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Original article

Genetic variation and recombination analysis of the *NSP11* gene of Porcine reproductive and respiratory syndrome-2 strains in China from 1996 to 2022

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is caused by the PRRS virus (PRRSV). It leads to reproductive disorders and respiratory failure in sows and piglets. As an endoribonuclease, the PRRSV non-structural protein 11 (NSP11) is crucial in replication and assists immune system evasion. NSP11, which is relatively more conserved than NSP2, could potentially cause a new round of PRRSV epidemics, given a major mutation occurs. Here, we aimed to analyze the PRRSV-2 NSP11 genetic diversity in China between 1996-2022. The NSP11 nucleotide sequence was analyzed in 60 PRRSV-2 strains, revealing a similarity of 83.6% - 100%. Similarly, amino acid sequences exhibited homology ranges of 91.0% - 100.0%. Amino acid sequence alignment analysis revealed multiple substitutions in NSP11. NSP11 phylogenetic analysis of 489 PRRSV-2 strains revealed that Lineages 8 and 1 were the predominant strains of PRRSV circulating in China. These two lineages exhibit relatively close genetic relationships. Although unsupported by SimPlot analysis, recombination analysis suggested a potential recombination event in the 489 PRRSV-2 NSP11 sequences. Recombination analysis and amino acid sequence alignment confirmed the PRRSV NSP11 conservation. Our findings provide genetic diversity of PRRSV-2 NSP11 in China and contribute to effective strategy development to prevent and control PRRSV.

Keywords: Porcine reproductive and respiratory syndrome virus, non-structural protein 11, genetic variation, recombination

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Introduction

Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), is a highly contagious viral disease that affects pigs. PRRSV primarily affects pigs, causing respiratory diseases and reproductive failure, including stillbirth, abortion, and mummified fetuses in pregnant sows (Chand et al. 2012). Global swine production has been adversely affected by PRRSV's emergence. In the United States alone, PRRSV has resulted in an estimated annual economic loss of $664 million (Dwivedi et al. 2012). In 1996, PRRSV was first discovered in China (Snijder and Meulenberg 1998), and has since become widespread. In 2006, there was the emergence of a remarkably highly pathogenic of PRRSV (HP-PRRSV) with a detectable deletion of 30 amino acids in NSP2. The outbreak spread rapidly across China, dealing a significant blow to the swine industry (Neumann et al. 2005, Tian et al. 2007).

PRRSV, a member of the Arteriviridae family, is an RNA virus with a positive-sense, single-stranded genome (Montaner-Tarbes et al. 2019). Its genome, approximately 15 kb in length, features a 5' cap structure and a 3' polyadenylated tail (Meulenberg et al. 1993, Nelsen et al. 1999, Wootton et al. 2000). PRRSV isolates can be categorized into two genotypes: PRRSV-1 (European type) and PRRSV-2 (North American type) (Meng et al. 1995, Kuhn et al. 2016). The nucleotide sequence homology of various genes between the two genotypes ranged between 55.0% - 70.0% with amino acid sequence homology of 50% - 80% (Darwich et al. 2011). Recently, PRRSV-1 and PRRSV-2 have been classified as two separate species, Betaarterivirus suid 1 and Betaarterivirus suid 2, respectively (https: //talk.ictvonline.org). There are 11 open reading frames (ORFs) included in the genome of PRRSV. The PRRSV genome encode 8 structural proteins and 16 non-structural proteins (NSPs), some of which are NSP1α, NSP1β, NSP2N, NSP2TF, NSP2-6, NSP7α, NSP7β, and NSP8-12 (Kappes and Faaberg 2015). Based on the sequence of ORF5, the global classification system for PRRSV identifies nine lineages for categorizing PRRSV-2 strains (Shi et al. 2010a, Shi et al. 2010b, Paploski et al. 2019). PRRSV-2 strains from China are predominantly classified as Lineage 5. The BJ-4 strain, first reported in 1996, also belongs to this lineage. Other notable strains of this lineage include ATCC VR2332 and Resp-PRRS MLV. In 2010, a new strain known as QYYZ-like strains or Lineage 3 strains arose in China, primarily affecting the southern regions, specifically Fujian and Guangdong provinces (Wenhui et al. 2012, Lu et al. 2015). These are represented by the FJFS, GM2, and QYYZ strains (Guo et al. 2018). Lineage 1 strains, with representatives including NADC30, CHsx1401, and HNyc15, appeared in China in 2013 and originated from the prevalent NADC30 strain in the United States (Li et al. 2016; Paploski et al. 2021; Zhou et al. 2015).

NSP11 consists of 223 amino acids and functions as an endoribonuclease (Nedialkova et al. 2009). Recent studies revealed that the crystal structure of NSP11 exhibits an asymmetric dimeric conformation consisting of subdomains A and B. Subdomain A is responsible for ribonuclease activity, while subdomain B contributes to the overall structural configuration (Shi et al. 2016). PRRSV NSP11 contains a highly conserved nidoviral endoribonuclease U (EndoU) domain at its C-terminal region (Sun et al. 2016). Recombination events primarily occur within NSP2, NSP11, NSP12, GP2, GP3, and GP5 (Yu et al. 2020). To understand its pathogenic mechanisms, it is crucial to comprehensively research PRRSV NSP11.

Here, 489 PRRSV-2 NSP11 sequences were collected between 1996–2022. An analysis was conducted by constructing a phylogenetic tree using these sequences. The nucleotide and amino acid sequences of NSP11 from representative strains of various lineages were also analyzed for homology. Furthermore, A comparative analysis was conducted on the amino acid sequences of NSP11, and recombination analysis was performed on the 489 PRRSV-2 NSP11 strains. Our findings contribute to understanding the epidemiology and genetic diversity of PRRSV-2 infections. Furthermore, we offer valuable insights that can contribute to the monitoring and control of PRRSV-2 in China.

Materials and Methods

Dataset

PRRSV-2 strains (n=489) were selected from nucleotide database. These included 476 Chinese and 16 American PRRSV-2 strains. The selection criteria for the strains were as follows: strains from different years between 1996-2022 with the vaccine and American PRRSV-2 strains as reference strains for sequence analysis. Sixty PRRSV-2 strains were selected from the 489 strains based on the following criteria to ensure representation of each lineage: commonly cited representative strains and equal representation.

NSP11 nucleotide homology analysis

To assess the resemblances between NSP11 nucleotide sequences of diverse PRRSV lineages, we analyzed the reference strain data utilizing the ClustalW tech-

nique within the MegAlign function of DNAStar software (version 7.0, Madison, WI).

NSP11 amino acid homology analysis and alignment

ClustalW analysis was utilized to analyze the similarity between amino acid sequences of NSP11. Multi-sequence alignment analysis was executed using the Megalign function.

Phylogenetic analysis

The phylogenetic analysis of NSP11 was conducted utilizing the sequence information of the reference strain. Sixteen American PRRSV-2 strains were used as outgroups to construct the rooted phylogenetic trees. Initially, the ClustalW method was employed to conduct a comparison in the DNAStar software MegA-lign function. Subsequently, the neighbor-joining (NJ) method of MEGA software (version 7.0.26, Mega Limited, Auckland, New Zealand) was used with 1000 bootstrap replicates. The phylogenetic tree was annotated using the online software Interactive Tree Of Life (https: //itol.embl.de).

Recombination analysis of NSP11

When analyzing and detecting potential recombination events with the RDP software (version 4.0, USA), several methods are employed, including RDP, GeneConv, SiScan, MaxChi, BootScan, Chimaera, and 3Seq. A genetic recombination event is considered when it is identified by four or more of these methods with a P-value of less than 0.05. Upon meeting this criterion, strains were considered recombinant. Recombination events were confirmed through the use of SimPlot (version 3.5.1).

Results

Nucleotide homology

Representative strains from different PRRSV-2 lineages and 60 prevalent strains were selected to explore the genetic diversity of NSP11 during the evolution of PRRSV. NSP11 sequences of the 60 strains were obtained for nucleotide sequence homology analysis. The results suggest that the NSP11 nucleotide homology among various PRRSV-2 strains varied significantly, ranging from 83.6% to 100.0%. The lowest homology (83.6 %) was observed between strains 15LN3-2015 and JS2021NADC34-2021. In contrast, the highest nucleotide sequences were found in the following strains (100.0% homology): CC-1-2006 and MLV RespPRRS Repro-1999, S1-2006, YN-2011; CH2002-2009 and CH2003-2009, CH2004-2009; DQYQC2-2016 and GDYDZZZ-2016; GDZQ-2017 and rJXA1-R-2020; and QY2010-2010 and QYYZ-2011. Nucleotide homology analysis across different lineages revealed that Lineage 1 displayed nucleotide homology ranging between 83.6% - 100.0%. Lineage 3 exhibited a range of 83.7% - 100.0%, Lineage 5 varied from 84.0% - 100.0%, and Lineage 8 varied from 89.7% - 100.0%. Notably, within Lineage 1 strains, the most significant disparity in nucleotide homology was detected, whereas the smallest difference was observed among Lineage 8 strains (Fig. 1).

Amino acid sequence homology

The genetic diversity in the PRRSV NSP11 at the amino acid level was also investigated in addition to nucleotide similarity analysis. This analysis was conducted using 60 representative strains from four lineages. The aim was to gain insight into the evolutionary relationships among the amino acid sequences within each lineage (Fig. 2). The NSP11 of different PRRSV-2 strains exhibited amino acid homology ranging between 91.0% - 100.0%, with the lowest homology (91.0 %) between strains SCcd17-2017 and MN184A-2005. Strains demonstrating remarkably similar amino acid sequences with 100.0% homology included CC-1-2006, BJ-4-2000, MLV RespPRRS Repro-1999, S1-2006, YN-2011, and SD1-100-2009; CH2002-2009, CH2003-2009, and CH2004-2009; and LN-2007, rJXA1-R-2020, and TJ-2006. Amino acid homology analysis was performed for each lineage. The results demonstrated that the amino acid homology within Lineage 1, 3, 5, 8 ranged between 91.0% - 100.0%, 91.5% - 100.0%, 91.5% - 100.0%, and 95.1% - 100.0%, respectively. The differences in amino acid homology were largest between strains of Lineage 1 and smallest between strains of Lineage 8. Interestingly, Lineages 3 and 5 exhibit identical amino acid homology. The amino acid homology analysis results were similar to those of the nucleotide homology analysis, indicating relatively high homology and a low frequency of amino acid substitutions.

Amino acid sequence alignment

The amino acid sequences were translated from the nucleotide sequences of NSP11 obtained from 60 selected strains for multiple sequence alignment and comparative analysis. Amino acid mutations were identified at various positions in the NSP11 sequence of PRRSV-2. Specifically, mutation sites were detected between positions 41-46aa, 92-98aa, 123-126aa, and 194-202aa, while single amino acid mutations or no

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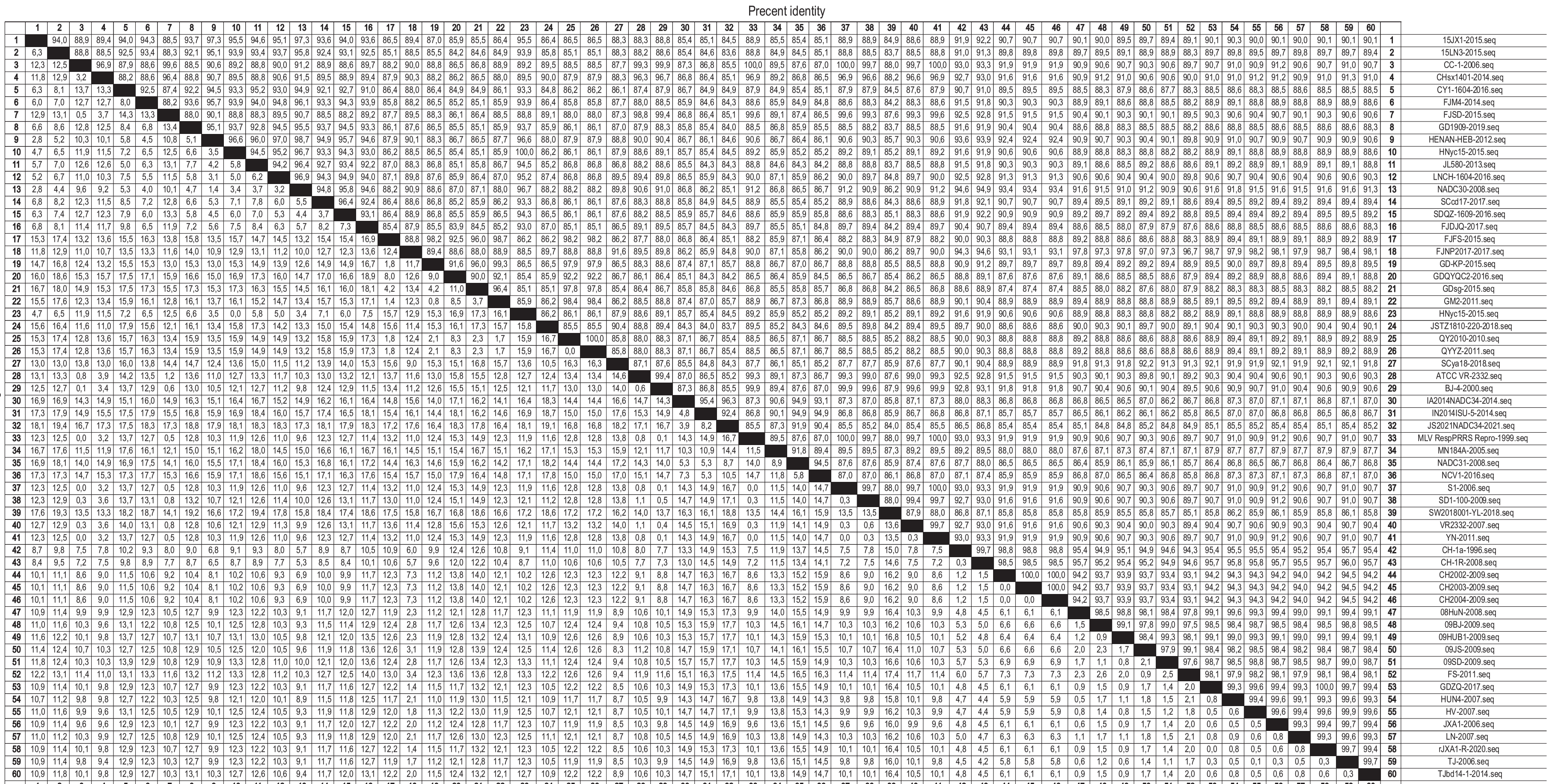

Fig. 1. The MegAlign feature in DNAStar software (version 7.0, Madison, WI) can be utilized to assess nucleotide homology among 60 porcine reproductive and respiratory syndrome (PRRSV) NSP11 sequences from lineages 1, 3, 5, and 8 using the Clustal W method. Among selected strains (n=60), representative strains presented from different PRRSV lineages, including NADC30-2008, CHsx1401-2014, and JL580-2013 for lineage 1; QYYZ-2011, GM2-2011, and FJFS-2012 for lineage 3; BJ-4-1996, VR2332-1992, and RespPRRS MLV-1998 for lineage 5; and CH-1R-2008, CH-1a-1996, and JXA1-2006 for lineage 8.

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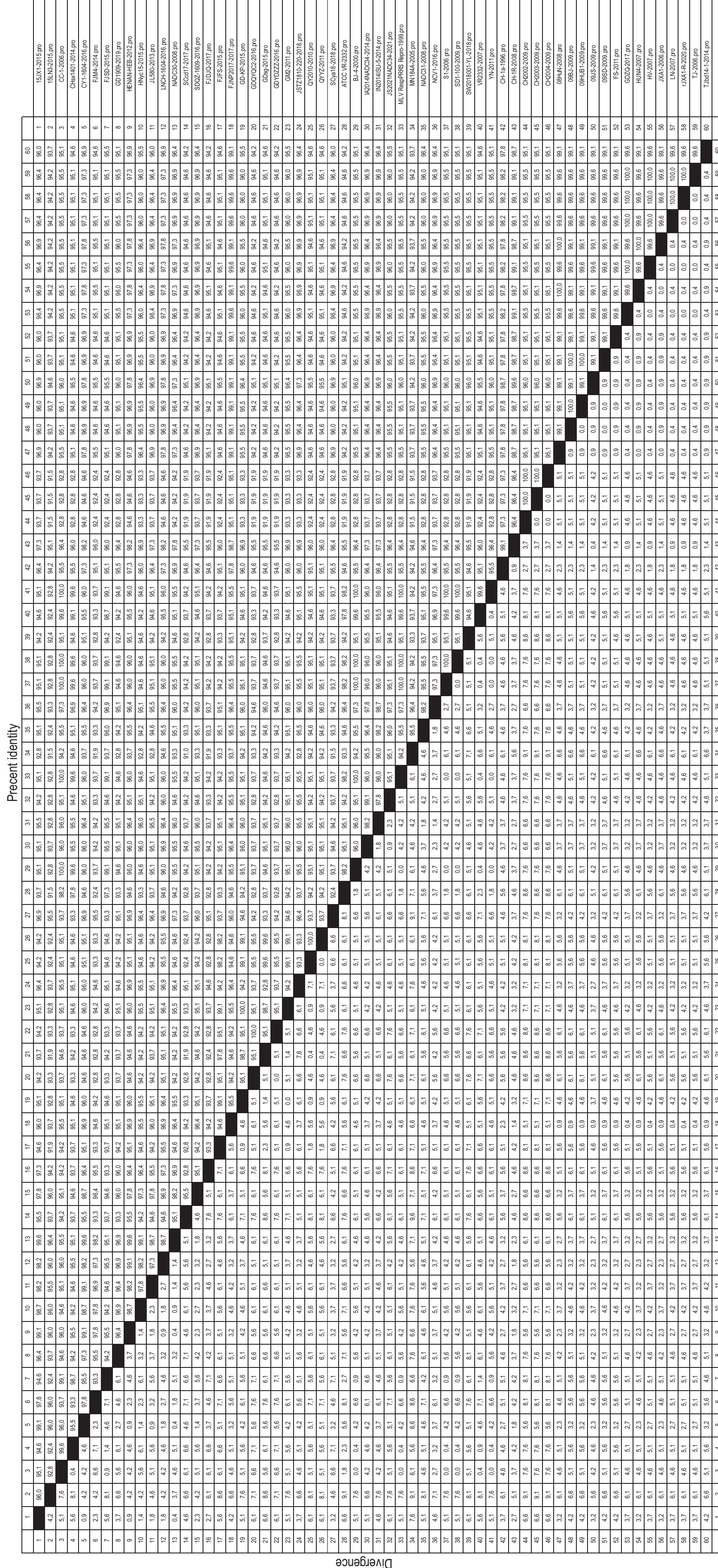

Fig. 2. The analysis of amino acid homology was conducted on 60 sequences of NSP11 from PRRSV in lineages 1, 3, 5, and 8. To analyze the amino acid sequence homology of PRRSV NSP11, we utilized the ClustalW method available in the MegAlign function of DNAStar software (version 7.0, Madison, WI). The 60 selected strains included representative strains from different PRRSV lineages, including NADC30-2008, CHsx1401-2014, and JL580-2013 for lineage 1; FJFS-2012, GM2-2011, and QYYZ-2011 for lineage 3; BJ-4-1996, RespPRRS MLV-1998, and VR2332-1992 for lineage 5; and CH-1R-2008, CH-1a-1996, and JXA1-2006 for lineage 8.

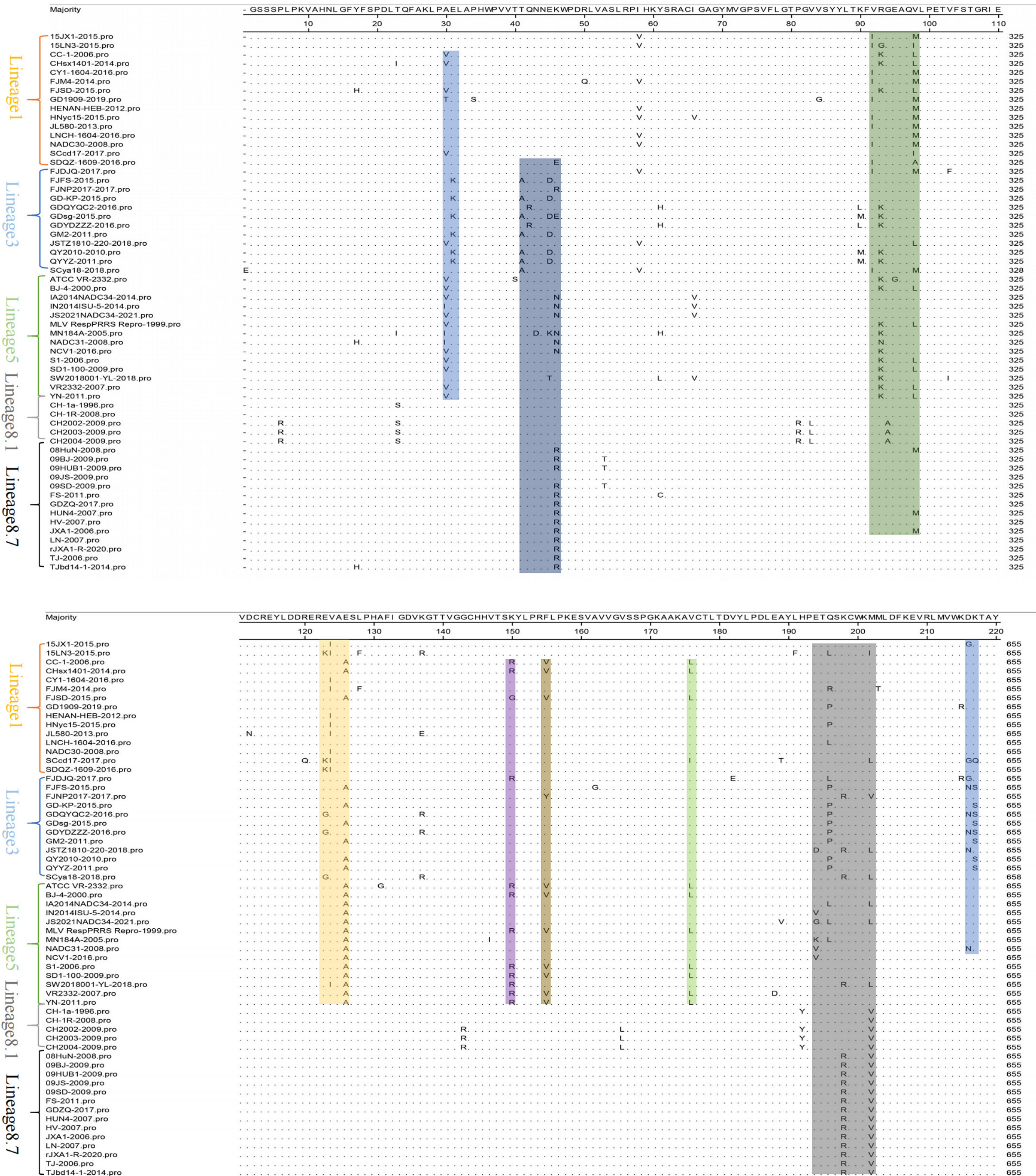

Fig. 3. The NSP11 amino acid sequences of 60 PRRSV strains aligned. The amino acid sequence of NSP11 was analyzed using the MegAlign sequence alignment editor in DNAStar software (version 7.0, Madison, WI). Blue, green, and gray represent regions with multiple amino acid mutations, whereas yellow indicates that only lineages 1, 3, and 5 have amino acid mutations.

mutations were observed at other positions. In this study, we identified several amino acid mutations in different lineages. In position 41–46, Lineage 8.7 showed a K46→R46 mutation, while Lineage 3 showed T40→A40 and E45→D45 mutations. In addition, the yellow region in Fig. 3 shows that multiple amino acid sites which underwent mutations in Lineages 1, 3, and 5. For example, Lineages 1, 3, and 5 showed E127→A127 and I162→V162 mutations, whereas half of the 14 strains belonging to Lineage 5 showed K150→R150, F156→V156, and V176→L176 amino acid mutations (Fig. 3). Notably, a mutation at position 202 was found, as shown in Fig. 3, where a mutation from M202 to V202 was observed in all the strains belonging to Lineage 8.

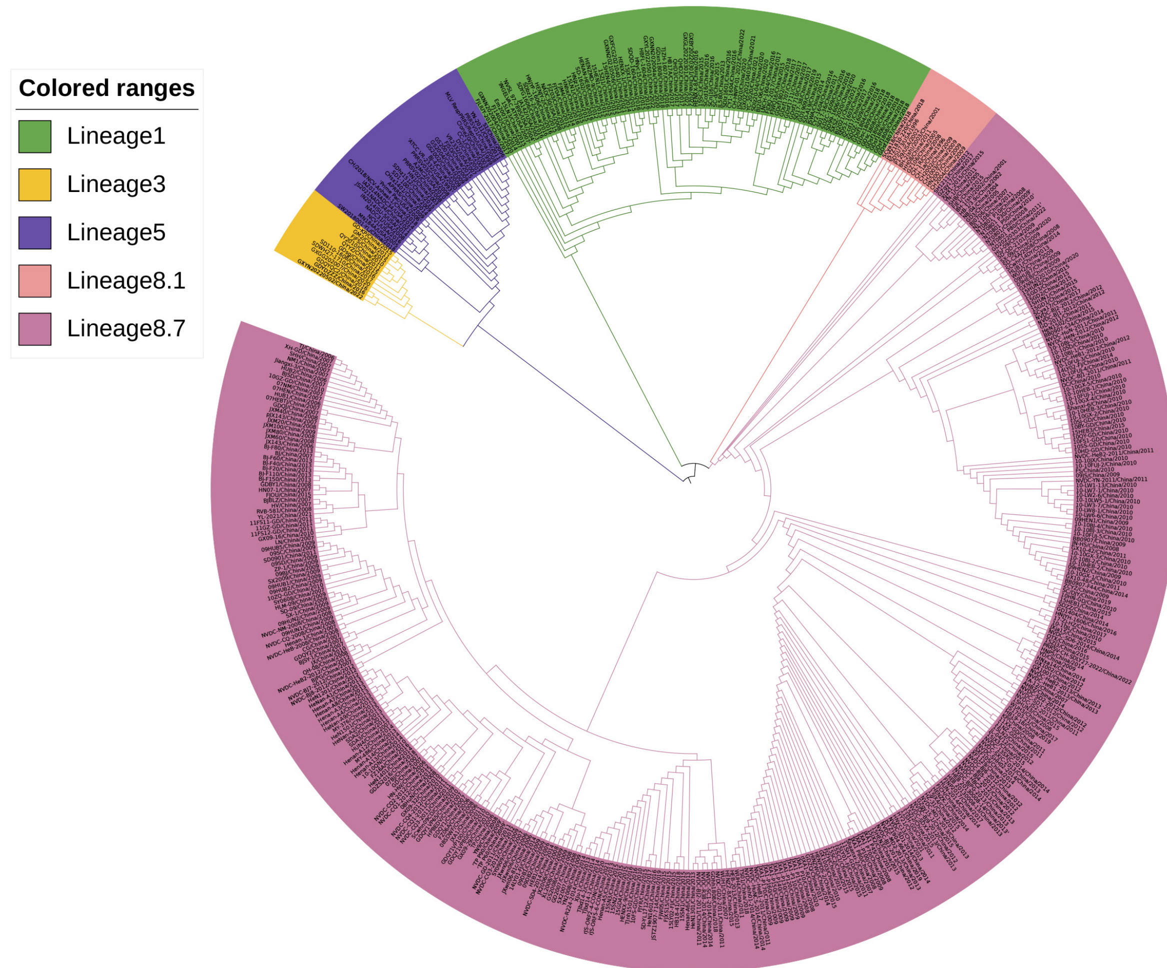

Fig. 4. With the utilization of MEGA software (version 7.0.26, Mega Limited, Auckland, New Zealand), a NJ phylogenetic tree was created with default parameters and 1000 bootstrap replicates. The analysis incorporated 476 NSP11 sequences from Chinese PRRSV-2 strains, along with a reference group of 16 PRRSV-2 strains from the United States.

Phylogenetic analysis

Under the guidance of global PRRSV classification system and NSP11 sequence information in GenBank database, we made phylogenetic analysis of NSP11 sequences of 489 PRRSV-2 strains. The findings indicate that the dominant PRRSV-2 strains in China are primarily found within four Lineages: 1, 3, 5, and 8. Lineage 8 can be further divided into sublineages 8.1 and 8.7 (Fig. 4). Lineages 3 and 5 exhibited relatively close genetic distances. Lineages 3 and 8 exhibited relatively large genetic distances from each other. Lineages 1 and 8 had close genetic distances and are the main lineages of the currently prevalent Chinese PRRSV-2 strains.

Recombination analysis

To gain a more comprehensive understanding of NSP11 recombination after the entry of PRRSV-2 into China, recombination analysis of 489 NSP11 genes was conducted using the RDP software (version 4.0, USA) (Fig. 5). The analysis revealed one potential recombination event (Table 1), which was supported by four algorithms with high credibility and a statistical significance of $p<0.05$. Potential recombination events occurred between two strains, PRRSV02-2018 from Lineage 5 and 15GD1-2015 from Lineage 8. The recombinant strain SH1211-2012 had 15GD1-2015 as the major parental strain and PRRSV02-2018 as the minor parental strain. SimPlot (version 3.5.1) was used to verify this potential recombination event, and the analysis indicated the absence of any observed recombination (Fig. 6).

Discussion

High variability in the PRRSV genome can lead to immune evasion, which promotes PRRSV spread. Currently, the NADC34-like strain has spread to 10 Chinese provinces. Therefore, it is important to monitor and control PRRSV-2 to prevent PRRS occurrence (Zhao et al. 2022). While there has been considerable

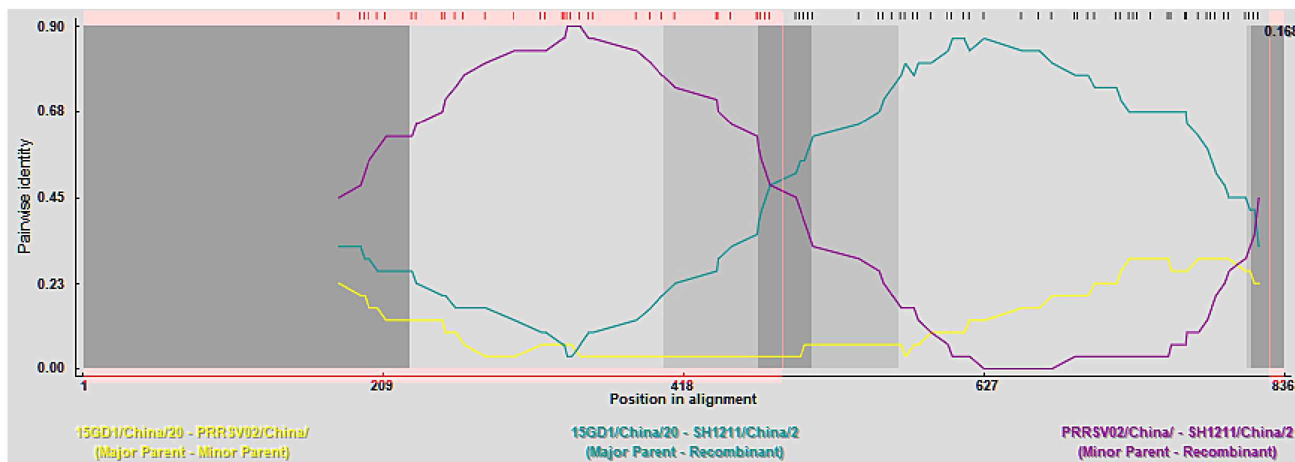

Fig. 5. NSP11 recombination event prediction of SH1211-2012 by RDP 4.0 (version 4.0). The alignment position is represented by the horizontal axis, whereas pairwise identity is depicted by the vertical axis.

Table 1. Statistical analysis of recombination events in PRRSV 2 *NSP11* gene.

Recombinant strains (lineages)	Main parental strain (lineages)	Minor parental strain (lineages)	Recombinant breakpoint	Recombination analysis method
SH1211-2012(8.7)	15GD1-2015(8.7)	PRRSV02-2018(5)	228~810 (404~568)	MaxChi (P = 3.827×10^{-04})
				Chi-maera (P = 4.071×10^{-04})
				SiScan (P = 1.489×10^{-04})
				3seq (P = 3.880×10^{-04})

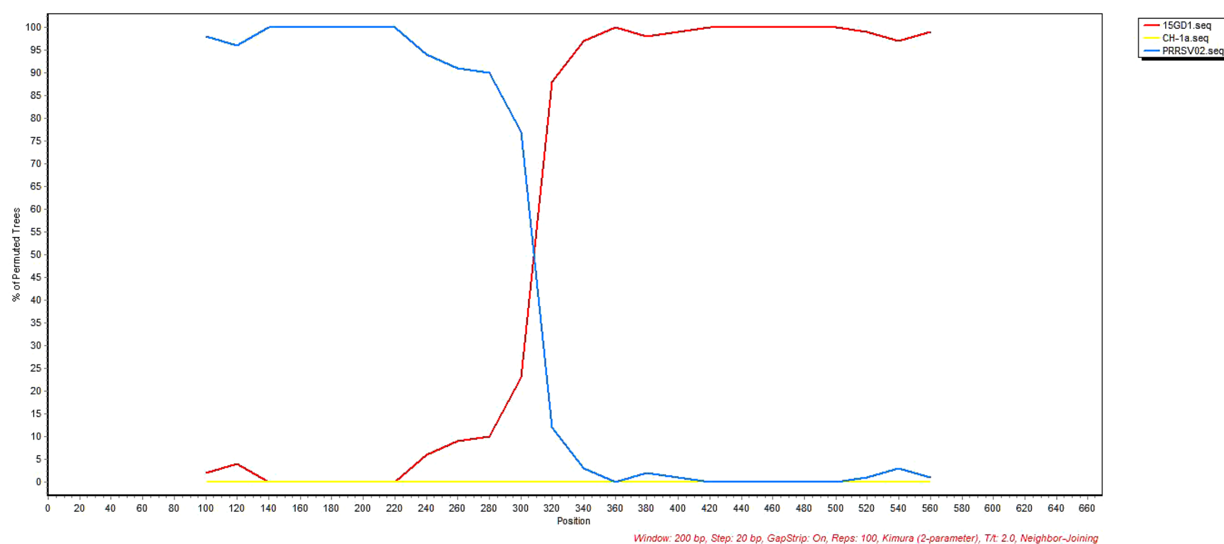

Fig. 6. NSP11 recombination event verification using SimPlot (version 3.5.1) as detected by RDP (version 4.0). In the graph, position is represented by the horizontal axis, while the percentage of the arrangement tree is represented by the vertical axis. Various strains of PRRSV are denoted by different colors. The main and minor parental strains are represented by the red and blue lines, respectively.

focus on studying amino acid changes in the highly variable regions of NSP2 and ORF5, the attention given to NSP11, a relatively conserved NSP in PRRSV, is limited. Regardless, mutations occurring in the conserved regions of NSP11 could potentially contribute to a new outbreak of PRRS. This emphasizes the importance of studying and monitoring NSP11 genetic variations.

To investigate the PRRSV-2 NSP11 genetic evolution, 60 PRRSV-2 NSP11 sequences were analyzed to determine their nucleotide and amino acid homology. The findings revealed that the nucleotide and amino

acid homologies among the PRRSV-2 NSP11 sequences varied between 86.3% - 100.0% and 91.0% to 100.0%, respectively. These results indicated a notable degree of homology in both the nucleotide and amino acid sequences of NSP11, with a low amino acid substitution frequency. Sha et al. (2023) evaluated the genetic evolution of the conserved gene PRRSV-2 NSP4 in China between 1996-2021. The results demonstrated significant nucleotide and amino acid conservation across the 123 PRRSV-2 NSP4 sequences. In their study, nucleotide and amino acid homologies also ranged between 86.3% - 100.0% and 91.0% - 100.0%, respectively; consistent with the nucleotide and amino acid homology analysis results for PRRSV-2 NSP11 in this study. Lineages 3 and 5 exhibited high nucleotide homology. We posit that there may be low genetic variation in PRRSV-2 NSP11, complicating PRRSV's host immune response evasion. However, additional research is required to fully understand the precise mechanisms underlying this phenomenon. Predominant circulating Chinese PRRSV strains belong to Lineages 1 and 8. Lineage 1 exhibited the highest nucleotide sequence divergence among the strains, indicating that these variants may have a higher likelihood of recombination and mutation. Lineages 1 and 8 exhibit a propensity for recombination and adaptation, contributing to their long-term evolutionary and mutational dynamics. As shown in Fig. 1, there was a high degree of nucleotide sequence similarity (91.2%) in the *NSP11* gene between the highly pathogenic Chinese PRRSV strain JXA1-2006 and the low-virulence NADC30-like strain CHsx1401-2014. This indicates that despite the differences in virulence, the *NSP11* gene remains highly conserved at the nucleotide level. The nucleotide sequences of NSP11 from PRRSV Lineages 3 and 5 showed significant similarity, suggesting little genetic variation in NSP11 between these two lineages. This finding indicates that NSP11 was conserved during the evolution of PRRSV.

In 2006, a new strain known as HP-PRRSV, emerged in China. These strains were characterized by a 30-amino acid deletion in the NSP2 protein. To understand the genetic variations in NSP11 amino acid sequences following the introduction of PRRSV-2 in China, 60 strains were selected for amino acid sequence alignment. This study found amino acid mutations in the NSP11 amino acid sequence at positions 41-46aa, 92-98aa, 123-126aa, and 194-202aa. A mutation at position 202 was observed, where all strains belonging to Lineage 8 showed a mutation M202→V202. This may be one reason why these strains were classified as Lineage 8. In contrast to NSP2 and GP5, which showed significant genetic variation, no regions with amino acid insertions or deletions were found in NSP1 in our study, and there was a lower likelihood of amino acid mutations. Overall, PRRSV-2 NSP11 remained relatively conserved during its evolution in China, with key amino acid sites showing resistance to mutations. Several studies have supported the view that certain sites in NSP11, such as Cys112, His144, Asp173, Lys180, and Tyr219, are essential for its Deubiquitinating Enzyme (DUB) activity, as elucidated by Dong et al. (2018). Additionally, the amino acid residues Ser74 and Phe76 in NSP11 have been identified as critical factors for Interleukin-17 (IL-17) production and viral replication (Wang et al. 2019). Shi et al. (2016) further demonstrated that the presence of Ser at position 74 and Phe at position 76 is crucial for NSP11 dimer formation, which enables NendoU function. These studies also indicated that the key amino acid sites responsible for NSP11 dimerization are highly conserved. These findings offer new insights for antiviral drug development (Zhang et al. 2017). Furthermore, these key amino acid sites in NSP11 that are crucial for its biological functions have not undergone any mutations, deletions, or insertions, indirectly highlighting their conservation.

As a conserved gene, PRRSV-2 NSP11 can be used to assess the evolutionary relationships and prevalence of various lineages in China by phylogenetic analysis. These findings indicate close genetic proximity between Lineages 1 and 8 in the evolutionary tree. Recently, Lineage 1 has been spreading rapidly in China, with clinical detection rates similar to those of Lineage 8 strains. Currently, these two lineages are the most prevalent lineages in China. They are more prone to recombination, allowing them to effectively evade immune detection. According to the results of phylogenetic tree analysis, the newly discovered PRRSV-2 strains in 2022 were distributed in Lineages 1 and 8, which aligned with the prevalence of PRRSV-2 strains in China. Lineages 3 and 5 were clustered within the same major clade and were genetically close. Lineage 3 primarily emerged after 2010, whereas Lineage 5 emerged as early as 1996. However, both the lineages have low clinical detection rates in China.

In this study, the constructed phylogenetic tree was similar to the system phylogenetic tree based on the NSP2 gene reported by Yin et al. (2021) in that Lineage 5 had a closer genetic relationship with Lineage 3. However, there was a difference in the system phylogenetic analysis results reported by Yin et al. (2021), who showed that Lineage 3 has a closer genetic relationship with Lineage 8. Likewise, Zhang et al. (2023) conducted an NJ analysis of 122 PRRSV-2 NSP2 sequences and found that Lineages 1 and 8 were dominant, whereas Lineages 3 and 5 were genetically close to each other, which is consistent with our findings. However, unlike

in our study, Zhang et al. (2023) found that Lineages 1 and 8 were genetically distant. The discrepancies between our phylogenetic reconstruction and previous analyses may be due to the use of different PRRSV NSP-coding genes, sequence datasets, and analytical methods. Our study included a larger sample of PRRSV strains spanning a longer period than previous studies. Inferred evolutionary tree analyses resolved the genetic distances between lineages, offering insights into the genetic diversification of PRRSV-2 NSP11 in China.

Since 1996, PRRSV has been continuously spreading and has undergone more than 20 years of recombination and mutations, resulting in the emergence of various new strains (Jiang et al. 2020). Lineages 1 and 8 are dominant in PRRSV-2 in China, with NADC30-like strains undergoing frequent recombination with other strains, thus changing their virulence and evading vaccine-mediated immunity (Tian 2017). Multiple recombination events have been demonstrated in Lineages 1 and 8, which help strains adapt and improve their survival rates during long-term evolution and mutation. The Em2007 strain had a unique 68 amino acid deletion in NSP2. Em2007 is a spontaneous recombination between an attenuated PRRSV vaccine strain CH-1R and an HP-PRRSV strain, WUH1 (Li et al. 2009). Zhao et al. (2015) further confirmed that NADC30-like PRRSV strains, introduced from North America, spread to several Chinese provinces and recombined with the local HP-PRRSV strains. These strains have high pathogenicity and a mixed genetic background that differs from that of local strains. HP-PRRSV emergence in China in 2006 highlighted the significance of recombination in the genetic evolution of PRRSVs. PRRSV NSP11 plays a crucial role because of its highly conserved and unique NendoU domain, which can affect both host innate immunity and viral replication. Consequently, the genetic evolution and recombination analyses of NSP11 are important. NSP11 recombination analysis of the 489 PRRSV strains revealed a potential recombination event supported by four algorithms with high credibility ($p<0.05$). This study suggests that a recombination event may have occurred between the strains PRRSV02-2018 of Lineage 5 and 15GD1-2015 of Lineage 8. The main parental strain of the recombinant strain SH1211-2012 was 15GD1-2015, whereas the minor parental strain was PRRSV02-2018. However, this event was not detected by SimPlot validation. There have been no reports of recombination events involving NSP11. Conserved genes are less likely to undergo recombination; however, monitoring gene conservation during the genetic evolution of PRRSV should not be ignored.

NSP11, a crucial protease of PRRSV, has been identified as playing a pivotal role in viral propagation. In their study, Sun et al. (Sun et al. 2014) found that NSP11 delayed the S phase of the cell cycle. Subsequent analysis revealed that the NendoU activity of NSP11 is responsible for processing RNA, ultimately influencing viral replication. These findings suggest that targeting NSP11 may be a viable strategy to control PRRSV replication. Serological tests demonstrated the effectiveness of the NSP11 recombinant protein in stimulating neutralizing antibody production against PRRSV. Recombinant NSP11 enhances the immunogenicity of pigs and displays high specificity for detecting antigens in PRRSV-positive pig farms (Contreras-Luna et al. 2022).

NSP11 conservation is a factor that ensures the fundamental PRRSV life characteristics and functions. Given the complex genetic diversity of PRRSV, NSP11 is a key target for drug and diagnostic reagent development owing to its minimal changes and significant biological functions. Continuous monitoring of NSP11 genetic variations is crucial for understanding the prevalence dynamics of PRRSV-2 in China, enabling timely prevention and control of future PRRS outbreaks.

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