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ORIGINAL ARTICLE

High-frequency mutation and recombination are responsible for the emergence of novel porcine reproductive and respiratory syndrome virus in northwest China

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is one of the most highly infectious diseases in the pig industry, resulting in enormous economic losses worldwide. In this study, a PRRS virus (PRRSV) strain was isolated from primary porcine alveolar macrophage cells in Xinjiang in northwest China. This new strain was sequenced and designated as XJzx1-2015, and its sequence was then compared to those of other representative PRRSV strains from around the world. Complete genomic characterisation showed that the full-length nucleotide sequence of XJzx1-2015 exhibited low-level similarity to NB/04 (91.6%), JXA1 (90.5%), CH-1a (90.2%), VR-2332 (86.9%), QYYZ (85.7%), and JL580 (82.2%), with the highest similarity to HK13 (91.7%) sequence identity. Nonstructural protein 2 (NSP2) and glycosylated protein (GP) 2 of XJzx1-2015 had deletions of five and two amino acids, respectively, corresponding to strain VR-2332 positions 475-479 and 173-174. Phylogenetic analysis based on complete genome sequences showed that XJzx1-2015 and four other strains from China formed a new subgenotype closely related to other sublineage 8.7 (JXA1-like) strains belonging to the North American genotype. However, phylogenetic analysis based on NSP2 and GP5 showed that XJzx1-2015 clustered with sublineage 8.7 (JXA1-like, CH-1a-like) and lineage 3 (QYYZ-like) strains, respectively. Recombination analysis indicated that XJzx1-2015 is an intersubgenotype recombinant of CH-1a-like and QYYZ-like strains. Overall, our findings demonstrate that XJzx1-2015 is a novel PRRSV strain with a significantly high frequency of mutation and a recombinant between lineage 3 and sublineage 8.7 identified in northwest China. These results provide important insights into PRRSV evolution.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by PRRS virus (PRRSV), which is responsible for economically devastating disease outbreaks in pigs worldwide. PRRSV was first reported in the early 1990s and was found to belong to the genus *Betaarterivirus*, family *Arteriviridae*, order *Nidovirales*. High mutation rates combined with viral recombination are responsible for the adaptation of viruses of this family, leading to extremely dynamic evolution and extraordinarily diverse viruses [1].

PRRSV is an enveloped RNA virus with a linear, positive-sense genome approximately 15 kb in length. The viral genome comprises a 5′ untranslated region (UTR), at least 10 open reading frames (ORFs; designated ORF1a, -1b, -2a, -2b, -3–5a, and -5–7), and a 3′-UTR [2–4]. Nearly 75% of the 5′ portion of the genome encodes ORFs 1a and b, which encode the viral 1a and 1ab replicase polyproteins. These

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polyproteins are proteolytically cleaved into 14 nonstructural proteins (NSPs). The remaining ORFs, located in the 3′ position of the genome, encode viral structural proteins, including the nucleocapsid protein, two major envelope proteins (GP5 and M), and five minor envelope proteins (GP2a, E, GP3, GP4, and GP5a) [5–7].

PRRSV is divided into type 1 (European; prototype strain Lelystad) and type 2 (North American; prototype strain VR-2332) genotypes, which have only about 50–60% nucleotide sequence identity [8–11]. In China, the classical type-2 PRRSV (C-PRRSV) has been circulating and predominant since 1996 (China: representative strains CH-1a and BJ-4) [12]. However, highly pathogenic PRRSV (HP-PRRSV), with a unique discontinuous 30 (1 + 29)-amino acid (aa) deletion in NSP2, has affected more than 20 million pigs and caused the loss of over 1,000,000 pigs since the initial outbreak in 2006 (China: representative strains HUN4 and JXA1) [13]. NADC30-like HP-PRRSV was first reported in 2015 and contains a unique discontinuous 131 (111 + 1 + 19)-aa deletion in NSP2 (China: representative strains JL580 and HENAN-XINX). According to the global PRRSV classification system based on phylogenetic analysis of the ORF5 sequence, type-2 PRRSV can be divided into nine monophyletic lineages, with each lineage possessing several sublineages [14]. Thus, type-2 PRRSV strains present in China can be divided into four lineages/sublineages: lineage 1 (NADC30-like and JL580-like), lineage 3 (QYYZ-like), sublineage 5.1 (VR-2332-like and BJ-4-like), and sublineage 8.7 (JXA1-like and CH-1a-like) [15]. Among these four lineages/sublineages, sublineage 5.1 and sublineage 8.7 are pandemic throughout China, whereas lineages 1 and 3 emerged in 2010 and are mainly circulating in southern China (e.g., Guang Dong), northeast China (e.g., Ji Lin), and central China (e.g., He Nan); no cases have been found in northwest China.

Genetically heterogeneous recombination between different lineages/sublineages is an important mechanism of PRRSV evolution [16, 17]. Accordingly, in this study, using MARC-145 cells and porcine alveolar macrophages (PAMs), the recombinant PRRSV strain XJzx1-2015, which exhibited a high frequency of mutation, was isolated from a piglet in northwest China; the strain was then characterised, and the evolutionary mechanism was examined.

Materials and methods

Ethics statement

Sampling procedures were performed in accordance with the guidelines of the Animal Ethics Committee of Shihezi University (approval no. AECSU2013-17) and were approved by the Institutional Committee of Post-Graduate Studies and Research at Shihezi University, China. This manuscript did not include any experiments with animals performed by any of the authors.

Sample collection and virus isolation

The XJzx1-2015 strain was isolated, using PAMs and MARC-145 cells, from the lung tissues of piglets from a Shihezi pig farm in the Xinjiang Uygur autonomous region, northwest China in 2015. The piglets showed significant clinical symptoms of PRRS, including high fever (40-42°C), labored breathing, petechiae and lethargy.

Reverse transcription polymerase chain reaction (RT-PCR) and nucleotide sequencing

Total RNA was extracted from virus cultures using an RNAprep Pure Cell Kit (TIANGEN, China), and reverse transcription was carried out using a PrimeScript 1st Strand cDNA Synthesis Kit (Takara Co., Dalian, China). The complete genome of XJzx1-2015 was amplified by LA Taq polymerase (TIANGEN) with nine pairs of primers (Table 1) designed based on HK13 (KF287140). Next, nine overlapping fragments were purified using a Gel Extraction Kit (GENEray Biotechnology, China) and cloned into the pLB-Simple vector (TIANGEN). Finally, recombinant clones were sent for sequencing (Sangon Biotech Shanghai Co., Ltd.), and at least three clones were sequenced per fragment.

Genome sequence alignment

The overlapping sequences of fragments were combined using DNAMAN (version 7.0) to produce the complete genomic sequence of strain XJzx1-2015. The nucleotide and aa sequence identities of the representative strains and XJzx1-2015 were determined using DNAStar Lasergene MegAlin (version 7.1). Multiple alignments of the amino acid sequences of NSP2, ORF2, and ORF5 were performed using Clustal W 2.0.

Phylogenetic analysis

All complete genome sequences of PRRSV reference strains were downloaded from the GenBank database (Table 2). These sequences comprised 60 field strains from various countries, two live attenuated vaccine strains (MLV Resp-PRRS/Repro and PrimePac), and our laboratory strain (XJzx1-2015). Phylogenetic trees of full-genome, NSP2, and ORF5 nucleotide sequences were constructed by the maximum-likelihood method using 100 bootstrap replicates under default settings with six random starts and subtree pruning regrafting improvement (http://www.atgc-montp

Table 1 Primers for generating the full-length genome sequence of XJzx1-2015

Fragment	Sequence of PCR primers (5′-3′)	Position in genome	Length of PCR product
A	ATGACGTATAGGTGTTGGCTCT CATAGCGAATTTCTTTATTCTG	1-1057	1057 bp
B	CCTGAAGGAAACTGCTGGTG CGGTTGGTTTCATTCTGACGG	985-2075	1090 bp
C	GCACAAGGGCGGTCCTGGTT GCTGAGCATCTTGGGCGTGT	1779-3239	1460 bp
D	GGCAAGTCAGACAACCGAACA ACGCCGAGAAGACCCAGAAA	2229-4050	1820 bp
E	GTGGTTATTCTCCAGGTGATTG CCTCCTCCTTGTTTGTTTGAT	3926-6026	2100 bp
F	GCCTATTGGCTGACATCCTC AAACCTGTTCCCATTTATTCC	5872-8568	2696 bp
G	CGGCTTTGAGTTATATGTGC ATTCTTACCAGGACACCAACC	8289-10266	1977 bp
H	ACACGCCAACTCACCAGACCA TTCGCAACCACAGCACAACA	10112-12755	2643 bp
I	TTGGAAGCAGGTGGTGAGTG TTTAATTTCGGCCGCATGGT	12436-15394	2958 bp

ellier.fr/phyml/) [18]. The final graphical representation of the tree was produced using FigTree (version 1.4.3) [19].

Recombination analysis

All type-2 PRRSV reference strains were aligned using Clustal W 2.0 and analysed using Recombination Detection Program (RDP) v4.66 [20] with six different algorithms (RDP, GENECONV, MaxChi, BootScan, SiScan, and 3Seq) [21]. The final results were presented using the RDP method.

Results

Virus isolation

PAMs and MARC-145 cells were used to isolate the virus present in the sample. The PAMs showed detachment and partial lysis after 48 h. In contrast, there were no observable cytopathic effects in MARC-145 cells after incubation for 5 days and 5 passages. The two cell lines were then tested by RT-PCR. The results showed that PAM cultures were PRRSV-positive, whereas the MARC-145 cell cultures were PRRSV-negative. The isolated virus was named "XJzx1-2015". Cell adaption experiments indicated that strain XJzx1-2015 could be isolated using PAMs but was nonviable in MARC-145 cells.

Comparison of full-length genome sequences

The full-length genome sequence of XJzx1-2015 was 15,388 nt, excluding the polyadenosine tail (GenBank accession no. KX689233). Nucleotide sequence alignment revealed that the full-genome sequence of XJzx1-2015 shared the highest level of nucleotide sequence identity with the HK13 strain (91.7%) and showed 82.2%/81.1%, 85.7%, 86.8%/86.9%, 90.2%/90.5%, and 57.9% sequence identity to JL580/HENAN-XINX (lineage 1), QYYZ (lineage 3), BJ-4/VR-2332 (sublineage 5.1), CH-1a/ JXA1 (sublineage 8.7), and Lelystad virus (type-1 prototype strain), respectively (Table 3). When compared with ten representative PRRSV strains, ORF1a and ORF1b of XJzx1-2015 shared the highest nucleotide sequence identity with NB/04 (91.9% and 94.0%, respectively), whereas ORF2–7 shared higher nucleotide sequence identity with the QYYZ strain (90.9%) than with the other type-2 strains (85.5–88.3%). This result revealed that XJzx1-2015 has a close relationship to the NB/04 and QYYZ strains.

Amino acid sequence analysis

An alignment of the amino acid sequence of NSP2 of the XJzx1-2015 isolate with those of representative strains showed that this isolate had a 5-aa continuous deletion corresponding to positions 475-479 in strain VR-2332. However, the deletion in NSP2 was different from the 30-aa

Table 2 PRRSV strains used in this study

No.	Name	Accession number	Year	Country
1	CH-1a	AY032626	1996	China
2	BJ-4	AF331831	2000	China
3	HB-1(sh)/2002	AY150312	2002	China
4	HB-2(sh)/2002	AY262352	2002	China
5	HN1	AY457635	2003	China
6	HUN4	EF6350061	2006	China
7	JXA1	EF112445	2006	China
8	HEB1	EF112447	2006	China
9	HUB1	EF075945	2006	China
10	HuN	EF517962	2007	China
11	HPBEDV	EU236259	2007	China
12	Henan-1	EU200962	2007	China
13	SHH	EU106888	2007	China
14	GD	EU109503	2007	China
15	07HEN	FJ393457	2008	China
16	HB-1/3.9	EU360130	2008	China
17	07NM	FJ393456	2008	China
18	08SDWF	GU168569	2009	China
19	CWZ-1-F3	FJ889130	2009	China
20	CBB-1-F3	FJ889129	2009	China
21	HN2007	EU880437	2009	China
22	GD2007	EU880433	2009	China
23	NB/04	FJ536165	2009	China
24	YD	JF748717	2009	China
25	SX2009	FJ895329	2009	China
26	HN-HW	FJ797690	2010	China
27	09SD	JF268678	2011	China
28	HLM-09	HQ843179	2011	China
39	HLJ-09	HQ843178	2011	China
30	Shaanxi-2	HQ401282	2011	China
31	JXwn06-81c	HQ233604	2011	China
32	QYYZ	JQ308798	2011	China
33	10-10FUJ-2	JQ663547	2012	China
34	GM2	JN662424	2012	China
35	SDA3	JX878380	2012	China
36	HV	JX317648	2012	China
37	DC	JF748718	2012	China
38	WUH4	JQ326271	2012	China
39	11SH-GD	JX235365	2013	China
40	GX1003	JX912249	2013	China
41	GX1002	JQ955658	2013	China
42	WUH3	HM853673	2013	China
43	SRV07	JX512910	2013	China
44	HK13	KF287140	2013	China
45	HK6	KF287135	2014	China
46	Henan-A14	KJ819936	2014	China
47	Henan-A6	KJ534541	2014	China
48	SH1211	KF678434	2014	China
49	HENAN-HEB	KJ143621	2014	China
50	JL580	KR706343	2015	China
51	HENAN-XINX	KF611905	2015	China
52	SCcd16	MF196905	2018	China
53	SCya17	MH324400	2018	China
54	GD1404	MF124329	2017	China
55	XJzx1-2015	KX689233	2015	China
56	JA142	AY424271	2004	USA
57	Prime Pac	DQ779791.1	2006	USA
58	VR-2332	U87392	1992	USA
59	MLV RespPRRS/Repro	AF159149.1	1998	USA
60	MN184A	DQ176019	2006	USA
61	MN184C	EF488739	2008	USA
62	NADC30	JN654459	2012	USA
63	Lelystad virus	M96262	1991	Europe

discontinuous deletion that is a characteristic feature of JXA1-like strains (Fig. 1A).

Sequence comparisons also showed a 2-aa continuous deletion (corresponding to positions 173-174 in strain VR-2332) in GP2 of XJzx1-2015. Moreover, five unique positions (F^{6}, L^{27}, F^{33}, T^{235}, and L^{237}) were found in the predicted signal peptide region (aa 1–40) and endodomain (aa 235–258), and there were two specific positions (D^{45}, Y^{49}) in antigenic epitope 1 that coincided with those in QYYZ-like and NADC30-like strains. However, no specific positions were found in epitope 2 or the transmembrane (TM) helix domains (Fig. 1B).

Through multiple alignment of the GP5 aa sequences of XJzx1-2015 and the PRRSV reference strains, four unique positions were found in the signal peptide (F^{10}, H^{13}, and S^{14}) and HVR2 (hypervariable region; G^{58}). Additionally, 12 specific positions were found in the signal peptide (S^{25}, I^{26}), decoy (S^{30}), HVR1 (G^{33}), primary neutralising epitope (PNE; Y^{38} and S^{39}), TM1 (C^{66}), TM2 (H^{102}), TM3 (F^{117} and I^{124}), T cell epitope 1 (V^{128}), and T cell epitope 2 (I^{152}), which coincided with the QYYZ and GM2 (QYYZ-like) strains. GP5 possessed two major epitopes: the decoy epitope (DE; aa 27–30) and PNE (aa 37–45) regions [22, 23]. Residues of $V^{27}L^{28}A^{29}N^{30}$ in the VR2332 strain, $V^{27}L^{28}V^{29}N^{30}$ in the JXA1-like, and $A^{27}L^{28}A^{29}N^{30}$ in the NADC30-like HP-PRRSV strain were found in the DE region. However, some residues of the XJzx1-2015 strain, such as $A^{27}L^{28}V^{29}S^{30}$, were identical to those in the QYYZ and GM2 (QYYZ-like) strains, while residues In the PNE region were predominantly $S^{37}H^{38}L^{39}Q^{40}L^{41}I^{42}Y^{43}N^{44}L^{45}$ in almost all of the other reference strains. Moreover, the XJzx1-2015, QYYZ, and GM2 strains had a unique position (S^{39}) and contained two conserved glycosylation sites (S^{30} and G^{33}), which differed from the putative glycosylation sites of the other strains (Fig. 1C) [24, 25]. This suggests

Table 3 Nucleotide and deduced amino acid sequence identities of different regions of XJzx1 compared with nine representative PRRSV strains

Sequence	VR-2332		BJ-4		CH-1a		NB/04		QYYZ		HUN4		JXA1		JL580		HENAN-XINX		Lelystad virus	
	nt/%	aa/%	nt/%	aa/%	nt/%	aa/%	nt/%	aa/%	nt/%	aa/%	nt/%	aa/%	nt/%	aa/%	nt/%	aa/%	nt/%	aa/%	nt/%	aa/%
Full-length	86.9		86.8		90.2		91.6		85.7		90.7		90.5		82.2		81.1		57.9	
5'UTR	88.2		86.1		95.2		94.7		93.0		94.7		94.7		90.4		84.0		49.5	
ORF1a	84.2	84.2	84.0	83.8	79.0	89.0	91.9	91.2	71.8	83.4	91.4	90.9	91.2	90.8	82.4	82.6	80.1	80.3	51.9	47.9
ORF1b	89.8	95.8	89.8	95.7	92.7	96.8	94.0	97.5	88.5	96.2	93.5	97.3	93.6	97.2	85.1	94.7	85.3	94.2	63.2	68.9
ORF2a	89.3	90.2	88.9	89.8	88.9	89.4	89.2	88.2	92.5	93.3	89.2	88.6	89.2	89.0	86.8	87.0	85.5	88.6	66.1	63.6
ORF2b	92.8	93.2	92.3	91.8	91.9	89.0	92.3	91.8	95.9	95.9	92.3	91.8	92.3	91.8	90.5	91.8	91.4	91.8	74.2	75.7
ORF3	87.6	88.6	87.5	89.0	86.4	88.2	86.4	89.8	86.8	86.6	86.0	87.4	85.8	87.0	84.6	87.0	84.1	83.5	64.8	58.9
ORF4	88.3	89.9	87.9	89.3	88.1	91.6	87.0	90.4	88.1	91.0	87.3	91.6	86.2	89.3	85.3	88.2	84.9	85.4	68.9	68.0
ORF5	83.1	82.0	82.9	82	84.6	83.5	82.6	80.0	92.9	94.5	83.1	82.0	83.1	82.0	85.4	85.0	84.1	84.5	62.8	56.6
ORF6	92.2	96.0	92.2	96.0	90.9	96.6	90.3	96.6	93.7	98.3	90.1	96.6	90.1	96.6	89.7	93.1	89.9	93.1	70.3	79.2
ORF7	92.5	97.6	92.5	97.6	89.2	95.9	90.6	95.9	91.9	95.9	90.3	94.3	90.3	94.3	89.7	91.9	88.4	92.7	66.5	64.2
3'UTR	96.2		96.2		91.7		90.9		92.5		91.7		91.7		92.5		91.7		69.5	

that the GP5 of XJzx1-2015 has functions similar to those of GP5 of QYYZ-like strains.

Phylogenetic analysis

As shown in Fig. 2A, phylogenetic trees based on the full-length nucleotide sequence of the viral genome revealed that XJzx1-2015 and five other isolates (SCcd16, SCya17, GD1404, and SH1211) from China formed a new minor branch within sublineage 8.7, which could be divided into two main branches (JXA1-like and CH-1a-like). Phylogenetic trees based on NSP2 nucleotide sequences showed that XJzx1-2015 belongs to sublineage 8.7 (Fig. 2B) and has a close relationship to the CH-1a-like strains NB/04 and HK13. However, phylogenetic trees based on ORF5 nucleotide sequences revealed that XJzx1-2015 and seven other isolates (QYYZ, GM2, SCcd16, SCya17, GD1404, SH1211, and HK6) belong to the QYYZ-like lineage (lineage 3) (Fig. 2C).

Recombination analysis

Recombination analysis of the complete genome sequence of strain XJzx1-2015 identified one event supported by the RDP (1.449×10^{-34}), MaxChi (9.045×10^{-9}, 4.972×10^{-5}), BootScan (7.632×10^{-34}), SiScan (7.778×10^{-34}), and 3Seq (1.449×10^{-34}) methods. As shown in Figure 3, potential breakpoints were identified with optimal *P*-values based on X^2 analysis by the RDP method and two potential breakpoints at nucleotide positions 14,014 and 14,876, located within ORF5 and ORF7, respectively. These two points separate the genome into three regions (1–14,013, 14,014–14,876, and 14,877–15,787), which are closely related to the NB/04 (CH-1a-like) strain, QYYZ strain, and NB/04, respectively. These findings revealed that XJzx1-2015 was the result of a recombination between lineage 3 and sublineage 8.7 strains circulating in China and suggested that, after introduction from the eastern and central regions of China, the virus gained variation by recombining with local CH-1a-like strains in the northwest region of China. Interestingly, three other related viruses (i.e., SCcd16, SCya17, and GD1404) were also found to be possible recombinants of QYYZ-like strains and other type-2 PRRSV strains.

Discussion

PRRSV is a major viral threat to the Chinese swine industry. During the last two decades, four lineages/sublineages (lineage 1, lineage 3, sublineage 5.1, and sublineage 8.7) of type-2 PRRSV have been reported in China based on the global PRRSV classification system. Among these four lineages/sublineages, lineage 1 (NADC30-like) and sublineage 8.7 (JXA1-like) were the most important variants due to their high pathogenicity; thus, many researchers have studied these lineages. Lineage 3 (QYYZ-like) was first reported in Hong Kong and Taiwan and has primarily circulated in southern China [14]. In recent years, phylogenetic analyses have revealed that lineage 3 viruses (e.g., SH1211, GD1404, SCcd16, and SCya17) re-emerged in eastern China (e.g., Shanghai) [26], southern China (e.g., Guangdong) [27], and southwest China (e.g., Sichuan) [28]. According to this study, the novel PRRSV isolate XJzx1-2015 was first found in northwest China based on genetic mutation analysis of the complete genome sequence.

Genetic differences caused by mutations are significant characteristics of the XJzx1-2015 strain. The higher

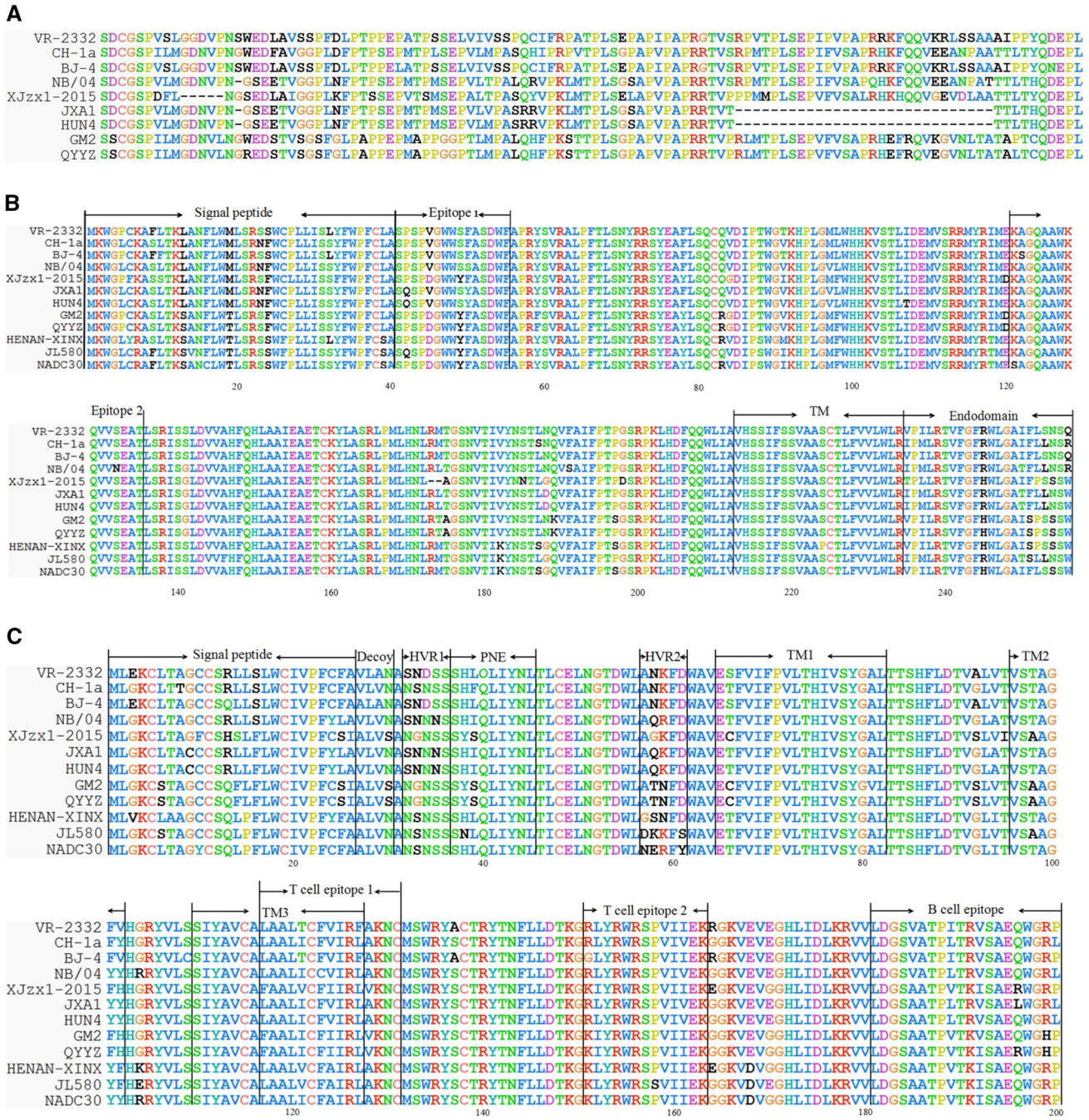

Fig. 1 Alignment of partial NPS2 and complete GP2 amino acid sequences of XJzx1-2015 with those of several representative PRRSV strains. (A) Five continuous amino acid deletions (471–482, corresponding to VR-2332) are shown as a black bar in NSP2 of XJzx1-2015. (B) Two continuous amino acid deletions (173–174, corresponding to VR-2332) are shown as a black bar in GP2 of XJzx1-2015. (C) Multiple alignment of GP5 amino acid sequences of XJzx1-2015 and 10 PRRSV reference strains. Different functional regions of the signal peptide, T cell epitope, B cell epitope, decoy epitope (DE), primary neutralising epitope (PNE), transmembrane (TM), endodomain, and hypervariable region (HVR) are labelled at the top of the alignment

full-length nucleotide sequence of this strain was most similar to those of the HK13 and NB/04 strain (91.7% and 91.6% identity) and was less similar to those of other representative type-2 PRRSV strains (81.1–90.7% identity). It was only 58% identical to that of the Lelystad virus strain. Moreover, unique amino acids were found in some positions in GP2 (F^{6}, L^{27}, F^{33}, T^{235} and L^{237}) and GP5 (F^{10}, H^{13}, S^{14} and G^{58}) of XJzx1-2015. These findings indicated that XJzx1-2015 had experienced a high frequency of mutation, which is uncommon in other type-2 PRRSV strains.

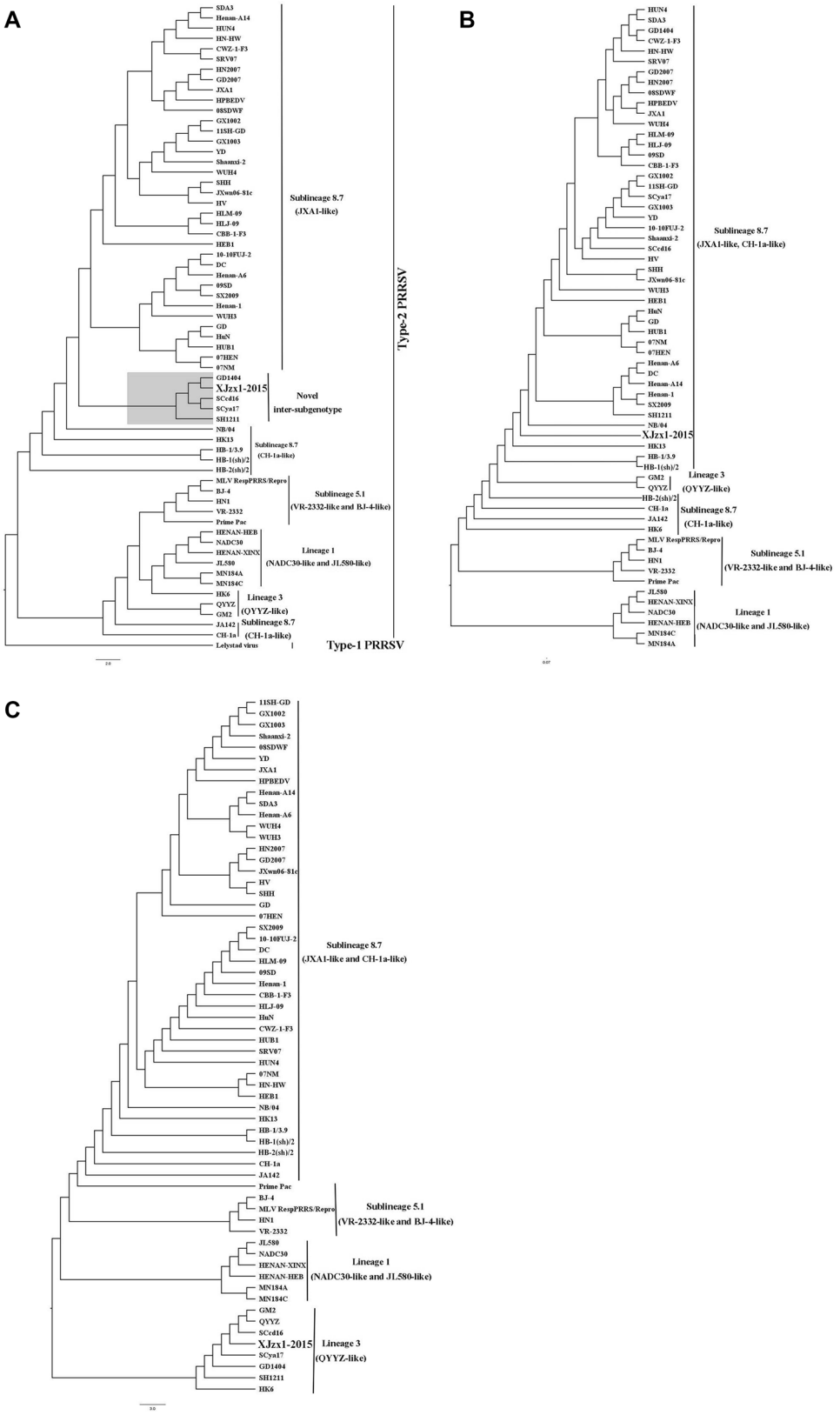

Fig. 2 Phylogenetic analysis based on nucleotide sequences of the full-length genome (A), NSP2 (B), and ORF5 (C) of XJzx1-2015 isolates and PRRSV reference strains. The XJzx1-2015 isolate from this study is shown in a larger font, and the novel intersubgenotype is shaded in dark grey. The evolutionary history was inferred using the maximum-likelihood method. The bootstrap test included 100 replicates. Evolutionary analysis was conducted in PhyML online, and the trees were drawn using FigTree v1.4.3

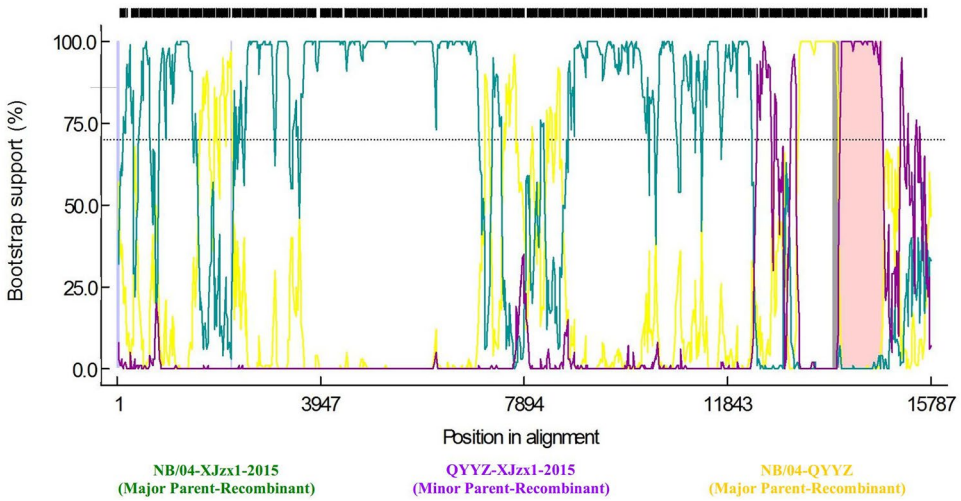

Fig. 3 Recombination analysis of XJzx1 and other PRRSV reference strains. Recombination analysis of strain XJzx1 was carried out using the RDP method in RDP4 software. The *x*-axis shows the position in the alignment, and the *y*-axis shows the bootstrap support (%). The major parent plot (blue line) and minor parent plot (purple line) cross in two areas (pink regions), which represent the recombinant regions. The bootstrap cutoff was 70%

The novel SCcd16, SCya17, and GD1404 strains exhibited rather low full-length nucleotide sequence identity to JXA1 (93.1%, 93.2%, and 95.2%, respectively), VR-2332 (86.9%, 87.0%, and 88.2%, respectively), QYYZ (85.3%, 85.7%, and 87.4%, respectively), and NADC30 (83.6% and 82.0%, respectively) [27, 28].

Genetically heterogeneous recombination between different lineages/sublineages is also an important evolutionary mechanism of PRRSV [29, 30]. Previous studies have shown that genomic recombination of PRRSV greatly affects both replication and pathogenicity. For example, the JL580 (NADC30-like) and novel GD1404 strains, when recombined with the moderately virulent NADC30 and JXA1 (HP-PRRSV) strains and the moderately virulent QYYZ and JXA1 strains, respectively, have pathogenicity similar to that of the HP-PRRSV strains. In this study, the XJzx1-2015 strain was identified as a recombinant derived from lineage 3 QYYZ-like strains (minor parent) and NB/04 strains (major parent, CH-1a-like). Interestingly, this recombination event was similar to that observed in the GD1404 and SH1211 strains, which resulted from recombination of QYYZ-like and JXA1-like strains [26, 27]. Moreover, analysis of nucleotide sequence showed that the NSPs (ORF1a and ORF1b) of the XJzx1-2015 strain have higher similarity to NB/04 (92.7% identity), but the structural proteins (ORF2-7) of XJzx1-2015 strain have higher similarity to the QYYZ strain (90.9% identity) than other type-2 strains (85.5–88.3% identity). Through multiple alignment of the GP5 aa sequences, 12 (S^{25}, I^{26}, S^{30}, G^{33}, Y^{38}, S^{39}, C^{66}, H^{102}, F^{117}, I^{124}, V^{128}, I^{152}) unique positions were found in GP5 that coincided with representative QYYZ-like strains. These results revealed that XJzx1-2015 is closely related to strains NB/04 and QYYZ.

In summary, this study describes and characterises the emergence of a QYYZ-like PRRSV strain that was reported for the first time in northwest China. Subsequent recombination with QYYZ-like and CH-1a-like strains resulted in a high frequency of mutation, unique substitutions, complex recombination, and variable adaption to cells. These results provide evolutionary and epidemiological evidence that the novel strain XJzx1-2015 is spreading throughout northwest China and challenge the previous evolutionary model in which evolution predominantly occurred through recombination. Therefore, we suggest that more surveillance data should be collected and vaccine strategies should be updated in order to prevent future epidemics.

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Compliance with ethical standards

Conflict of interest The authors confirm that this article content is associated with no conflict of interest.

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