Polymorphism Identification and Genetic Diversity Studies of IRF3 Gene in White Fulani, Muturu and N'Dama Cattle

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Abstract

Interferon regulatory factor 3 (IRF3) is one of the strongest positional candidate genes implicated in a host of health-related phenotypes such as general disease resistance. The study conducted genetic characterization of the IRF3 gene in the Muturu, N'Dama and White Fulani cattle. DNA was extracted from the blood samples using the Zymo-spin extraction kit. ARLEQUIN 2.0001 software was used to estimate the basic population genetic statistics while DnaSP version 5.10.01 was used to estimate genetic diversity indices and test for deviation from neutrality. A total number of 18 and 29 Single nucleotide polymorphisms (SNPs) were detected using the codon code aligner software in exons 1-2 and 5-6 of the IRF3 gene respectively in the three cattle breeds after polymerase chain reaction and sequencing. In exons 1-2, the Muturu cattle had the highest value for number of SNPs (16) and genetic diversity indices, while the N'Dama breed had the least (1). In exons 5-6, the White Fulani cattle had the highest value for number of SNPs (14) and also genetic diversity indices, while the Muturu had the least. Some of the SNPs identified at these loci were shared by the breeds, suggesting the likelihood of shared ancestral alleles and similar functions. Analysis of molecular variation (AMOVA) carried out for the loci

under consideration revealed a higher level of variation among populations than within populations. The study concluded that the IRF3 gene was polymorphic and highly diverse in Nigerian cattle breeds.

Key words: Cattle, IRF3, Polymorphism, Genetic diversity.

INTRODUCTION

The interferon regulatory factors (IRF) are made up of developing family of similar transcription proteins recognized firstly as controllers of the IFN-alpha/beta gene promoters, as well as the IFN-stimulated response element (ISRE) of some IFN-stimulated genes (Hiscott *et al*., 1999). It has been reported that mammalian members which are nine in numbers of the interferon regulatory factor (IRF) family, IRF-1 to IRF-9 have been identified (Taniguchi *et al*., 2001).
The Interferon regulatory factor 3 (IRF3) gene encodes interferon regulatory factor 3, a member of the interferon regulatory transcription factor (IRF) family and is much revealed and is found in the cytoplasm of undiseased cells (Jann *et al*., 2009). Prior to infection, the micro-organism virus triggers phosphorylation of C-terminal serine/threonine residues and consequently leads to a conformational transformation in IRF3 with exposure of both the DNA binding domain (DBD) and Interferon associated domains (IAD), which leads to homo- or hetero-dimerization, , association with CBP/p300 coactivators, cytoplasm-to-nucleus transsfer, activation of multiple target genes and stimulation of DNA binding to the IFN-stimulated response elements (ISREs). This gene has also been reported to play a major role in the stimulation of type I IFNs following virus infection and is a constitutively expressed phosphoprotein of 427 amino acids in humans (Au *et al.*, 1995). The cattle IRF3 gene is made up of 8 exons and 7 introns and encodes a 417-amino acid protein. It is a phospho-protein and is made up of an N-terminal DNA binding domain (DBD domain), a C-terminal IRF-associated domain (IAD) and a transactivation domain, coupled with its critical roles in host defense and cell survival, the activity of IRF3 is strictly controlled. This gene is one of the strongest positional candidate genes implicated in a host of health-related phenotypes such as general disease resistance not only in cattle but in humans and mice as well, likewise this gene has also been reported to play an important role in the ability to adapt to infections caused by protozoans in mice and cattle (Jann *et al*., 2009).
Cattle populations have several polymorphisms at the IRF3 locus that change single amino acids. Some of the most polymorphic regions of this gene in cattle are exons 2, 5 and 6 (Ensembl cow release 92). Considering the importance of this gene, characterisation was done in three Nigerian cattle breeds which include; Muturu, White Fulani and N'Dama cattle breeds.

MATERIALS AND METHODS

Animals and Sampling

A total number of 190 animals were purposively sampled in this study. These comprised 85 White Fulani cattle, 72 Muturu and 33 N'Dama cattle. Samples of White Fulani cattle were collected at three locations; the Cattle Production Venture of the Federal University of Agriculture, Abeokuta (FUNAAB), Ajani Farms, Ogbomosho and Odeda Local Government, Ogun State, all located in Nigeria. The N'Dama breed was sampled at the Institute of Agricultural Research and Training Moor Plantation, Ibadan, off-site ranch at Ilora in Oyo State and the Federal Department of Livestock N'Dama Conservation Programme Ranch at Fashola in Oyo State while the Muturu breed was sampled at Odeda Local Government in Ogun State, Ipokia Local Government in Ogun State and Institute of Food Security, Environmental Resources and Agricultural Research facility at the FUNAAB all located in Nigeria.

DNA Extraction

Genomic DNA was extracted from the blood samples using Zymo-Spin IIC^{TM} extraction kit at the Central Biotechnology Laboratory of the Federal University of Agriculture, Abeokuta, Ogun State in Nigeria, using the manufacturer's protocol after which the extracted DNA was quantified for concentration and purity using Nanodrop spectrophotometer in congruent with protocol reported by Desjaldins and Conklin (2010). After quantification the samples were kept at -4^{o}C for further analyses.

Primer design and DNA amplification

Bovine exons 1-2 and exons 5-6 IRF3 gene specific primers were designed at Stab vida genetic laboratory situated in Caprica-Portugal using Fast PCR software. Primer sequence, primer length, annealing temperatures and the product sizes of the amplicons are presented in Table 1. For amplification, 10-20ng of genomic DNA was added to the reaction containing 0.4mM of primers forward and reverse, 1mM of each dNTPs, 1.5mM of MgCl2 and 1.5u Taq polymerase and amplified by Magnetic Beads carboxylate cycler at following conditions; one cycle of initial denaturation of 15 minutes at 96°C, final denaturation of 30 seconds at 95°C , optimum annealing temperature of 60°C for 30 seconds, extension at 70°C for 2 minutes in 35 cycles with one cycle of the final extension performed at 70°C for 5 minutes.

Table 1: Primer sequences used in the amplification of Bovine Interferon Regulatory Factor 3 gene exons 1-2 and exons 5-6

Primer	Length	Primer sequence	Amplicon size	TmºC
Exons1-2 (f)	19 bp	5' TCGGAAAACCTAAGAAGGG -3'	1335 bp	60º
Exons1-2 (r)	19 bp	5' ACCAGCCAACACAAATACC -3'		
Exons5-6 (f)	22 bp	5' CTGTCTTTTACTGTGCTGGTGG -3'	1029 bp	60º
Exons5-6 (r)	22 bp	5' CAGGTAAGGAGAGGGAGGAGAC-3'		

Sequencing of PCR products

The PCR products were purified using commercial kit (Magnetic Beads Carboxylate MC Lab, USA). The purified product were subjected to sequencing using BigDye® terminator cycle sequencing kit on ABI 3730xl (Applied Biosystems) DNA analyzer at Stab vida genetic laboratory situated in Caprica-Portugal.

IRF3 exons 1-2 and exons 5-6 DNA sequence analysis

The analysis of exons 1-2 and surrounding introns in 62 animals (WF: 22; MT: 23 and ND: 17) of the three Nigerian cattle breeds covered a sequence length of one thousand and twenty five base pairs (1025bp) each after trimming and cleaning of the sequences, while that of the exons 5-6 and their surrounding introns in 64 animals (WF: 20; MT: 22 and ND: 22) of the three Nigerian

cattle breeds covered a sequence length of seven hundred base pairs (700bp) each after trimming and cleaning of the sequences with Bioedit and MEGA 5. Multiple sequence alignment was carried out on all the obtained sequences of nucleotide using CLUSTAL W software (Thompson *et al.*, 1994). The SNPs in the bovine IRF3 gene exons 1-2 and exons 5-6 of each breed were identified using Codon code aligner software (htpp;//www.codon code. com/aligner).

The DNA sequence polymophism programme (DnaSP) version 5.10.01 was used to estimate haplotype frequencies, nucleotide diversity and sequence conservation. Tajima D and Fu's Fs was also performed to test for deviation from neutrality (Tajima, 1989; Fu, 1997) using the same software. ARLEQUIN 2.0001 software (Excoffier *et al.*, 1992) was used to estimate the basic population genetic statistics such as Analysis of molecular variance (AMOVA), population pairwise F*st* values and Standard genetic distances among the populations.

RESULTS AND DISCUSSION

Single nucleotide polymorphisms identified in exons 1-2 of cattle IRF3 gene

A total number of 18 SNPs were detected in exons 1-2 and the surrounding introns of the IRF 3 gene in all the three cattle breeds used for the study (presented inTable 2). Sixteen SNPs were identified in the Muturu, four were identified in the White Fulani and only one was identified in the N'Dama. Two of the SNPs were shared by the White Fulani and Muturu while one SNP was shared by the White Fulani and N'Dama. Majority of the SNPs identified were transversion type mutations.

Table 2: Single Nucleotide Polymorphisms Identified in exons 1-2 of Bovine Interferon Regulatory Factor 3 gene

	Breed Present			
SNP	**WF**	**MT**	**ND**	**Type of Mutation**
758A>C	√	-	√	Transversion
815C>T	-	√	-	Transition
816T>A	-	√	-	Transversion
823T>G	-	√	-	Transversion
897C>A	-	√	-	Transversion
909C>T	-	√	-	Transversion

Table 2 contd: Single Nucleotide Polymorphisms Identified in exons 1-2 of Bovine Interferon Regulatory Factor 3 gene

	Breed Present			
SNP	**WF**	**MT**	**ND**	**Type of Mutation**
975T>C	√	√	-	Transition
979T>G	-	√	-	Transversion
981T>C	-	√	-	Transition
986T>C	-	√	-	Transition
987A>T	-	√	-	Transversion
995G>C	√	-	-	Transversion
1003G>T	-	√	-	Transversion
1009C>G	-	√	-	Transversion
1013G>A	-	√	-	Transition
1019C>T	-	√	-	Transition
1024G>T	-	√	-	Transversion
1025G>T	√	√	-	Transversion

WF-White Fulani; MT- Muturu; ND-N'Dama.

Genetic diversity of exons 1-2 of IRF3 gene of the three cattle breeds

Table 3 represents the result of the genetic diversity study of exons 1 and 2 of the IRF 3 gene in the three breeds of cattle. The numbers of sequences used were twenty two for the White Fulani, twenty three for the Muturu and seventeen for the N'Dama cattle respectively. A total number of

four polymorphic sites (3 singletons and 1 parsimony informative site) were identified in this region of the IRF 3 gene in the White Fulani cattle.

Sixteen polymorphic sites (16 parsimony informative sites) were identified in the Muturu cattle, while only one polymorphic site which was a parsimony informative site was identified in the N'Dama cattle. The highest number of haplotypes were found in the Muturu cattle (4), followed by the White Fulani (3), while the N'Dama had the least. The Muturu cattle had the highest haplotype diversity value of 0.628, followed by the White Fulani (0.382), while the N'Dama had the lowest value of 0.260.

The Muturu cattle had the highest value of 0.0075 for nucleotide diversity while the N'Dama had the lowest value of 0.0004. With respect to the average number of nucleotide differences, the Muturu breed recorded the highest value of 7.570; the White Fulani cattle came second with a value of 0.519, while the N'Dama cattle had the lowest value of 0.382. It could be observed that there was very high sequence conservation across board, with the N'Dama cattle having the highest value of 0.999, the White Fulani was in second place with 0.996, while the Muturu cattle recorded 0.980.

Table 3: Genetic diversity of exons 1-2 of the IRF3 gene in three breeds of Nigerian Cattle.

	Breed		
Diversity Indices	**WF**	**MT**	**ND**
No. of sequences	22	23	17
No. of sites	1025	1025	1025
No. of polymorphic sites	4	16	1
No. of singleton variable sites	3	0	0
No. of parsimony informative sites	1	16	1
No. of haplotypes	3	4	2
Haplotype diversity	0.382	0.628	0.260
Nucleotide diversity	0.0005	0.0075	0.0004
Average number of nucleotide differences	0.519	7.570	0.382
Sequence conservation	0.996	0.980	0.999

WF-White Fulani, MT-Muturu, ND-N'Dama

Test for deviation from neutrality

Table 4 showed the result of the test of neutrality of the exons 1-2 of the IRF 3 gene in the three breeds of cattle. The Tajima's D and Fu's F statistics were both insignificant ($p>0.05$) for both White Fulani cattle (-1.479 and -1.390) and the N'Dama (0.5655 and 0.834), but were both observed to be positive and significant ($p<0.05$) for Muturu cattle (2.679 and 11.654).

Table 4: Test of Neutrality of exons 1-2 of bovine IRF3 gene in White Fulani, Muturu and N'Dama Cattle

Breed	Tajima's D	Fu's F statisitics
WF	-1.479^{NS}	-1.390^{NS}
MT	2.679^{**}	11.654^{*}
ND	0.5655^{NS}	0.834^{NS}

WF-white Fulani, MT-Muturu, ND-N'dama, * $P<0.05$, ** $P<0.01$, NS-not significant ($P>0.10$)

Table 5: Analysis of molecular variation for exons 1-2 of bovine IRF3 gene in the three cattle breeds used for the study

Source	df	SS	Var. Comp.	% Var.	F_{ST}
Among pop.	2	854.45	20.76	93.03	0.93
Within pop.	59	91.73	1.56	6.97	
Total	61	946.18	22.32		

Df-degree of freedom; SS-sum of squares, F_{ST}- Fixation index

Table 5 revealed the result of the analysis of molecular variance (AMOVA) for exons 1-2 of the IRF 3 gene in the three breeds of cattle. The variation amongst the three breeds was much higher (93.03%) than that of the variation within populations (6.97%). The AMOVA also had an F_{ST} value of 0.93.

Table 6: Single Nucleotide Polymorphisms Identified in exons 5-6 of Bovine Interferon Regulatory Factor 3 gene

SNP	Breed Present			Type of Mutation
	WF	MT	ND	
10T>A	-	-	√	Transversion
22G>A	√	-	-	Transition
62G>C	√	-	√	Transversion
125C>G	√	-	√	Transversion
126C>G	√	-	-	Transversion
207C>G	-	-	√	Transversion
241T>G	√	√	√	Transversion
262A>C	√	-	√	Transversion
271G>A	√	-	-	Transition
290C>T	-	√	√	Transition
365G>T	-	-	√	Transversion

Table 6 contd: Single Nucleotide Polymorphisms Identified in exons 5-6 of Bovine Interferon Regulatory Factor 3 gene√

SNP	Breed Present			Type of Mutation
	WF	MT	ND	
371C>G	-	-	√	Transversion
391C>T	√	-	-	Transition
392T>C	-	-	√	Transition
403A>G	√	-	√	Transition
404G>A	-	-	√	Transition
538C>T	-	√	-	Transition
539C>G	√	-	-	Transversion
542G>T	√	-	-	Transversion
617A>T	-	-	√	Transversion
653C>T	-	√	-	Transition
654T>G	-	√	-	Transversion
657G>A	√	-	-	Transition
659C>G	-	√	-	Transversion
660C>G	√	-	-	Transversion
678A>G	-	√	-	Transition
679G>A	-	-	√	Transition
681G>A	-	√	-	Transition
682G>A	√	-	-	Transition

WF-White Fulani; MT- Muturu; ND-N'Dama.

A total number of 29 SNPs were detected in exon 5-6 of the IRF 3 gene in all the three cattle breeds used for the study (Table 6). Fourteen SNPs were present in the White Fulani cattle; thirteen were identified in the N'Dama and eight in the Muturu.

Four SNPs (62G>C, 125C>G, 262C>A and 403A>G) were shared between the White Fulani and N'Dama cattle, one SNP (290C>T) was shared between the Muturu and N'Dama cattle, while one SNP (241T>G) was observed to be shared by the three cattle breeds at the exon 5-6 region of the IRF3 gene. Fifteen of the identified SNPs were transversion mutations while the remaining 14 were transition in nature.

Table 7: Genetic diversity of exons 5-6 of the Interferon Regulatory Factor 3 gene in the three breeds of Nigerian Cattle

	Breed		
Diversity Indices	**WF**	**MT**	**ND**
No. of sequences	20	22	22
No. of sites	700	700	700
No. of polymorphic sites	14	8	13
No. of singleton variable sites	7	5	5
No. of parsimony informative sites	7	3	8
No. of haplotypes	12	6	11
Haplotype diversity	0.93	0.54	0.87
Nucleotide diversity	0.0049	0.0015	0.0043
Average number of nucleotide differences	3.337	1.048	2.913

WF-White Fulani, MT-Muturu, ND-N'Dama.

Table 7 represented the results of the genetic diversity study of exons 5-6 of the IRF 3 gene in the three breeds of cattle used for the study. A total number of fourteen polymorphic sites (7 singleton variable sites and 7 parsimony informative site) were identified in this region of the IRF 3 gene in the White Fulani cattle, 8 polymorphic sites (3 parsimony informative sites and 5 singleton variable sites) were identified in the Muturu cattle while 13 polymorpic sites (8 parsimony informative sites and 5 singleton variable sites) were identified in the N'Dama Cattle.

The highest number of haplotypes were found in the White Fulani cattle (12), followed by the N'Dama (11), with the Muturu having the least of six (6) haplotypes in these regions. The White Fulani Cattle had the highest haplotype diversity value of 0.93, followed by the N'Dama (0.87), while the Muturu had the lowest value of 0.54. The White Fulani Cattle had the highest value of 0.0049 for nucleotide diversity; N'Dama had 0.0043 while Muturu had the lowest value of 0.0015. With respect to the average number of nucleotide differences, the White Fulani Cattle recorded the highest value of 3.337; the N'Dama had 2.913 while the Muturu cattle had the lowest value of 1.048.

Table 8 –Test of Neutrality for exons 5-6 of the IRF3 gene in the three cattle breeds

Breed	Tajima's D	Fu's F statisitics
WF	-0.78^{NS}	-6.06^{NS}
MT	-1.72^{NS}	-3.16^{NS}
ND	-0.65^{NS}	-4.81^{NS}

WF-White Fulani, MT-Muturu, ND-N'Dama, NS-Not significant (p>0.05)

Table 8 showed the result of the test of neutrality of the exons 5-6 of the IRF 3 gene in the three breeds of cattle used for the study. The Tajima's D and Fu's F statistics were both insignificant (p>0.05) for the three cattle breeds. The three breeds had negative values (White Fulani -0.78 and -6.06; Muturu -1.72 and -3.16; N'Dama -0.65 and -4.81) for Tajima's D and Fu's F statistics respectively.

Table 9: Analysis of molecular variation for exons 5-6 of bovine IRF3 gene in the three cattle breeds used for the study

Source	df	SS	Var. Comp.	% Var.	F_{ST}
Among pop.	2	3739.47	87.68	98.76	0.99
Within pop.	61	66.93	1.10	1.24	
Total	63	3806.40	88.78		

Df-degree of freedom; SS-sum of squares, F_{ST}- Fixation index

Table 9 revealed the result of the analysis of molecular variation (AMOVA) for exons 5-6 of the IRF 3 gene in the three breeds of cattle. The variation among the three breeds was much higher (98.76%) than that of the variation within populations (1.24%) in this region. The AMOVA had an F*st* value of 0.99.

DISCUSSION

This study was carried out to identify polymorphisms in exons 1-2 and exons 5-6 of the IRF3 gene in the White Fulani, Muturu and N'Dama cattle breeds. These exons have been identified to be some of the most polymorphic regions of the candidate gene in Cattle (Ensembl cow release 92). A total number of 18 nucleotide polymorphisms were identified in exons 1-2 and the surrounding introns in the three cattle breeds, suggesting this region is polymorphic, especially in the Muturu and White Fulani breeds both having a higher number of SNPs than the N'Dama which had only one, but generally this region of the gene in the three breeds was quite

conserved. According to Dobzhansky (1970), polymorphisms are common in nature; they are related to biodiversity, genetic variation and adaptation; thereby playing a functional role in retaining a variety of form in a population living in a varied environment. The highly polymorphic nature of this region in the Muturu breed could also suggest the genes involvement in various physiological activities like immune responses, considering this breed has been reported to be trypanotolerant but highly susceptible to heat stress (Adebambo, 2001; Styslinger, 2011; Udeh *et al*., 2011).

A single mutation discovered to be common to both the White Fulani and the N'Dama suggests a shared common ancestor in their evolutionary development. This particular mutation could also play a role in growth and development, likewise heat tolerance considering the fact that these two breeds are larger animals and have been reported to be more heat tolerant than the Muturu (Behl *et al*., 2010; Udeh *et al*., 2011). Two mutations were also observed to be common to both the White Fulani and the Muturu, which likewise suggests not just a common ancestor but the likelihood that these mutations could also be responsible for immunological activities such as tolerance and resistance to intestinal helminthes, ticks and tick-borne diseases which are some of the characteristics shared by these two breeds (Claxton and Laperre, 1991; Mattioli *et al*., 2000).

In exons 5-6 and the surrounding introns of the candidate gene, a total number of 29 polymorphic sites were identified across the three cattle breeds, suggesting this region of the cattle IRF3 gene is also polymorphic with the Muturu breed having fewer mutated sites. It should be noted that variations in the DNA sequences of individuals have been reported to affect how they develop diseases and respond to pathogens, chemicals, drugs, vaccines, and other agents (Carlson, 2008). The presence of more SNPs in exons 5-6 of White Fulani could be related to their involvement in transhumance activities which has exposed them to varying climatic and different adaptive pressures. A single mutated site was identified which was common to the three breeds; suggesting a common ancestor in their evolutionary development or an ancestral allele which could have arisen due to introgression or crossbreeding.

The four mutated sites in exons 5-6 of the candidate gene, being shared by the White Fulani and N'Dama, still suggested a common ancestor at a point, with these mutations also likely to be involved in growth and heat tolerance activities, considering the fact that the two breeds are larger animals and have been reported to be more heat-tolerant than the Muturu (Behl *et al*., 2010; Styslinger, 2011). A single mutated site in exons 5-6 was also identified as being shared by the Muturu and the N'Dama. The two breeds are both Taurines and some level of ancestral relationship would be expected between them. This shared SNP could play a vital role in immune response in the two breeds, which have been extensively reported to be the major trypano-tolerant breeds of cattle in Nigeria; making them thrive in areas highly-infested by tsetse flies (Akinwunmi and Ikpi, 1985; Charles,1991; Tawah and Rege, 1996; Adebambo, 2001). The absence of this particular mutation in the White Fulani cattle which is susceptible to trypanosomosis reinforces the likely implication of this SNP in resistance to the disease.

Analysis of genetic diversity of the IRF3 gene could be a great asset for improvement of various traits being influenced by the gene through marker-assisted selection. A total number of seven haplotypes were identified in exons 1-2 of the IRF3 gene across the three breeds. The highest number of haplotypes (4) was identified in the Muturu, which also had the highest haplotype and nucleotide diversity; this is an indication of high genetic diversity in this region of the gene in Muturu which will respond better to selection when compared with other breeds used for the study. The higher genetic variation in exons 5-6 of the IRF3 gene of White Fulani must have contributed to its adaptability, as genetic variation is important in helping organisms to adapt to an ever changing environment. This higher genetic variation in this breed could also be attributed to the lack of artificial selection pressure when compared to N'Dama and Muturu which are mostly bred in breeding stations and by subsistent farmers.

Tajima D and Fu' Fs test are commonly used test of neutrality in population genetic studies. It summaries the sequence data into a single value (Tajima, 1989). The test of neutrality carried out on exons 1-2 region of the candidate gene in the White Fulani produced negative results which could be interpreted as a signal of population expansion in the absence of selection, the negative Tajima value is also related to the presence of singletons which were detected in the White Fulani cattle. The test of neutrality on exons 1-2 of the Muturu and N'Dama cattle yielded positive results for both Tajima's D and Fu's Fs, suggesting a situation of balancing selection and lack of singletons as was observed in these two breeds.

The same test of neutrality was carried out on exons 5-6 of the candidate gene in the three breeds, the result yielded negative Tajima D and Fu Fs values suggesting some level of population expansion in the absence of selection with respect to this particular region, an excess amount of singletons were also detected in this region across the three breeds which is also related to the negative values obtained for the test of neutrality (William *et al*., 1995). In recent times, it has been argued that singletons or rare variants located in different genes could play a more important role in disease susceptibility than common variants (Bodmer and Bonilla, 2008; Saint Pierre and Génin, 2014). These rare genetic variants not initially captured by genome-wide association studies using single nucleotide polymorphism-chips have now become detectable with the advent of next-generation sequencing technologies. Goldstein *et al.,* (2013) also believed that the process of purifying selection played a major role in maintaining a low frequency of such rare variants capable of strongly predisposing individuals to diseases in populations. The presence of singletons in exons 1-2 and 5-6 of the IRF3 gene in the White Fulani cattle could therefore play a significant role in this particular breeds' susceptibility to diseases. Singletons were also observed to be present in exons 5-6 of the candidate gene in the Muturu and N'Dama which would also suggest the likelihood of such playing critical roles in disease susceptibility and probably poor heat tolerance. Analysis of molecular variance (AMOVA) is a statistical model for the molecular variation in a single species (Excoffier *et al.*, 1992). It is widely used in population genetics to test the hypothesis that genetic diversity within two populations is not significantly different from that which would result from pooling the two

populations (Excoffier *et al.*, 1992; Anderson, 2001). Analysis of exons 1-2 and 5-6 of the IRF3 gene in N'Dama, Muturu and White Fulani based on AMOVA demonstrated more variation among population compared to within population indicating that gene exchange or crossbreeding among the populations used for the study was low.

Conclusion

This study to the best of our knowledge would be the first to report polymorphisms and genetic diversity of the IRF3 gene in the Nigerian White Fulani, Muturu and N'Dama cattle. The results of different analyses carried out in the study revealed the Muturu breed had the highest values for genetic diversity indices for exons 1-2 of the IRF3 gene while the White Fulani cattle breed had the highest genetic diversity indices for exons 5-6 of the IRF3 gene which we believe must have contributed to its adaptability considering the transhumance activities it is exposed to. The study also revealed some of the polymorphisms identified were shared by the different breeds suggesting the possibility of common ancestors and similar functions. The polymorphic nature of the candidate gene in the three cattle breeds would also suggest a form of nature's response to ability of pathogens to evolve to evade the immune system, considering the gene has been reported to perform various immune functions. The SNPs detected within exons 1-2 and 5-6 of the IRF3 gene can therefore be used as genetic markers for association studies in further researches.

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