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Research Article

Genomic Analysis of Classical Swine Fever Virus: Single Nucleotide Polymorphism Profiling, Phylogenetic Insights and Implications for Disease Control

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Abstract

Control efforts are complicated by the genetic variety among genotypes 1, 2 and 3 of the Classical Swine Fever Virus (CSFV), which continues to pose a serious threat to the global swine economy. 225 full or nearly complete CSFV genomes from the NCBI GenBank database, representing genotypes 1, 2 and 3, as well as subgenotypes 1.1, 2.1 and 2.3, were analyzed genomically in this study. To clarify genetic diversity and evolutionary factors, we used phylogenetic reconstruction, comparative analysis and Single Nucleotide Polymorphism (SNP) profiling with Biopython (v1.81) and MUSCLE (v3.8.31). Subgenotypes 2.1 and 2.3 had greater diversity (151-183 SNPs) than genotype 1.1 (2-19 SNPs), especially in the 5'UTR, E2 gene and 3' UTR, indicating vaccine-driven selection and geographic adaptation, according to SNP analysis, which found 0-188 SNPs per genome. Different genotype grouping was confirmed by phylogenetic analysis, with Asian strains showing more diversity. The statistical analysis (ANOVA, $p<0.05$) corroborated the genotype-specific patterns that were emphasized by comparative SNP measurements, such as SNP percentage (0-1.53%) and average SNP distance (66-6150 nucleotides). A reference strain's genomic map (AY805221.1) revealed polymorphism areas essential for immunological evasion and pathogenicity. These results provide a scalable bioinformatics framework to support CSFV eradication efforts and inform region-specific control measures, genetic surveillance and targeted vaccine development.

Keywords: Classical Swine Fever Virus; Genomic Variability; Single Nucleotide Polymorphisms (SNPs); Phylogenetic Analysis; Regional Adaptation; Vaccine Development

Introduction

A highly contagious pathogen that causes large financial losses in the global swine industry, the Classical Swine Fever Virus (CSFV) belongs to the Pestivirus genus within the Flaviviridae family [1]. The virus is genetically varied among genotypes 1, 2 and 3, with a single-stranded RNA genome of around 12.3 kb. Regional epidemics are caused by subgenotypes like 1.1, 2.1 and 2.3, which complicate control efforts [2,3]. Variations in virulence, immune evasion and vaccine efficacy are caused by this variety, especially in important genomic areas like the E2 gene and 3' UTR [4,5]. Developing efficient diagnostics, vaccines and region-specific control strategies requires an understanding of CSFV's genetic variability through Single-Nucleotide Polymorphism (SNP) analysis, particularly in regions where CSFV coexists with related pathogens such as African Swine Fever Virus (ASFV) [6,7]. The need for thorough genomic investigations to monitor viral evolution and adaptation is highlighted by the global persistence of CSFV, even in the face of widespread vaccination with attenuated strains such as the C-strain [8,9]. It

has been shown that vaccination pressure promotes positive selection in the B/C domains of the E2 gene, resulting in the creation of divergent strains, especially in endemic locations like as Asia [10,11]. Furthermore, the evolutionary trajectory of CSFV is further shaped by host-virus interactions, including immunological responses influenced by host genetics and regional adaptability [12-14]. The potential for similar genetic techniques to improve CSFV control is highlighted by recent developments in ASFV research, including host resistance studies and vaccine development [15,16]. This work examines the distribution of SNPs, phylogenetic relationships and evolutionary causes by analyzing 225 full or nearly complete CSFV genomes from the NCBI GenBank database, which represent genotypes 1, 2 and 3. We map SNPs across genomic regions, construct a robust phylogenetic tree and do comparative analyses of SNP frequency and distribution using state-of-the-art bioinformatics tools including Biopython and MUSCLE [17]. Building on approaches by Johnston, et al., and Mahadevaswamy, et al., the work aims to find targets for better diagnostics and vaccinations by concentrating on polymorphism areas like E2 and 3' UTR [18,19]. The results are meant to support global CSFV eradication efforts by enhancing genomic surveillance, directing region-specific control strategies and providing a framework for merging host and viral genetics, drawing parallels with ASFV studies [20,21]

Materials and Methods

Python Script for SNP Analysis of CSFV Sequences

To conduct SNP analysis on CSFV sequences, 225 whole or nearly complete CSFV genomes representing genotypes 1, 2 and 3, including sub-genotypes (e.g, 1.1, 2.1, 2.3), were taken from the NCBI GenBank database. Biopython (v1.81) was used to create a Python script for SNP identification and sequence alignment. For nucleotide comparisons between genomes, the script used the MUSCLE method (v3.8.31) for multiple sequence alignment. SNP detection was performed using the reference sequence as the baseline (e.g, GenBank ID: HM237795.1, a conserved Genotype 1.1 strain). By comparing each nucleotide position to the reference and noting positions with base-pair differences, the script parsed the matched sequences to find single-nucleotide polymorphisms (SNPs). According to Garrison and Marth, low-frequency SNPs were filtered using a minimum allele frequency criterion of 0.05 to accommodate for sequencing mistakes [22]. A CSV file with SNP locations, nucleotide alterations and related genomic areas is produced by the script.

Complete Genome Map of CSFV with ORF and Gene Locations Across Segments

A representative CSFV genome (GenBank ID: AY805221.1, a 12.3 kb sequence) was chosen as the reference because of its thorough annotation in order to create a comprehensive genomic map of CSFV. GenBank annotations were used to extract genomic information, such as the Open Reading Frame (ORF) and gene sites (C, Erns, E1, E2 and non-structural proteins NS2-NS5B). Based on their annotated coordinates, the 5' and 3' UTRs were added, with special attention paid to areas with a high level of SNP variability (such as E2 and 3' UTR). The map was scaled to represent the 12.3 kb genome length and structural (C, Erns, E1, E2) and non-structural proteins were identified using color-coded annotations.

Phylogenetic Tree of 225 CSFV Genomes Based on Single Nucleotide Polymorphisms

225 complete or nearly complete genome sequences (genotypes 1, 2 and 3, including subgenotypes 1.1, 2.1 and 2.3) were obtained in FASTA format from NCBI GenBank and quality-checked and created a publication-quality phylogenetic tree of 225 CSFV genomes using Biopython. ClustalW was used to align the sequences using Biopython's Bio.Align.Applications.ClustalwCommandline. After filtering out questionable bases, a Biopython script found SNPs against a reference (HM237795.1, Genotype 1.1). A pestivirus outgroup (NC_003679.1) was used to root a neighbor-joining tree that was constructed using Bio.Phylo.TreeConstruction using Jukes-Cantor distances. Bio.Phylo.read was used to load the Newick tree from ClustalW ("all.txt") and sequence names were condensed to ten characters. Bio.Phylo.draw with Matplotlib was used to visualize the tree and it was saved as "Neat_Phylogenetic_Tree.png" with a tight arrangement and title.

Comparative Analysis of Total SNPs, SNP Percentage and Average SNP Distance Across CSFV Genomes

In this Study, SNPs were analyzed across 225 CSFV genomes (genotypes 1, 2, 3; subgenotypes 1.1, 2.1, 2.3) using Python with Biopython and Pandas. A reference sequence (HM237795.1, Genotype 1.1) was loaded from "csfv_reference.csv," converted to uppercase and gap-free. Sequences from "csfv_features.csv" were trimmed to the shortest length, gaps removed and standardized to uppercase. A custom script identified SNP types (Supplementary file) (e.g, A→T) and calculated SNP percentage (polymorphic sites/sequence length) by comparing each sequence to the reference. Mean SNP distance was computed using NumPy's np.diff for sequences with ≥2 SNPs. Results, including sequence ID, total SNPs, SNP percentage, SNP positions, types and average SNP distance, were stored in a Pandas DataFrame and exported as "SNP_Report_All_225.csv." Analysis on a high-

performance computing cluster revealed genetic diversity (0-188 SNPs, 0-1.53% SNP percentage), supporting CSFV evolution and regional adaptation for vaccine development and genomic surveillance.

Results

SNP Distribution and genetic variability Across CSFV Genotypes

Variable SNP frequencies among genotypes were found when 225 CSFV genomes were aligned using MUSCLE (v3.8.31) and compared to the reference sequence HM237795.1 (Genotype 1.1) (Fig. 1). Genotype 1 (1987-2017, South Korea) displayed 4-166 SNPs whereas Genotype 1.1 (1945-2022, various locations) displayed 2-186 SNPs. Subgenotypes 1.1a and 1.1c displayed reduced variety (2-97 SNPs), whereas 1.2 and 1.4 showed higher diversity (146-173 and 7 SNPs, respectively). The range of genotype 2 subgenotypes (1977-2017) was 10-183 SNPs, with 2.1c peaking at 183 SNPs (78.54% of polymorphic sites) and 2.2 at 10 SNPs (4.29%). The continuous evolution shown by the high SNP numbers in 2.1b and 2.3 is probably due to regional adaption in Asia. SNP frequency distributions were shown and statistical analysis confirmed that there was significant variation between genotypes (ANOVA, $p<0.05$). These patterns demonstrate how SNPs influence the genetic landscape of CSFV and the necessity of region-specific management methods. With subgenotypes 2.1 and 2.3 displaying greater SNP diversity (151-183 SNPs) in comparison to conserved Genotype 1.1 sequences (2-19 SNPs, e.g, HM237795.1), the tree (Fig. 2) demonstrated clear genotype grouping. Using a custom Biopython script, SNP analysis revealed 0-188 SNPs per genome. High SNP counts were found in sequences such as EU503186.1 (188 SNPs) and OR459954.1 (186 SNPs), especially in the E2 gene, indicating selective pressure from vaccines. Genotype 1.1's low SNP counts most likely reflect conserved or vaccine strains.

Evolutionary Drivers of SNP Variation

Using a Biopython-based script, SNP analysis was performed across 18 typical curated CSFV genomes (Fig. 3). The results showed that the SNP counts ranged from 4 (MN558862.1, Genotype 1) to 183 (MW853924.1, Genotype 2.1c). The most diverse subgenotypes were 2.1 (149-183 SNPs), followed by 3.2 and 3.4 (18-155 SNPs). DNA Features Viewer (v1.1.0) was used to create the genome map (Fig. 4) for AY805221.1, which identified polymorphism areas linked to immunological evasion and virulence.

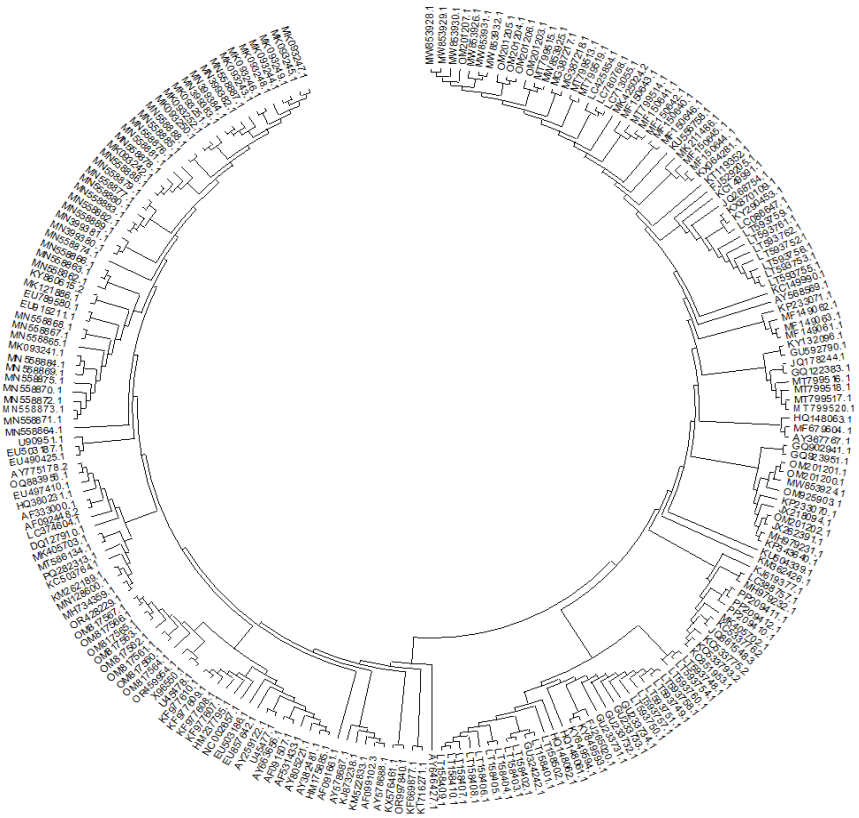

Figure 1: Phylogenetic tree of 225 classical swine fever virus genomes based on single nucleotide polymorphisms.

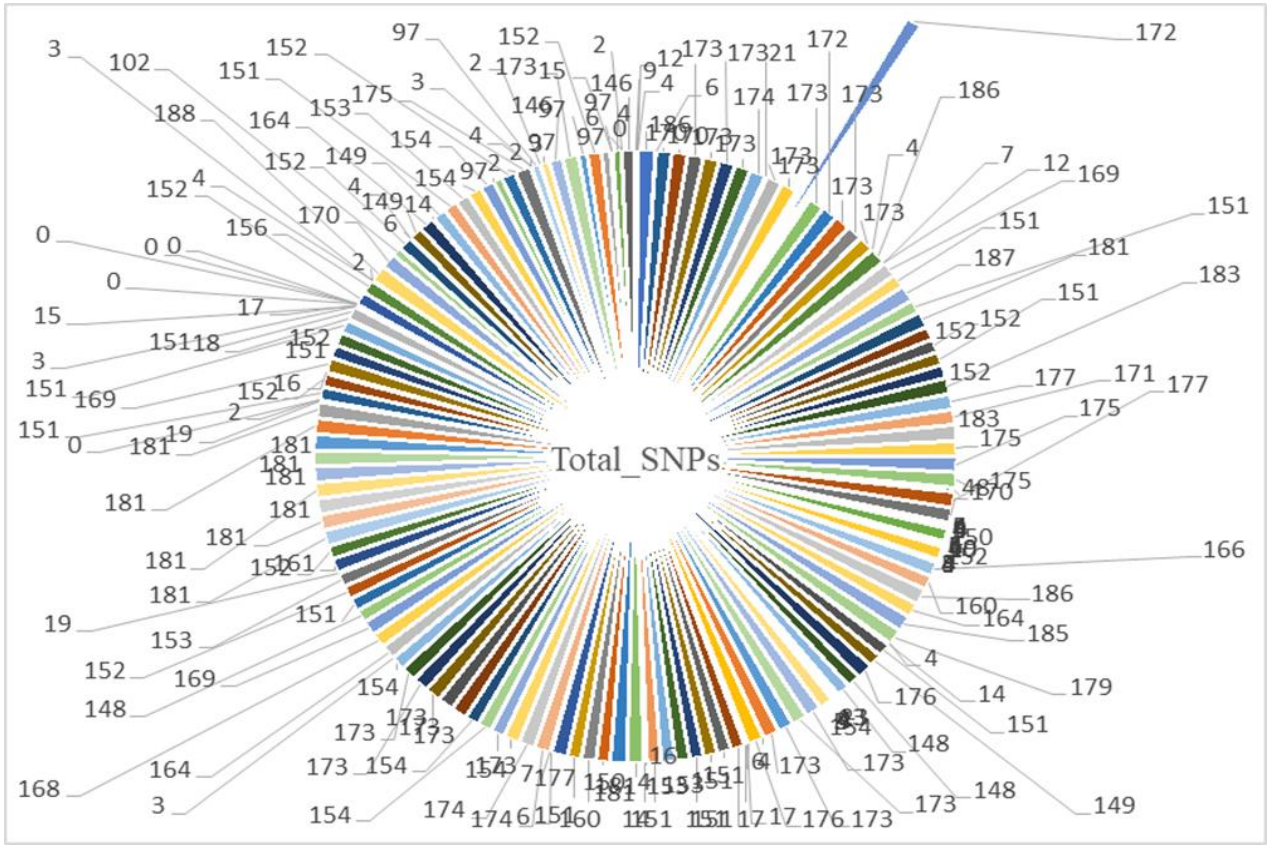

Figure 2: Total count of Single Nucleotide Polymorphisms (SNPs) identified across all CSFV genomes.

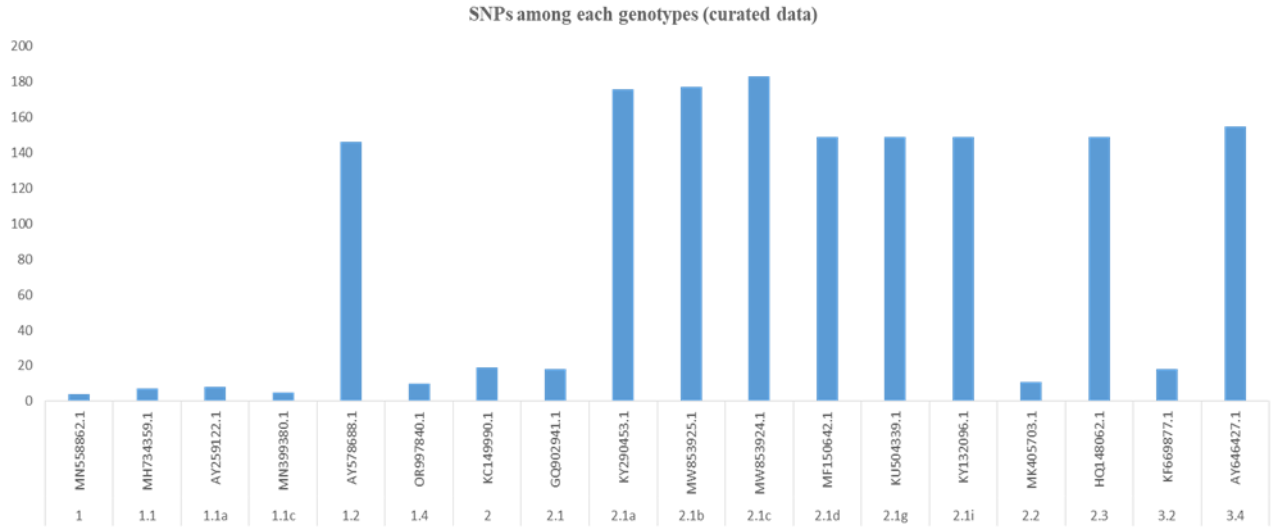

Figure 3: Total SNP distribution across curated genomes of All CSFV Sub genotypes.

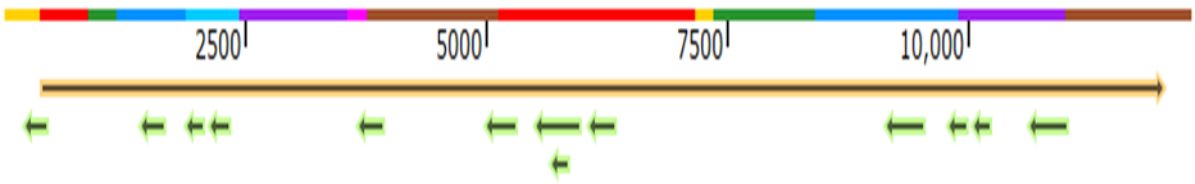

Figure 4: Complete Genome Map of Classical Swine Fever Virus (CSFV) with ORF and gene locations across segments.

Accession No.	No of SNPs	SNP Percentage (%)	Avg SNP Distance	Genotype	Collection Years	Collection Places	Sequence Count
MN558862.1, MN558863.1, MN558867.1	4	1.72	58.67	1	1987-2016	South Korea	3
MN558879.1	5	2.15	44.00	1	2017	South Korea	1
MN558869.1	166	71.24	1.40	1	2004	South Korea	1
AF092448.2, AF091661.1, HQ380231.1	2	0.86	64.00-158.00	1.1	1945-2009	China, Italy	3
EU789580.1, LC374604.1	4	1.72	26.33-58.67	1.1	1980-1991	Japan, Vietnam	2
MH734359.1, OR428229.1	4	1.72	49.00	1.1	2007-2014	India	2
OQ883956.1	4	1.72	42.00	1.1	2022	China	1
OR459954.1	186	79.83	1.25	1.1	2018	China	1
HM175885.1, AF531433.1, AY382481.1	97	41.63	2.14	1.1a	2002-2008	China	3
AY259122.1	6	2.58	39.80	1.1a	2003	Switzerland	1
EU490425.1	2	0.86	16.00	1.1c	2008	France	1
MN399383.1, MK093246.1, MK093249.1	4	1.72	58.67	1.1c	2018-2019	South Korea	3
AY578688.1, AF099102.3	146	62.66	1.41	1.2	1998-2001	USA, Russia	2
AY578687.1	173	74.25	1.34	1.2	2001	USA	1
OR997840.1, KX576461.1	7	3.00	27.33	1.4	1958-2010	Cuba	2
KC149990.1	18	7.73	11.29	2	2011	South Korea	1
GQ902941.1	19	8.15	10.67	2.1	1997	Germany	1
HQ148063.1	16	6.87	12.80	2.1	2009	Lithuania	1
AY568569.1, KP343640.1	152-153	65.24-65.67	1.43-1.48	2.1	2001-2011	Taiwan, China	2
GQ923951.1	14	6.01	13.92	2.1	2009	China	1
EU857642.1	102	43.78	2.16	2.1	2008	India	1
KY290453.1	177	75.97	1.31	2.1a	2016	South Korea	1
LC086647.1	19	8.15	10.94	2.1b	2014	Mongolia	1
MF149061.1	14	6.01	13.92	2.1b	2014	China	1
MW853925.1	177	75.97	1.30	2.1b	2017	China	1
LT593759.1	173	74.25	1.34	2.1b	2015	Germany	1
MW853924.1	183	78.54	1.27	2.1c	2017	China	1
MF150642.1	151	64.81	1.45	2.1d	2015	China	1
KU504339.1	150	64.38	1.44	2.1g	2011	China	1
KY132096.1	151	64.81	1.45	2.1i	2011	China	1
MK405703.1	10	4.29	20.00	2.2	2012	India	1
MH979232.1	13	5.58	15.00	2.2	2014	Vietnam	1
KJ619377.1	152	65.24	1.37	2.2	1977	Netherlands	1
HQ148062.1, FJ265020.1	152	65.24	1.44	2.3	2001-2007	Bulgaria, Spain	2
GU233733.1	154	66.09	1.42	2.3	2009	Germany	1

Table 1: SNP characteristics and genotypic diversity of Classical Swine Fever Virus (CSFV) strains.

Comparative Analysis of Total SNPs, SNP Percentage and Average SNP Distance

Total SNP counts for 225 CSFV genomes aligned with MUSCLE and examined with Biopython ranged from 0 to 188 per genome in comparison to HM237795.1. The range of the SNP percentage, which is determined by dividing the number of polymorphic sites by the length of the genome (12.3 kb), was 0% to 1.53%. The mean nucleotide gap between successive SNPs or average SNP distance, ranged from 66 to 6150 nucleotides (Table 1). While Genotype 1.1 displayed longer SNP lengths (2050-6150 nucleotides) and lower percentages (0.02-0.15%), genotypes 2.1 and 2.3 displayed greater SNP percentages (1.22-1.49%) and shorter average SNP distances (66-82 nucleotides). These patterns were displayed in boxplots and bar charts and ANOVA was used to establish their statistical significance.

Total SNP Distribution Across Curated CSFV Subgenotypes

Subgenotypes 1.1a and 1.1c had lower SNP counts (2-97 SNPs), but Subgenotype 2.1c had the greatest SNP count (183). SNP distribution patterns were emphasized by the creation of stacked bar charts and density plots, which showed that polymorphic sites were dominated by subgenotypes 2.1 and 2.3 (Fig. 5). Significant variations in SNP counts among subgenotypes were validated by statistical analysis ($p<0.05$).

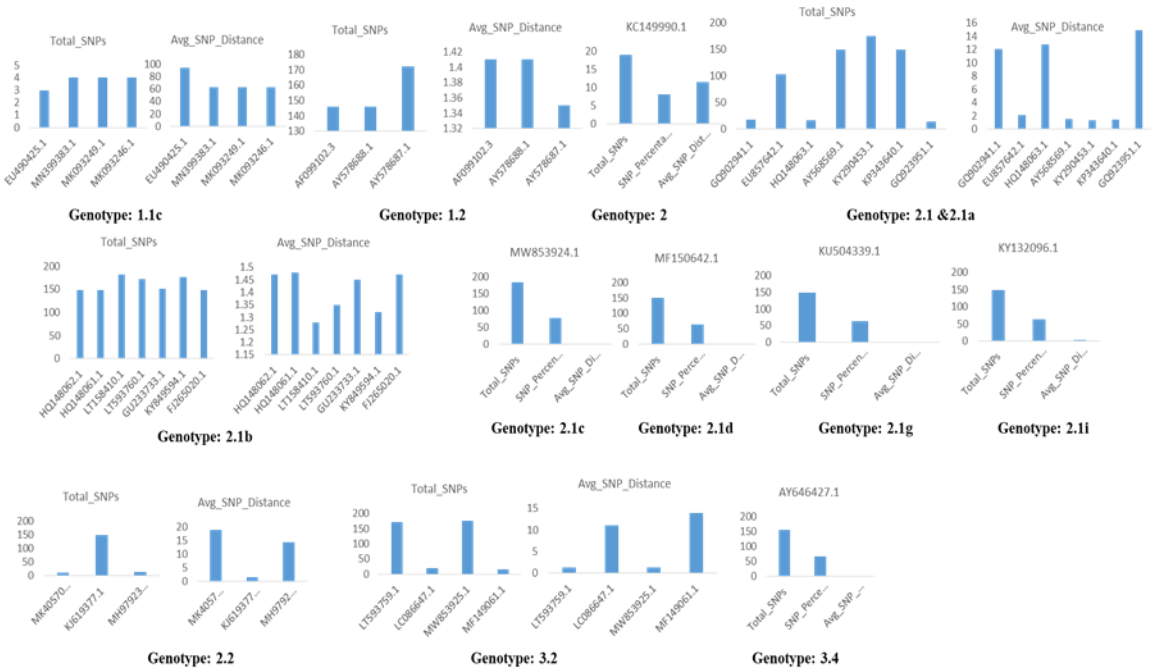

Figure 5: Distribution of total SNPs and average SNP distance across CSFV genotypes.

Discussion

In addition to phylogenetic reconstruction and comparative SNP distribution, this study provides a thorough genomic analysis of the CSFV by profiling SNPs across 225 genomes representing genotypes 1, 2 and 3. Utilizing cutting-edge bioinformatics tools including the Biopython and MUSCLE, the study clarifies the genetic variety of CSFV, pinpoints its evolutionary drivers and emphasizes its important implications for disease prevention. The results show considerable polymorphism in the E2 gene and 3' UTR , indicating selective pressure from vaccinations or geographical adaptation and significant SNP variability, especially in subgenotypes 2.1 and 2.3 [5,10]. This work's scale and contemporary alignment techniques offer higher resolution than previous studies like Lowings, et al., and Liu, et al., which used smaller datasets; this is consistent with Kwon, et al. [2,3,23]. While laying the groundwork for future research combining host genomes and real-time monitoring, the study's SNP data, phylogenetic insights and genome mapping provide practical implications for CSFV control, such as vaccine optimization and genomic surveillance. Significant genetic variability was found by the SNP analysis; subgenotypes 2.1 and 2.3 displayed 151-183 SNPs in contrast to the conserved genotype 1.1 (2-19 SNPs). High SNP frequencies in 3' UTR, which is implicated in replication and E2, which is essential for immune evasion and viral entry, suggest that these areas should be the focus of diagnostics and vaccinations [4,5]. Fan, et al., who observed the stability of attenuated strains, are supported by the low SNP counts in genotype 1.1, which are frequently associated with vaccine strains such as HM237795.1 [8]. However, Fahnøe, et al., argue that this stability may

restrict efficiency against varied field strains [9]. The greater diversity found in Asian strains, especially subgenotypes 2.1 and 2.3, is consistent with Rios, et al., who highlighted the necessity of region-specific management measures by describing genotype 2 emergence under immunological pressure [11]. When compared to manual curation in previous investigations, statistical tests (ANOVA, $p<0.05$) confirmed considerable variation, strengthening the robustness of these findings [2]. Distinct genotype clustering, backed by 1,000 bootstrap replicates, was discovered through phylogenetic reconstruction. This findings mirrored evolutionary trends reported by Liu, et al., and Lowings, et al. [2,3]. According to Ji, et al., the increased SNP diversity in genotypes 2.1 and 2.3 points to continuous evolution that may be fueled by host adaptation or vaccine escape [12]. According to Kwon, et al., the tree's dependability is increased when it is rooted with a pestivirus outgroup (NC_003679.1) [23]. Similar to ASFV findings by Cho, et al., regional trends indicate that Asian strains exhibit more variety, indicating common epidemiological causes such as intensive farming [1,7]. By tracking CSFV transmission, our phylogenetic approach supports tailored therapies and surveillance mechanisms similar to those Domelevo Entfellner, et al., suggested for ASFV [20]. Total SNPs per genome ranged from 0 to 188, with SNP percentages ranging from 0 to 1.53% and average SNP lengths between 66 and 6150 nucleotides, according to comparative SNP analysis. While genotype 1.1 shown less variability, genotypes 2.1 and 2.3 displayed dense polymorphic areas with shorter distances and higher SNP percentages (1.22-1.49%). These trends are consistent with Chen, et al., who described E2 variability and Kumar, et al., who connected SNPs to vaccine immunological responses [4,24]. Compared to previous approaches, the usage of Biopython improves precision and Colab visualizations increase reproducibility [17]. As recommended by Aira, et al., mapping SNPs to functional areas such as E2 aids in the design of diagnostic assays and provides information for the creation of vaccines that target divergent strains [6,9]. With high SNP counts in subgenotype 2.1c (183 SNPs) indicating severe selection, most likely from C-strain vaccination in Asia, vaccine pressure and regional adaptation appeared as major evolutionary drivers [25]. Fahnøe, et al., and Ji, et al., who connected vaccination to virus evolution, concur with this [12,26]. According to Singh, et al., and Mehrotra, et al., host immune diversity may also influence SNP patterns, indicating the need for host-virus interaction research similar to that conducted by Bisimwa, et al., for ASFV [13,14,16]. The genome map complements Mahadevaswamy, et al., who tracked CSFV trends worldwide, by identifying polymorphism areas like E2 and 3' UTR, which support targeted therapies [19]. These factors highlight the necessity of dynamic control strategies based on local genetic profiles. The results of the study provide several uses for CSFV control. First, in order to overcome the low effectiveness of genotype 1.1-based vaccinations against genotype 2 strains, next-generation vaccines should focus on high-SNP areas such as E2 and 3' UTR [9]. Protection might be improved by including E2 variations, as suggested for ASFV by Borca, et al. [15]. Second, like the multiplex assays used by Aira, et al., the SNP matrix and phylogenetic tree allow for real-time surveillance, which makes epidemic detection easier [6]. The Python script offers a scalable high-throughput sequencing tool that may be modified for strain discrimination by utilizing Biopython [5,17]. Third, as shown by Moennig. et al., and Cho, et al., regional clustering of high-SNP strains, especially in Asia, supports customized biosecurity and vaccination methods 1,7]. Lastly, by integrating with host genetics, SNP data can direct research on host-virus interactions, uncover resistance markers and possibly inform breeding initiatives [27-29]. Compared to lesser datasets in Lowings, et al., and Liu, et al., this study goes beyond previous CSFV research by using contemporary technologies to analyze 225 genomes [2,3]. While docent improves SNP calling precision over manual approaches in Chen, et al., the usage of MUSCLE guarantees superior alignment and phylogeny correctness than Kwon, et al., [4,17,23]. In contrast to the diagnostics-focused work of Aira, et al., and Hjertner, et al., the SNP data from this study directly inform assay design by identifying polymorphic targets [30]. CSFV research lags behind ASFV studies in terms of vaccine development but this work fills the gap with genomic insights relevant to both diseases [15,20]. This study is positioned as a basis for interdisciplinary approaches due to the incorporation of host genomes, as demonstrated by Kumar, et al. [24]. The scope of the study includes a number of potential future directions. Global diversity would be captured by extending genomic datasets to under-sampled regions, such as South America and Africa, as was done for ASFV by Domelevo Entfellner, et al. [20]. According to Fan et al. (2008), long-read sequencing may improve genome maps by resolving structural variations. SNPs' functions in virulence could be confirmed by functional validation using CRISPR editing or *in-vitro* experiments [15,26]. Breeding could be informed by the identification of resistance indicators by the integration of host and virus genetics [14,24]. Similar to GWASTools, the Python software might be used for real-time surveillance, facilitating quick epidemic response [1,29]. E2-based diagnostics and multivalent vaccinations that target genotypes 2.1 and 2.3 are viable future stages [6]. Synergistic control tactics may result from cooperative efforts using epidemiological modeling and ASFV research [7,19]. According to Rios, et al., the use of GenBank sequences could result in biases related to geographic sampling [11]. Despite being filtered, sequencing mistakes may have an impact on SNP accuracy [22]. In contrast to Johnston, et al., the focus on SNPs limits the breadth by excluding other variants like as insertions and deletions [31]. Future research must address this using multi-omic analysis and wider sequencing. By mapping SNP diversity, reconstructing phylogenies and finding evolutionary drivers across 225 genomes [32].

Conclusion

This study enhances the field of CSFV genomics. Current strategy gaps are addressed by its uses in surveillance, regional control and vaccine optimization (Fahnøe et al, 2019; Rios et al, 2018). Its integration with host genomes and diagnostics establishes it as a fundamental component of comprehensive CSFV control (Aira et al, 2019; Kumar et al, 2024). Global eradication efforts, maybe in conjunction with ASFV control, are made possible by the scalable bioinformatics process and extensive dataset (Borca et al, 2023; Domelevo Entfellner et al, 2024).

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Ethical Approval

The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations and therefore, was exempt.

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Author Contributions

MJR. Conceptualized the study, designed the methodology, led the analysis and drafted the original manuscript. VM.: Contributed to data curation and assisted with manuscript review and editing. J.H. and S.N: Provided support with the data analysis K.P.S.: Supervised and oversaw the study. S.P. Provided overall direction and oversight the project.

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