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Mutational load and deleterious mutations in goat genome from Kenya

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Abstract
Different domestication processes such as selection and effective population size are among other factors responsible for the accumulation of mutations in an animal. This study investigated the mutation load and accumulation of deleterious mutations in Kenyan goat populations using Single Nucleotide Polymorphism (SNP) data obtained from local (Galla = 12) and exotic (Saanen = 24, Alpine = 28, and Toggenburg = 30) goat genotypes. The SNP annotation was done on the ENSEMBLE goat (*Capra hircus*) using the Variant Effect Predictor (VEP). Sorting Intolerant from Tolerant (SIFT) was done on the annotated file using a score of > 0.01 to describe highly deleterious mutations. Biomart tool in the ENSEMBL goat *C. hircus* was used for Gene Ontology (GO) for the genes identified from highly deleterious mutations. The analysis disclosed no differences in mutation load among the studied genotypes. Overall, the synonymous mutations were in abundance compared to missense mutations, totaling 693 and 258 representing 1.01% and 0.37%, respectively. The calculated mutation load was similar (0.37) suggesting exposure of goats to similar domestication processes that are creating similar effects on the animal's genome. Further analysis of the missense variants revealed 126 deleterious mutations and 132 tolerated mutations. A total of 111 genes were identified from the deleterious mutations and only 5 were among the highly deleterious mutations (SIFT score > 0.01) which include PROS1, EHBP1, LTN1, LRRN4, and FNDC3A. Gene Ontology describes these genes as responsible for different functions associated with animal reproduction, diseases, and memory. This study's results have confirmed the levels of mutation load and the presence of deleterious mutations in Kenyan goats. This can be used as a base to predict the future rate of mutation load to ensure better implementation of conservation programs to avoid an accumulation of deleterious mutations.

Keywords: Annotation, deleterious mutations, goats, genes, mutation load

Introduction
Mutation is one of the processes of evolution that involve a change in the nucleotide sequence of the genome in an organism. Mutation load is defined as the loss of fitness in animals due to deleterious alleles that are maintained by mutation-selection balance [1]. The mutation occurs every generation and can be beneficial or harmful in a population. Similarly, deleterious mutations can also either be beneficial or harmful. Harmful deleterious mutations are not required in any animal genome because they affect an animal's productivity in a population [19]. Generally, individuals with harmful deleterious mutations are more vulnerable to diseases or phenotypic disorders. Occurrences of deleterious mutations in a population is a function of many factors including selection level, domestication, geographical history, and effective population size which are exposed to different plant and animal populations [5, 24].
A number of studies have been conducted on different livestock species to analyse deleterious variants including goats and cattle [26, 8]. Deleterious mutation in *CWC15* and *SLC37A2* genes are reported to be possible causes of low reproductive efficiency in Jersey cattle and fetus abortion in Vorderwald cattle [23, 21]. Also, a retinal genetic defect in European cattle breeds was discovered to be caused by PR1gene mutation [15]. Accumulation of deleterious mutations in goat populations were reported in different studies including nine goat breeds of Asian countries [16], the Alpine ibex population [8], and worldwide domesticated goats [27]. Domesticated goats are exposed to many processes that facilitate the occurrences of deleterious mutations in their populations. For instance, continuous artificial selection for improved traits, reduction in effective population size, and high inbreeding levels in

domesticated animals were proved to create a conducive environment for the presence of deleterious mutations due to increased homozygous alleles in a genome [14, 5, 13]. In most animal populations, phenotypic variations are observed while their mutational load are not well understood.

In every population, new mutations are introduced each generation. Therefore, it is not an exception for the goat populations in Kenya to have some deleterious variants since they are also exposed to different domestication processes. This study investigated the variations in mutation load and deleterious mutations between Kenyan local and exotic goat genotypes, and identified affected genes with their associated functions. The findings will help in understanding the effects of existing domestication processes of goats in Kenya and how to maintain them to minimize the overload of deleterious mutations.

Materials and Method

Sampling

The study used Single Nucleotide Polymorphism (SNP) data from Kenya obtained from 94 goats from four goat genotypes (Galla, Saanen, Alpine and Toggenburg) randomly selected in different sub-counties. The areas include; Nyeri (Mukurweini Sub-county), Meru (Central Imenti Sub-County) and Homa Bay (Homa Bay town) located in the Central (wet-dry), Eastern (wet) and Western regions (Wet area) of Kenya.

DNA extraction, genotyping and Quality control

Blood samples were collected using EDTA tubes followed by DNA extraction using the Qiagen DNeasy Blood and Tissue Kits. Purified DNA quality and quantity were validated using the Qubit dsDNA BR (Broad-Range) Assay Kit on the Qubit 2.0 and Nanodrop Spectrophotometer (Nanodrop ND-1000). Genotyping was conducted using the Illumina goat SNP50 Bead chip developed by International Goat Genome Consortium (IGGC). Quality control procedures of SNPs was done in PLINK v 1.9 [4]. The standard parameters of SNP filtering was applied; all SNPs less than 95 % call rate, less than 0.05 Minor Allele Frequency (MAF < 0.05), Hardy-Weinberg Equilibrium (< 0.001) and more than 10 % missing genotypes were removed remaining with 48, 808 SNPs for downward analysis.

Data analysis

Functional annotation was done on the ENSEMBLE goat (*C. hircus*) using the Variant Effect Predictor (VEP) based on a method described by [10]. A genome annotation file was downloaded from the Ensemble-VEP containing all the functional consequences information. The number of missense and synonymous variants were calculated from the downloaded file in excel, and they were used in calculating the ratio of missense and synonymous to get the mutation load for the population [5]. Sorting Intolerant from Tolerant (SIFT) algorithm was used to identify the mutation effect on protein function and understand the effect of changes in amino acids. The SIFT score ranged from 0 to 1 where a SIFT of 0 to 0.05 is regarded to be deleterious while a score of greater than 0.05 is tolerable [11], [22]. All deleterious variant genes with SIFT score of less than 0.01 were extracted and analysed for their functions using the Biomart tool in the ENSEMBL goat *C. hircus*.

Results

Annotation of SNPs in ENSEMBLE-VEP revealed the presence of different variants located within and outside the genes. The variants located outside the genes (intergenic and up/downstream variants) accounted for 48.57% while the remaining percentages were variants located within the genes (Table 1). Classification of variants to be missense (mutations that affect amino acid sequence in a protein) or synonymous (mutations with no effect on the amino-acid produced) showed that the synonymous variants were in abundance compared to missense variants totaling 693 and 258 representing 1.01% and 0.37%, respectively (Figure 1). Further classification of the 258 missense variants showed that 126 variants were deleterious while 132 are tolerated. Only 0.02 % of the variants were found to be eligible for loss or gain of the stop codon. Generally, the ratio of missense to synonymous variants was found to be 0.37 across the populations.

A total of 111 genes were identified under missense variants. Using a SIFT score of less than 0.01, five genes were identified from 21 highly deleterious variants (Table 1). The identified genes include PROS1, EHBP1, LTN1, LRRN4, and FNDC3A which are associated with different functionalities according to Gene Ontology analysis (Table 2). Some of the gene functions analysed include; negative regulation of blood coagulation (PROS1), mediation of spermatid-sertili adhesion during spermatogenesis (FNDC3A), regulation of vesicular trafficking, and linking endosomes to the actin cytoskeleton (EHBP1), animal memory of cells (LRRN4) and regulation of translation, elongation and proteasome-mediated ubiquitin-dependent protein catabolic process (LTN1).

Table 1: Summary of SNP annotation for goat genotypes in Kenya

Annotation Category	Numbers	Percentage (%)
Intergenic	25792	37.42
Intronic	30701	44.54
UTR3'/UTR5'	560	0.81
Upstream/downstream	7683	11.15
Exonic		
-Nonsynonymous	258	0.37
-Nonsynonymous deleterious	126	0.18
-Nonsynonymous deleterious (>0.01)	21	0.03
-Nonsynonymous tolerated	132	0.19
-Stop gain/loss	13	0.02
Synonymous	693	1.01
Splicing	91	0.13
Intron non-coding regions	2996	4.35
Protein non-coding regions	128	0.19
Stop retained variants	12	0.02

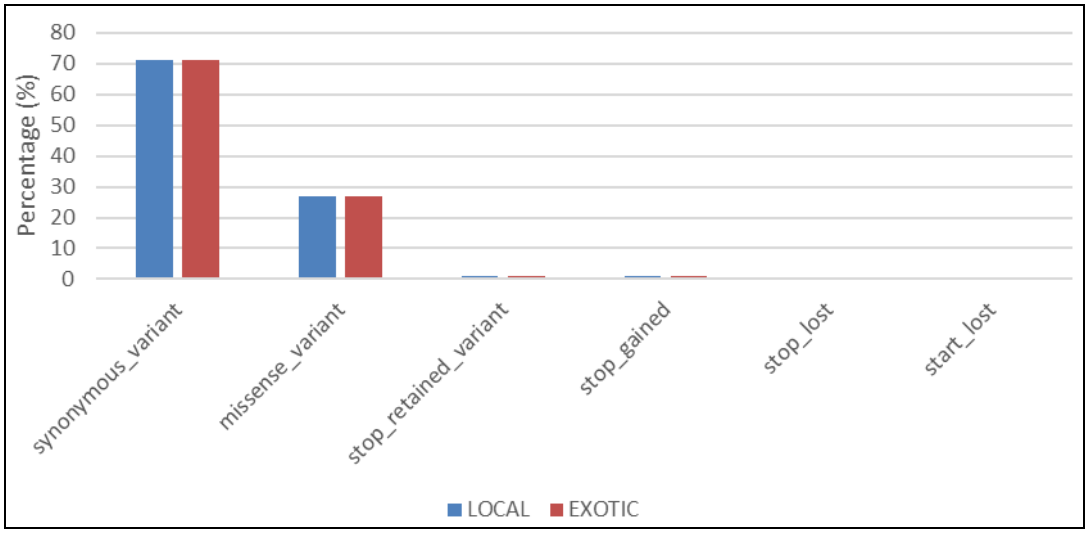

Fig 1: Coding consequences for local and exotic goats in Kenya

Table 2: GO terms enrichment for non-synonymous deleterious variants in goat populations based on SIFT prediction

Gene description	GO term name	GO term accession	GO domain	COUNT
Protein S (PROS1)	Negative regulation of blood coagulation	GO:0030195	Biological-process	2
	calcium ion binding	GO:0005509	Molecular-function	2
	Fibrinolysis	GO:0042730	Biological-process	1
EH domain-binding protein 1 (EHBP1)	Cytosol	GO:0005829	Cellular-component	1
	plasma membrane	GO:0005886	Cellular-component	1
Fibronectin type III domain-containing 3A (FNDC3A)	Membrane	GO:0016020	Cellular-component	4
	An integral component of membrane	GO:0016021	Cellular-component	4
	protein binding	GO:0005515	Molecular-function	4
	Golgi apparatus	GO:0005794	Cellular-component	1
Listerin E3 ubiquitin protein ligase 1 (LTN 1)	protein autoubiquitination	GO:0051865	Biological-process	1
	RQC complex	GO:1990112	Cellular-component	3
	zinc ion binding	GO:0008270	Molecular-function	3
	Cytosol	GO:0005829	Cellular-component	3
	metal ion binding	GO:0046872	Molecular-function	2
	transferase activity	GO:0016740	Molecular-function	2
	rescue of stalled ribosome	GO:0072344	Biological-process	2
leucine rich repeat neuronal 4 (LRRN4)	long-term memory	GO:0007616	Biological-process	1
	visual learning	GO:0008542	Biological-process	1
	protein binding	GO:0005515	Molecular-function	1

Discussion

Some mutations can persist in a population for a long time before being eliminated through selection. Regardless of the mutation levels in a population, the presence of deleterious mutations can cause a reduction in population size and possible species extinction if not properly managed [1]. Surprisingly, more studies on genomes report only the deleterious mutations observed in a population and not the levels of mutation load making it difficult to compare mutation load across populations [9]. Conclusions on levels of mutation load are mostly based on the comparison between populations within or across the study since there are no recommended estimates to base on. The levels of mutation load obtained in this study through the ratio of missense to synonymous mutations were similar (0.37) across the studied genotypes (Figure 1). This suggests that mutations are present in all Kenyan goat populations regardless of the genotype. The similarities can be attributed to the exposure of these animals to almost similar selection criteria towards the same breeding goal creating similar effects on the genome [17, 25].

This study followed the principle of [5] who described the mutation load of 0.025 and 0.03 to be higher in the European pig genome than the 0.02 load observed in the Asian pig genome. In the same study, a mutation load of 0.018 in commercial chickens was described as higher compared to 0.014 observed in the village chicken. Also [13], described the mutation load of 0.83 and 0.46 for domesticated rabbits and chickens respectively as higher than their wild counterparts with lower values. It is reported that mutation load is common in domesticated animals compared to their wild counterparts [14]. Concerning the results of other species mentioned above, it can be concluded that the mutation load of goat genotypes from Kenya is lower than the reported domesticated dogs, rabbits, and chickens but higher than the mutation load of the pig's genome.

Recent mutation studies are focusing on deleterious mutations due to their effects on population fitness. The deleterious mutations observed in this study accounted for 0.18 %, and only 0.03 % were considered to be more deleterious. However, this is lower than the 0.17 % reported in seven Capra species [8]. It is reported that deleterious alleles occur at a very low frequency as observed in domesticated goats [27]. This can lead to the conclusion that different forces that contribute to deleterious mutations have a limited impact on the accumulation of deleterious mutations in the studied goats.

Out of 111 genes identified from the deleterious mutations, five genes were found within the highly deleterious mutations

that are associated with diseases, reproduction, vision, and memory (Table 2). Information on functional annotation in any population helps in understanding the genetic basis of phenotypic variations among individuals hence ensuring accurate selection during breeding. According to [2], the PROS1 gene help in protecting mammals from different diseases such as bronchial asthma in humans. Currently, the effect of the PROS1 gene in goats has not been reported however it can also contribute to cardiac disorders but further research needs to be done to verify.

Naturally, goats have good term memory regardless of sex, age, or breed whose mechanism is unclear [4]. It is reported that goats can remember any visual shape within 42 days and a doe can recognize its kids' voice 12 months after weaning [12, 4]. The LRRN4 gene found in this study might be the contributing factor to memory, natural feeding, and social interaction behaviour in goats during management. Goats with difficulties in visual and memory can be due to mutations in the LRRN4 gene in their genome among other contributing factors. Furthermore, the LTN1 gene is involved in the biological process of rescuing stalled ribosomes. Stalled ribosomes can occur during protein synthesis due to several factors like depletion of amino acids leading to incomplete protein synthesis. This causes the accumulation of proteotoxic components that lead to cellular stress and neurodegenerative diseases in animals. Scrapie is one of the reported fatal neurodegenerative diseases associated with various clinical signs and it is difficult to diagnose in goats and sheep [3].

Male infertility can be due to several factors including genetic disorders. According to [18], the FNDC3A gene which is found in testis tissues helps in spermatogenesis by mediating the adhesion between spermatids and sertoli cells. The mutation of the FNDC3A gene causes male sterility by failure to release mature spermatids. Some male goats in a population might not be reproductive due to unknown causes, this study has therefore provided insight into the possible cause of infertility which requires further studies to validate. Finally, the EHBP1 gene is essential for several functions in mammals, which include vesicular trafficking, early development as well as cancer [20]. However, the biochemical processes associated with this function are still unknown.

Conclusion

The phenotypic differences among individuals can be a factor of many genes working together not the function of a single gene. Domestication is associated with several processes that contribute to the accumulation of deleterious variants in a population. This study has proven that goat genotypes in Kenya are exposed to similar domestication processes hence the similarities in mutations and accumulation of deleterious variants. Therefore, the identification of mutations, genes, and their associated functions in goat populations should be prioritised for strategic implementation of breeding programs to avoid increasing gene mutations.

Ethics approval

This study was approved by the Egerton University Research Ethics Committee, Nakuru, Kenya Approval No. EUREC/APP/138/2021 and also the National Commission for Science, Technology and Innovation (NACOSTI), license No. NACOSTI/P/21/14174

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