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Computational Molecular Analysis of the 5 Candidate Genes of Suidae Family Related to African Swine Fever Occurrence

O. O. Oluwole[1*]

[1]Institute of Agricultural Research and Training, Obafemi Awolowo University, Nigeria.

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Suidae family consisting, domestic pig, warthog and babyrousa are identified with even hooves and snout nose. They have been reported to be tolerant to African swine fever virus (ASFV) with the exception of the domestic pig. Domestic pig is valuable for humans as a source of protein all over the world, but is more susceptible to this disease. ASFV has been found in warthog and bush pig. Five candidate genes, RELA, PPP3CB, PPIA, NFKBIA, and NFATC1 have been suggested to be responsible for the genetic variation between the components of Suidae family, which may contribute to the species-specific responses to ASFV infection. This study aims to investigate the evolution, differentiation and functional function of these five genes in three species of Suidae family that are either susceptible or tolerant to ASFV using molecular computational genetics.

The nucleotide and amino acid sequence (aas) of the 5 genes of pig, warthog and babyrousa were downloaded from NCBI, Essembel geneome browser, and Uniprot database. They were aligned and analysed to predict functional effect of amino acid substitution as deleterious or neutral for small insertions and deletions.

The candidate genes were observed to have variants with the exception of NFKBIA. They were observed to have deleterious variants. These deleterious variants may be responsible for the susceptibility or tolerance of this family to ASF disease.

**Corresponding author: E-mail: oluwafunmike@yahoo.co.uk, oluwoleolufunke1@gmail.com;*

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1. INTRODUCTION

Suidae family is a family from Animal kingdom with ungulate hooves that has large head with distinct long snout nose, small neck, small eyes, short tassel tail and bristly coat. It consists of the pig, warthog, babyrousa, bush pig, and river hog [1]. It has been reported that African Swine Fever virus (ASFV) is a pathogen that affects this family and some members are resistant to the virus [2]. warthog, babyrousa and riverhog have been reported to be tolerant while the domestic pigs are more susceptible [3,4]. ASFV has been found in the warthog [5] and in the bushpig [6]. This infectious disease causes haemorrhagic fever, weakness, skin redness, especially ear and the abdomen, loss of appetite and weight, swollen joints, pneumonia and skin ulcer and death after few days [7]. It has been reported that some local breeds of domestic pigs in Africa are also resistant [8]. Oluwole and Omitogun [9] reported the presence of ASFV in Nigerian pigs while Adeoye and Adebambo [10] carried out an investigation on the ASF outbreak survivors in Nigeria, their offspring and F_2 showed reduced antibody levels against ASF from 100% to 18.79%. Palgrave et al. [11] also reported the ability of the virus to survive in one species and killing other species in the same family where it is likely to be due to their genetic variations. The authors investigated on the species-specific variation in RELA (p65; v-rel reticuloendotheliosis viral oncogene homolog A) using differences in nf-κb activity in the candidate- gene approach based on known signaling pathways that interact with the virus-encoded immunomodulatory protein A238L. A238L has three target proteins that are encoded by RELA, PPP3CB (protein phosphatase 3 catalytic subunit beta), PPIA (peptidylprolyl isomerase A), NFKBIA (nuclear factor-kappa-B inhibitor alpha) and NFATC1 (nuclear factor of activated T cells 1) genes. They are candidate genes for the genetic variation between Suidae family which may contribute to species-specific responses to ASFV infection [11]. Palgrave et al. 2011 reported that there was difference between the pig RELA and warthog RELA amino acid sequence (aas) at position 151. Oluwole et al. [12] also identified this difference using the bioinformatics tool and deduced the protein structure of RELA, PPP3CB, PPIA, NFKBIA and NFATC1 and drew phylogenetic tree of each one of them.

The aim of this study is to investigate the evolution, differentiation and functional function of five genes of three members of Suidae family (pig, warthog and babyrousa) that are either susceptible or tolerant to ASFV using molecular computational genetics.

2. MATERIALS AND METHODS

2.1 Retrieval of Amino Acids Sequences

The nucleotide and amino acid sequences (AAS) of RELA gene of pig, warthog and that of the babyrousa were downloaded from the National Center for Biotechnology information (NCBI) data base, United State of America [13], Universal protein resources (Uniprot) database, United Kingdom [14] and Essembel geneome browser [15].

2.2 Sequence Alignment

Sequence alignment and comparison were done with ClustalW using IUB substitution matrix, gap open penalty of 10 and gap extension penalty of 0.2 in MEGA 7 [16].

2.3 Estimates of Evolutionary Divergence between Sequences

Analyses were conducted using the pairwise method and p-distance model with a bootstrap of 1000 replicates. The analysis involved 3 amino acid sequences of 3 members of Suidae family (pig, warthog and babyrousa). All positions containing gaps and missing data were eliminated. There were a total of 551 (RELA), 492 (PPP2CB), 164 (PPIA), 40 (NFKBIA), 250 (NFATC1) positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [16].

2.4 Phylogenetic Analysis

The phylogenetic tree was 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 Maximum Likelihood method based on the JTT matrix-based model (Jones et al. 1996) and the tree with the highest log likelihood (-1753.9556) for PPP3CB and (-762.3946) for PPIA displayed. 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 was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 18 and 27 amino acid sequences for PPP3CB and PPIA. All positions containing gaps and missing data were eliminated. There were a total of 479 (PPP3CB) and 163 (PPIA) positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [16].

2.5 Functional Analysis of 5 Genes of Suidae Family

Functional analyses of the RELA, PPP3CB, PPIA, NFKBIA and NFATC1 genes of Suidae family were obtained by using Protein variation effect analyser PROVEAN online [17] with threshold value of -2.5 in order to predict functional effect of amino acids substitution as deleterious or neutral for small insertions and deletions. Any protein sequence variants having a PROVEAN score below the threshold value of -2.5 were termed deleterious and a PROVEAN score above the same threshold value were considered neutral.

3. RESULTS AND DISCUSSION

RELA gene was found on the chromosome 2 and has 10 exons. It is a protein coding with forward strand type of 1,635 base pairs (bp) and 544 amino acids (aa). It has 2 transcripts (splice variants) and is a member of Ensembl protein family with 127 orthologous.

The genetic distance of RELA gene of pig and warthog were closer genetically (0.005) to each other than the genetic looseness between the pig and the babyrousa with genetic value of 0.015.

The PPP3CB gene was located on chromosome 14 and has 20 exons. It is a protein coding with negative strand type of 4,989 bp and 527 aa. It is a member of Ensembl protein family with 60 orthologous and 2 paralogues.

The genetic distance of PPP3CB gene between the species shows the warthog and babyrousa closer genetically (0.000) to each other than that of pig with genetic value of 0.002.

PPIA gene was located on chromosome 18 and has only 5 exons. It is a protein coding with negative strand type of 1,953 bp and 164 aa. It has 1 transcript and is a member of Ensembl protein family with 58 orthologous and 5 paralogues. There is genetic closeness between the pig and warthog PPIA gene (0.000) than that of babyrousa.

The NFKBIA gene was located on chromosome 7 and has 6 exons. It is a protein coding with forward or positive strand type of 1,871 bp and 314 aa. It has 1 transcript and is a member of Ensembl protein family with 56 orthologous [15]. The NFKBIA genes of all the species are closer to each other.

NFATC1 gene was located on chromosome 1 and has only 10 exons. It is a protein coding with negative strand type of 6,692 bp and 891 aa. It has 6 transcripts and it is a member of Ensembl protein family with 173 orthologous [15]. In NFATC1 gene, there was genetic closeness between the pig and babyrousa than that of warthog.

The neighbor joining phylogenetic tree results below (Figs. 1-5) show that the RELA and PPIA gene of domestic pig and that of warthog clustered together meaning that they are more genetically related than the babyrousa. It also reveals that the PPP3CB and NFKBIA gene of warthog and babyrousa clustered together. NFATC1 gene of babyrousa and pig also clustered together. These results tally with the results obtained by Oluwole et al. [12] where human was used as an out-group.

From the eleven variants analyzed, only one was suspected to be deleterious (K219E) while others were neutral. The deleterious result from PROVEAN score (<-2.5 or -2.5) show that the aa mutation or substitution may be harmful while the neutral result (>-2.5) show that the aa substitution does not damage the protein function. The variant K219E that was deleterious from PROVEAN results as shown in Table 1, where there was SNP substitution or mutation of aa, where there was Glutamic acid in Babyrousa instead of Lysine (K) that was present in pig and warthog. The variant P529S in domestic pig, which is a phosphorylation site present in all mammals except the warthog, was found to be neutral (-0.543). This shows that it was found not to be harmful but it activates a set of genes as reported by Palgrave et al. [11]. The activation of these genes in the domestic pig may be responsible for its susceptibility to ASFV and its absence in the warthog resulting in the Warthog's tolerance to ASFV. Oluwole et al. [12]

also reported polymorphic variation observed in Warthog at sequence 531 where there was insertion of P instead of S. The result obtained from the PROVEAN score in this study showed the possibility of high degree of closeness between the warthog and the pig.

The PPP3CB gene has 12 variants and all of them were deleterious with the exception of C146Y variant that was neutral as shown on the Table 2. The cysteine aa was replaced with threonine aa at position 146, where both the warthog and babyrousa have threonine aa while the pig has cysteine. This variant causes no defect in the protein function of the pig. The deletion at position 363 and from 134 to 433 in both the domestic pig and babyrousa were deleterious and this means these can cause defect in protein structure and functions. The PPP3CB gene activates the T cell of the immune system which attacks foreign organisms such as ASFV. These positions can be suspected to be part of the reasons for susceptibility to ASFV because they only occurred in both pig and babyrousa but the warthog and babyrousa are more genetically closer than the pig.

There was only one out of two variants that was deleterious in PPIA gene where there was mutation of Threonine replaced by Alanine at the position 107 as shown in Table 3. The warthog and the pig have threonine while the babyrousa has alanine at the same position. This same result tallied with Oluwole et al. [12] findings.

There was no variant of NFKBIA gene observed among the 3 species of the family of Suidae family as illustrated by the genetic distance but the warthog and the babyrousa were genetically related than that of the pig NFKBIA gene. Out of the 4 variants of the NFATC1 gene, only one was deleterious where the Asparagine replaced T (theonine). The pig and babyrousa were more related compared to warthog.

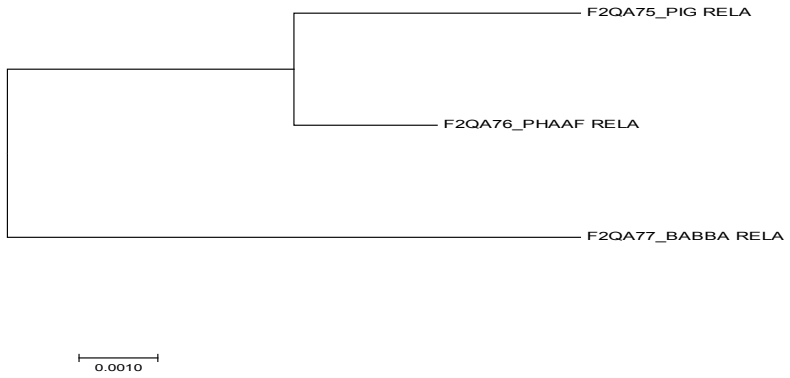

Fig. 1. Phylogenetic tree of the RELA gene of domestic pig, warthog and babyrousa

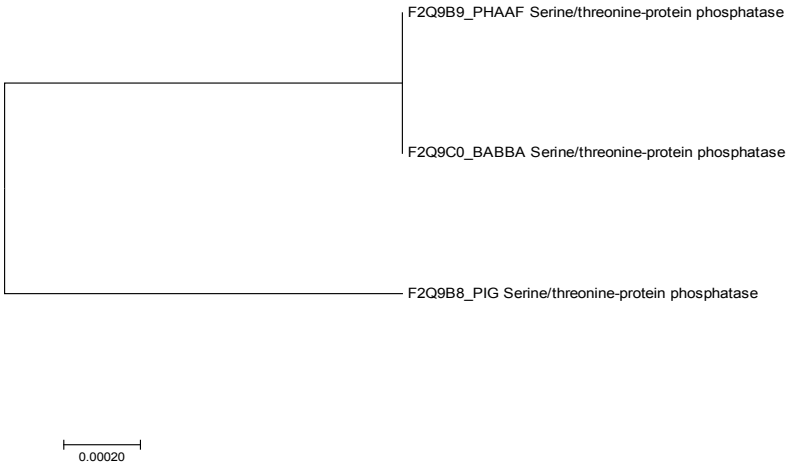

Fig. 2. Phylogenetic tree of the PPP3CB gene of domestic pig, warthog and babyrousa

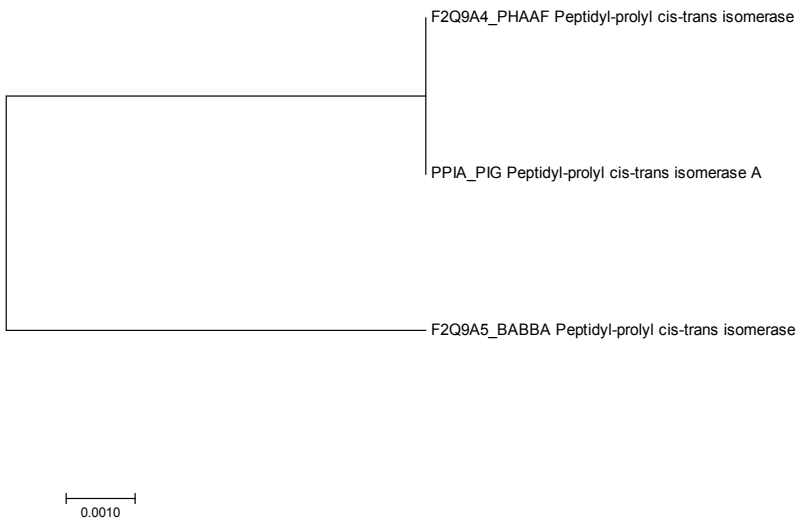

Fig. 3. Phylogenetic tree of the PPIA gene of domestic pig, warthog and babyrousa

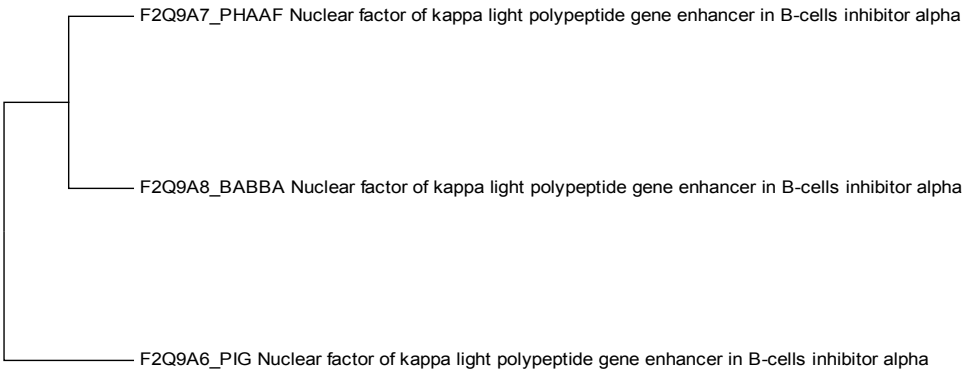

Fig. 4. Phylogenetic tree of the NFKBIA gene of domestic pig, warthog and babyrousa

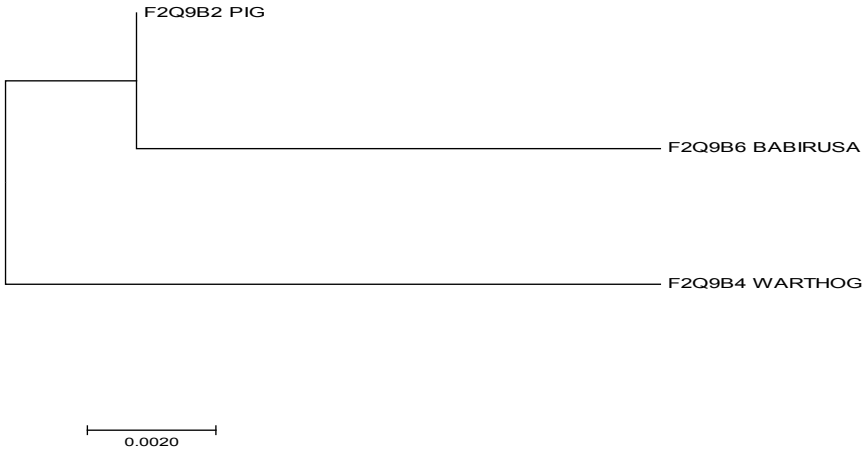

Fig. 5. Phylogenetic tree of the NFATC1 gene of domestic pig, warthog and babyrousa

Table 1. Provean score of RELA gene of suidae family

Variants	Provean score	Prediction (cutoff= -2.5)
K219E	-3.655	Deleterious

E = Glutamic acid, K = Lysine

Table 2. Provean score of PP3CB gene of suidae family

Variants	Provean score	Prediction (cutoff= -2.5)
G363del	-3.000	Deleterious
I424del	-6.034	Deleterious
R425del	-6.151	Deleterious
G426del	-8.979	Deleterious
F427del	-7.478	Deleterious
S428del	-6.445	Deleterious
P429del	-4.494	Deleterious
P430del	-4.494	Deleterious
H431del	-8.877	Deleterious
R432del	-5.894	Deleterious
I433del	-7.213	Deleterious

V = Valine, E = Glutamic acid, L = Leucine, D = Aspartic acid, T = Threonine, N = Asparagine, A = Alanine, W = Tryptophan, S = Serine, R = Arginine, G = Glycine, H = Histidine, K = Lysine, del=delete, ins- Insertion

Table 3. Provean score of PPIA gene of suidae family

Variants	Provean score	Prediction (cutoff= -2.5)
T107A	-4.559	Deleterious

M = Methionine, A = Alanine, L = Leucine, S = Serine,E = Glutamic acid, F = Phenylalanine, C = Cysteine, T= Threonine, V = Valine, P = Proline, G = Glycine, D = Aspartic acid., Q= Glutamine, K = Lysine, R =Arginine, P= Proline

Table 4. Provean score of NFATC1 gene of Suidae family

Variants	Provean score	Prediction (cutoff= -2.5)
T245N	-3.880	Deleterious

M = Methionine, A = Alanine, L = Leucine, S = Serine,E = Glutamic acid, F = Phenylalanine, C = Cysteine, T= Threonine, V = Valine, P = Proline, G = Glycine, D = Aspartic acid., Q= Glutamine, K = Lysine, R =Arginine, P= Proline

4. CONCLUSION

In conclusion, all tested genes with the exception of NFKBIA, have variants scored by PROVEAN. They also have deleterious variants. These deleterious variants detected may be responsible for the susceptibility or tolerance to the ASFV disease. This shows that there is possibility of mutation occurrence that can cause change in immune response to the disease. Further studies are still going on but this result shows that these variations between the pig and warthog can be the factor why pig are susceptible to ASFV and warthog resistant to ASFV. This study could give genomics bases to the discovery of a potent vaccine for prevention and control of the disease. The early detection of these genes can be used as genetic markers for selection against ASF diseases.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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