Characterization of Transmembrane Bax Inhibitor Motif-6 Gene in Nigerian Goats Based on Single Nucleotide Polymorphisms

John Odunayo ADEBAYO[1,4]
Martha Nchang BEMJI[2] (✉)
Mathew WHETO[2]
Monsuru Oladimeji ABIOJA[3]
Ismail MURITALA[5]

Summary

Variation in Transmembrane Bax Inhibitor Motif 6 (*TMBIM6*) gene influences heat tolerance levels in eukaryotes. Characterizing genes in different goat breeds would provide valuable insights into animal genetic variation, potential adaptive significance and implications for breeding strategies aimed at improving stress tolerance and overall productivity in goats. Thus, this study characterized Nigerian goat populations by analyzing single nucleotide polymorphisms (SNPs) from exon 4 to intron 6 of *TMBIM6* gene. Genomic DNA of 77 Nigerian goats: Red Sokoto (41), Sahel (16) and West African Dwarf (WAD) (20) from semi-arid, Sahel and humid regions, respectively, were isolated, sequenced and analyzed for number of alleles, allele frequency, heterozygosity (*He*) and other genetic indices. Fifteen SNPs (8, 5 and 2 for Sahel, WAD and Red Sokoto, respectively) were characterized. Allele frequencies of the SNPs ranged from 0.89 in the Sahel and WAD breeds to 0.63 in the Sahel breed. SNPs at exon 5 (11857A>G) in Sahel and intron 6 (12221C>T) in Red Sokoto had the highest *He* (0.47), while the lowest (0.20) for intron 4 (11714C>G) and exon 6 (12134G>A) in WAD and Red Sokoto, respectively. Phylogenetic analysis showed that WAD and Sahel goats remained more closely related. This first genetic study at exon 4 to intron 6 of *TMBIM6* gene in Nigerian goats has preliminarily established that Nigerian goat breeds are highly diverse. Further research is needed to establish links between identified SNPs and heat tolerance in goats.

Key words

genetic diversity, *TMBIM6* gene, phylogenetic relationship, SNPs, goat

[1] Livestock Science and Sustainable Environment, Centre for Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, Nigeria

[2] Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria

[3] Department of Animal Physiology, Federal University of Agriculture, Abeokuta, Nigeria

[4] Department of Animal Sciences, North Carolina Agricultural and Technical State University, Greensboro, North Carolina, United States

[5] Department of Agriculture, Faculty of Science, Agriculture and Engineering, University of Zululand, KwaDlangezwa, South Africa

✉ Corresponding author: bemjimn@funaab.edu.ng

Received: July 30, 2024 | Accepted: April 2, 2025 | Online first version published: April 25, 2025

Introduction

Goats (*Capra hircus*) are widely distributed all over the world due to their adaptability to different environmental and climatic conditions (Mahmoud 2010). They live in dry regions to humid tropical rain forests and cold, low-oxygen high-altitude regions (Hirst 2017; Lohani and Bhandari 2021). Goat breeds from various regions differ in size, shape and types of production (Adu 1979; Lohani and Bhandari 2021). In Nigeria, three distinct goat breeds have been characterized: the short-legged West African Dwarf (WAD), predominantly distributed in the humid forest belt of the southern region; the moderately small-sized Red Sokoto, primarily found in the semi-arid zones; and the long-limbed Sahel breed, native to the savannah regions of the country (Muema et al. 2009; Muritala et al. 2015). The variations in goats' responses to heat stress are influenced by their distinct physical characteristics (Ugwu 2007). Archana et al. (2017) reported a relationship between diversity in the ability of individual animals to cope with heat stress and the nucleotide changes occurring in the Transmembrane Bax Inhibitor Motif 6 (*TMBIM6*) gene in goats. Two unique single nucleotide polymorphisms (SNPs) were reported in exon 5 of *TMBIM6* gene of heat stress-tolerant and stress-susceptible individuals in Barbari and Jakhrana which include 55 (T>C) and 96 (A>C). *TMBIM6* gene plays a key role in regulating cell survival in eukaryotes by responding to various stressors, both biotic and abiotic, and likely acts as a critical protector of cellular integrity (Singh et al. 2019). *TMBIM6* gene is associated with thermo-tolerance traits, enhancing cellular or organismal resistance and adaptation to heat stress following prior sub-lethal heat exposure, as reported by Singh et al. (2019). Knowledge of genetic variation among goat breeds is crucial for genetic improvement, understanding environmental adaptation, and optimizing both the utilization and conservation of these breeds (Berihulay et al. 2019). The increasing availability of molecular markers across various farm animal species, along with advancements in techniques to assess molecular variation, is significantly enhancing the ability to characterize the genetic diversity of breeds (Okpeku et al. 2011; Muritala et al. 2015). According to Lipshutz et al. (1999) and Salisu et al. (2018), there are four main reasons for the increasing interest in using single nucleotide polymorphisms (SNPs) as genetic markers: SNPs are abundant and offer more potential markers near or within loci of interest compared to other types of polymorphisms; some SNPs are located within coding regions and directly influence protein function, making them responsible for differences in economically important traits; SNPs exhibit greater inheritance stability than microsatellites, making them more promising for long-term selection markers; SNPs are also more reliable than microsatellites for high-throughput genetic analysis, particularly with DNA microarray technology. Genetic characterization of diverse goat populations in Nigeria is essential for the effective utilization of goat genetic resources. This characterization would establish a database that identifies which populations represent homogeneous breeds and which are genetically distinct. Additionally, genetic characterization will provide insights into the genetic composition of Nigerian goat breeds and enhance our understanding of the evolutionary history of goats in Nigeria, as well as inform the future management of goat genetic resources. Therefore, this study was designed to characterize Nigerian goat populations by analyzing single nucleotide polymorphisms from exon 4 to intron 6 of the *TMBIM6* gene.

Materials and Methods

Experimental Animals, Location and Management

A total of 77 goats comprising Red Sokoto (n = 41, 22 males and 19 females), Sahel (n = 16, 3 males and 13 females), and West African Dwarf (n = 20, 3 males and 17 females) were used for the study. The experimental animals included the three Nigerian goat breeds with age range between 2 and 3 years, apparently disease-free, selected from Sahel and Red Sokoto goats kept semi-intensively at the National Animal Production Research Institute, Ahmadu Bello University, Zaria, and WAD goats kept at the SELEMA Farms, Iwo, Osun State, Nigeria.

Blood Sample Collection, DNA Extraction and Quantification

Blood samples were collected from jugular veins into vacutainer tubes containing ethylene diamine tetra acetic (EDTA) anticoagulant, homogenized and stored at -20 °C until further analysis. Genomic DNA was isolated from blood using Zymo Research extraction kit according to the manufacturer's protocol. The extracted genomic DNA was quantified using Nanodrop spectrophotometer (ND-1000; NanoDrop Technologies, USA) according to Desjardins & Conkin (2010). The quality of the amplicons was checked using gel electrophoresis (Fig. 1).

Primer Design and Amplification of *TMBIM6* Gene

A pair of primer (Table 1) specific to exon 4 - intron 6 of *TMBIM6* gene designed by STAB VIDA laboratory in Portugal was used for DNA amplification. The primer sequence, lengths, annealing temperature and size of amplicon are shown in Table 1. Polymerase chain reaction was carried out in a total volume of 20 µl containing 50 ng genomic DNA added to a reaction mix containing12.8µl of H_2O MQ, 2.5 µl of 1×PCR reaction buffer, 1µl dNTPs, 1.5µl $MgCl_2$, pH 9, 1µl each of forward and reverse primers and 0.2µl of *Taq* DNA polymerase. The cycling protocol was implemented according to the following programme: initial denaturation for 15 minutes at 96 °C, 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 53 °C for 30 seconds, extension at 70 °C for 1 minute and 40 seconds and final extension at 70 °C for 5 minutes.

DNA Sequencing, Trimming and Alignment

The genomic DNA was purified from the gel (Fig. 1) using a commercial kit following manufacturer's procedure and sequenced on ABI 3730XI gene sequencer. Trimming and editing of nucleotide sequences were done using Bioedit 7.2 software to remove noise and copied into notepads in FASTA format. The sequences obtained for exon 4 to intron 6 were aligned with reference sequence (Accession number: NC 030812.1). The alignment was done using CLUSTAL W (Thompson et al. 1994) implemented in Molecular Evolutionary Genomic Analysis software XI (Tamura et al. 2013).

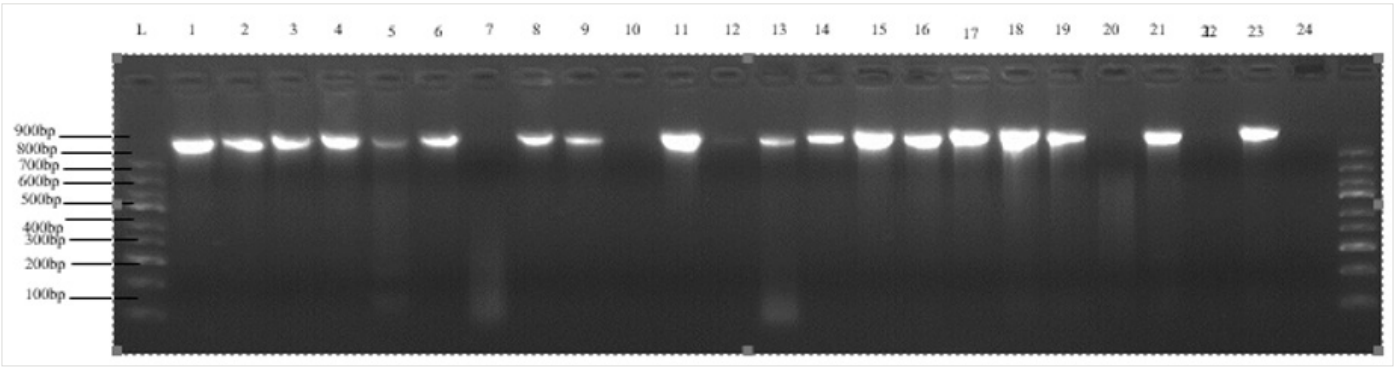


Figure 1. Genomic DNA isolated from Nigerian indigenous goats

Table 1. Primer Sequences, Annealing Temperature and Product Size of Exon 4 - Intron 6 of *TMBIM6* gene

Target sequence	Primer sequence (5' – 3')	Amplicon size (bp)	Annealing Temperature (°C)
Goat *TMBIM6*	Forward – TGAGGATGGAAGGGAGG	1109.000	53.000
(Exon 4 – intron 6)	Reverse – CAAAGGAAGAATGAGGCG		

Identification and Analyses of Single Nucleotide Polymorphisms

The SNPs were confirmed using DnaSP (Librado & Rozas 2009). The allele frequency of each SNP was determined by dividing the frequency of each allele with total sample size for each genotype (Falconer & Mackay 1996). Heterozygosity (*He*) was calculated using the formula by Guo & Elston (1999):

$$Heterozygosity\ (He) = 1 - (p^2 + q^2),$$

where p is the major allele frequency and q is the minor allele frequency. The Polymorphic Information Content (*PIC*) was calculated using the formula proposed by Bostein et al. (1980):

$$PIC = He - 2\,p^2 q^2,$$

where p is the major allele frequency and q is the minor allele frequency.

Phylogenetic Relationship

The nucleotide sequences for exon 5 of *TMBIM6* gene of Nigerian goat breeds and retrieved sequences of some selected goat and sheep breeds (Table 2) which included Jamunapari, Barbari, Jakhrana, Sirohi, Ganjam, Black Bengal, Raighar, Kermani sheep, Chinese Merino sheep, and San Clemente goats (Fig. 2) were from National Center for Biotechnology Information nucleotide webpage. MEGA XI software was used to infer a phylogenetic relationship using Neighbor-joining method (*p-distance* model), Fasta files (~750 bp) and a 10000 bootstrap replication confidence level. Geographical distribution (Fig. 3) of the goats and breeds included in phylogenetic analysis was constructed using Matplotlib (Hunter 2007).

Table 2. Breed, country of origin and accession number of goats and sheep breeds used for phylogenetic study

Breed	Country of Origin	Accession Number
Raighar goat	India	MF673128
Jakrahna goat	India	KT820013
Black Bengal goat	Bangladesh / India	MF673129
Kermani Sheep	Iran	ASM2243283v1
Xinong Saanen goat	Switzerland	ASM2665220v1
Chinese Merino Sheep	Spain	ASM2243282v1
Sirohi goat	India	KT820014
Ganjam goat	Eastern India	MF673127
San Clemente goat	California, United States	NC_030812


Xinong Saanen goat (source: livestockmarket.com)


Ganjam goat (source: indiamart.com)


Black Bengal (source: ar.inspiredpencil.com)

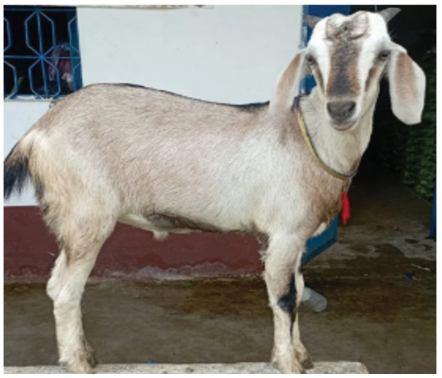

Sirohi goat (source: goatwala.com)


Jakrahna goat (source:blogspot.com)


San Clemente goat (source: livestockconservancy.org)


Chinese Merino sheep (source: Ma et al., (2021))


Kermani sheep (source: journeytopersia.com)


Raighar goat (source: indiamart.com)


Sahel goat (source: naijadazz.com)


Red Sokoto goat (source: youtube.com)


WAD goat (source: animalia-life.club)

Figure 2. Photographic representation of goats and sheep (outgroup) breeds included in neighbor-joining tree

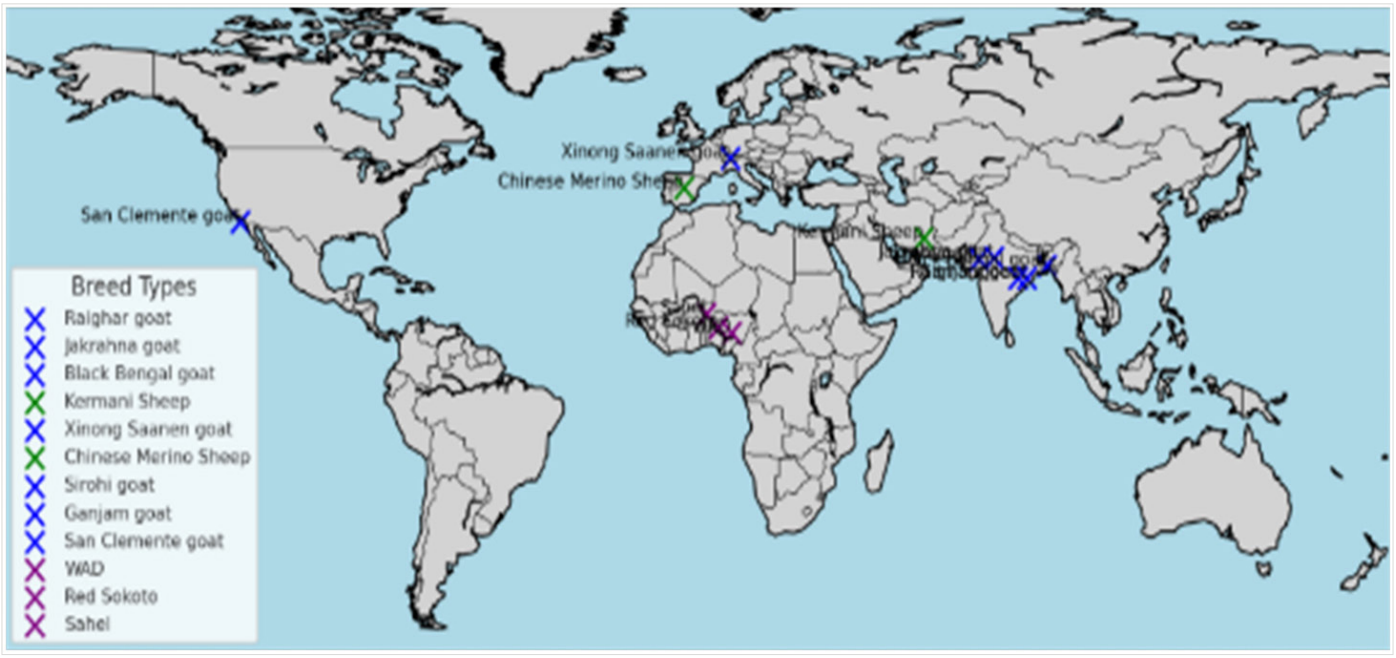


Figure 3. Geographical map showing the distribution of goats and sheep breeds included in phylogenetic analysis

Results

Fifteen single nucleotide polymorphisms (SNPs) were found in the *TMBIM6* gene of Nigerian goat breeds, covering exon 4 to intron 6. Of these, 13 had been previously reported, while 2 were novel. The distribution of SNPs varied among the Nigerian breeds: Red Sokoto goats had 2, WAD goats had 5 and Sahel goats, 8 SNPs (Table 3). The major allele frequencies (MAF) ranged from 0.63 for 12134G>A (exon 5) in Sahel to 0.89 for 11714C>G (intron 4) in WAD and 12134G>A (exon 6) in Red Sokoto. The lowest heterozygosity (0.20) was found in intron 4 (11714C>G) in WAD and exon 6 (12134G>A) in Red Sokoto, while the highest heterozygosity (0.47) was observed in exon 5 (11857A>G) in Sahel and intron 6 (12221C>T) in Red Sokoto. The polymorphic information content (PIC) ranged from 0.18 in intron 4 (11714C>G) in Red Sokoto and exon 6 (12134G>A) to 0.36 in exon 5 (11857A>G) in Sahel and intron 6 (12221C>T) in Red Sokoto (Table 3).

All three breeds showed 49 nucleotide sites, but only Sahel goats had a polymorphic site, a parsimony-informative site and a singleton variable site, with two haplotypes observed at exon 4 (Table 4). However, at intron 4, all breeds had 107 nucleotide sites, with WAD and Sahel each exhibiting one polymorphic site and WAD having a singleton variable site (Table 5). Two polymorphic sites were identified in Sahel, while none were found in Red Sokoto or WAD at exon 5. Sahel was the only breed with a singleton variable site and a parsimony-informative site. The number of haplotypes in WAD, Red Sokoto and Sahel were 0, 1, and 3, respectively (Table 6).

Diversity at exon 6 of the *TMBIM6* gene (Table 7) revealed polymorphic sites in Nigerian goats: WAD (1), Red Sokoto (1) and Sahel (2). Singleton variable sites were 1 in Red Sokoto, 2 in Sahel, and none in WAD. However, only WAD exhibited a parsimony-informative site.

At intron 6, WAD recorded the highest number of polymorphic sites (3), while Red Sokoto had the lowest (1). Singleton variable sites for WAD and Sahel were one each, while none were found in Red Sokoto. Parsimony-informative sites were 1 in Red Sokoto and Sahel, and 2 in WAD. The number of haplotypes recorded were 2 for Red Sokoto, 3 for Sahel, and 4 for WAD. However, WAD had the highest (0.750) haplotype diversity while lowest (0.536) was found for Red Sokoto. Nucleotide diversities were 0.001 for Red Sokoto and 0.002 for Sahel and WAD. Red Sokoto had 0.536 average nucleotide differences, while 0.867 and 1.107 were found in Sahel and WAD, respectively (Table 8).

Most of the SNPs identified in *TMBIM6* gene of Nigerian goats were non-synonymous, except 11635C>T in exon 4, which led to a synonymous amino acid change (Table 9). The phylogenetic analysis showed that the three Nigerian goat breeds (WAD, Sahel, and Red Sokoto goats) had evolved distinct yet related genetic lineages (Fig. 4), while WAD and Sahel goats remained most closely related (Fig. 4). Nigerian goat breeds shared a relatively common ancestor with other goat breeds (Jakrahna, San Clemente, Raighar, Xinong Saanen, Ganjam, Black Bengal and Sirohi goats) compared to the ancestral level with the outgroup species (Kermani and Chinese merino sheep).

Table 3. Major allele frequency, heterozygosity and polymorphic information content for identified SNPs at exon 4 to intron 6 of *TMBIM6* gene in Nigerian goats

Region	SNP	Breed	Major allele frequency	Heterozygosity	PIC
Exon 4	11635C>T	Sahel	0.86	0.24	0.21
Intron 4	11723C>G	Sahel	0.75	0.38	0.3
	11714C>G	WAD	0.89	0.2	0.18
Exon 5	11857A>G	Sahel	0.63	0.47	0.36
	11909G>A	Sahel	0.88	0.21	0.19
Exon 6	12134G>A	Red Sokoto	0.89	0.2	0.18
	12136C>T	Sahel	0.88	0.21	0.19
	12145G>A	Sahel	0.88	0.21	0.19
	12131G>A	WAD	0.78	0.34	0.28
Intron 6	12221C>T	Red Sokoto	0.63	0.47	0.36
	12243C>T	Sahel	0.67	0.44	0.34
	12655G>A	Sahel	0.83	0.28	0.24
	12220C>T	WAD	0.75	0.38	0.3
	12266A>T	WAD	0.88	0.21	0.19
	12616G>A	WAD	0.75	0.38	0.31

Note: PIC - Polymorphic Information Content; WAD – West African Dwarf

Table 4. Diversity of exon 4 of *TMBIM6* gene in Red Sokoto, Sahel and West African Dwarf goats

Diversity Indices	Breed		
	Red Sokoto	Sahel	WAD
Nucleotide sites	49	49	49
Polymorphic site	0	1	0
Singleton variable site	0	1	0
Parsimony informative site	0	0	0
Number of Haplotype	1	2	0
Haplotype diversity	0.000	0.286 ± 0.196	0.000
Nucleotide diversity	0.000	0.00583 ± 0.008	0.000
Average number of nucleotide differences	0.000	0.286	0.000

Table 5. Diversity of intron 4 of *TMB1M6* gene in Red Sokoto, Sahel and West African Dwarf goats

Diversity Indices	Breed		
	Red Sokoto	Sahel	WAD
Nucleotide sites	107	107	107
Polymorphic site	0	1	1
Singleton variable site	0	0	1
Parsimony informative site	0	1	0
Number of Haplotype	1	2	2
Haplotype diversity	0.000	0.429 ± 0.169	0.222 ± 0.166
Nucleotide diversity	0.000	0.00401 ± 0.004	0.002 ± 0.003
Average number of nucleotide differences	0.000	0.429	0.222

Table 6. Diversity of Exon 5 of *TMBIM6* Gene in Red Sokoto, Sahel and West African Dwarf goats

Diversity Indices	Breed		
	Red Sokoto	Sahel	WAD
Nucleotide sites	98	98	98
Polymorphic site	0	2	0
Singleton variable site	0	1	0
Parsimony informative site	0	1	0
Number of Haplotype	1	3	0
Haplotype diversity	0.000	0.679 ± 0.122	0.000
Nucleotide diversity	0.000	0.00802 ± 0.00787	0.000
Average number of nucleotide differences	0.000	0.786	0.000

Table 7. Diversity of Exon 6 of *TMBIM6* gene in Red Sokoto, Sahel and West African Dwarf goats

Diversity Indices	Breed		
	Red Sokoto	Sahel	WAD
Nucleotide sites	80	80	80
Polymorphic site	1	2	1
Singleton variable site	1	2	0
Parsimony informative site	0	0	1
Number of Haplotype	2	3	2
Haplotype diversity	0.222 ± 0.166	0.417 ± 0.191	0.389 ± 0.164
Nucleotide diversity	0.00278 ± 0.00460	0.00556 ± 0.00920	0.00486 ± 0.00460
Average number of nucleotide differences	0.222	0.444	0.389

Table 8. Diversity of Intron 6 of *TMBIM6* gene in Red Sokoto, Sahel and West African Dwarf goats

Diversity Indices	Breed		
	Red Sokoto	Sahel	WAD
Nucleotide sites	412	430	446
Polymorphic site	1	2	3
Singleton variable site	0	1	1
Parsimony informative site	1	1	2
Number of Haplotype	2	3	4
Haplotype diversity	0.536 ± 0.123	0.600 ± 0.215	0.750 ± 0.139
Nucleotide diversity	0.00130 ± 0.00094	0.00202 ± 0.00204	0.00248 ± 0.00259
Average number of nucleotide differences	0.536	0.867	1.107

Table 9. Amino acid variations of SNPs identified in exon 4 to exon 6 of *TMBIM6* gene in Nigerian goats

Region	SNP	Codon Change	Type of mutation	Amino acid variation	Effect of amino acid change
Exon 4	11635C>T	ACC>ACT	Transversion	Threonine > Threonine	Synonymous
Intron 4	11723C>G	ACT>AGT	Transversion	Non-coding	
	11714C>G	ACT>AGT	Transversion	Non-coding	
Exon 5	11857A>G	AAT>AGT	Transition	Asparagine > Serine	Non-Synonymous
	11909G>A	TAG>TAA	Transition	Stop Codon	Stop Codon
Exon 6	12134G>A	CGG>CAG	Transition	Arginine > Gluthamine	Non-Synonymous
	12136C>T	TCT>TTT	Transversion	Serine > Phenylalanine	Non-Synonymous
	12145G>A	CGG>CAG	Transition	Arginine > Gluthamine	Non-Synonymous
	12131G>A	CGG>CAG	Transition	Arginine > Gluthamine	Non-Synonymous
Intron 6	12221C>T	CCG>CTG	Transition	Non-coding	
	12243C>T	CCG>CTG	Transition	Non-coding	
	12655G>A	GAG>AAG	Transition	Non-coding	
	12220C>T	CCG>CTG	Transition	Non-coding	
	12266A>T	AAA>AAT	Transversion	Non-coding	
	12616G>A	AGA>AAA	Transition	Non-coding	

Figure 4. Phylogenetic relationship between different goat populations with bootstrap values indicated in the nodes

Discussion

The results of this study revealed a substantial number of SNPs within the *TMBIM6* gene across the three Nigerian goat populations, which are being investigated for the first time. Previous research has shown that the *TMBIM6* gene is evolutionarily conserved and plays a crucial role in regulating cell survival in response to stress in both animals and plants (Singh et al. 2019). Intron 6 was identified as the most polymorphic region within the gene segment studied. Archana et al. (2017) report that individual animals exhibit varying capacities to adapt to stress, with natural nucleotide variations in different regions of the *TMBIM6* gene contributing to diverse stress tolerance levels among goats. Two (2) novel SNPs (55 T>C and 96 A>C) were earlier reported in exon 5 of *TMBIM6* gene of heat stress-tolerant and stress-susceptible individuals in Barbari and Jakhrana goat breeds while in this study two novel SNPs (11857A>G; 11909G>A) were found in exon 5 in Sahel population. Major allele frequencies found in exon 4 to intron 6 of the *TMBIM6* gene are quite high, an indication that the alleles are becoming stable in different populations of Nigerian goats. Allele frequency depicts the proportion of gene copies of a particular allele in a defined population (Silver 2001; Stephenson 2016). Allele frequency ranges from 0, indicating the absence of the variant in the population, to 1, where the observed variant is the only allele present. When the allele frequency reaches 1, the population is considered fixed for that allele (Aquadro 2001). In this study, observed heterozygosity ranged from 0.21 to 0.47, reflecting low to moderate genetic variation among Nigerian goat breeds. Heterozygosity quantifies individual genetic diversity and is also associated with inbreeding. Low heterozygosity can affect fitness in natural settings (Walling et al. 2011). The PICs recorded in this study ranged from 0.18-0.36. Seven (7) of the 13 SNPs showed median polymorphisms, implying that these SNPs had large genetic variations and selection potentials. According to Tian et al. (2014), PIC is classified as low polymorphism (PIC value < 0.25), median polymorphism (0.25 < PIC value < 0.5), and high polymorphism (PIC value > 0.5). The Polymorphic Information Content is useful in measuring the informativeness of a genetic marker for linkage studies (Guo & Elson 1999). A total of twenty-eight (28) haplotypes were found between Exon 4 to Intron 6 of the *TMBIM6* gene in Nigerian goats with the highest (13) number recorded in the Sahel population. Sahel goats had the highest haplotype diversity, nucleotide diversity, average number of nucleotide differences, number of polymorphic (segregating) sites, parsimony informative site and singleton variable site when the sequences were pooled. This showed that the Sahel *TMBIM6* gene had the highest rate of mutation and degree of allelic variation compared to the other breeds of goat examined in this study. The extent of allelic variation contributes to the overall genetic diversity of the gene. Greater genetic diversity enhances the adaptive capacity of these species by providing evolutionary potential to respond to rapidly changing environmental conditions in tropical climates (Wheto et al. 2021). It is therefore likely that the higher genetic diversity observed in Sahel breed compared to WAD and Red Sokoto might not be unconnected with its potential to adapt to the arid region which is its predominant environment. Phylogenetic analysis obtained in this study reveals that Sahel goats are closer to WAD goats than Red Sokoto goat although the three breeds share common evolutionary ancestor and are distributed across various environments in Nigeria indicating their adaptability and resilience (Ejogugua & Okonkwo 2022). According to Etta et al. (2020), WAD and Sahel goats exhibit a high degree of genetic similarity and may have originated from a common ancestor. West African goat breeds such as WAD, Sahel, and Red Sokoto exhibit superior heat resilience due to their long-term adaptation to the hot and humid environments of sub-Saharan Africa, a trait shaped by natural selection under persistent thermal stress and limited resources (Chineke et al., 2006). In contrast, breeds like Jamunapari and Barbari have evolved in less thermally challenging environments and therefore possess lower levels of heat tolerance (Rout et al. 2017). Further investigation is needed to substantiate the relationship between the SNPs identified with heat tolerance and production traits in goats using larger datasets.

Conclusion

Considerable number of single nucleotide polymorphisms (SNPs), both previously reported, and novel were found in *TMBIM6* gene in Nigerian goats. Most of the SNPs were non-synonymous while only one was synonymous. The major alleles of the SNPs had higher frequencies. Distributions of the SNPs showed that Nigerian goats are diverse genetically and thus could translate to differences in the breeds' potential to adapt to heat stress. Further investigation on association between SNPs in *TMBIM6* gene and functional or productive traits in goats is therefore required to establish the markers that could be used for selection toward genetic improvement of the breeds.

Acknowledgements

The authors are grateful to the Management of National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Zaria and SELEMA Oloba Ranch, Iwo, Osun State, Nigeria for providing the experimental animals used for this study.

Ethical Statement

The experiment was approved by the Federal University of Agriculture Abeokuta (FUNAAB), Nigeria, and carried out according to the standard of animal care and handling committee of the College of Livestock Production and Animal Science, FUNAAB, Nigeria.

CRediT Authorship Contribution Statement

Adebayo John Odunayo: Conceptualized, investigated, conducted experiments, data analysis, and drafting and revision of the manuscript. **Bemji Martha Nchang:** Conceived the project and supervised the study. **Wheto Mathew:** Co-supervised the experiment and assisted in data analyses. **Abioja Monsuru Oladimeji:** Co-supervised the experiment and contributed to the correction of the manuscript. **Muritala Ismaila:** Contributed to the drafting and revision of the manuscript.

Declaration of Competing Interest

The authors declare no competing financial or personal interests that could have influenced the work reported in this study.

References

Adu I. F., Ngere L. O. (1979). The Indigenous Sheep of Nigeria. World Review Animal Production 15 (3): 51-67.

Altschul S. F., Gish W., Miller W., Myers E. W. (1990). Basic Local Alignment Search Tool. J Mol Biol 215 (3): 403-410. doi: 10.1016/S0022-2836(05)80360-2

Archana P. R., Aleena J., Pragna P., Vidya M. K., Abdul Niyas P. A., Bagath M., Krishnan G., Manimaran A., Beena V., Kurien E. K., Sejian V., Bhatta R. (2017). Role of Heat Shock Proteins in Livestock Adaptation to Heat Stress. J Dairy Vet Anim Res 5 (1): 13-19. doi: 10.15406/jdvar.2017.05.00127

Aquadro C. F. (2001). Gene Frequency. Encyclopedia of Genetics. (Maloy S., Hughes K., eds), Brenner's Encyclopedia of Genetics (Second Edition), Academic Press, pp. 197-198 doi: 10.1016/B978-0-12-374984-0.00590-8

Berihulay H., Li Y., Liu X., Gebreselassie G., Islam R., Liu W., Jiang L., Ma Y. (2019). Genetic Diversity and Population Structure in Multiple Chinese Goat Populations Using a SNP Panel. Anim Genet 50 (3): 242-249. doi: 10.1111/age.12776

Botstein D., White R. L., Skolnick M., Davis R. W. (1980). Construction of a Genetic Linkage Map in Man Using Restriction Fragment Polymorphisms. Am J Hum Genet 32 (3): 314-331.

Chineke, C. A., Ologun, A. G., Ikeobi, C. O. N. (2006). Hematological Parameters in Rabbit Breeds and Crosses in Humid Tropics. Pak J Biol Sci 9 (11): 2102–2106. doi: 10.3923/pjbs.2006.2102.2106

Ejogugua V. C., Okonkwo J. C. (2022). Phylogenic Relationship between West African Dwarf Goat and Red Sokoto Goat in Anambra, Nigeria. Acta Scientific Biotechnology 3 (5): 18-20.

Etta H. E., Ikpeme E. V., Ukegbu K. N. (2020). Phylogenetic Analyses of Three Goat (Capra Aegagrus Hircus) Breeds in Cross River State, Nigeria. J Transl Sci Res 3: 010. doi: 10.24966/TSR-6899/100010

Falconer D. S., Mackay T. F. C. (1996). Introduction to Quantitative Genetics. 4th Edition, Benjamin Cummings, London, UK. pp 464.

FAO. (2015). Inclusive Business Models: Guidelines for Improving Linkages between Producer Groups and Buyers of Agricultural Produce. FAO, Rome. Available at: www.fao.org/3/i5068e/i5068e.pdf [Accessed 6 May 2024]

Guo X., Elston R. (1999). Linkage Information Content of Polymorphic Markers. Human Heredity 49 (2): 112-118. doi: 10.1159/000022855

Hall T. A. (2004). BioEdit: A User-Friendly Biological Sequence Alignment Program for Windows 95/98/NT. Nucleic Acids Symposium Series. 41: 95-98.

Hirst K. K. (2017). The Domestication History of Goats (*Capra hircus*). Available at: https://www.thoughtco.com/the-domestication-history-of-goats-170661. [Accessed 12 December 2021]

Hunter, J. D. (2007). Matplotlib: A 2D Graphics Environment. Computing in Science & Engineering 9 (3): 90-95. doi: 10.1109/MCSE.2007.55

Lipshutz R. J., Fodor S. P. A., Lockhart D. J. (1999). High Density Synthetic Oligonucleotide Arrays. Nature Genetics 21: 20-24. doi: 10.1038/4447

Lohani M., Bhandari D. (2021). The Importance of Goats in the World. Professional Agricultural Workers Journal 6: 1-4. doi: 10.22004/ag.econ.319686

Mahmoud A. A. (2010). The Present Status of the World Goat Populations and Their Productivity. Lohman Information 45 (2): 42.

Mirkena T., Duguma G., Haile A., Tibbo M., Okeyo A., Wurzinger M., Sölkner J. (2010). Genetics of Adaptation in Domestic Farm Animals: A Review. Livest Sci 132 (1): 1-12. doi. 10.1016/j.livsci.2010.05.003

Mueme E. K., Wakhungu J. W., Hanotte O., Jianlin H. (2009). Genetic Diversity and Relationship of Indigenous Goats of Sub-Saharan Africa Using Microsatellite DNA Markers. Livest ResRural Devel 21 (28): 1-6. Available at: http://www.lrrd.org/lrrd21/2/muem21028.htm [Accessed 18 February 2022].

Muritala I., Afolayan O., Bemji M. N., Dadi O., Landi V., Martinez A., Delgado J. V., Adebambo O. A., Aina A. B. J., Adebambo A. O. (2015). Genetic Diversity and Population Structure of Nigerian Indigenous Goat Using DNA Microsatellite Markers. Archivos de Zootecnia 64 (246): 93-98.

Okpeku M., Yakubu A., Peters S., Ozoje M. O., Ikeobi C. O. N., Adebambo O. A., Imumorin I. (2011). Application of Multivariate Principal Component Analyses to Morphological Characterization of Indigenous Goats in Southern Nigeria. Acta Agriculturae Slovenica 98 (2): 101-109. doi: 10.14720/aas.2011.98.2.14580

Salisu I. B., Olawale A. S., Jabba B., Koloko B. L., Abdulrahaman S. L., Amin A. B., Ali Q. (2018). Molecular Markers and Their Potential in Animal Breeding and Genetics. Niger J Anim Sci 20 (3): 29-48.

Schlötterer C. (2000). Evolutionary Dynamics of Microsatellite DNA. Chromosoma 109 (6): 365-371. doi 10.1007/s004120000089

Singh R., Kaushik R., Dige M. S., Rout P. K. (2019). Identification of Mutation in TMB1M6 Gene in Response to Heat Stress in Goats. Biological Rhytm Research 51 (7): 995-1005. doi. 10.1080/09291016.2018.1563322

Silver L. (2001). Allele Frequency. Encyclopedia of Genetics. (In: Brenner S., Miller H. J., Broughton W. J., eds), Academic Press, pp 37. doi: 10.1006/rwgn. 2001.0027

Singh R., Kaushik R., Dige M. S., Rout P. K. (2019). Identification of Mutation in TMB1M6 Gene in Response to Heat Stress in Goats. Biological Rhythm Research 51: 995-1005. doi: 10.1080/09291016.2018.1563322.

Stephenson F. H. (2016). Forensics and Paternity. In: Calculation for Molecular Biology and Biotechnology, 3rd Edition, Academic Press, pp. 439 – 463.

Sunnucks P. (2000). Efficient Genetic Markers for Population Biology. Trends in Ecology Evolution 15 (5): 199-203.

Swain L. L., Mishra C., Sahoo S. S., Nayak G., Pradhan S. K., Mishra S. R., Dige M. (2020). An *in vivo* and *in silico* Analysis of Novel Variation in *TMBIM6* Gene Affecting Cardiopulmonary Traits of Indian Goats. Journal of Thermodynamic Biology 88: 102491. doi: 10.1016/j.jtherbio.2019.102491

Tamura, K., Stecher, G., and Kumar, S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38 (7): 3022-3027. doi: 10.1093/molbev/msab120

Tian C., Yang M., Lv L., Yuan Y., Liang X., Guo W., Song Y., Zhao C. (2014). Single Nucleotide Polymorphisms in Growth Hormone Gene and Their Association with Growth Traits in Siniperca chuatsi (Basilewsky). Int J Mol Sci 15 (4): 7029–7036. doi. 10.3390/ijms15047029

Ugwu D. S. (2007). The Role of Small Ruminants in the Household of Economy of Southeast Zone in Nigeria. J Applied Sci 2 (6): 726-732.

Umesh S., Arun K., Khanna A. (2010). Non-Genetic Factors Affecting Age at First Calving and First Lactation Traits in Hariana Cows. Indian Veterinary Journal 87 (1): 91-93.

Walling C. A., Nussey D. H., Morris A, Clutton-Brock T. H, EB Kruuk L, Pemberton J. M. (2011). Inbreeding Depression in Red Deer Calves. BMC Evol Biol 11: 318. doi: 10.1186/1471-2148-11-318

Wheto M., Ismaila O. O., Adeleke M. A., Adenaike A. S., Peters S. O., Yakubu A., Adebambo., A. O., Ikeobi C. O. N., Adebambo O. A. (2021). Sequence Analyses of Insulin-like Growth Factor 1 Gene in Nigerian Indigenous and Arbor Acre Chicken. Genetika 53 (1): 271-282. doi: 10.2298/GENSR2101271W

Yadav A. K., Tomar S., Jha A. K., Singh J. (2017). Importance of Molecular Markers in Livestock Improvement: A Review. International Journal of Agriculture Innovations and Research 5 (4): 614-621.

aCS90_14