	5424–5441	656
L-GSP2	CAA GTA AGT ACT ATC ATC TAA	6033–6053	
L-Nested GSP	CCC TCG AGA CAG TCC CTA AA	5771–5790	
L-F1	ACA CAG AGA CGC CCA GAT GA	1–20	760
L-R1	GCT CAA TGT CTG CAT CCA TAG A	635–656	
L-F2	CAG GAT GAG GCA GAG GAG CT	569–588	
L-R2	CTG CCA TTC TCT TCC ACT TCT TC	1306–1328	676
L-F3	GTG GAG GGG AAG AAG TGG AAG	1298–1318	
L-R3	GAC TGA TCA GGA AGG TCA GGC	1953–1973	
L-F4	GGC TCC ATG GCT GGT TCG AA	1903–1922	634
L-R4	CTT GGT CCC AAT CTC TCT CAG	2516–2536	
L-F5	GGA AGC AGA ACC TTG AGG AGC	2490–2510	
L-R5	GGA AGG CRT CTG TCA TTG CTT C	3164–3185	702
L-F6	CTG AGT CTA GGT CAT CTG ATC C	3135–3156	
L-R6	CTA GGG CTG GAT CTG GGT CT	3817–3836	
L-F7	CTR CAC CAC TAC ATG CTT CTA GG	3746–3769	711
L-R7	CTT CCT CAA GCA ATG TGC ATA C	4436–4457	
L-F8	GAG CGA ATG CTC TTC CCT CAG	4397–4417	
L-R8	GCC CTC TCA ATC TGG TCT AGA A	5079–5100	675
L-F9	CCW CAG CCA TCA GAG GTC AT	4994–5013	
L-R9	GAT CCA GCT GCA GGA TGG YTC	5648–5668	
L-F10	GCA TAC CTC TGG AGC AAT CGA	5603–5623	766
L-R10	ACA CAA AGA CCG CCC AGA TCT TA	6346–6368	
M-GSP1	CTA CTC TGC CAT ACC CAC	2577–2594	775
M-GSP2	TTC TTC TTA ATC ATG TCT TTG T	3169–3190	
M-Nested GSP	AGC TAT GAA TCC CCT CCA GGA CAC	2870–2893	
M-F1	ACA CAG AGA CGG CCA ACA ATG ATG A	1–25	758
M-R1	ATG AGC TCT CCT GTA CCC ATT TGG T	751–775	
M-F2	GAG AGT CTA CCT CAG CCC TTT GAT G	673–697	
M-R2	GTG AGT AGC ATG AGG CCT GCA TAT C	1406–1430	780
M-F3	AGT GGA CTG CAC ATT CTG TCG TGA G	1314–1338	
M-R3	CCA CAT CCA TCA GAG CAG CCA CT	2071–2093	
M-F4	ATC AGT GAG GAG ATG TCG TTG GGC	1953–1976	685
M-R4	CTG GCT GTC ATG GCT TCT CTC AAA C	2613–2637	
M-F5	AGA GCT ACG AGT GGA TGA CGC TG	2514–2536	
M-R5	ACA CAA AGA CCG GCC AAC ACT TC	3356–3378	865
S-GSP1	TGA TGA AGA GCT GAG AAC	826–843	
S-GSP2	TTC TGC CCT CTC AAC CAG	1412–1429	
S-Nested GSP	AGGGATCCTCTCCATGC	1095–1111	615
S-F1	ACA CAA AGA CCC CCT TCA TTT G	1–23	
S-R1	CCT CTT AGC CTT GCA AGT GAC	595–615	
S-F2	GCG AAG RCT TAG ACT TGG GT	514–533	651
S-R2	CTA TCA ATG TGA AGA TGC GYG	1144–1164	
S-F3	GAA GAC AGA GTT CAC AGC AGC	1076–1096	
S-R3	ACA CAA AGA CCC CCA AAA AAG G	1723–1744	669
3'-Oligo (dT)-anchor-R	GAG GAC TCG AGC TCA AGC TTT TTT TTT TTT	3'-end poly A tail	
3'-Anchor-R	GAG GAC TCG AGC TCA AGC		

^a According to GenBank accession number MH937372–MH937374.

from SFTS in Jeju Island, South Korea in October 2017 and compared it to those of other human SFTSV strains to determine the genetic and evolutionary characteristics of this important pathogen. We expect that these data will prove useful, not only for advancing research in the molecular biology of SFTSV, but also for basic epidemiological analyses and tracking the international spread of the virus.

Materials and Methods

Ethics statement

Jeju Halla General Hospital received written consent for sample collection from each patient with SFTSV infection. All participants were adults, and the study was approved by the institutional review board (IRB) of the Catholic Medical Center Office of Human Research Protection Program (CMC OHRP) of South Korea (approval no. MC18SESI0077). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Virus propagation, cell lines, and viral RNA extraction

For propagation, the virus was passaged five times on confluent monolayers of Vero cells (KCLB 10081; Korean Cell Line Bank) in Dulbecco's Modified Eagle Medium (DMEM; Hyclone) containing 10% fetal bovine serum (FBS; Hyclone) with penicillin (100 IU/ml)/streptomycin (100 µg/ml; P/S, Hyclone) in a 37°C incubator supplemented with 5% CO₂. Cell culture supernatants were collected after seven days and stored at -80°C as working virus stocks for whole-genome sequencing. Viral RNA was extracted from 140 µl of the viral stocks using QIAamp Viral RNA Mini Kits (Qiagen) according to the manufacturer's instructions. Single stranded complementary DNA (cDNA) was synthesized from the viral RNA as template using primers specific for each segment and a cDNA Synthesis Kit (TaKaRa Bio) according to the manufacturer's instructions.

Whole-genome sequencing

For whole-genome sequencing, one to six overlapping PCR fragments covering the SFTSV genome, including full-length L, M, and S segments, were amplified by polymerase chain reaction (PCR) using the cDNA as template and Taq DNA polymerase (Qiagen) on an S1000 thermal cycler (Bio-Rad). The PCR primers were designed based on the full-genome sequence of the detected SFTSV (Table 1). PCR amplification of each segment was performed using a profile of 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec, annealing for 30 sec at 53 or 60°C according to the primer sets for each segment, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. All PCR products were examined by electrophoresis on 2% agarose gels stained with ethidium bromide.

Determination of the 5'- and 3'-ends of the SFTSV genomic RNA

To determine the 5'-ends of the SFTSV genomic RNA, rapid

amplification of cDNA ends (RACE) was performed using the 5' RACE System for Rapid Amplification of cDNA Ends Version 2.0 Kit (Invitrogen) according to the manufacturer's recommendations. Three primers, GSP1, GSP2, and nested GSP, were designed for 5' RACE PCR based on the L sequence (Table 1). To obtain the exact sequence of the 3'-end of the SFTSV genomic RNA, cDNA was synthesized by reverse transcription using 3'-oligo (dT)-anchor-R (Table 1). The second PCR was conducted using the S-F and 3'-anchor-R primers (Table 1) under the following conditions: 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min followed by 72°C for 10 min.

Cloning and sequencing of the complete genome

All PCR products were extracted using a HiYield Gel/PCR DNA Fragments Extraction Kit (RBC Bioscience) and cloned into pGEM-T Easy Vectors (Promega). The cloned vectors were transformed into *Escherichia coli* DH5α competent cells (RBC Bioscience) according to the manufacturer's instructions and selected at 37°C for 16–18 h on Luria-Bertani agar plates (Duchefa) containing 40 mg/ml X-gal, 0.1 mM isopropyl-β-D-thiogalactoside, and 50 mg/ml ampicillin. Selected clones were inoculated into Luria-Bertani broth (Duchefa) and incubated overnight in a shaking incubator (IS-971R, Jeiotech) at 37°C and 200 rpm. Plasmid DNA was purified using a HiYield Plasmid Mini Kit (RBC Bioscience) and sequenced (Macrogen). The sequencing results were analyzed using the Basic Local Alignment Search Tool (BLAST) provided by the National Center for Biotechnology Information (NCBI; National Institutes of Health).

Phylogenetic analysis

Comparative sequence analyses, including sequence alignments and estimation of genetic distances, were performed using the ClustalW program of the Molecular Evolutionary Genetic Analysis (MEGA) software, version 7.0 (Tamura *et al.*, 2013). Phylogenetic trees were constructed using the neighbor-joining method with a Kimura two-parameter model provided in the MEGA software (Saitou and Nei, 1987) and branch support was calculated based on 1,000 bootstrap replicates. The complete-genome sequences and partial-genome sequences were obtained from the NCBI database.

Results

Nucleotide sequence identities

The genome of the human-derived South Korean SFTSV strain CUK-JJ01 (GenBank no. MH937372–MH937374) analyzed in this study consisted of L, M, and S segments of 6368, 3378, and 1744 nucleotides (nt), respectively. The L segment had a 6255-nt long open reading frame (ORF) for the RNA-dependent RNA polymerase gene that encoded a predicted 2084 amino acid (aa) protein. The M segment contained an ORF for a 3222-nt precursor of the glycoprotein gene encoding for proteins Gn (516 aa) and Gc (511 aa). The S segment contained 882- and 738-nt long ORFs, which encoded the NS protein (293 aa) and NP (245 aa), respectively. The non-coding regions (NCRs) of the L, M, and S segments

at the 5'-termini were 16-, 18-, and 42-nt long, respectively, and at the 3'-termini were 97-, 138-, and 29-nt long, respectively. Segment analysis of CUK-JJ01 revealed that the L segment was 97.49% similar to that of KAJNH2/2013/Korea (GenBank accession no. KU507547), the M segment was 97.18% similar to that of ZJZSHS-FDE/2012/China (GenBank accession no. KR017859), and the S segment was 97.94% similar to that of KADGH/2013/Korea (GenBank accession no. KU507553). Moreover, NP-encoding region of the S segment of strain CUK-JJ01 demonstrated 98.24% sequence identity at the nt level with the genotype D strain KP663731

(KASJH). Surprisingly, sequence comparison of the NP ORFs of the S segment indicated that CUK-JJ01 was most closely related to SFTSV genotype B reference strains at 95.25–98.64% identity (Table 2). The highest sequence identity (98.64%) was observed with the reference strain KU507543 (KADGH), which was isolated in South Korea. A comparative analysis of the CUK-JJ01 nt sequence of the NS ORF of the S segment with that of the SFTSV reference sequences indicated that CUK-JJ01 was most closely related to the South Korean reference strain KU507543 (KADGH) at 97.28% identity (Table 2).

Table 2. Nucleotide sequence identities of the full-length genome and L, M, and S-NP/NSs genes between the SFTSV CUK-JJ01 strain and reference strains

Reference strain	Accession number	Genotype	Collection date	Country	Nucleotide sequence identity (%)			
					L	M	S-NP	S-NSs
AH15	HQ141592	A	2010	China	95.98%	93.82%	95.79%	94.67%
CB2	KY789434	A	2015	Korea	96.11%	93.92%	95.79%	95.35%
HL_Adult_G2	KF791962	A	2013	China	95.97%	93.85%	95.12%	94.78%
HL_Egg_G2	KF791959	A	2013	China	95.97%	93.92%	95.12%	94.78%
HL_Injected	KF791958	A	2013	China	95.97%	93.92%	95.12%	94.78%
HL_Larvae_G2	KF791960	A	2013	China	95.97%	93.85%	95.12%	94.78%
HL_Nymph_G2	KF791961	A	2013	China	95.97%	93.85%	95.12%	94.78%
JS3	HQ141601	A	2010	China	96.00%	93.85%	95.52%	95.01%
LN3	HQ141610	A	2010	China	96.11%	93.89%	94.57%	95.46%
SPL087A	AB983515	A	2013	Japan	95.79%	93.70%	94.84%	94.67%
01_Zhejiang	KJ597825	B	2011	China	97.31%	97.11%	97.96%	96.94%
CB1	KY789433	B	2014	Korea	97.12%	96.46%	95.79%	95.80%
CB3	KY789435	B	2016	Korea	96.24%	95.81%	95.93%	95.69%
KACNH3	KP663743	B	2014	Korea	96.08%	95.56%	96.74%	95.57%
KADGH	KU507543	B	2013	Korea	97.20%	96.46%	98.64%	97.28%
KAGBH5	KP663737	B	2014	Korea	96.37%	95.90%	96.20%	96.94%
KAGBH6	KP663740	B	2014	Korea	96.42%	95.59%	95.25%	96.94%
KAGNH	KU507545	B	2013	Korea	96.19%	95.28%	96.07%	96.37%
KAGNH4	KU507546	B	2013	Korea	96.22%	95.65%	97.15%	97.28%
KAGWT	KY273136	B	2013	Korea	96.42%	95.62%	96.20%	96.48%
KAJNH2	KU507547	B	2013	Korea	97.49%	96.87%	96.07%	96.37%
Zhao	KF374682	B	2013	China	97.38%	97.14%	97.83%	96.82%
ZJZSHS-FDE	KR017840	B	2012	China	97.39%	97.18%	97.96%	96.82%
ZJZSHS-LWL	KR017844	B	2014	China	97.30%	97.05%	97.96%	96.94%
ZJZSHS-WRF	KR017845	B	2014	China	97.31%	97.14%	97.83%	96.59%
2011YPQ17	KF711887	D	2011	China	95.47%	93.51%	95.12%	94.55%
AHL	JQ670934	D	2011	China	95.58%	94.29%	95.66%	94.67%
HB154	JQ733561	D	2011	China	95.60%	93.48%	94.71%	95.01%
HB155	JQ733564	D	2011	China	95.54%	93.45%	94.84%	94.78%
HB156	JQ733567	D	2011	China	95.55%	93.58%	94.71%	95.01%
KASJH	KP663731	D	2014	Korea	95.79%	93.67%	98.24%	96.71%
SD24	HM802200	D	2010	China	95.58%	93.58%	94.57%	94.55%
JS4	HQ141604	E	2010	China	95.71%	94.17%	96.20%	95.12%
SD4	HM802202	E	2010	China	95.74%	94.01%	94.84%	95.12%
AH12	HQ116417	F	2010	China	96.08%	94.01%	94.84%	94.67%
AH-YTY_	KR017830	F	2012	China	95.97%	94.04%	94.84%	94.67%
AHZ	JQ670929	F	2011	China	95.92%	93.76%	94.57%	95.12%
Gangwon	KF358691	F	2012	Korea	96.03%	94.01%	94.84%	94.55%
HN6	HQ141595	F	2010	China	95.86%	93.82%	94.57%	94.67%
JS2012-tick01	KC473540	F	2012	China	95.84%	93.73%	94.71%	95.12%
LN2	HQ141607	F	2010	China	95.92%	93.79%	95.25%	94.67%
QD7	KR706567	F	2014	China	95.76%	93.64%	95.39%	95.35%
SDLZtick12	JQ684871	F	2012	China	95.86%	93.82%	96.20%	94.78%

SFTSV, severe fever with thrombocytopenia syndrome virus.

Genotype	L segment									
	448	449	580	586	587	987	1371	1633	1795	
Type B	CUK-JJ01	E	T	S	D	A	E	I	S	V
	KADGH Korea(2013) KU507543	-	-	T	-	T	K	V	L	I
	CB1 Korea(2014) KY789433	-	-	T	-	T	K	V	L	I
	KAGWT Korea(2013) KY273136	-	-	T	-	T	K	V	L	I
	KAGBH5 South Korea(2014) KP663737	-	-	T	-	T	K	V	L	I
	KAGNH Korea(2013) KU507545	-	-	T	-	T	K	V	L	I
	KAJNH2 Korea(2013) KU507547	-	-	T	-	T	K	V	L	I
	ZJZSH-WRF China(2014) KR017845	-	-	T	-	T	K	V	L	I
	Zhao China(2013) KF374682	-	-	T	-	T	K	V	L	I
	ZJZSH-LWL China(2014) KR017844	-	-	T	-	T	K	V	L	I
	ZJZSH-FDE China(2012) KR017840	-	-	T	-	T	K	V	L	I
	01 Zhejiang(2011) KJ597825	-	-	T	-	T	K	V	L	I
	KAGNH4 Korea(2013) KU507546	-	-	T	-	T	K	V	L	I
	CB3 Korea(2016) KY789435	-	-	T	-	T	R	V	L	I
Type A	SPL087A Japan(2013) AB983515	-	-	T	-	T	K	V	L	I
	HL Adult G2 China(2013) KF791962	D	A	T	E	T	K	V	L	I
	HL Egg G2 China(2013) KF791959	D	A	T	E	T	K	V	L	I
	HL Injected China(2013) KF791958	D	A	T	E	T	K	V	L	I
	HL Larvae G2 China(2013) KF791960	D	A	T	E	T	K	V	L	I
	HL Nymph G2 China(2013) KF791961	D	A	T	E	T	K	V	L	I
	CB2 Korea(2015) KY789434	-	-	T	-	T	K	V	L	I
Type D	KASJH South Korea(2014) KP663731	-	-	T	-	T	K	V	L	I
	SD24 Shandong(2010) HM802200	-	-	T	-	T	K	V	L	I
	HB154 China(2011) JQ733561	-	-	T	-	T	K	V	L	I
	HB155 China(2011) JQ733564	-	-	T	-	T	K	V	L	I
Type E	HB156 China(2011) JQ733567	-	-	T	-	T	K	V	L	I
	AHL Anhui(2011) JQ670934	-	-	T	-	T	K	V	L	I
	JS4 Jiangsu(2010) HQ141604	-	-	T	-	T	K	V	L	I
	SD4 Shandong(2010) HM802202	-	-	T	-	T	K	V	L	I
Type F	AHZ China(2011) JQ670929	-	-	T	-	T	K	V	L	I
	SDLZick12 Shandong(2012) JQ684871	-	-	T	-	T	K	V	L	I
	AH-YTY China(2012) KR017830	-	-	T	-	T	K	V	L	I
	Gangwon South Korea(2012) KF358691	-	-	T	-	T	K	V	L	I
	AH12 Anhui(2010) HQ116417	-	-	T	-	T	K	V	L	I
	JS2012-tick01 China(2012) KC473540	-	-	T	-	T	K	V	L	I
	QD7 China(2014) KR706567	-	-	T	-	T	K	V	L	I

Genotype	M segment									
	7	47	218	264	272	354	580	662	921	956
Type B	CUK-JJ01	L	R	S	E	R	I	G	S	S
	KADGH Korea(2013) KU507548	F	K	-	G	K	L	S	-	C
	CB1 Korea(2014) KY789436	F	K	-	G	E	L	S	-	C
	KAGWT Korea(2013) KY273137	F	K	-	G	K	L	S	-	C
	KAGBH5 South Korea(2014) KP663738	F	K	-	G	K	L	S	-	C
	KAGBH6 South Korea(2014) KP663741	F	K	-	G	K	L	S	-	C
	KAGNH Korea(2013) KU507550	F	K	-	G	K	L	S	-	C
	KAJNH2 Korea(2013) KU507552	F	K	-	G	K	L	S	-	C
	ZJZSH-WRF China(2014) KR017864	F	K	-	G	K	L	S	-	C
	Zhao China(2013) KF374684	F	K	-	G	K	L	S	-	C
	ZJZSH-LWL China(2014) KR017863	F	K	-	G	K	L	S	-	C
	ZJZSH-FDE China(2012) KR017859	F	K	-	G	K	L	S	-	C
	01 Zhejiang(2011) KJ597824	F	K	-	G	K	L	S	-	C
	KAGNH4 Korea(2013) KU507551	F	K	-	G	K	L	S	-	C
Type A	CB3 Korea(2016) KY789438	F	K	-	G	K	L	S	-	C
	KACHH3 South Korea(2014) KP663744	F	K	-	G	K	L	S	-	C
	SPL087A Japan(2013) AB983509	F	K	G	G	K	L	S	P	C
	HL Adult G2 China(2013) KF791957	F	K	G	G	K	L	S	P	C
	HL Egg G2 China(2013) KF791954	F	K	G	G	K	L	S	P	C
	HL Injected China(2013) KF791953	F	K	G	G	K	L	S	P	C
	HL Larvae G2 China(2013) KF791955	F	K	G	G	K	L	S	P	C
Type D	HL Nymph G2 China(2013) KF791956	F	K	G	G	K	L	S	P	C
	CB2 Korea(2015) KY789437	F	K	G	G	K	L	S	P	C
	JS3 China(2010) HQ141602	F	K	G	G	K	L	S	P	C
	LN3 Liaoning(2010) HQ141611	F	K	G	G	K	L	S	P	C
Type E	AH15 Anhui(2010) HQ141593	F	K	G	G	K	L	S	P	C
	KASJH South Korea(2014) KP663732	F	K	G	G	K	L	S	P	C
	SD24 Shandong(2010) HM802201	F	K	G	G	K	L	S	P	C
	HB154 China(2011) JQ733560	F	K	G	G	K	L	S	P	C
Type F	HB155 China(2011) JQ733563	F	K	G	G	K	L	S	P	C
	HB156 China(2010) JQ733566	F	K	G	G	K	L	S	P	C
	AHL Anhui(2011) JQ670930	F	K	G	G	K	L	S	P	C
	2011YQ17 Henan(2011) KF711923	F	K	G	G	K	L	S	P	C
Type F	JS4 Jiangsu(2010) HQ141605	F	K	G	G	K	L	S	P	C
	SD4 Shandong(2010) HM802203	F	K	G	G	K	L	S	P	C
	AHZ China(2011) JQ670931	F	K	G	G	K	L	S	P	C
	SDLZick12 Shandong(2010) JQ684872	F	K	G	G	K	L	S	P	C
	AH-YTY China(2012) KR017849	F	K	G	G	K	L	S	P	C
Type F	Gangwon South Korea(2012) KF358692	F	K	G	G	K	L	S	P	C
	AH12 Anhui(2010) HQ141590	F	K	G	G	K	L	S	P	C
	JS2012-tick01 China(2012) KC473541	F	K	G	G	K	L	S	P	C
	QD7 China(2014) KR706566	F	K	G	G	K	L	S	P	C
	HB6 China(2010) HQ141596	F	K	G	G	K	L	S	P	C
	LN2 Liaoning(2010) HQ141608	F	K	G	G	K	L	S	P	C

Fig. 1. Comparison of RNA-dependent RNA polymerase, glycoprotein/nucleoprotein, and nonstructural protein amino acid substitutions among SFTSV genotypes. Representative strains for each SFTSV genotype were selected. The red borders indicate the sites of amino acid changes. The portion marked in yellow indicates a location where a specific sequence change occurred for the specific genotype. SFTSV, severe fever with thrombocytopenia syndrome virus.

Genotype		S-NP segment					
		55	84	116	128	135	240
Type B	CUK-JJ01	L	R	S	S	R	Y
	KADGH Korea(2013) KU507553	-	-	-	C	G	N
	CB1 Korea(2014) KY789439	-	-	-	C	G	N
	KAGWT Korea(2013) KY273138	-	-	-	C	G	N
	KAGBH5 South Korea(2014) KP663739	-	-	-	C	G	N
	KAGBH6 South Korea(2014) KP663742	-	-	-	C	G	N
	KAGNH Korea(2013) KU507555	-	-	-	C	G	N
	KAJNH2 Korea(2013) KU507557	-	-	-	C	G	N
	ZJZSH-WRF China(2014) KR017826	-	-	-	C	G	N
	Zhao China(2013) KF374683	-	-	-	C	G	N
	ZJZSH-LWL China(2014) KR017825	-	-	-	C	G	N
	ZJZSH-FDE China(2012) KR017821	-	-	-	C	G	N
	01 Zhejiang(2011) KJ597823	-	-	-	C	G	N
	KAGNH4 Korea(2013) KU507556	-	-	-	C	G	N
	CB3 Korea(2016) KY789441	-	-	-	C	G	N
	KACNH3 South Korea(2014) KP663745	-	-	-	C	G	N
Type A	HL Adult G2 China(2013) KF791952	F	G	G	C	G	N
	HL Egg G2 China(2013) KF791949	F	G	G	C	G	N
	HL Injected China(2013) KF791948	F	G	G	C	G	N
	HL Larvae G2 China(2013) KF791950	F	G	G	C	G	N
	HL Nymph G2 China(2013) KF791951	F	G	G	C	G	N
	CB2 Kroea(2015) KY789440	F	G	G	C	G	N
	JS3 China(2010) HQ141603	F	G	G	C	G	N
	LN3 Liaoning(2010) HQ141612	F	G	G	C	G	N
	AH15 Anhui(2010) HQ141594	F	G	G	C	G	N
	KASJH South Korea(2014) KP663733	F	G	G	C	G	N
	SD24 Shandong(2010) HM802205	F	G	G	C	G	N
	HB154 China(2011) JQ744462	F	G	G	C	G	N
	HB155 China(2011) JQ733565	F	G	G	C	G	N
	HB156 China(2011) JQ733568	F	G	G	C	G	N
	AHL Anhui(2011) JQ670932	F	G	G	C	G	N
	2011YPQ17 Henan(2011) KF711896	F	G	G	C	G	N
Type E	JS4 Jiangsu(2010) HQ141606	F	G	G	C	G	N
	SD4 Shandong(2010) HM802204	F	G	G	C	G	N
	AHZ China(2011) JQ670933	F	G	G	C	G	N
	SDLZick12 Shandong(2010) JQ684873	F	G	G	C	G	N
	AH-YTY China(2012) KR017811	F	G	G	C	G	N
	Gangwon South Korea(2012) KF358693	F	G	G	C	G	N
Type F	AH12 Anhui(2010) HQ141591	F	G	G	C	G	N
	JS2012-tick01 China(2012) KC473542	F	G	G	C	G	N
	QD7 China(2014) KR706565	F	G	G	C	G	N
	HN6 China(2010) HQ141597	F	G	G	C	G	N
	LN2 Liaoning(2010) HQ141609	F	G	G	C	G	N
Genotype		S-Ns segment					
		123	235				
Type B	CUK-JJ01	N	M				
	KADGH Korea(2013) KU507553	S	L				
	CB1 Korea(2014) KY789439	S	L				
	KAGWT Korea(2013) KY273138	S	L				
	KAGBH5 South Korea(2014) KP663739	S	L				
	KAGBH6 South Korea(2014) KP663742	S	L				
	KAGNH Korea(2013) KU507555	S	L				
	KAJNH2 Korea(2013) KU507557	S	L				
	ZJZSH-WRF China(2014) KR017826	S	L				
	Zhao China(2013) KF374683	S	L				
	ZJZSH-LWL China(2014) KR017825	S	L				
	ZJZSH-FDE China(2012) KR017821	S	L				
	01 Zhejiang(2011) KJ597823	S	L				
	KAGNH4 Korea(2013) KU507556	S	L				
	CB3 Korea(2016) KY789441	S	L				
	KACNH3 South Korea(2014) KP663745	S	L				
Type A	SPL087A Japan(2013) AB985541	S	L				
	HL Adult G2 China(2013) KF791952	S	L				
	HL Egg G2 China(2013) KF791949	S	L				
	HL Injected China(2013) KF791948	S	L				
	HL Larvae G2 China(2013) KF791950	S	L				
	HL Nymph G2 China(2013) KF791951	S	L				
	CB2 Kroea(2015) KY789440	S	L				
	JS3 China(2010) HQ141603	S	L				
	LN3 Liaoning(2010) HQ141612	S	L				
	AH15 Anhui(2010) HQ141594	S	L				
Type D	KASJH South Korea(2014) KP663733	S	L				
	SD24 Shandong(2010) HM802205	S	L				
	HB154 China(2011) JQ744462	S	L				
	HB155 China(2011) JQ733565	S	L				
	HB156 China(2011) JQ733568	S	L				
	AHL Anhui(2011) JQ670932	S	L				
Type E	2011YPQ17 Henan(2011) KF711896	S	L				
	JS4 Jiangsu(2010) HQ141606	S	L				
	SD4 Shandong(2010) HM802204	S	L				
	AHZ China(2011) JQ670933	S	L				
Type F	SDLZick12 Shandong(2010) JQ684873	S	L				
	AH-YTY China(2012) KR017811	S	L				
	Gangwon South Korea(2012) KF358693	S	L				
	AH12 Anhui(2010) HQ141591	S	L				
	JS2012-tick01 China(2012) KC473542	S	L				
	QD7 China(2014) KR706565	S	L				
	HN6 China(2010) HQ141597	S	L				
	LN2 Liaoning(2010) HQ141609	S	L				

Fig. 1. Continued

Fig. 2. Phylogenetic analysis using the complete nucleotide sequences of the L segments of SFTSV strains. We used an ML method based on the Kimura 2-parameter model, and the numbers on the branches indicate bootstrap percentages based on 1,000 replicates. The scale bar indicates the number of nucleotide substitutions per site. The phylogenetic branches were supported with greater than 70% bootstrap values. The human-derived South Korean SFTSV strains analyzed in this study are indicated by the red closed circles. ML, maximum likelihood; SFTSV, severe fever with thrombocytopenia syndrome virus.

Fig. 3. Phylogenetic analysis using the complete nucleotide sequences of the M segments of SFTSV strains. We used an ML method based on the Kimura 2-parameter model, and the numbers on the branches indicate bootstrap percentages based on 1,000 replicates. The scale bar indicates the number of nucleotide substitutions per site. The phylogenetic branches were supported with greater than 70% bootstrap values. The human-derived South Korean SFTSV strains analyzed in this study are indicated by the red closed circles. ML, maximum likelihood; SFTSV, severe fever with thrombocytopenia syndrome virus.

Fig. 4. Phylogenetic analysis using the complete nucleotide sequences of the S segments of SFTSV strains. We used an ML method based on the Kimura 2-parameter model, and the numbers on the branches indicate bootstrap percentages based on 1,000 replicates. The scale bar indicates the number of nucleotide substitutions per site. The phylogenetic branches were supported with greater than 70% bootstrap values. The human-derived South Korean SFTSV strains analyzed in this study are indicated by the red closed circles. ML, maximum likelihood; SFTSV, severe fever with thrombocytopenia syndrome virus.

Nucleotide and amino acid polymorphisms

Based on sequence comparison of the three SFTSV RNA segments (L, M, and S) of CUK-JJ01 and the complete genomes of 43 SFTSV strains, six of the 6255 nt of the CUK-JJ01 L segment displayed polymorphisms with five (90%)

being transitions and one (10%) being a transversion; 12 of the 3222 nt of the M segment displayed polymorphisms with 10 (83%) being transitions and two (17%) being transversion; and eight of the 1620 nt of the S segment displayed polymorphisms with four (50%) being transitions and four (50%)

being transversion (Fig. 1). Specifically, we identified three unique amino acid substitutions in the L segment of CUK-JJ01 when compared to that of viruses from *Haemaphysalis longicornis*; the L segment of *Haemaphysalis longicornis*-derived viruses had Asp in aa 448, Ala in aa 449, and Glu in aa 586, while the L segment of CUK-JJ01 had Glu at aa 448, Thr at aa 449, and Asp at aa 586. These genotype-specific substitutions distinguished the different SFTSV genotypes. Among the 1074 aa of the M segment, three amino acid substitutions were detected in CUK-JJ01: aa 218 (Glu→Gly), aa 662 (Ser→Pro), and aa 1011 (Ser→Thr). Three amino acid substitutions were detected among the 540 aa of the S segment of CUK-JJ01: aa 55 (Leu→Phe), aa 84 (Arg→Gly), and aa 116 (Ser→Gly).

Phylogenetic analysis

To investigate genetically the evolutionary origins and the relationship between the tick-derived and human-derived South Korean SFTSV strains, phylogenetic analyses were conducted using complete full-length sequences of CUK-JJ01 and previously isolated SFTSV strains from China, Japan, and South Korea. Phylogenetic analyses of the L, M, and S segments indicated that the tick-derived South Korean SFTSV strain KAGWT clustered with genotype B SFTSV strains that circulate in humans in China, Japan, and South Korea (Fig. 2). The CUK-JJ01 human isolate also clustered with genotype B viruses, although it was separated into a different sub-node than KAGWT and was closely related to a recent South Korean SFTSV human isolate KADGH, as well as the Chinese isolate ZJZSH-FDE and Korean isolate KAJNH2. Overall, the phylogenetic trees revealed that South Korean SFTSV strains could be classified with high branch support into four distinct genotypes out of the six genotypes described previously (A, B, D, and F). No apparent genotypic differences were evident among the phylogenetic trees based on the SFTSV L, M, and S segments and the complete genomes of 51 SFTSV strains (Figs. 2, 3, and 4). As previously reported, the L segment appeared to be the most highly conserved, whereas the S segment had the greatest number of nt polymorphisms. Moreover, the phylogenetic trees based on the S segment and the complete genome were nearly identical, which indicated that the S segment may be used to analyze the genetic relationships among SFTSV strains instead of the complete genome.

Discussion

SFTS was first recognized in 2007 and its causative agent SFTSV was identified in 2011 as a novel *Phlebovirus* (Xu *et al.*, 2011; Yu *et al.*, 2011; Zhang *et al.*, 2011). SFTSV circulates in seven central-eastern provinces of China and was more recently also found in South Korea, Japan, and Vietnam (Takahashi *et al.*, 2014; Yun *et al.*, 2014; Yoshikawa *et al.*, 2015; Tran *et al.*, 2019), which are separated from China by ocean straits. Recently, virus-bearing *Amblyomma testudinarium* ticks and migratory birds were shown to play a role in dispersing SFTSV to Vietnam (Tran *et al.*, 2019). Thus, SFTSV poses a threat to public health due to its wide range of animal hosts and its ability to cause severe infections in humans, especially the elderly. Continuous monitoring and the mo-

lecular detection of this virus are important for the correct diagnoses of SFTSV infections.

Since animals fail to show overt clinical symptoms upon infection and clinical symptoms of SFTSV infection in humans resemble those of other diseases, it is possible SFTSV is currently present in countries other than China, South Korea, Japan, and Vietnam. SFTSV genotypes A, D, and F are the most commonly detected genotypes in mainland China, while genotype B is most commonly found circulating in South Korea and Japan. Moreover, genotypes B, D, and F are all known to co-circulate in South Korea.

Due to the segmented nature of the SFTSV genome, genetic reassortments have occurred in strains from China and Japan, resulting in at least ten strains with unique genotypes (Fu *et al.*, 2016; Shi *et al.*, 2017). In the current study, the genome sequence of a virus isolated from a patient who died from SFTS in Jeju Island in October 2017 was analyzed and compared to the whole-genome sequences of other human-derived strains. The analysis revealed that consistent with all SFTSV strains, CUK-JJ01 consisted of three genomic segments, including a 6368-nt L segment, a 3378-nt M segment, and a 1744-nt S segment. Furthermore, CUK-JJ01 belongs to genotype B and showed no evidence of intra- or inter-genogroup recombination of the L, M, or S segment. Sequence analysis of CUK-JJ01 revealed that the L segment was 97.49% similar to that of KAJNH2, the M segment was 97.18% similar to that of ZJZSH-FDE, and the S segment was 97.94% similar to that of KADGH. Genetic and phylogenetic analyses of full-length genome sequences revealed that tick-derived and human-derived South Korean SFTSV strains are closely related and that at least four different genotypes of SFTSV are co-circulating in South Korea.

To date, only few studies have been conducted on SFTSV in Korean population, and most are limited to serological analysis. In China and Japan, the number of patients with SFTSV is increasing, and various genotypes have been detected since 2010. In particular, new SFTSV genotypes, reassortments, and recombination isolates have been identified by long-term molecular epidemiological analysis.

Although the present study has limitations in terms of not analyzing multiple samples, the findings should serve as basis for the development of methods for diagnosis and to detect viral recombinants, as well as to elucidate the transmission and spread of SFTSV by comparing the whole genome sequence of SFTSV isolated in South Korea and that of foreign isolates. In the future, it is anticipated that gene and mutation analyses using animal-derived viruses can shed light into inter-species propagation and emergence of new viruses.

Although there have been several studies focusing on the pathogenesis and transmission of SFTSV, there is currently no vaccine available to prevent its spread. Initial studies indicate a higher mortality rate in patients 50 years of age and above, suggesting that susceptibility to SFTSV infection may vary and be dependent upon several risk factors, including the age and immune status of the patient (Robles *et al.*, 2018). Nonetheless, with initial indications of overall mortality rates from 30–40% in China, South Korea, and Japan, and a wide range of host species that have direct human contact but display limited clinical illnesses, vaccine development will be critical for the prevention of SFTS and any hope for the

eradication of SFTSV.

The results from the current study provide insight into the genetic origins of human of SFTSV strains, as well as shed light on the molecular epidemiology, genetic diversity, and evolution of SFTSV. These associations have important implications in the design and development of diagnostic procedures and vaccines against SFTSV. Moreover, the information acquired from the whole-genome sequence of SFTSV strain CUK-JJ01 may prove useful for obtaining more accurate diagnoses of SFTSV and for advancing research toward the elucidation of genetic functions, the prediction of newly appearing pandemic variants via comparison with SFTSVs in neighboring countries, and in vaccine development. Overall, broadening the information base and genetic resources regarding SFTSVs circulating globally will provide important benefits for public health and help to identify new emerging strains of SFTSV.

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Conflicts of Interest

The authors declare no conflicts of interest.

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