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Characterization, Prediction and Analysis of Cytosine Phospho Guanine Islands Genes Related to African Swine Fever Resistance In Pigs

OLUWOLE, OO;[*1] ADEOLA, AC;[2] OLALERE, MO;[1] OLADEJO, OA;[3] TIAMIYU, AK;[1]BOLAJI, AA;[1] BOLADURO, BA; OLORUNGBOUNMI, TO[1]

[1]*Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan, Nigeria*
[2] *State Key Laboratory of Genetic Resources and Evolution & Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China.*
[3]*Bowen University, Iwo, Osun State, Nigeria*

**Corresponding Author Email: oluwoleolufunke@gmail.co*
**ORCID: https://orcid.org/0000-0003-3459-8111*
**Tel: +234(0)8037422069*

Co-Authors Email: Oluwoleolufunke@gmail.com; chadeola@mail.kiz.ac.cn; olalereolaitan13@gmail.com; ope_oladejo@yahoo.com; tiamiyu7@yahoo.com; bolajiafolarin@gmail.com;deleboladuro@gmail.com; temmyluv256@gmail.com

ABSTRACT: African swine fever (ASF) has devastating effects on pig populations and the farming economy. Cytosine phospho Guanine islands (CGIs) are short, unmethylated CpG-rich sequences of many vertebrate genes. They could be linked to ASF disease resistance. Consequently, the objective of this paper was to characterize, predict and analyze Cytosine phospho Guanine islands (CpGIs) genes related to African Swine Fever (ASF) resistance in pigs. Eight genes related to African Swine Fever (ASF) resistance in pigs were analyzed for CGI presence, promoter sites, and phylogenetic relationships with other species. Eight genes involved in ASF disease resistance were selected and downloaded from online databases. Their promoters and CpG islands in their upstream regions were predicted and analyzed using bioinformatic tools. Their genetic distances and phylogenetic trees were also constructed. Bioinformatic tools revealed that the CGIs of these genes were well-conserved, with more exons correlating with a higher number of CGIs and promoters. Phylogenetic analysis showed that warthogs and camels were most closely related to pigs for most genes, except for RFXANK, where goats were the closest. The findings emphasize the role of CGIs in ASF resistance and their potential for epigenetic modifications, aiding in the selection of ASF-resistant pigs to improve production, health, and welfare. The presence of CGIs in these genes associated with resistance to ASF disease highlights the potential for epigenetic modifications to induce beneficial phenotypic changes. The knowledge of these genes are predisposed to such modifications is crucial for researchers, breeding companies, and commercial farmers aiming to select ASF-resistant animals for optimal pig production, health, and welfare.

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African swine fever (ASF) is a highly contagious and deadly disease that rapidly kills domestic pigs. It was first identified in Kenya but has now spread globally, leading to significant economic losses (Montgomery, 1921). The disease is caused by the African swine fever virus (ASFV), a large and complex double-stranded DNA virus with a genome encoding 150-167 open reading frames (Dixon *et al.*, 2023). ASF

**Corresponding Author Email: oluwoleolufunke@gmail.co*
**ORCID: https://orcid.org/0000-0003-3459-8111*
**Tel: +234(0)8037422069*

has been reported to have seven polymorphic events in the coding region of the RELA gene, which may play a role in enhancing pigs' tolerance to African swine fever virus (ASFV). It is also identified as a major challenge for pig farming in Sub all over the world (Bisimiwa *et al*., 2024). Animal genetics plays a vital role in livestock development, including the characterization, conservation, and genetic enhancement of animals across different scales (FAO, 2024). CpG islands are short DNA sequences rich in guanine and cytosine (GC), typically remaining non-methylated (McQueen *et al.*, 1997; Deaton and Bird, 2011; Baylin and Jones, 2011). The role of CpG islands (CGIs) highlights specific genes in livestock genetics, particularly their involvement in resistance to African Swine Fever (ASF) (Yang *et al*., 2021; Gujar *et al*., 2019). CGIs, short unmethylated DNA regions rich in guanine and cytosine, are crucial in regulating gene expression and are linked to many vertebrate genes (Angeloni and Bogdanovic, 2021), play a key role in gene silencing and are useful for gene prediction and annotation (Li and Zhang 2014), animal reproductive performance (Jhamart *et al.*, 2020; Usman *et al.*, 2021; Wang *et al.*, 2020; Gross *et al.*, 2020), growth and development ((Horvath *et al.*, 2018; Horvath *et al.*, 2022; Johnson *et al.*, 2019) and disease resistance (Zhang *et al.*, 2021; de soutello *et al.*, 2022). Centromeric and pericentromeric regions, along with repetitive elements, exhibit heavy methylation in mammals and plants. While genic regions also display significant methylation, promoter regions are mostly devoid of DNA methylation. DNA methylation levels varied across different tissues, with percentages ranging from approximately 50% to 54%. These variations suggest potential links to gene expression during tissue differentiation, growth, and development (Young *et al.*, 2011). Yu *et al.* (2022) reported that very little is known about the characteristics and methylation status of CpG islands within the ASFV.

Genetic analyses revealed polymorphisms in the *RELA* gene (Palgrave *et al.*, 2011; Oluwole *et al*., 2017) and variations in tyrosine kinase genes that contribute to ASF resilience in pigs (Enguita-Germán *et al*., 2011; Palkina *et al*., 2023). The *AXL* gene, a key receptor for ASFV entry, shows potential as a target for antiviral strategies (Chen *et al.*, 2023). Phylogenetic studies further highlighted similarities between pigs and species like warthogs and camels in these resistance genes (Choudhary *et al.*, 2022). The findings emphasize the potential of leveraging CGIs, DNA methylation, and specific gene markers to enhance ASF resistance through breeding programs. This approach could improve pig health, welfare, and productivity, mitigating the devastating impact of ASF on the global pig farming industry. The study focuses on eight ASF-related genes, including *RELA*, *SLADMA*, and tyrosine kinase genes like *SRC*, *FYN*, and *AXL* has not been done before. These genes play critical roles in immune response, viral replication, and disease resistance, making them valuable targets for genetic improvement. Hence, there is limited knowledge about the association between ASF-resistance genes. their (CpGIs) and promoter sites, and their phylogenetic relationships with orthologous genes in livestock. Consequently, the objective of this paper is to characterize, predict and analyze Cytosine phospho Guanine islands (CpGIs) genes related to African Swine Fever (ASF) resistance in pigs

MATERIALS AND METHODS

Retrieval of nucleotide sequences and prediction of CPGI and promoter sites: The nucleotide and protein sequences of Pig special traits for disease resistance in ASF diseases were obtained from the databases NCBI and Ensembl. Furthermore, the 2000 bp upstream sequences of the eight ASF-resistance-associated genes (*SLADMA*, *SLADMB*, *RELA*, *Kit*, *ACOX3*, *RFXANK*, *RFXAP*, and *CIITA*) were extracted and used as the promoter prediction.

Table 1: The genomic structure of each gene

Gene	Accession Number NCBI	Ensembl	Exons	Chromo-some Number	Protein
SLADMA	AB117619.1	ENSSSCG00000001470	5	7	290aa
SLADMB	DQ431246.1	ENSSSCG00000001469	6	7	296aa
ACOX3 Gene	XR_002346548.1	ENSSSCG00000008724	21	8	722aa
RELA	NM_001114281.1	ENSSSCG00000012981	10	2	499aa
AXL receptorTyrosine kinase gene	AB364504.1	ENSSSCG00000038806	21	6	887aa
RFXANK	NM_001243347.1	Ensembl:ENSSSCG00000036310	10	2	277aa
RFXAP	LUXQ01077536.1	ENSSSCG00040062983	4	11	233aa
CIITA		ENSSSCG00000007901	22	3	1127aa

Promoter sites were predicted using the online prediction software, Berkeley Drosophila Genome Project (http://www.fruitfly.org/seq_tools/promoter.html). CpGis analysis was performed on these eight genes and on the upstream 2000 bp sequences of the above

genes. The online software database CpG island (DBCAT) was used to predict the CpG islands of the above genes from (http://www.http://dbcat.cgm.ntu.edu.tw/).

This method predicts CpG islands by detecting the GC content (50 %) where any CpG island which has GC content less than 50% is filtered out, and the observation/expected value (observation-to-expected CpG dinucleotide ratio (0.60)) of a sequence window were determined.

The default setting of the CpGi was used: sequence window length not less than 200 bases; GC content not less than 50% and observation/expected value not less than 60%. The user-friendly interface of each of the CpG islands to select parameters were followed.

Multiple sequence alignment : The nucleotide, amino acid sequences (AAS) and structure of the eight genes were retrieved from the National Center for Biotechnology Information, United States of America (NCBI) database, (http://www.ncbi.nlm.nih.gov/protein/) and Ensembl geneome browser (www.ensembl.org/). Sequence alignment and comparison were done with Muscle using IUB substitution matrix, gap open penalty of 10 and gap extension penalty of 0.2.

The evolutionary divergence between the sequences of each gene was estimated by using the Poisson correction model (Zuckerkand *et al.,* 1965). All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted by using MEGA6 (Kumar *et al.,* 2016). The phylogenetic trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary history was inferred by using the Neighbour-Joining method based on the JTT matrix-based model (Jones *et al.,* 1996).

The tree with the highest log likelihood (-4968.9605) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value.

The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted using MEGA7.

Table 2A: The CPG Island locations and lengths within the eight genes sequences

Gene	CpG Islands counts	Start	End	CpG Length (s)	GC%
SLADMA	5	2654	2955	302	55
		3045	3265	221	51
		3498	4235	738	53
		4377	4813	437	62
		4936	5156	221	57
SLADMB	5	2285	2505	221	53
		9084	9338	255	59
		12642	12862	221	53
		16135	16355	221	53
		21435	21689	255	59
ACOX3 Gene	27	199	1267	1069	61
		1793	2159	367	59
		2726	4650	1925	61
		4866	6252	1387	56
		6893	7174	282	60
		7278	8754	1477	63
		8932	9685	754	58
		9789	10602	814	53
		11467	12239	773	53
		12341	13889	1549	65
		13979	18834	4856	64
		19056	19444	389	52
		19628	20000	373	51
		20292	27810	7519	64
		28269	29299	1031	56
		29395	30823	1429	61
		31190	35216	4027	63
		35350	36361	1012	62
		38149	42462	4314	62
		36481	37973	1493	61
		42550	44744	2195	61
		44944	48614	3671	64
		49331	49661	331	55
		50000	51007	1008	68
		51759	52370	612	68
		52184	54666	2083	59
		54754	55163	410	50
RELA	9	79	1343	1265	69
		1466	2143	678	66
		3295	4301	1007	60
		4840	5503	664	62
		5732	6433	702	58
		6803	7092	290	52
		7526	7937	412	57
		9105	9325	221	61
		10465	10727	263	56

RESULTS AND DISCUSSION

The genomic structure of each eight genes was presented in Table 1, where the numbers of exons, chromosome locations and amino acid numbers were presented. The predicted CpG islands in the eight genes and their numbers, location, length, and distribution characteristics were analyzed. The results showed that these analyzed genes for ASF resistance in pigs have CpG islands ranged from 5-30 with lengths ranged from 221 to 7519 (Table 2). From these results, it was discovered that the genes with larger exons have the largest CGI.

The genes ACOX3, AXL and CIIAT have the largest exons of 21-22 and CGI of 27-30 as shown in Tables 2A and 2B. Li *et al* (2018) provides insights into how methylation affects exon usage during transcription, alternative splicing, and cancer development in human tissues. According to Dong *et al.* (2024), the host cell can initiate DNA methylation as a defense mechanism against viral infections, where upon recognizing a virus, the innate immune system activates signaling pathways that stimulate the production of interferons and other antiviral factors.

Yu *et al*. (2024) reported that methylation may serve as a host defense, suggesting that drugs promoting viral DNA methylation could be a potential antiviral strategy for ASFV. The varying distribution of CpG islands of these genes may influence their methylation status, thereby affecting gene expression regulation and potentially leading to resistance to the ASF disease.

According to Yu *et al*. (2024), methylation can either directly block transcription initiation factors from binding to promoters or recruit transcription inhibition complexes, which compete with transcription factors, resulting in gene silencing.

Methylation may serve as a host defense mechanism, suggesting that drugs promoting host DNA methylation could be a potential treatment against ASF disease.

The results from promoter activities after running the software, generated predicted results, with reliability increasing as the score rose. Scores ranging from 0.8 to 1.0 indicated that the predictions were trustworthy, and the predicted promoter activity was credible. Additionally, the larger font in each predicted promoter sequence marked the transcription start site.

The predicted CpG islands in the 2000 bp upstream gene sequence analyzed their number, location and length, can either directly block transcription initiation factors from binding to promoters or recruit transcription inhibition complexes, which compete with transcription factors, resulting in gene silencing as reported by Kitazawa *et al. (*2022). LaMere *et al*. (2019) also reported that viral gene expression is believed to be partially regulated by DNA methylation, which contributes to transcriptional silencing and gene suppression through various mechanisms in host tissues, though the effects of viral infection on host genome methylation remain unclear. Genome-wide bisulfite sequencing can detect methylation in both host and viral CpG islands, as well as in non-CpG islands as reported by LaMere *et al*. (2019) and Gu *et al*. (2022), which provide insights into these changes.

Table 3B: The CPG Island locations and lengths within the eight genes sequences

Gene	CpG Islands counts	Start	End	CpG Length (s)	GC%
AXL	27	173	993	821	67
receptor		1637	2172	536	66
Tyrosine		2372	2760	389	61
kinase		3006	3254	249	56
gene		3670	4149	480	61
		6111	6954	844	67
		10245	10572	328	51
		11307	11977	671	51
		12653	12873	221	53
		13364	13768	405	62
		13902	14389	488	60
		16920	17914	995	58
		18024	18244	221	52
		18725	18945	221	51
		20196	20416	221	53
		20264	20899	276	59
		21247	21479	233	59
		22308	22654	347	61
		22780	23106	327	58
		23589	23809	221	62
		24705	25037	333	50
		27364	28014	651	58
		28584	28963	380	52
		29322	29567	246	53
		29765	30161	397	52
		30552	30794	243	55
		30960	31367	408	61
RFXANK	3	154	1164	1011	58
		4196	4882	687	63
		5202	5443	242	59
RFXAP	1	203	1614	1412	64
CIITA	30	905	1144	240	60
		1559	1779	221	54
		2589	2823	235	56
		2939	3168	230	57
		3285	3638	354	57
		3952	4484	533	55
		5614	6281	668	56
		9793	10099	307	51
		14862	15202	341	53
		16648	16868	221	55
		17329	17805	477	54
		25318	25858	541	50
		26546	27176	631	60
		28110	28330	221	55
		28608	28973	366	53
		29974	30202	229	54
		32049	32269	221	53
		34697	34917	221	61
		35094	35314	221	61
		36801	37381	581	58
		38473	38909	437	53
		39124	41007	1884	66
		47540	47760	221	54
		48081	48301	221	52
		49378	49614	237	63
		51238	51753	516	50
		52817	53037	221	61
		54340	55307	968	62
		57707	59166	1460	56

The phylogenetic trees of the eight genes were shown in Figure 8.

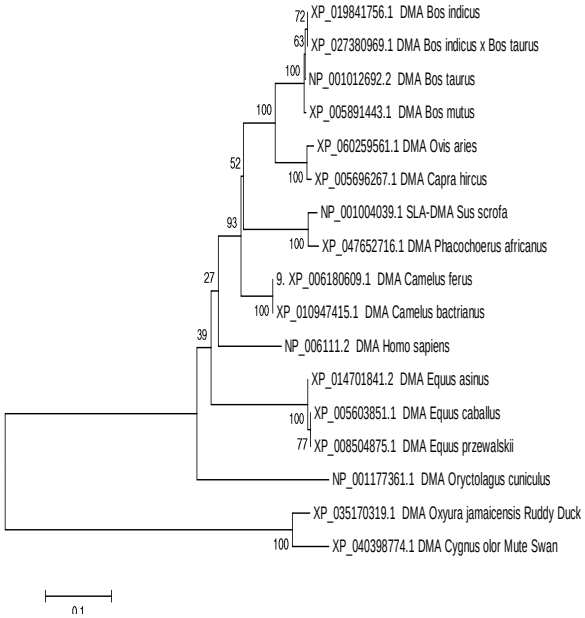

Fig. 1: Phylogenetic tree of DMA gene in pigs and other orthologous species making avian as an outgroup

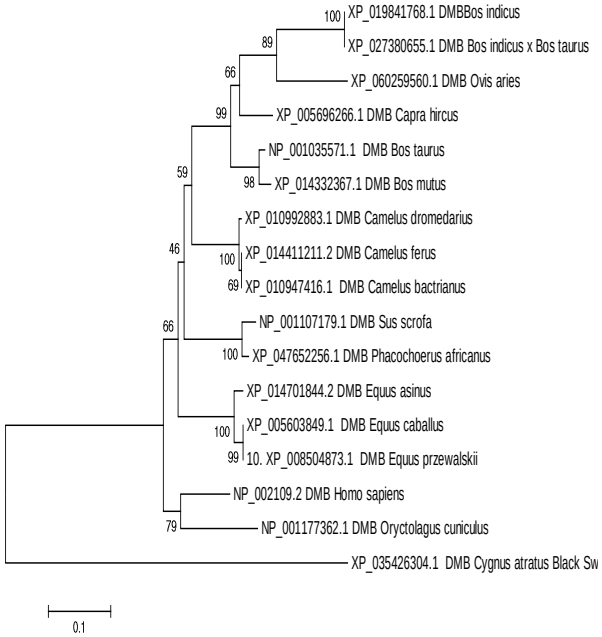

Fig. 2: Phylogenetic tree of DMB gene in pigs and other orthologous species making avian as an outgroup

The pig, in all the genes, was observed to have low genetic distance, thereby closer to the warthog and the camel than other mammalian species (Table 3) with the exception of the RXANK gene, where it was much closer to the goat than any other species.

This result corroborated with Premraj *et al.* (2015) and Chouldhary *et al.* (2022) where the dromedary camel leptin gene has high amino acid similarity percentage with domestic species such as pigs, sheep, cattle, etc.

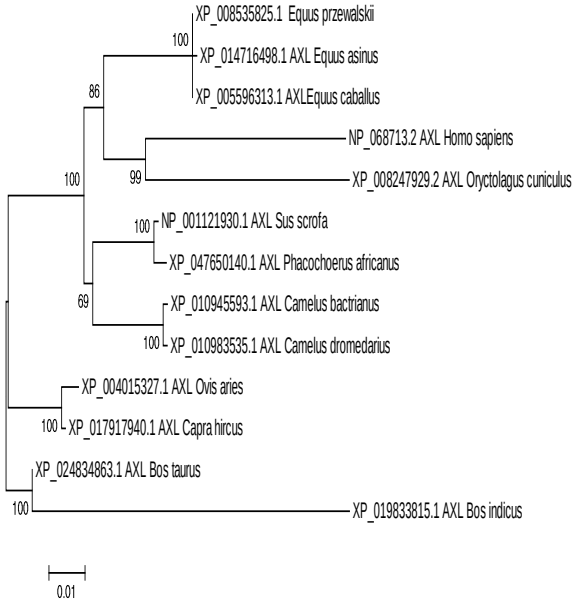

Fig. 3 Phylogenetic tree of AXL gene in pigs and other orthologous species making avian as an outgroup

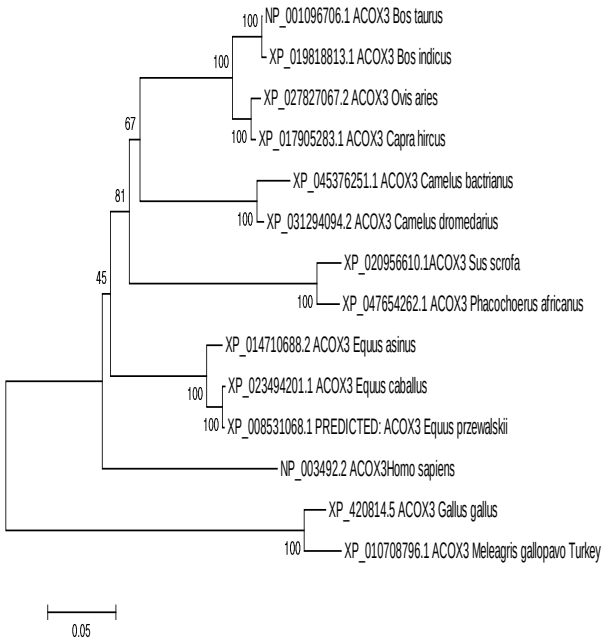

Fig. 4: Phylogenetic tree of ACOX3 gene in pigs and other orthologous species making avian as an outgroup

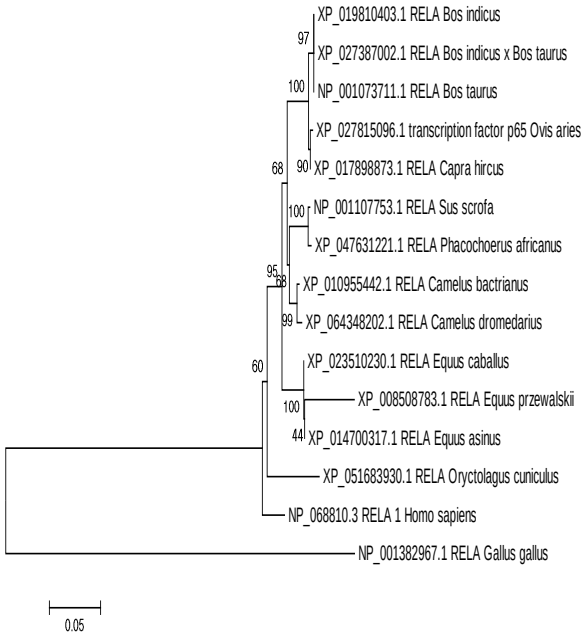

Fig. 5: Phylogenetic tree of RELA gene in pigs and other orthologous species making avian as an outgroup

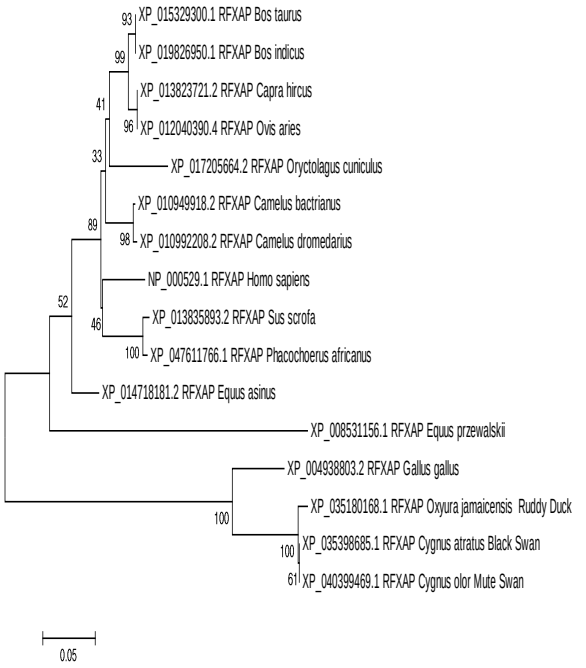

Fig. 6: Phylogenetic tree of RFXAP gene in pigs and other orthologous species making avian as an outgroup

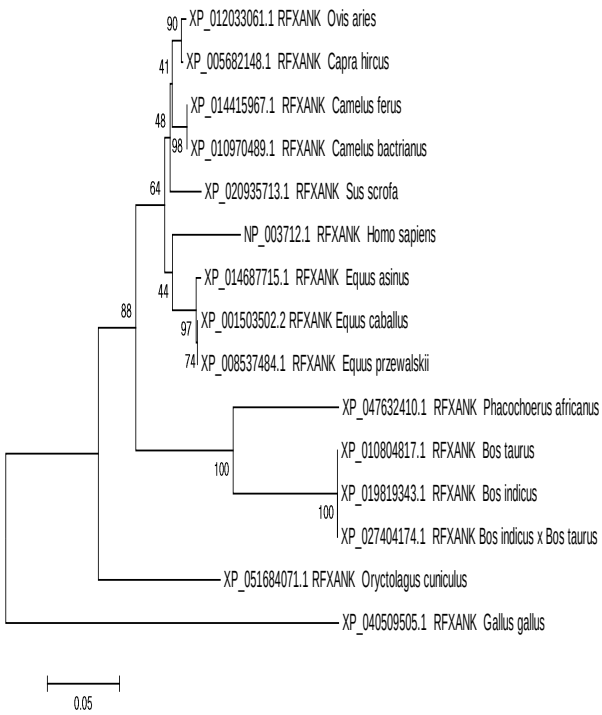

Fig 7: Phylogenetic tree of RFXANK gene in pigs and other orthologous species making avian as an outgroup

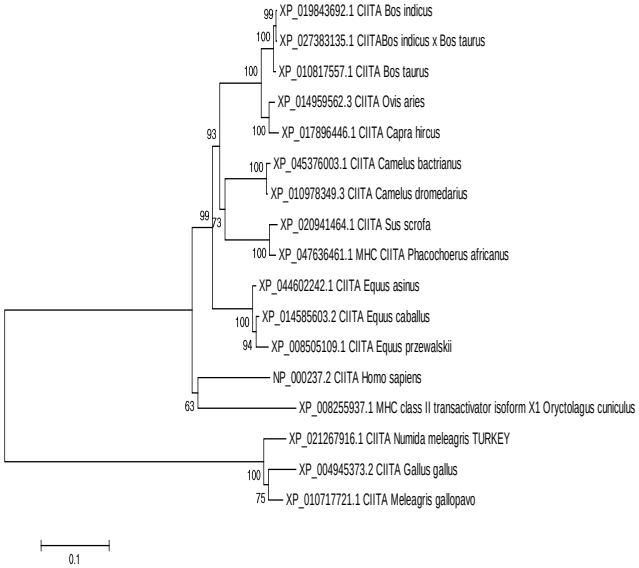

Fig. 8: Phylogenetic tree of CIITA gene in pigs and other orthologous species making avian as an out group

Table 4: Genetic distances between pig, warthog and camel

Species	DMA	DMB	ACOX3	RELA	AXL	RFXANK	RFXAP	CIITA
Warthog	0.0029	0.032	0.034	0.005	0.004	0.164	0.010	0.021
Carmel	0.165	0.198	0.245	0.030	0.0039	0.033	0.073	0.143
Goat	-	-	-		-	0.029		
Sheep						0.033		

The evolutionary histories for the eight genes were inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length for each gene = 0.33696723, DMA=1.66373794; DMB=1.89057029; AXL=0.33696723 RFXANK= 0.75844819; RFXAP=0.92069117, is shown.

The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965) and were in the units of the number of amino acid substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).

Conclusion: This study analyzed the promoter and CpG islands within the 2000 bp region upstream of eight genes associated with ASF resistance. It can be concluded that at the promoter sites, there is an overlap with CpG islands, the methylation at these sites might have hindered transcription factors from binding to promoters, thereby inhibiting gene activation and restriction of viral protein synthesis. This may be associated with disease resistance, therefore genes can be used as biomarkers for the selection of ASF disease resistance in pigs. Phylogenetic tree of all the genes shows the evolutionary relationship between amino acid sequences of pig and other orthologous species, with the exception of the RFXNAK gene.

Declaration of Conflict of Interest: The authors declare no competing interests

Data Availability Statement: Data are available upon request from the corresponding author or any of the other authors.

Abbreviations
African swine fever virus: (ASF)
African swine fever virus: (ASFV)
Cytosine phospho Guanine islands: (CGIs)
Swine leucocyte antigen DM alpha chain: (SLADMA) gene,
Swine leucocyte antigen DM beta chain: (SLADMB) gene,
v-rel avian reticuloendotheliosis viral oncogene homolog A: (RELA) gene,
AXL (Anexelekto): gene,
Acyl-CoA oxidase 3 gene (ACOX3),
Regulatory factor X associated ankyrin containing protein (RFXANK) gene, Regulatory factor X associated protein (RFXAP) gene
CIITA: (Class II, Major Histocompatibility Complex, Transactivator) gene

REFERENCES

Barazandeh, A; Mohammadabadi, M; Ghaderi-Zefrehei, M; Rafeie, F; Imumorin, IG. (2019). Whole genome comparative analysis of CpG islands in camelid and other mammalian genomes. *Mamm. Bio.*, 98, 73-79. https://doi.org/10.1016/j.mambio.2019.07.007

Bisimwa, PN; Ongus, JR; Tonui, R et al. (2024). Resistance to African swine fever virus among African domestic pigs appears to be associated with a distinct polymorphic signature in the RelA gene and upregulation of RelA transcription. *Virol J.* 21, 93 (2024). https://doi.org/10.1186/s12985-024-02351-9

Chen, X; Zheng, J; Li, T; Liu, C; Bao, M; Wang, X; Li, X; Li, J; Huang, L; Zhang, Z; Weng, C. (2023). Coreceptor AXL Facilitates African Swine Fever Virus Entry via Apoptotic Mimicry. *J. Virol*. 27; 97(7):e0061623.

Choudhary, N; Prakash, V; Joshi, R.K; Pannu, UR. (2022). Characterization of Leptin (LEP) gene in Indian camel (Camelus dromedarius). *Indian J. Anim. Sci*. 92 (7): 853–857. https://doi.org/10.56093/ijans.v92i7.121297

Cross, S.H; Bird, AP. (1995). CpG islands and genes. *Curr Opin Genet Dev*. 1995;5:309–314.

Deaton, AM; Bird, A. (2011). CpG islands and the regulation of transcription. *Genes Dev*. 25(10):1010-22..

De Soutello, RVG; Rodrigues, MGF; Goncalves, JA; Bello, HJS; Pavan, BE; Ramos, ES. (2022). Global genomic methylation related to the degree of parasitism in cattle. *Sci. Rep*. 12:18135.

Dong, W; Wang, H; Li, M; Li, P; Ji, S. (2024). Virus-induced host genomicremodeling dysregulates gene expression, triggering tumorigenesis. *Front. Cell. Infect. Microbiol.*, 14, 1359766. https://doi.org/10.3389/ fcimb.2024. 1359766

Dixon, LK; Chapman, DA; Netherton, CL; Upton, C. (2012). African swine fever virus replication and genomics. *Virus Res*. 173(1):314

Enguita-Germán, M; Gurrea, M; Schiapparelli, P; Zhu, TS; Crowley, JG; Hamm, LL; Costello, MA; He, X; Talsma, CE; Flack, CG; Hervey-Jumper, SL; Heth, JA; Muraszko, K.M; Rey, JA, Fan, X; Castresana, JS. (2010). KIT expression and methylation in medulloblastoma and PNET cell lines and tumors. *J. Neurooncol.* 103(2):247-53.

Fang, L; Zhou, Y., Liu, S; Jiang, J; Bickhart, DM; Null, DJ; Li, B; Schroeder, SG., Rosen, BD; Cole, JB., et al. (2019). Comparative analyses of sperm DNA methylomes among human, mouse and cattle provide insights into epigenomic evolution and complex traits. *Epigenetics*. 14:260–276.

FAO, Food and Agriculture Organization of United Nations. 2024. Why is animal genetics important? https://www.fao.org/animal-genetics/en/

Feng, W; Zhou, L; Zhao, P; Du H; Diao, C; Zhang, Y; Liu, Z; Jin, W; Yu, J; Han, J; Okoth. E; Mrode, R; Liu, JF. (2023). Comparative Genomic Analysis of Warthog and Sus Scrofa Identifies Adaptive Genes Associated with African Swine Fever. *Biology* (Basel). 14: 12(7):1001.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolut.* 39:783-791.

Gebert, C; Wrenzycki C., Herrmann D; Groger D; Reinhardt R; Hajkova P; Lucas-Hahn A; Carnwath J; Lehrach H; Niemann H. (2006). The bovine IGF2 gene is differentially methylated in oocyte and sperm DNA. *Genomics.* 88:222–229.

Goel, N; Karir, P; Garg, VK. (2017). Role of DNA methylation in human age prediction. *Mech. Ageing Dev.*, 166, 33-41.

Gross, N., Penagaricano, F; Khatib, H. (2020). Integration of whole-genome DNA methylation data with RNA sequencing data to identify markers for bull fertility. *Anim. Genet*. 2020;51:502–510. doi: 10.1111/age.12941.

Gu, Z., Yang J;Yang, M, Jiao, Y. (2022). Exploring crucial molecular events in pearl oyster after pre-grafting conditioning by genome-wide bisulfite sequencing for DNA methylation analysis. *Fish Shellfish Immunol* 123: 10-19.

Horvath, S; Raj, K. (2018). DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat. Rev. Genet.* 19:371–384.

Horvath S; Lu, AT., Haghani A; Zoller JA; Li CZ; Lim AR; Brooke RT; Raj K; Serres-Armero A; Dreger DL et al (2022). DNA methylation clocks for dogs and humans. Proc. *Natl. Acad. Sci. USA.* 119:e2120887119. doi: 10.1073/pnas.2120887119.

Illingworth, RS; Gruenewald-Schneider, U; Webb, S; Kerr AR; James KD; Turner DJ et al (2010). Orphan CpG islands identify numerous conserved promoters in the mammalian genome. *PLoS Genet* 6: e1001134.

Jhamat, N; Niazi, A; Guo, Y; Chanrot, M; Ivanova, E; Kelsey, G; Bongcam-Rudloff, E; Andersson, G; Humblot, P. (2020). LPS-treatment of bovine endometrial epithelial ells causes differential DNA methylation of genes associated with inflammation and endometrial function. *BMC Genom.* 2020;21:385. doi: 10.1186/s12864-020-06777-7.

Johnson, ND; Conneely, KN. (2019). The role of DNA methylation and hydroxymethylation in immunosenescence. *Ageing Res. Rev.* 51:11–23. doi: 10.1016/j.arr.2019.01.011.

Kitazawa, S., Ohno, T; Haraguchi, R; Kitazawa R. (2022) Histochemistry, Cytochemistry and Epigenetics. *Acta Histochem et Cytochem* 55: 1-7.

LaMere, S.A; Chaillon, A; Huynh, C; Smith, DM; Gianella, S. (2019) Challenges in Quantifying Cytosine Methylation in the HIV Provirus. *mBio* 10: e02268-18.

Larsen, F; Gundersen, G; Lopez, R; Prydz, H.(1992). CpG islands as gene markers in the human genome. *Genomics.* 13:1095–1107

Liu, S; Fang, L; Zhou, Y; Santos, DJA; Xiang, R; Daetwyler. HD; Chamberlain, AJ; Cole, JB; Li CJ; Yu Y; et al. (2019). Analyses of inter-individual variations of sperm DNA methylation and their potential implications in cattle. *BMC Genom.* 20:888. doi: 10.1186/s12864-019-6228-6.

Marcelle, M; Nascimento, José, A; Lemos, JA; Vanessa, K; Lin, RA; Burne. (2008). Role of RelA of Streptococcus mutans in global control of gene expression. *J. Bacteriol.* 190(1):28-36. D

Ma, X; Jia, C; Chu, M; Fu, D; Lei, Q; Ding, X;Wu, X; Guo, X; Pei, J; Bao, P;et al. (2019). Transcriptome and DNA Methylation Analyses of the Molecular Mechanisms Underlying with Longissimus dorsi Muscles at Different Stages of Development in the Polled Yak. *Genes*.10: 970.

McQueen, HA; Clark, VH; Bird, AP; Yerle, M; Archibald, AL. (1997). CpGislands of the pig. *Genome Res.* 7(9):924-31.

Miguel, A; Gama-Sosa, R; Valerie, A; Slagel, S; Kenneth, C; Kuo, Charles WG; Melanie E. (1983). Tissue-specific differences in DNA methylation in various mammals, Biochimica et Biophysica Acta (BBA) G*ene Structure and Express.* 740(2), 212-219,

Mikesell, S. 2023. Newly identified key gene is critical for African swine fever infection.*The pigsite.* https://www.thepigsite.com/articles/newly-identified-key-gene-is-critical-for-african-swine-fever-infection

Momparler, R; Bovenzi V. (2020). DNA methylation and cancer. *J Cell Physiol.* 2000;183:145–154.

Montgomery, RE. (1921). On a form of swine fever occurring in British East Africa (Kenya Colony). *J Compar. Pathol.* 4:159–91.

Palgrave, CJ; Gilmour, L; Lowden S; Simon, G; Martha, L; Mellencamp, A; Whitelaw, CA. (2011). Pathogenesis Potential Role in African SwineFever underlies differences in NF- kB Activity: a Species-Specific Variation in RELA. *J. Virol*. 85(12): 6008.

Palkina, N; Tyumentseva, A; Ruksha, T. (2023). Novel somatic Missense Mutations in Exon 11 of the KIT Gene are Detected in Melanoma. *Asian Pac J Cancer Prev*. 24(9):3015-3020.

Pannhorst, K; Carlson, J; Hölper, JE. et al. (2023) The non-classical major histocompatibility complex II protein SLA-DM is crucial for African swine fever virus replication. *Sci. Rep.* 13, 10342

Penrith, ML; Thomson, GR; Bastos ADS. (2004). African swine fever. In: Coetzer JAW, Tustin RC, editors. Infectious diseases of livestock. Volume 2, 2nd ed. Oxford, United Kingdom: Oxford University Press1088–119.

Perrier, J.P; Sellem, E; Prezelin, A; Gasselin, M; Jouneau, L; Piumi, F; Adhami, H;Weber, M; Fritz, S; Boichard, D. et al. (2018). A multi-scale analysis of bull sperm methylome revealed both species peculiarities and conserved tissue-specific features. *BMC Genom.* 19:404. doi: 10.1186/s12864-018-4764-0.

Premraj, A; Aleyas, A.G; Nautiyal, B; Rasool, TJ. (2015). Characterization of dromedary leptin gene–Prospects in camel Husbandry. Proceedings of 4thconference of ISOCARD on Silk Road Camel: The Camelids, Main Stakes for Sustainable. Development, Almaty, Kazakhstan June 8–12.

Razin, A. (1998). CpG methylation, chromatin structure and gene silencing-a three-way connection. *EMBO J.* 17:4905–4908.

Robertson, KD. (2005). DNA methylation and human disease. *Nat Rev Genet.* 2005;6:597–610.

Saeed-Zidane, M; Tesfaye, D; Mohammed, SY; Tholen, E; Neuhoff, C; Rings, F; Held, E; Hoelker, M; Schellander, K; Salilew-Wondim, D. (2019). Hyaluronic acid and epidermal growth factor improved the bovine *PLoS ONE* . 14:e0223753.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molec. Biol. Evolut.* 4:406-425.

Tamura, K; Stecher, G; Peterson, D; Filipski, A; Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecu. Biol. Evolut.* 30: 2725-2729.

Usman, T; Ali, N; Wang, Y; Yu, Y. (2021) .Association of Aberrant DNA Methylation Level in the CD4 and JAK-STAT-Pathway-Related Genes with Mastitis Indicator Traits in Chinese Holstein Dairy Cattle. *Animals.* 2021;12:65.

Wang, M; Liang, Y; Ibeagha-Awemu, E.M; Li, M; Zhang, H; Chen, Z; Sun, Y; Karrow, N.A; Yang, Z; Mao, Y. (2020). Genome-Wide DNA Methylation Analysis of Mammary Gland Tissues from Chinese Holstein Cows with Staphylococcus aureus Induced Mastitis. *Front. Genet.* 11:550515. doi: 10.3389/fgene.2020.550515.

Yang, Y; Fan, X; Yan, J; Chen, M; Zhu, M; Tang, Y; Liu, S; Tang, Z.A. (2021).Comprehensive epigenome atlas reveals DNA methylation

regulating skeletal muscle development. *Nucleic Acids Res.* 49:1313–1329doi: 10.1093/nar/gkaa1203.

Yu, YY; X, MS; Liu, Q. (2022). Comparative analysis of CpG islands in two genotypes of African swine fever virus. *Pol J Vet Sci*. 25(3):455-462. doi: 10.24425/pjvs.2022.142030. PMID: 36156102.

Zhang, J; Han, B; Zheng, W; Lin S; Li H; Gao Y. (2021). Sun D. Genome-Wide DNA Methylation Profile in Jejunum Reveals the Potential Genes Associated ith Paratuberculosis in Dairy Cattle. *Front. Genet*. 12:735147.

Zhou, Y; Connor, E.E; Bickhart, DM; Li, C; Baldwin, RL; Schroeder, SG; Rosen .B.D; Yang, L; Van, TCP; Liu, GE. (2022). Comparative whole genome DNA methylation profiling of cattle sperm and somatic tissues reveals striking hypomethylated patterns in sperm. *Gigascienc.* 7:giy039. doi: 10.1093/gigascience/giy039.

Zuckerkandl, E; Pauling, L. (1965). Evolutionary divergence and convergence in proteins. Edited in Evolving Genes and Proteins by V. Bryson and H.J. Vogel, pp. 97-166. *Academic Press*, New York.