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Original Research Paper

Genetic polymorphism in the *POU1F1* gene in Kalahari Red and two Nigerian goat breeds and their relationship with litter size

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Abstract
POU1F1 gene controls cell differentiation and animal growth by binding to target DNA promoter sequence, thereby auto-regulating its own expression and expression of growth hormone (GH), prolactin (PRL) and thyroid-stimulating hormone beta sub-unit (TSHβ) genes. Therefore, the exploration of caprine POU1F1 gene polymorphisms may be vital in the formulation of conservation and breed improvement strategies. In this study, POU1F1 gene was characterized for sequence polymorphisms in 366 individuals from two Nigerian goat breeds ((West African Dwarf (WAD) and Red Sokoto (RS)) and one South African goat breed (Kalahari (KR)). The effects of polymorphisms on litter size were investigated using linear mixed model. Two intronic mutations (g.306G>A and g.11236C>T) were identified. However, no significant association was found between the Single Nucleotide Polymorphisms (SNPs) and litter size in the three populations. The genetic distance based on POU1F1 investigated region revealed that the two Nigerian breeds and the South African breed were identical (pairwise genetic distance of 0.00). Phylogenetic tree constructed from the pairwise distance clustered the three breeds into a single clade with the two Nigerian goat breeds having a more recent common ancestor. Structural analysis of the POU1F1 protein confirmed that Pit-Oct-Unc transcription factors domain (POU) and Homeodomain (HOX) domains are conserved in mammals, with several overlapping sub-domains across the same region in all the three populations. We found a subdomain Subfamily of SANT domain or myb/SANT-like domain in Adf-1 (MADF) in goat, cattle, buffalo and camel that has not been reported in mammals.
Keywords: *POU1F1* gene; mutation; West African Dwarf; Red Sokoto; Kalahari Re

Introduction

Among domesticated ruminants reared in the tropical and subtropical regions, goat (*Capra hircus*) is regarded as the most prolific (Yadav and Yadav, 2008). Their ability to survive under low input production system as well as adapt to harsh environmental condition makes them a favorite choice for farmers in the tropics (Fajemilehin and Salako, 2008; Serrano et al., 2009). In Nigeria, the two most important goat breeds are West African Dwarf (WAD) and Red Sokoto (RS) (Adah et al., 1993; Yakubu et al., 2010a). These

breeds are widely spread in the country with both having unique features and adaptability to different climatic conditions of the country (Adah et al., 1993; Yakubu et al., 2010b; Obua et al., 2012). The WAD goats are trypanotolerant and more adapted to the humid climatic condition of Southern Nigeria whereas, the RS goats are widely popular for their high-quality skin and ability to adapt well to the tropical hot-dry climatic conditions of northern Nigeria (Hoste et al., 1992; Akpa et al., 1998). The Kalahari Red (KR) goat, on the other hand, is a meat-producing goat which originates from South Africa (Kotze et al., 2004). It was introduced to Nigeria in 2011 for breeding purposes (Bemji et al., 2014). The KR goat is also renowned for its ability to adapt to a wider range of climatic conditions (Kotze et al., 2004).

Litter size, i.e., the number of young born alive per kidding, is an important factor driving the profitability in the goat industry (Lai et al., 2016). In Nigeria, the WAD and RS goats are the most prolific breeds with average litter size of 1.60 and 1.45, respectively (Abubakar et al., 2013; Oga, 2016). Information based on limited data showed that mean litter size of KR goats bred in Nigeria was 1.67 for dams fed with grass supplemented with low protein diets (Oderinwale et al., 2017). Over the past decades, the application of traditional selective breeding to improve litter size resulted in limited success (An et al., 2010) due to its low heritability (Otuma and Onu, 2013). Thus, the application of candidate gene approach could lead to accelerated improvement for this trait in selecting breeding stocks with high reproductive potential (Li et al., 2011).

In breeding indigenous goats, the critical research problem that necessitates urgent attention is the conservation and use of these breeds of animals (Wang et al., 2011). Genetic characterization allows the assessment of genetic variability and understanding animal evolutionary history (Muritala et al., 2015), which is crucial for breed conservation priorities and sustainable management programs (Aggarwal et al., 2007; Glowatzki-Mullis et al., 2008; Kevorkian et al., 2010). Several studies have characterized Nigerian breeds and Kalahari Red goat using microsatellite markers (Okpeku et al., 2011; Agaviezor et al., 2012; Ojo et al., 2015, Murital et al., 2015; Ojo et al., 2018). Information is currently limited on characterization of Nigerian goat breeds based on candidate genes that affect economic traits.

The *POU1F1* gene is a member of the pituitary-specific POU-containing transcription factor family, which contains POU DNA-binding domain (Ozmen et al., 2014). It consists of the N-terminal transactivating domain (TAD) and Pou-Homeo domain which are involved in protein–protein interactions, DNA binding and interactions with transcription co-factors (Andersen and Rosenfeld, 2001). The *POU1F1* gene controls cell differentiation and animal growth and development by binding to target DNA promoter sequence, thereby auto-regulating its own expression (Andersen and Rosenfeld, 2001; Ho et al., 2015). It also regulates the expression of three other genes that code for hormones, namely; growth hormone (*GH*), prolactin (*PRL*) and thyroid stimulating hormone beta sub-unit (*TSHβ*) genes (Sobrier et al., 2016). These three genes function within the hypothalamo-pituitary gonadal axis that regulates ovulation and litter size in small ruminants (Zhang et al., 2011; An et al., 2015; Huang et al., 2015). The *POU1F1* gene contains 6 exons and 5 introns and encodes a protein with 291 amino acids. Mutations in the gene have been associated with economically important traits in livestock (Carsai et al., 2012; Feng et al., 2012; Daga et al., 2013; Korkmaz-Ağaoğlu et al., 2019; Putra et al., 2019). However, no study has examined polymorphisms of this gene and their relationship with litter size in African goat breeds. This study was therefore conducted to characterize its genetic polymorphisms in two Nigerian goat breeds and Kalahari Red goat and also investigate its association with litter size.

Materials and Methods

Experimental animals, management and location

In this study, 226 WAD, 70 RS and 70 KR goats were used. Animals were managed semi-intensively at Ipokia Local Government area of Ogun State by local farmers (WAD), National Animal Production

Research Institute (NAPRI), Shika-Zaria (RS) and Institute of Food Security, Environmental Resources and Agricultural Research of the Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria (KR). Only animals that kidded within the same season were used with parity of does ranging from 1-5. The average litter size and parities of the three populations are presented in Table 1. The locations of the sampled herds are indicated in Figure. 1.

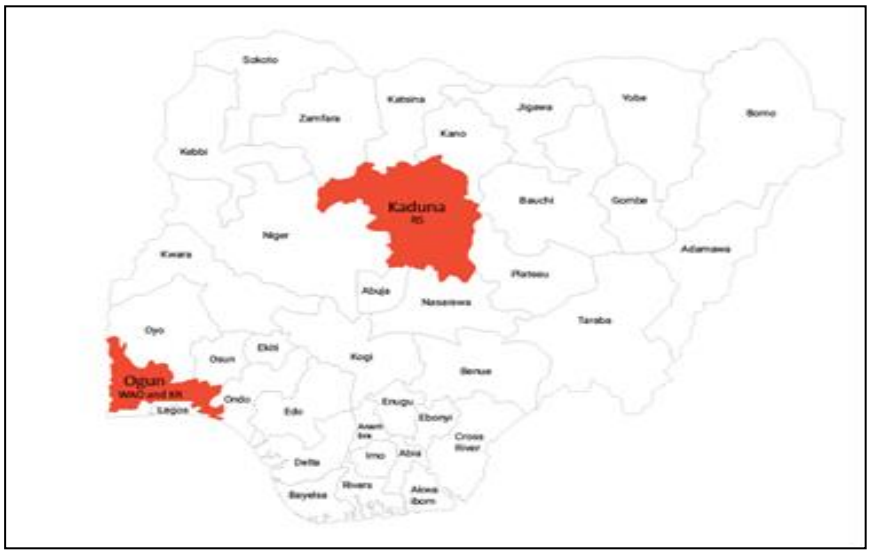

Figure. 1. Map of the Federal Republic of Nigeria showing the locations of the study area

Table 1. Parity and the average litter size of the three populations

Breed	N	Parity	Litter size
WAD	70	1	1.25±0.06
	56	2	1.62±0.09
	78	3	1.94±0.07
	16	4	2.15±0.10
	6	5	2.0±0.23
RS	70	3	1.59±0.14
KR	52	1	1.35±0.11
	18	2	1.29±0.18

N= No of animal; WAD= West African Dwarf; RS= Red Sokoto; KR= Kalahari

Blood sample collection and DNA isolation

The ethical guidelines and approval of the College of Animal Science and Livestock Production of the Federal University of Agriculture, Abeokuta, Nigeria were followed in the research. Blood samples (about 5 mL/animal) were collected aseptically from the jugular vein of does into BD vacutainer tubes (Becton, Dickinson and Company, USA) containing EDTA anticoagulant. The samples were snap frozen and transported to the laboratory and kept in a freezer at -20°C until DNA purification. Genomic DNA was extracted from whole blood using NucleoSpin® Genomic DNA extraction kit (MACHEREY-NAGEL GmBh & Co. KG, Germany) based on manufacturer's procedure. The NanoDrop ND-1000 spectrophotometer (Nano Drop Technolgies, Wilmington, DE, USA) was used to assess the quantity and quality of extracted DNA.

Primer design, PCR amplification and sequencing

Caprine POU1F1 gene sequence (NC_030808.1, position 34235896 to 34251973) was used to design six

pairs of primers (Table 2) with Primer3 software (Untergrasser et al., 2012) to amplify the promoter region and exons, including ~250 bps of surrounding introns. PCR reactions were performed in a 25 µL volume containing 1 µM each primer (forward and reverse), 10X PCR buffer (including 1.5 mM MgCl2), 200 µM dNTPs and 1 unit of Taq DNA polymerase (New England Biolabs Ltd, Whitby, ON, Canada). About 60 ng of genomic DNA was used as template. Thermocycling conditions consisted of 35 cycles of initial denaturation at 95 °C for 5 min, denaturation at 94 °C for 30 s; primer annealing at 58 to 62°C for 1 min; primer extension at 72°C for 1 min with final extension at 72°C for 10 min. PCR products were separated in 1.5% agarose gel in 1 x TBE electrophoresis buffer. The gels were stained with Safe View (Applied Biological Materials Inc, Richmond, BC, Canada) and viewed under UV light using Alphamager® 2200 version 5.5 gel documentation systems (Alpha Innotech, San Leandro, CA, USA). Sequencing of pooled PCR products (10 samples per pool) for polymorphism detection was carried out with the Big Dye® Terminator chemistry on ABI3730XI (Applied Biosystems, Foster City, CA, USA) DNA analyzer by Genome Quebec and McGill University Innovation Center, Quebec, Canada (https://genomequebec.mcgill.ca/).

Table 2. Primer sequences used in the amplification of the coding regions and surrounding intronic sequences of the *POU1F1* gene

Primer	Primer length	Primer sequence (5' → 3)	Region	Length (bp)	TA (°C)
POU1F1_80F	20	TTGCCTTCATTCCCTACCCA	Promoter region and exon 1	837	58.62
POU1F1_4073F	22	ACGAATGTGTCTTGAATCCTCAT	Exon 2 + introns	493	58.93
POU1F1_11091F	20	GCTTCAGAAAACCGAATGTC	Exon 3 + introns	**943**	59.50
POU1F1_13635F	23	TGAATGGCAGATGTTCCTATCTG	Exon 4 + introns	682	58.29
POU1F1_15754F	20	GGAAACGGAGAACAACTATC	Exon 5 + introns	802	57.52
POU1F1_15754F	20	GCTTGGAAGGTGTTTGCAGA	Exon 6 + introns	788	59.89

SNP identification and genotyping

Single nucleotide polymorphisms were identified in POU1F1 gene from chromatographs of pooled samples with the aid of Codon Code aligner (Codon code Corporation Dedham, MA, USA) and verification was done using Chromas version 2.31 software (Technelsium Pty Ltd., Tewantin, Queensland, Australia). SNP genotyping of individual samples at the identified SNP loci was accomplished by the method of SequenumiPLEX Gold Technology on a MassARRAY platform (Sequenom Inc. San Diego, CA, USA) by McGill University and Genome Quebec Innovation Center (https://genomequebec.mcgill.ca/). A total of 366 does were genotyped at the two identified SNP loci. Diversity indices including allelic and genotypic frequencies, heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg equilibrium were estimated using Genetics package (https://cran.r-project.org/web/packages/ genetics/index.html) in R environment. The effect of SNP genotype on litter size was evaluated for separate populations using the following mixed model:

$$\mathbf{y = Xb + Za + e}$$

Where; **y** is the vector for observation on litter size, **b** is the vector of fixed effects of genotype (1, 2, 3) and parity (1, 2, 3, 4, 5), **a** is the vector of random effect of doe, **e** is the random error. X and Z are incidence matrices relating records to their respective effects. Analysis of variance was done using SAS version 9 (SAS Institute, Cary, NC, USA).

Sequence analysis

Nucleotide sequences of different PCR fragments of the investigated region of the POU1F1 gene were assembled into contigs in WAD, RS and KR breeds. The nucleotide sequences were then aligned with that of

other mammalian species available in GenBank using the ClustalW program (Thompson et al., 1994), implemented in MEGA-X. Pairwise genetic distances among the three goat breeds and other mammalian species were estimated using the various aligned sequences. Phylogenetic tree was constructed using the Neighbor-joining method (Saitou and Nei, 1987) to classify the populations into different clades. The reliability of the phylogenetic tree was assessed using bootstrap values computed after 10,000 replications of re-sampling loci.

Domain Structure Analysis

To delineate the functional domains of the complete open reading frame (ORF) of goat (representing WAD, RS and KR) and other mammalian species (ORF retrieved from GenBank), POU1F1 gene ORFs from goat and other mammals were submitted into Simple Modular Architecture Research Tool software (SMART; http://smartemblheidelberg.de).

Results

Polymorphisms in the POU1F1 gene and genetic diversity of the populations

Two transition mutations (g.306G>A and g.11236C>T) were identified within POU1F1 gene in the goat populations (Figure. 2). The SNPs were detected in introns 1 (g.306G>A) and 3 (g.11236C>T) (Table 3). KR goats were non-polymorphic at g.306G>A loci. The polymorphic sites were numbered according to caprine POU1F1 sequence (GeneBank: NC_030808.1) relative to the transcription start site.

Individuals with homozygous AA genotype at g.306G>A were predominant in WAD and RS goats at frequencies of 0.70 and 0.58, respectively. At g.11236C>T locus, CC genotype was the most abundant with a frequency of 0.67 in both WAD and KR goats. Conversely, heterozygote (CT) was the predominant genotype (0.41) in RS goats (Table 4).

High heterozygosity estimates were observed in RS (0.40 and 0.50 at g.306G>A and g.11236C>T, respectively). WAD and KR breeds recorded lower heterozygosity estimates (≤0.30) at the two SNP loci. The three breeds had moderate genetic diversity (0.25 ≤ PIC ≤ 0.50) at the polymorphic sites and were all in HWE (P > 0.05).

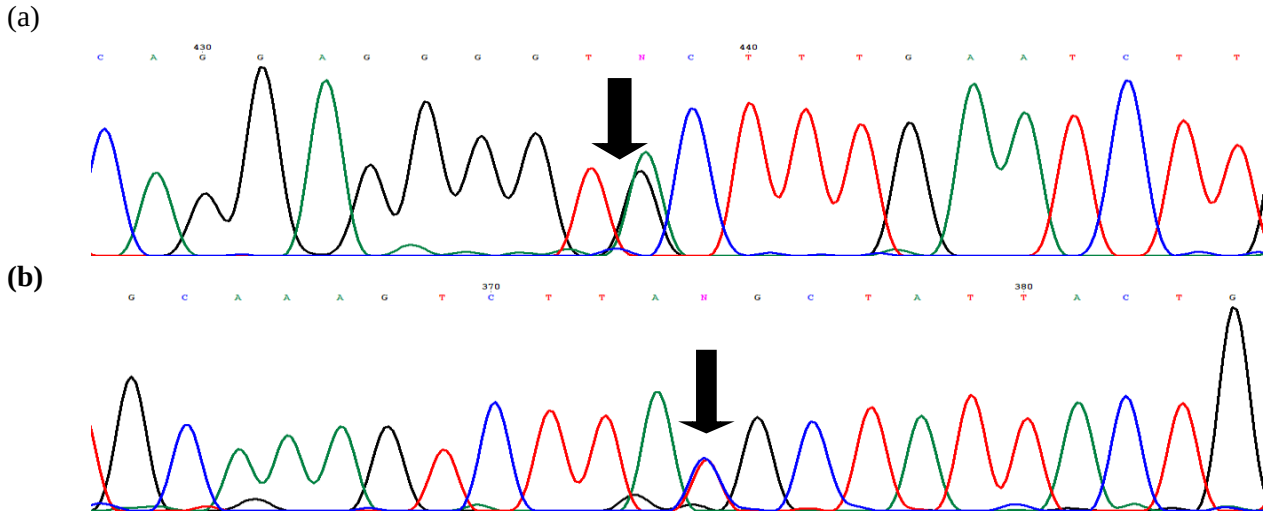

Figure 2. Sequence chromatogram showing the polymorphic sites in the *POU1F1* gene. (a) g.306G>A and (b) g.11236C>T.

Table 3. Single nucleotide polymorphisms identified in the *POU1F1* gene.

Site	Chromosome	Mutation	Genomic region
Intron 1	6	G > A	g.306G>A
Intron 3	6	C > T	g.11236C>T

Table 4. Genetic Diversity indices based on the identified mutations in the *POU1F1* gene of West African Dwarf, Red Sokoto and Kalahari Red goats.

SNP	Breed	Genotypic frequency			Allelic	frequency	He	PIC	HWE exact test (p-value)
g.306G>A		*GG*	*GA*	*AA*	A	G			
	WAD	*0.01*	*0.29*	*0.70*	0.84	0.16	0.29	0.25	0.61
	RS	*0.12*	*0.31*	*0.58*	0.73	0.27	0.40	0.31	0.32
g.11236C>T		*CC*	*CT*	*TT*	C	T			
	WAD	*0.67*	*0.28*	*0.04*	0.81	0.19	0.30	0.25	0.46
	RS	*0.33*	*0.41*	*0.26*	0.54	0.46	0.50	0.37	0.44
	KR	*0.67*	*0.23*	*0.00*	0.83	0.17	0.29	0.24	1.00

He = Heterozygosity; PIC = Polymorphic information content; HWE = Hardy-Weinberg equilibrium

Association analysis

The effect of SNP genotype at two SNPs loci on litter size is given in Table 4. No significant association between the intronic SNPs and litter size was found in the studied populations (Table 5).

Table 5. Least square mean for litter size for genotypes at SNP loci in the *POU1F1* gene of West African Dwarf, Red Sokoto and Kalahari Red goats

SNP	Breed	Genotype	Litter size ± SE	*P*-adjusted
g.306G>A	*WAD*	GG	1.75±0.48	0.86
		GA	1.68±0.09	
		AA	1.67±0.05	
	RS	GG	1.33±0.48	0.61
		GA	2.00±0.09	
		AA	1.47±0.05	
g.11236C>T	*WAD*	CC	1.66±0.05	0.87
		CT	1.65±0.09	
		TT	1.47±0.25	0.47
	RS	CC	1.33±0.24	0.38
		CT	1.82±0.26	
	KR	TT	1.57±0.32	
		CC	1.50±0.20	
		CT	1.00±0.36	

Genetic distance among mammalian species

Pair-wise genetic distances based on investigated region of the POU1F1 gene among the three populations are presented in Table 6. Results revealed the closest genetic distance of 0.00 among the three goat populations and the farthest genetic distance of 0.280 between Horse and the goat populations studied. The standard error of the estimate for all the genetic distances ranged from 0.000 to 0.015.

Table 6. Genetic distances among WAD, RS and KR goats and other mammalian species

	1	2	3	4	5	6	7	8	9	10	11
1		0.000	0.000	0.015	0.014	0.014	0.014	0.015	0.015	0.015	0.015
2	0.000		0.000	0.015	0.014	0.014	0.015	0.015	0.015	0.015	0.015
3	0.000	0.000		0.015	0.014	0.014	0.014	0.015	0.015	0.015	0.015
4	0.224	0.224	0.224		0.004	0.004	0.008	0.009	0.009	0.010	0.009
5	0.226	0.227	0.226	0.016		0.002	0.008	0.008	0.009	0.010	0.009
6	0.211	0.212	0.211	0.015	0.002		0.008	0.008	0.009	0.010	0.009
7	0.259	0.260	0.259	0.065	0.058	0.057		0.008	0.009	0.009	0.009
8	0.272	0.272	0.272	0.072	0.065	0.064	0.062		0.008	0.009	0.008
9	0.269	0.269	0.269	0.081	0.080	0.077	0.073	0.072		0.002	0.009
10	0.272	0.272	0.272	0.086	0.084	0.082	0.078	0.076	0.05		0.009
11	0.280	0.280	0.280	0.094	0.086	0.085	0.087	0.073	0.076	0.081	

1-WAD, 2-RS, 3-KR, 4- Sheep, 5-Cattle, 6-Buffalo, 7-Pig, 8-Camel, 9-Chimpanzee, 10-Human, 11-Horse
Standard genetic distances (below diagonal) and standard errors (above diagonal)

Domain structure analysis of POU1F1 gene

Structural analysis of predicted POU1F1 protein based on the ORF revealed similarity in domain structure among the three goat breeds, distinct from other mammalian species (Supplementary Figure 1). Some domains were not displayed in the supplementary figure 1 because the priority for display by the tool was SMART > PFAM > PROSPERO repeats > Signal peptide > Transmembrane > Coiled-coil > Unstructured regions > Low complexity. The domains not displayed because they overlapped with more prioritized domains include subfamily of SANT domain (myb/SANT-like domain in Adf-1) (MADF), membrane-attack complex/perforin (MACPF), helix_turn_helix multiple antibiotic resistance protein (HTH_MARR), domain in helicases and associated with SANT domains (HAS), helix loop helix domain (HLH), Fish-specific NACHT associated domain (FISNA), laminin N-terminal domain (domain VI) (LamNT), c-SKI Smad4 binding domain (c-SKI_SMAD_bind) (Supplementary Table 1). The goat populations had POU and HOX domains with the MADF sub-domain within the POU domain. All other mammalian species included in the analysis had additional sub-domains within the HOX domain that are different from the goat populations (Supplementary Table 1).

Phylogenetic analysis

The Neighbor-joining phylogenetic tree (Figure 3) revealed that all the three breeds belong to the same clade with the Nigerian goats (WAD and RS) having the most recent common ancestor. All the other mammalian species formed different clades as expected.

Discussion

The present work is the first attempt to characterize genetic polymorphisms in the POU1F1 gene in two Nigerian and Kalahari Red goat breeds. Results revealed two intronic mutations in POU1F1 gene of the two Nigerian goats and South African breed. These mutations were reported in Jining Grey goats (Feng et al., 2012). Daga et al. (2013) reported seven intronic SNPs in POU1F1 gene in Sarda goats. Similar to our results, no significant association was found between the intronic SNPs and litter size in the study of Feng et al. (2012). However, Getmantseva et al. (2017) reported significant association between an intronic SNP with reproductive traits in pigs. Hong and Park (2012) noted that larger sample size is required for population-based studies to increase statistical power in detecting significant variants. The inability to detect significant association between SNPs and litter size in our study could have been due to lower statistical power limited by sample size, especially for RS and KR populations. The importance of intronic mutations have been widely reviewed in the literature. Introns play key roles in transcription initiation and termination (Antoniou et al., 1998; Petit et al., 2008; Chorev and Carmel, 2012), regulation of alternative splicing (Sorek and Ast, 2003; Pan et al., 2008; Roy et al., 2015), genome organization (Liu et al., 1995; Vinogradov,

2006), regulation of nonsense-mediated decay (Maquat, 2004; Chang et al., 2007; Silva and Romao, 2009) and most importantly, positive regulation of gene expression (Clark et al., 1993; Juneau et al., 2006; Shabalina et al., 2010).

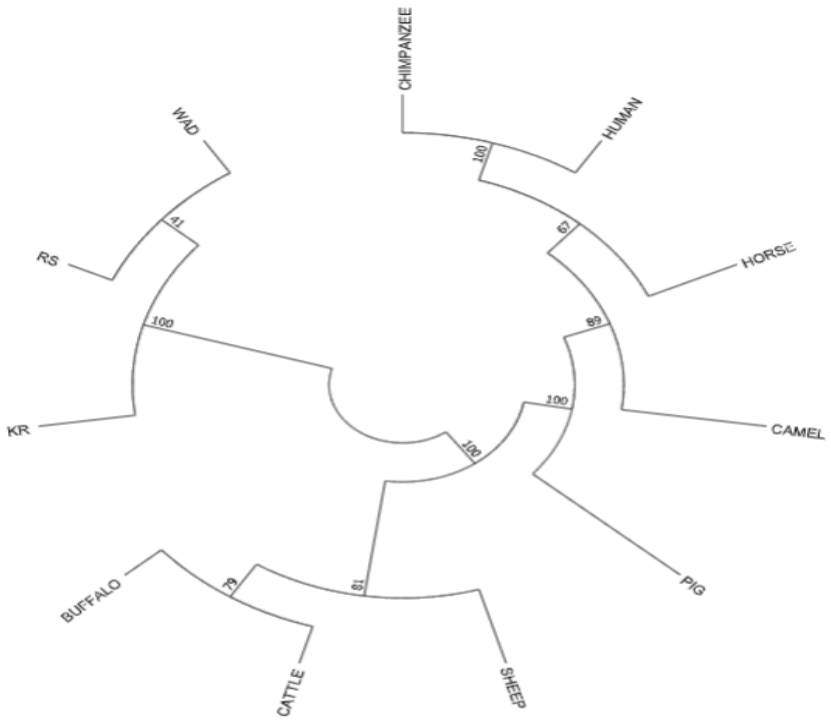

Figure. 3. Phylogenetic relationships among WAD, RS, KR and other mammalian species using bootstrap value computed after 10,000 replications

The estimate of genetic distance is a measure of genetic diversity between species or between populations within a species (Nei, 1987). Genetic distance and distribution of alleles between populations characterizes the evolutionary relatedness of populations to explicitly measure their genotype differentiation (Vieira et al., 2016). These distances, when used to construct phylogenetic trees, depict the genotypic relatedness between populations (Pritchard et al., 2000). The genetic distance observed between WAD and RS indicates that POU1F1 is highly conserved in the two Nigerian goat breeds. Higher genetic distance ranging from 0.11 to 0.39 was reported between WAD and RS using microsatellite markers (Adebambo, 2003; Okpeku et al., 2011, Muriatal et al., 2015; Ojo et al., 2018). This is expected because microsatellite markers are highly polymorphic than SNP mutation (Weber and Wong, 1993; Xu et al., 2000). Moreover, two loci were used to infer the genetic distance, coupled with the small sample size used in our study. The genetic similarity observed between the two goat populations in this study could be attributed to gene flow perhaps due to indiscriminate breeding between the populations, similar to what was reported between cattle populations reared under the nomadic husbandry system (Ibeagha-Awemu and Erhardt, 2005; Ibeagha-Awemu and Erhardt, 2006). The phylogenetic tree based on POU1F1 investigated regioin revealed a distinct clade for the two Nigerian breeds which might be due to common alleles shared between the two goat populations, another indication of interbreeding between the two breeds. This is expected due to free movement of the breeds in Nigeria as northern herdsmen, especially in the rainy season, usually migrate southwards with RS goats in search of pasture and premium price from selling the animals and their products (Ojo et al., 2015). This may have facilitated gene flow between the two distinct breeds. Although the two Nigerian goat breeds

and the South African goat clustered on the same clade, there is indication that WAD and RS are most closely related since both shared the most recent common ancestor compared with Kalahari Red. This is further supported by the study of Murital et al. (2015). The separation of the Kalahari Red from the two Nigerian breeds could also be attributed to differences in geographical locations of origin (Awotunde et al., 2015).

The domain structural analysis of POU1F1 ORFs in the studied goat breeds and other species revealed two conserved domains (POU and HOX) in mammals. POU domain is a sequence-specific DNA binding transcription factor and it is highly conserved in all mammalian species (Wallis, 2018), while the HOX domain is a DNA-binding factor that controls differential genetic programs along the anterior-posterior axis of animal bodies (Alonso, 2002). The POU domain transcription factors have many functions including regulation of neural development in both vertebrate and invertebrate sensory systems (Assa-Munt et al., 1993; Erkman et al., 1996; McEvilly et al., 2002; Komiyama et al., 2003; Corty et al., 2016) and organismal development (Andersen and Rosenfeld, 2001). In mammals, including goats, the POU domain is expressed in the germ-line cells and early embryogenesis, indicating its role in early development (Rosenfeld 1991). The POU domain is also widely expressed in mammalian fore and mid-brain suggesting its role in the development of brain structure (Rosenfeld 1991) and is one of the controlling components of the cell-cell signaling process underlying the hypothalamic regulation of female puberty (Ojeda et al., 1999) perhaps via interaction with estrogen receptor (Bourguignon et al., 1997). HOX domain, on the other hand, is responsible for shaping animal structures by inducing different developmental programs along the anteroposterior body axis (Alonso, 2002). This is achieved by the activation of cell death, promoting gene reaper to maintain the boundaries between the maxillary and mandibular head lobes (Lohmann et al., 2002).

Despite POU and HOX domains being conserved within the mammals, some sub-domains are overlapping them. Our analysis suggests that subfamily of SANT domain (MADF) is present in caprine, ovine and bovine species. The role of this domain has been reported in the literature, including transcriptional regulation of essential target genes that play key roles in germ cell development (Zimmermann et al., 2006; Rao et al., 2016). The MADF domain has been identified in numerous organisms, including worms (Rao et al., 2016), insects (Vidal et al., 2016), mites (Ljunggren et al., 2006), flies and fish (Zimmermann et al., 2006; Shukla et al., 2014). To the best of our knowledge, this domain has not been identified in mammalian genomes, which may be due to lack of complete experimental characterization of ORF predictions of major genome sequencing projects in mammals (Ljunggren et al., 2006). Well-characterized protein-coding loci could produce transcripts with the potential for encoding novel protein species (Denoeud et al., 2007; Rozowsky et al., 2007).

Conclusion

This is the first report of sequence characterization of the POU1F1 gene in African goat breeds. Two intronic SNPs were identified in the Nigerian breeds, but only one SNP was identified in KR goats. We were unable to detect association between the SNPs and litter size in the three populations. Larger sample size with its consequent improvement of statistical power should be used in future studies. Close genetic relationship observed based on the POU1F1 region investigated between the two Nigerian breeds and a distinct separation from the South African (KR) breed are attributed to relatively wide divide in geographical area of origin. This study thus confirms the conserved POU and HOX domain structures of POU1F1 in mammalian species with an underlying sub-domain overlapping the same region of the domain features. Further research should, however, completely characterize the ORF predictions of the whole genome further to unravel hidden domain structures of economic importance in goats.

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Conflict of interest

None

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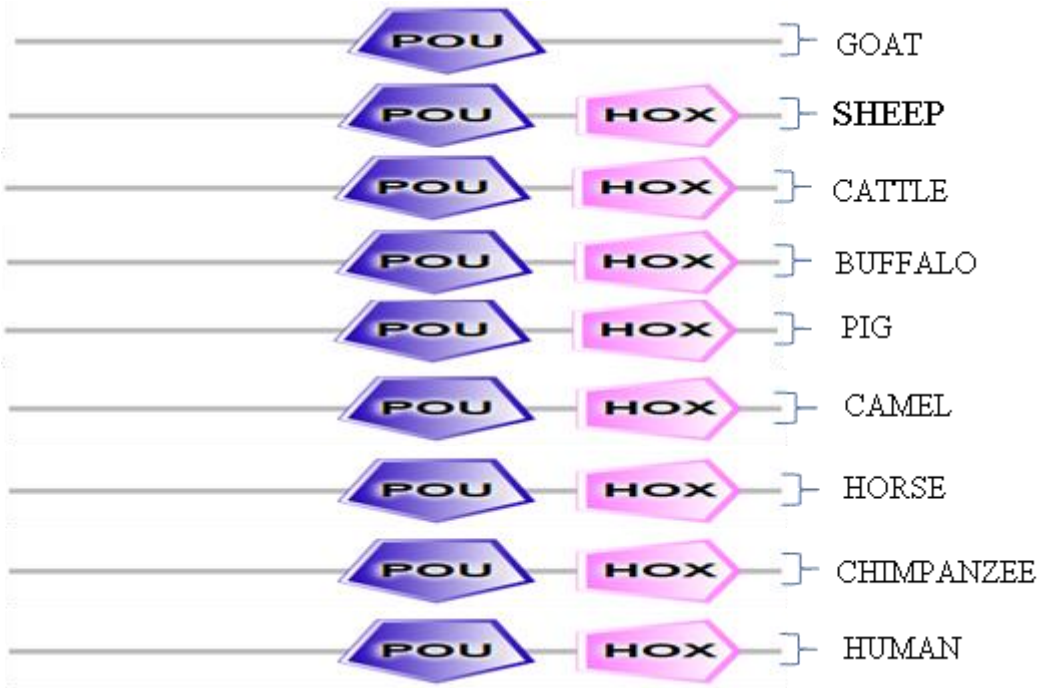
Supplementary materials



Supplementary Figure. 1. Comparison of the domain structures of *POU1F1* gene obtained from the amino acid sequence of different species based on SMART analysis.

Supplementary Table 1. Structural domains of WAD, RS, KR and other mammalian species

Animal	Name of domain	Definition	Start	End	E-value
WAD	POU	Pit-Oct-Unc transcription factors	150	224	4.04e-51
	HOX	Homeodomain	240	302	1.17e-18
	MADF	Subfamily of SANT domain (myb/SANT-like domain in Adf-1)	160	231	1430
Sheep	POU	Pit-Oct-Unc transcription factors	124	198	4.04e-51
	HOX	Homeodomain	214	276	1.02e-18
Cattle	POU	Pit-Oct-Unc transcription factors	124	198	4.04e-51
	HOX	Homeodomain	214	276	1.17e-18
	MACPF	Membrane-attack complex / perforin	109	281	668
	MADF	Subfamily of SANT domain (myb/SANT-like domain in Adf-1)	134	205	1430

	HTH_MARR	Helix_turn_helix multiple antibiotic resistance protein	139	256	895
	HAS	domain in helicases and associated with SANT domains	207	274	2880
	HLH	helix loop helix domain	211	257	189
	FISNA	Fish-specific NACHT associated domain	224	282	108000
Buffalo	POU	Pit-Oct-Unc transcription factors	124	198	4.04e-51
	HOX	Homeodomain	214	276	1.17e-18
	MACPF	Membrane-attack complex / perforin	109	281	668
	MADF	Subfamily of SANT domain (myb/SANT-like domain in Adf-1)	134	205	1430
	HTH_MARR	Helix_turn_helix multiple antibiotic resistance protein	139	256	895
	HAS	Domain in helicases and associated with SANT domains	207	274	2880
	HLH	Helix loop helix domain	211	257	189
	FISNA	Fish-specific NACHT associated domain	224	282	108000
Pig	POU	Pit-Oct-Unc transcription factors	124	198	4.04e-51
	HOX	Homeodomain	214	276	1.17e-18
Camel	POU	Pit-Oct-Unc transcription factors	124	198	4.04e-51
	HOX	Homeodomain	214	276	1.17e-18
	LamNT	Laminin N-terminal domain (domain VI)	28	143	1300
	c-SKI_SMAD_bind	c-SKI Smad4 binding domain	71	144	88000
	MACPF	Membrane-attack complex / perforin	109	281	668
	MADF	Subfamily of SANT domain (myb/SANT-like domain in Adf-1)	134	205	1430
	HTH_MARR	helix_turn_helix multiple antibiotic resistance protein	139	256	895
	HAS	Domain in helicases and associated with SANT	207	274	2880

		domains			
	HLH	Helix loop helix domain	211	257	189
	FISNA	Fish-specific NACHT associated domain	224	282	108000
Horse	POU	Pit-Oct-Unc transcription factors	124	198	4.04e-51
	HOX	Homeodomain	214	276	1.02e-18
Chimpanzee	POU	Pit-Oct-Unc transcription factors	124	198	4.04e-51
	HOX	Homeodomain	214	276	1.02e-18
Human	POU	Pit-Oct-Unc transcription factors	124	198	4.04e-51
	HOX	Homeodomain	214	276	1.02e-18