

Detection and Evaluation of Breed-specific SNPs and Minor allele frequency in Ethiopian Indigenous and European Beef Cattle Breeds

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Abstract

Introduction

Understanding the genetic foundation of locally adapted indigenous cattle breeds is critical information for developing appropriate genetic improvement and conservation methods and initiatives.

Methods

To investigate breed-specific SNPs, and minor allele frequency in three Ethiopian cattle breeds Begait, (n = 40), Boran (n = 40), and Fogera (n = 43) were genotyped with a high-density 80K SNP array. Three European beef cattle breeds (Angus, n = 42), Hereford (n = 35), and Charolais (n = 37) were also used for comparison. The SNP markers were screened for a call rate of $\geq 90\%$, a minor allele frequency (MAF) of > 0.01 , and a sample call rate of $> 90\%$.

Results

The average minor allele frequency was 0.19 ± 0.17 , 0.20 ± 0.17 , 0.21 ± 0.17 , 0.31 ± 0.13 , 0.32 ± 0.12 , 0.32 ± 0.13 for Angus, Herford, Charolais, Boran, Fogera, and Begait cattle, respectively. Minor allele frequency significance difference was observed between Ethiopian indigenous and European beef cattle breeds. Across the Ethiopian and European cattle breeds, a common variant minor allele frequency (≥ 0.10 and ≤ 0.5) accounted for an overall estimated 94% and 62%, of the SNPs respectively. A total of 7759 and 48 SNPs were identified as breed-specific in the Ethiopian cattle breeds and European beef cattle respectively. These specific SNPs resided with 3364 genes for Ethiopian cattle breeds and 17 genes for European beef cattle breeds. According to Gene Ontology analysis, interestingly important biological processes and pathways related to tropical adaptation were detected in Ethiopian cattle breeds.

Conclusions

The higher minor allele frequency and breed-specific SNPs detected in Ethiopian indigenous breeds show the presence of high genetic variability. This genetic variation in Ethiopian cattle breeds is used as a potential source for future breeding programs.

1. Introduction

Ethiopia's smallholder farming system is supported by indigenous cattle. They have potential contributions to rural livelihoods, food security, organic agriculture, sociocultural and historical events. Furthermore, local farmers prefer indigenous cattle because they are more adaptable and reproducible under low-input management approaches (1). In Africa, Ethiopia has the largest cattle population (70 million) Central statistical agency (CSA), 2020/21. The country is home to over 28 cattle breeds or

populations, which can be further grouped into four categories: zebu (*B. indicus*), sanga (*zebu* × *B. taurus*), zenga (*sanga* × *zebu*), and the humpless *B. taurus*. East Africa including Ethiopia considered the cradle of the Near-East *B. taurus* as well as the Arabian and Indian *B. indicus* cattle migration corridors, and are often referred to as the secondary hybridization zone (2). The country is strategically located near the Horn and the East Coast, which serve as cattle entry points into Africa, and microsatellite data analysis showed Ethiopian cattle to be hybrid populations (3). Many of them are named after the community maintaining the population or geographical location where they predominate, the true genetic relationship between the main populations has not yet been well defined or documented (4). Many livestock species have evolved distinct breeds as a result of natural and artificial selection, as well as genetic drift due to limited population sizes. As a result, breed names are becoming more commercialized and used as brand names and breed validation of livestock products is becoming increasingly important in determining food safety and authenticity in global and domestic markets quickly, easily, reliably, accurately, and economically by testing an individual's genotype at unique marker loci. (5).

The long-term research strategy (2015) of the Ethiopian livestock master plan focuses on the development of inside-breed selection programs for beef and milk breeds; the crossbreeding of superior indigenous breeds; the evaluation of indigenous breed potential for agro-economically significant traits; the development of a strategy and program for genetic enhancement for each major breed of livestock. Genomic identification of functional variations such as missense variants and variants in indigenous cattle within downstream and upstream genic regions would allow these variants to be checked for their effects on complex traits (6). In the absence of phenotypic evidence, comparisons of breed variabilities that have been subjected to different selective pressures may help different genomic regions and genes regulate qualitative and complex traits (7). Therefore, the investigation of differences between and within cattle breed is an important initial guide for promoting the best use of genetic resources for farm animals and allows successful genetic enhancement to meet the needs of production strategies and to plan and integrate enhancement programs in the context of the unique efficiency of a population (8).

In indigenous cattle, gene mapping and identification of candidate genes associated with agro-economically significant traits help to attain rapid genetic gain (9). Single nucleotide polymorphism (SNP) markers analysis has become the standard approach in recent years for genome-wide studies. Fine mapping and identification of genes controlling complex quantitative traits have allowed a wide range of genome-wide SNP panels to be commercially available (10). The present study investigates minor allele frequency (MAF) and breed-specific SNPs of Ethiopian indigenous cattle and European beef cattle breeds using bovine GeneSeek Genomic Profiler (GGP-80K) bead chip.

2. Materials And Methods

2.1. Study breeds, sample collection, and DNA extraction

DNA samples were collected from three Ethiopian indigenous cattle populations (n = 123) that were kept in different government ranches: Begait (n = 40), Boran (n = 40) and Fogera (n = 43). The Begait, Boran,

and Fogera cattle were sampled from Humera, Dida Tiyura, and Andassa Ranches, respectively. Unrelated female and male animals were sampled based on available pedigree information. Nasal samples (Front part of your nostrils) were collected using Performagene livestock's nasal swab DNA collection kit and DNA was extracted from nasal samples with nasal sample extraction kit according to the manufacturer's recommendations (DNA Genotek Inc., 2012). Three European beef breeds (n = 114) (Angus, n = 42), Hereford (n = 35), and Charolais (n = 37) were used as reference breeds.

2.2. Breed descriptions

Boran is a zebu (*B. indicus*) breed maintained in southern Ethiopia and neighboring areas. Usually, Boran cattle are white or grey fawns (11). They are very flexible and adapt well to different conditions. Depending on the improvement status, Boran bulls in Ethiopia, weight ranges from 318 to 680 kg in males and 225 to 454 kg in females (11). **Fogera** is Zenga (*Zebu x Sanga*) breed distinguished and well known for its black-and-white or black-and-grey pied coat; short, stumpy, pointed horns; hump ranges from thoracic to cervicothoracic; used for milk, and meat (12). **Begait** cattle belong to the Zebu community of North Sudan and are used for both milk and beef and reared in northwest Ethiopia. The breed is characterized by well-developed udder, long legs, large humps, long teats, and higher milk, 5 ± 0.5 liters/day, a live body weight of 333 ± 51 kg, and 278 ± 41 kg for males and females, respectively.

Angus is a top-ranking beef breed. In the early 19th century, several constructive breeders developed the breed and fixed the existing type of cattle. The mature cows are 500–550 kg in weight and the live weight of the bulls can reach up to 1000 kg with a fattening weight of 750–950 kg (<https://www.Livestockoftheworld.com/Cattle/>). **Charolais** is a breed of large light-colored cattle kept for the production of beef and used for crossbreeding. Large carcass yield and distinct muscularity with relatively low-fat deposits on the carcass and bulls' live body weight ranged from 1,000 to 1,650 kg, cows from 700 to 1,200 kg, are features of the Charolais (<http://afs.okstate.edu/breeds/cattle/Charolais/index.html/>). **Hereford** breeds are medium to large and mainly meat cattle. Colored with a white mask, they are usually dark red to red-yellow. They are available with both horned and polled variants. They are muscular, with medium to long side lengths <https://www.roysfarm.com/Hereford-cattle/>. The mean live body weight of mature cows is approximately 800 kg and bulls weigh about 1200 kg on average (<https://www.michiganstatefairllc.com/beef-cattle/>).

2.3. Genotyping and quality control

The samples were genotyped with 80K SNP Bead Chip (Gene Seek Genomic Profiler). The SNP markers were screened for a call rate of $\geq 90\%$, a minor allele frequency (MAF) of > 0.01 , and a sample call rate of $> 90\%$. After the above quality management parameters had been applied, the autosomal SNP markers obtained were used for downstream analysis. Two hundred thirty-one animals were kept after removing six animals with a genotype completion rate of less than 90%. From an initial set of 67,491 SNPs, a subset of 67477, 67468, 67414, 66811, 66934, and 65460 SNPs for Angus, Herford, Charolais, Boran, Fogera, and Begait respectively were kept for breed-specific SNPs and MAF analysis.

2.4. Data analysis

To examine within-breed genetic variability minor allele frequencies were estimated using PLINK (13). Breed-specific SNP, an SNP has been declared to be breed-specific if it has an allele that is present in only one breed (14). SNPs filtered which it is fixed to one breed and not yet to the other considered as breed-specific SNPs. The Bovine UMD.3.1 genome assembly was used to annotate breed-specific SNPs. The Database for Annotation, Visualization, and Integrated Discovery (*DAVID*) v6.8 was used to functionally annotate genes to detect major biological process significance through the FUNCTIONAL ANNOTATION CLUSTER tool based on the GO annotation function. To generate confidence enrichment scores, high stringency easy score criteria were used. Kyoto encyclopedia of genes and genomes (KEGG) pathway analyses were performed using DAVID to map clusters of genes involved in common pathways and processes.

3. Results And Discussions

3.1. Minor allele frequency

Minor allele frequency (MAF) was estimated from genotypic data for autosomal markers (Table 1). The mean MAF was 0.19 ± 0.17 , 0.20 ± 0.17 , 0.21 ± 0.17 , 0.31 ± 0.13 , 0.32 ± 0.12 , 0.32 ± 0.13 for Angus, Herford, Charolais, Boran, Fogera, and Begait breeds respectively. A significant difference was detected between the Ethiopian indigenous breeds and European beef cattle breeds. The three Ethiopian cattle breeds had a higher MAF value (32%) than the European beef cattle breeds (20%). The total MAF for the indigenous Ethiopian cattle breeds in this study was higher than the previous report value reported for most taurine breeds (1,15, 16,). The value found for the Boran cattle breed was higher than previous results (0.21 ± 0.150) (1). The Angus and Herford average values are different from the previous result (0.27 ± 0.14 and 0.29 ± 0.14) reported by (17) respectively. This may be due to the different marker densities used in the previous study, which used a lower density marker (Illumina Bovine 8K and 50k SNP Bead Chip). The higher values for Ethiopian breeds can be explained by the fact that the SNP loci used in this study were discovered in the indicine breeds, and their average minor allele frequency was much lower in taurine breeds. The MAF revealed in this analysis corresponded to different markers density (Illumina Bovine 8K, 10K, 50K, 80K, and 700K) used in previous studies in various cattle breeds around the world, with the majority of these breeds samples not being used before or during the design of these chips (15, 18, 19).

Table 1
Average MAF in Ethiopian and European
cattle breeds.

Breed/Population	n	Mean \pm SD
Boran	39	0.31 \pm 0.13
Begait	38	0.32 \pm 0.13
Fogera	40	0.32 \pm 0.12
Overall	117	0.32 \pm 0.13
Angus	42	0.19 \pm 0.17
Herford	35	0.20 \pm 0.17
Charolaise	37	0.21 \pm 0.17
Overall	114	0.20 \pm 0.17

SNP variation in Ethiopian and European cattle breeds was also studied (Table 2). At common variants (≥ 0.10 and ≤ 0.5), the minor allele frequency (MAFs) distribution for both Ethiopian and European breeds accounts for 94% and 62% respectively of the total. Angus cattle had the lowest proportion of common variants among these breeds (59%). Within Ethiopian breeds, 94% of SNP markers were found to be polymorphic, while the remaining 6% were considered monomorphic markers. The overall Ethiopian and European breed MAF variation at rare variants (> 0 and 0.05) were found to be 2% and 9% respectively. The European beef breeds showed a high percentage of rare variation because they are highly selected. Inbreeding is indicated by the higher proportion of alleles (fixed) in selected cattle populations, which is due to unregulated breeding management (15). The distribution of SNPs at a fixed level (0) was also investigated, and an average of 1% for Ethiopian and 23% for the European cattle breeds. Angus breed had the highest proportion of fixed SNPs (29%), whereas all Ethiopian cattle breeds had the lowest fixed SNPs (1%). Selection practices could explain the comparatively higher proportion of fixed alleles in European cattle breeds. These results differ significantly from the reported fixed SNP proportion of Ethiopian breeds (1).

Table 2
MAF distribution of 80K SNP Bead Chip in Ethiopian and European cattle breeds.

Breed	n	Fixed (0)		Rare (> 0 and < 0.05)		Intermediate (≥ 0.05 and < 0.10)		Common (≥ 0.1 and ≤ 0.5)	
		SNP	Prop	SNP	Prop	SNP	Prop	SNP	Prop
		Boran	39	328	0.01	1385	0.02	2806	0.04
Begait	38	350	0.01	1077	0.02	2693	0.04	61340	0.94
Fogera	40	690	0.01	1763	0.03	2287	0.03	63176	0.94
Overall	117	456	0.01	1408	0.02	2595	0.04	62269	0.94
Angus	42	19737	0.29	4321	0.06	3551	0.05	39868	0.59
Herford	35	18298	0.27	3634	0.05	3127	0.05	42413	0.63
Charolais	37	9032	0.13	10739	0.16	4641	0.07	43004	0.64
Overall	114	15687	0.23	6231	0.09	3773	0.06	41761	0.62

3.2. Density and Distribution of SNPs across the Autosomal-chromosome

Allele frequencies differ among breeds. The following figures provide a visual illustration of this difference with a bar graph of autosomal-chromosome allele frequencies for the Ethiopian and European breeds. Among Ethiopian breeds, the highest polymorphism was observed on BTA.4, BTA.7, BTA.8, and BTA.11 in the Begait breed (Fig. 1). The Boran cattle showed the lowest polymorphisms than Begait and Fogera across all the autosomal chromosomes. In European beef breeds, the highest polymorphism was observed in the Charolais breed and the lowest polymorphism scored at the Angus breed in all chromosomes (Fig. 2).

Polymorphisms were also compared between Ethiopian cattle breeds and European beef cattle breeds and the highest polymorphism was observed in the Ethiopian cattle population and the lowest polymorphism was shown at European beef cattle especially the Angus breed across all autosomal chromosomes (Fig. 3).

3.3. Breed-specific Single nucleotide polymorphism

Breed-specific SNPs are only polymorphic within a single breed, and in other breeds, one of the alleles is fixed (5, 14). To assess the protection and authenticity of livestock products in global and domestic markets, breed validation has become increasingly important. Breed-specific markers with different alleles fixed within each breed had the greatest discriminatory strength (5). In these studies, the allele

frequencies in the Ethiopian cattle breeds and European beef cattle breeds were reported to be breed-specific, with a minor allele frequency of $\geq 1\%$ in both breeds.

The total number of breed-specific SNPs detected and the average frequency of SNPs are shown in Table 3. From the Ethiopian population the highest total number of breed-specific SNPs was observed in the Boran breed (90) the lowest number of SNPs was scored in Fogera (28) and Begait was (76). The total commonly fixed Ethiopian cattle breed SNPs was (53). Similarly, breed-specific SNPs within European beef breeds were calculated (Table 4). Charolais breed had the highest number of breed-specific SNPs (8903) and the Angus breed had the lowest breed-specific SNPs (324), Herford scored (378). Angus and Herford showed a similar average frequency (0.06), but Charolais showed the lowest average frequency (0.02). European beef breeds share a high number of fixed SNPs (8125). In each breed SNPs unique to Ethiopian and European cattle breeds identified (Table 5); Boran (8038), Fogera (9031), and Begait (7869) had a higher number of unique SNPs, while all European beef cattle breeds showed a lower and similar number of unique SNPs (48).

Table 3
Breed-specific SNPs detected in Ethiopian cattle breeds.

Cattle breed	Number of SNPs	MAF		
		Minimum	Maximum	Average
Boran	90	0.012	0.23	0.04
Fogera	28	0.012	0.14	0.04
Begait	76	0.13	0.22	0.05

Table 4
Descriptive analysis of European beef cattle breeds, breed-specific SNPs.

Cattle breed	Number of SNPs	MAF		
		Minimum	Maximum	Average
Angus	251	0.01	0.44	0.06
Herford	377	0.01	0.42	0.06
Charolais	8902	0.01	0.45	0.02

Table 5
Breed-specific SNPs were detected in the comparison of Ethiopian cattle breeds and European beef cattle breeds.

Breed	Number of SNPs	MAF		
		Minimum	Maximum	Average
Boran	8038	0.013	0.50	0.32
Begait	7869	0.013	0.50	0.33
Fogera	8031	0.013	0.50	0.33
Angus	48	0.06	0.49	0.33
Herford	48	0.10	0.49	0.33
Charolais	48	0.03	0.50	0.32

3.4. Annotation of European beef cattle specific SNPs

European beef cattle-specific SNPs (48) and Ethiopian cattle-specific SNPs (7759) were genomically annotated to the UMD3.1 reference. Of the total European cattle breeds SNPs, 17 genes were identified. Thirty-seven (37) (0.70%) SNPs were found to be intergenic; 2 (0.04%) SNPs were upstream of genes, 3 (0.06%) SNPs were found downstream of the genes and the remaining 9(0.17%) SNPs were located within the coding regions (Fig. 4).

European cattle-specific SNPs corresponding genes were: - *LOC618554* gene associated with the olfactory transduction pathway. Animals employ both visual and olfactory inputs to direct survival behaviors including detection of food sources, finding mates, and predator avoidance (20). *C1H21orf62* gene is a POLL locus mutant and candidate for longhorn growth. Poll locus is responsible for lack of horns (21). In pure-bred zebu cattle, it has not yet been mapped (22). *DCBLD2* has been linked to the epidermal growth factor receptor, a tumor suppressor, vascular repair and angiogenesis, as well as glucose uptake and thrombus formation (23). The *GALNTL6* gene is linked to growth and feed production (24). *HACE1* gene linked to host defenses against pathogens. *MGRN1* gene identified as component of homeostasis control system in neurons, and aggregates the toxic stress (26). *MIR34A* gene is a possible immune cytokine regulator that is expressed in heat stress. In chickens, *MIR34A* targets the genes cytokine–cytokine receptor interleukin 2 (IL-2) and interleukin 12 (IL-12) (27). Ciliaogenesis and Hedgehog signaling pathways are regulated by *RFX3* gene, which are linked to ciliopathies (28). The *SCN2A* gene controls the voltage-gated sodium channel and keeps the cell's physiology in check (29). The *Rad54B* gene regulates DNA damage and repair (30) (Table 6).

Table 6

European beef breeds, specific SNPs, and the corresponding genes and their associated traits.

BTA	Position	Genes	rs-number	Traits/ Gene function	Reference
1	2049400	<i>C1H21orf62</i>	rs110875985	poll locus mutant	(21)
1	42749580	<i>DCBLD2</i>	rs109175475	Angiogenesis	(23)
2	31080979	<i>SCN2A</i>	rs110334343	Voltage-gated sodium channel regulation	(29)
8	4270697	<i>GALNTL6</i>	rs29015318	Growth and feed consumption	(24)
8	41527418	<i>RFX3</i>	rs42495334	Ciliaogenesis	(28)
9	45784946	<i>HACE1</i>	rs137743222	Host defenses against pathogens	(25)
14	72255136	<i>RAD54B</i>	rs109689318	DNA damage and repair	(30)
25	39154079	<i>LOC618554</i>	rs110798174	Olfactory transduction pathway	(20)
25	3768108	<i>MGRN1</i>	rs108945685	Homeostasis	(26)

3.5. Functional analysis of Ethiopian cattle specific SNPs

We annotated (7759) SNPs identified as Ethiopian cattle breed-specific SNPs. These SNPs were residing within 3364 genes (Table 7). Interestingly *ST8SIA1*, *ANO1*, *C27H8orf4*, *FGF1*, *HSF1*, *MYOF*, and *SCARA5* genes have been identified as involved in biological process of cellular response to heat (GO:0034605). Effects of heat stress in tropical cattle affect all domesticated animals (31). Genes also associated with cellular response to forskolin (GO:1904322) were, *EPHA5*, *GNAI1*, *ADCY1*, *ADCY3* identified. In pituitary gland development (GO:0021983), the pituitary gland plays a critical role in tropical cattle adaptation in the regulation of a wide range of basic physiological processes. Genes involved in pituitary gland development were *GATA2*, *GLI2*, *TBX19*, *BMPT1A*, *KDM1A*, *PAX6*. Genes involved in the regulation of p^H (GO:0006885), were *ATP12A*, *EDNRB*, *SLC9A1*, *SLC9A9*. Cell morphogenesis (GO:0000902), is the process through which a cell's size or form is determined and organized during development and the determined genes were *CAP2*, *FRY*, *NOX4*, *SOX6*, *SS18*, *TBCCD1*, *CAPZB*, *COL4A3BP*, *DMRT1*, *EGFR*, *IL7R*, *MAEL*, *NRG1*, *STK4*. *ACACB*, *AMPD2*, *LEPR*, *MRAP2*, *NR4A3* genes identified as involved in energy homeostasis (GO:0097009). Tropical cattle highly control the energy-demanding process by making off and on, due to a shortage of feed and water (32). Similarly, *KITLG*, *RASGRP1*, *SHOC2*, *IGF1*, *MMD2*, *NRG1*, *NOTCH2* genes were identified as involved in positive regulation of Ras protein signal transduction (GO:0046579). Ras protein controls activation of a variety of signaling molecules by translocating them to the plasma membrane (33). *ATP12A*, *ATP1A1*, *ATP1B1*, *KCNMA1* genes were identified as cellular potassium ion homeostasis (GO:0030007). The most abundant cation in the intracellular fluid is potassium, and maintaining adequate potassium distribution across the cell membrane is essential for normal cell function (34).

3.6. Pathway analyses of candidate genes corresponding with Ethiopian Cattle specific SNPs

A total of two hundred eleven (211) genes were identified as involved in various pathways (Table 8). One of the identified pathways was melanogenesis is process by which melanocytes produce the pigment melanin in melanosomes. Tropical cattle breeds adapted to arid and semi-arid environments and acquire genes that have unique physical characteristics, such as a thicker skin coat color, which helps to protect them from direct solar radiation (35). Both breeders and researchers have been interested in the color of cattle's coats because genes that control pigmentation have economic ramifications in the event of genetic abnormalities (36). The melanocortin receptor 1 is recognized to be the principal regulator of the switch between the two-coat color pigments: eumelanin (black pigment) and phaeomelanin (white pigment) in cattle (37). Ethiopian cattle specific SNPs corresponding candidate genes identified under melanogenesis pathway were, *GNAI1, GNAQ, HRAS, KITLG, WNT1, WNT10A, WNT16, WNT3A, WNT7A, ADCY1, ADCY3, ADCY8, CREB3L2, CREB3L4, CREB3, CAMK2A, CAMK2B, CAMK2D, EDNRB, FZD3, FZD8, LEF1, MAPK1, PLCB1, PLCB2, PLCB4, TCF7*. *KITLG* gene selected for roan hear pigment (38).

ADCYAP1R1, ATP1A1, ATP1B1, GNAQ, RAPGEF4, ADCY1, ADCY3, ADCY8, CREB3L2, CREB3L4, CREB3, CREB5, CACNA1D, CAMK2A, CAMK2B, CAMK2D, GLP1R, PLCB1, PLCB2, PLCB4, KCNMA1, KCNMB1, KCNN1, KCNN3, KGNU1, and SNAP25) were detected as involved in insulin secretion pathway. Food restriction in tropical cattle is often accompanied by a decrease in basal insulin concentrations (39). Toll-like receptor signaling pathway (TLR), play a key role in host survival through identification of pathogen-associated molecular patterns in mammals. Genes, *AKT3, FOS, RELA, TBK1, TRAF6, CTSK, IKBKB, IL12A, LBP, MAPK1, MAPK10, MAPK9, MAP2K6, MAP3K8, PIK3CB, PIK3R2, RIPK1, SPP1* were detected as novel SNPs in Toll-like receptor signaling pathway. Platelet activation signaling is important for platelet function in hemostasis. The identified genes involved in Platelet activation were *AKT3, GNAI1, GNAQ, LYN, RASGRP1, ADCY1, ADCY3, ADCY8, APBB1IP, COL1A2, COL11A1, GUCY1A2, ITPR2, ITGB1, MAPK1, MYLK4, MYL12B, PIK3CB, PIK3R2, PLCB1, PLCB2, PLCB4, PRKG1, PPP1CB, P2RY1, TLN1, TLN2, TBXAS1*. Calcium signaling is a key early aspect in immune cell activation (41). The genes *ATP2B1, ATP2A2, GNAL, GNAQ, ADCY1, ADCY3, ADCY8, ADRA1D, ADRB3, AGTR1, AVPR1A, CACNA1B, CACNA1D, CACNA1H, CAMK2A, CAMK2B, CAMK2D, DRD5, EDNRB, EGFR, ERBB3, ITPR2, LHCGR, MYLK4, PLCB1, PLCB2, PLCB4, PLCD3, PLCE1, PLCZ1, PHKB, PPP3CA, PPP3CC, PTK2B, P2RX3, SLC8A1, SLC8A3, VDAC3* were identified as involved in the Calcium signaling pathway.

The identified genes detected as involved in the Rap1 signaling pathway were *AKT3, EPHA2, GNAI1, GNAQ, HRAS, KITLG, MET, RAPGEF4, RAPGEF5, TIAM1, ADCY1, ADCY3, ADCY8, APBB1IP, EFNA3, EGFR, FGF1, FGF18, FGFR1, FGFR2, FGFR3, FLT1, IGF1, ITGB1, ITGB2, MAGI3, MAPK1, MAP2K6, PARD3, PIK3CB, PIK3R2, PLCB1, PLCB2, PLCB4, PLCE1, PDGFD, P2RY1, SIPA1L1, SKAP1, TLN1, TLN2*. Genes detected as involved in Endocytosis pathway were *RF1, ARFGEF1, ARAP2, CBLB, CBL, GRK5, GRK7, HRAS, RAB10, RAB11FIP3, RAB22A, SH3GL2, TRAF6, AMPH, BIN1, CAPZA2, CAPZB, CHMP4B, CCDC53, DNMT3,*

EGFR, FGFR2, FGFR3, HSPA2, IL2RA, KIF5A, KIF5B, LDLRAP1, PARD3, PIP5K1B, SNX6, TFRC, TGFBR1, VPS36, VTA1.

Table 7
The biological process of Ethiopian cattle specific SNPs corresponding candidate genes.

GO Terms	Gene count	P-Value
(GO:0000902) cell morphogenesis	14	0.0000037
(GO:0034605) cellular response to heat	7	0.000032
(GO:0030007) cellular potassium ion homeostasis	4	0.00083
(GO:1904322) cellular response to forskolin	4	0.00034
(GO:0008152) metabolic process	23	0.00011
(GO:0097009) energy homeostasis	5	0.0060
(GO:0006885) regulation of pH	4	0.027
(GO:0046579) positive regulation of Ras protein signal transduction	7	0.034
(GO:0021983), pituitary gland development	6	0.049

Table 8
KEGG pathway analysis of genes associated with SNPs detected as Ethiopian Cattle populations specific.

KEGG pathway	Gene count	P-value
Insulin secretion	26	4.1E-7
Rap1 signaling pathway	38	2.1E-5
Calcium signaling pathway	38	9.7E-4
Toll-like receptor signaling pathway	18	1.5E-3
Melanogenesis	28	1.3E-2
Platelet activation	28	2.8E-3
Endocytosis	35	2.4E-2

4. Conclusion

This work provides a concise overview of existing concepts and applications of genetic diversities in livestock breeding. The levels of genetic variation for SNPs on the Bovines GGP-80K assays identified in this study indicate that these assays have utility for genetic studies in Ethiopian indigenous cattle breeds. The higher average MAF in the indigenous Ethiopian breed increases the effectiveness of the assays for the selection of breed-informative markers. The highest breed-specific SNPs detected in Ethiopian cattle

breed shows the presence of high variability. The observed variations in the biological process and pathway analyses are most likely related to the level of selection and selection pressure that happens during their evolution. To prevent the biases inherent in SNP assays, detection of SNPs with breed-specific fixation of alternative alleles tends to involve whole-genome sequencing of pools of DNA from individuals from local cattle breeds. The identification of breed-specific SNPs provides the livestock industry with an easy, rapid, economic, and reliable method to validate the breed of livestock individuals and products.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors state that the publishing of this paper does not include any conflicts of interest.

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Authors' contributions

Dejenie Mengistie Zewdu Edea Tesfaye Sisay Tesema and Hailu Dadi conceptualized and designed the study.

Genet Dejene Jeilu Jemal Taddelle Dessie Kwan Suk Kim and Behailu Samuel conceptualize this study

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Availability of data and materials

All the data can be obtained, and the conclusion can be drawn through the analysis of the relevant software. We do have all datasets generated and/or analyzed during the current study.

Ethics approval and consent to participate

Not applicable

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Figures

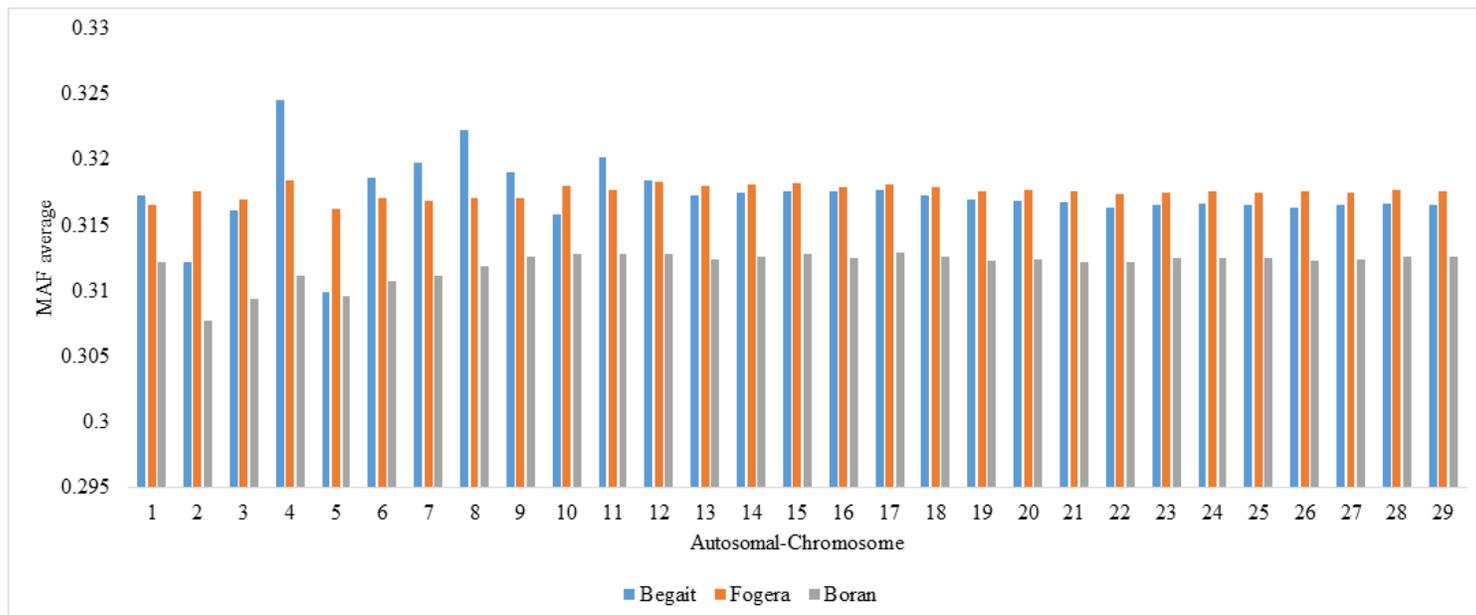


Figure 1

MAF distribution of Ethiopian cattle breeds across the autosomal chromosome, single nucleotide polymorphism.

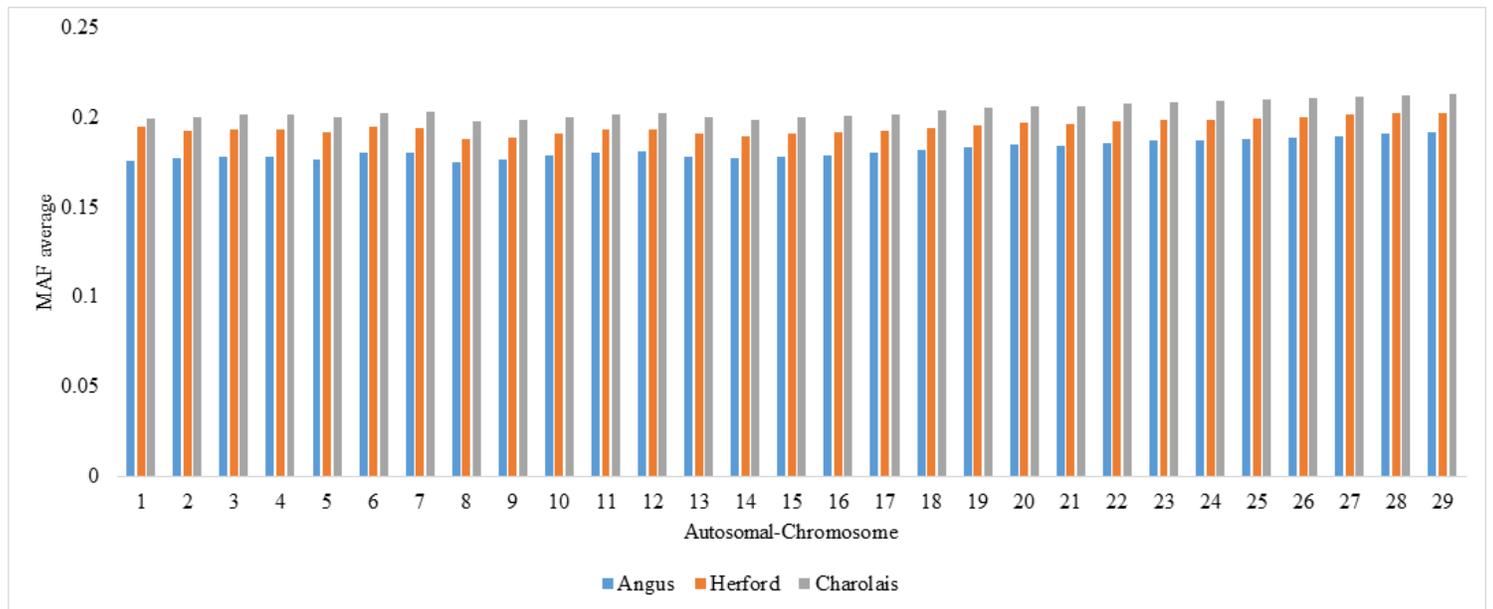


Figure 2

MAF distributions in European cattle breeds across the autosomal-chromosomes.

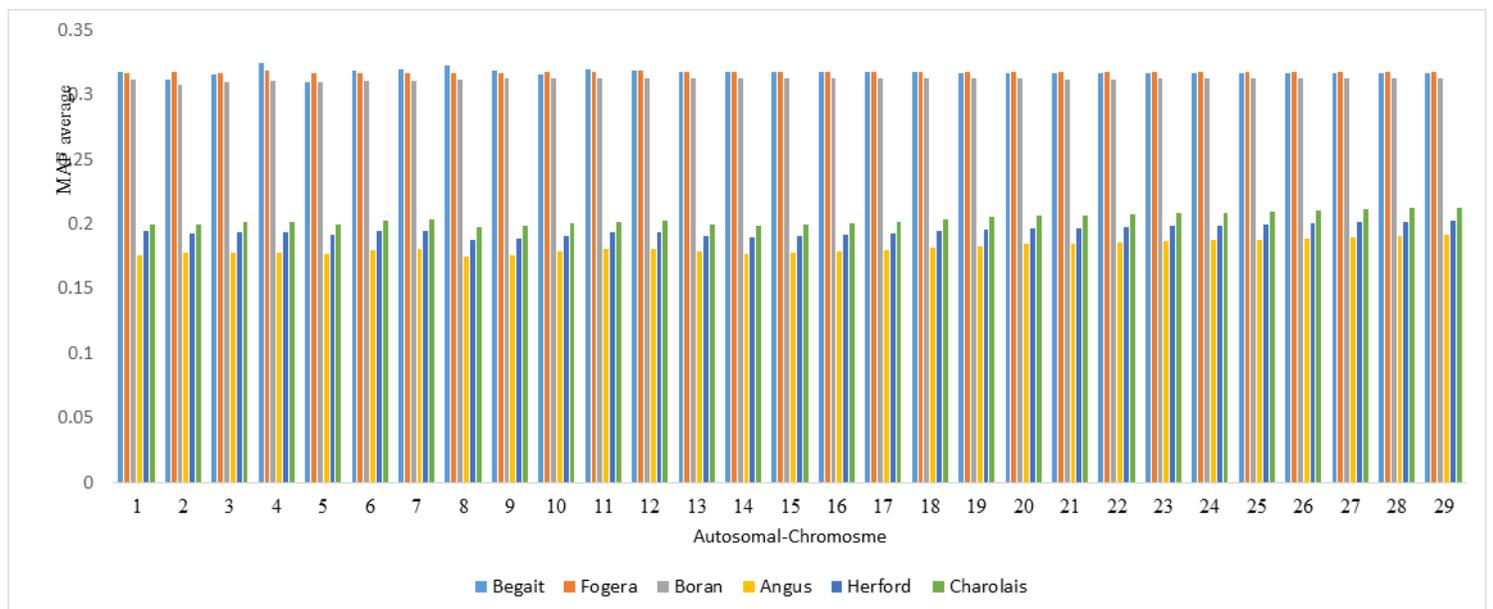


Figure 3

MAF distribution in Ethiopian and European breeds across the autosomal chromosomes.

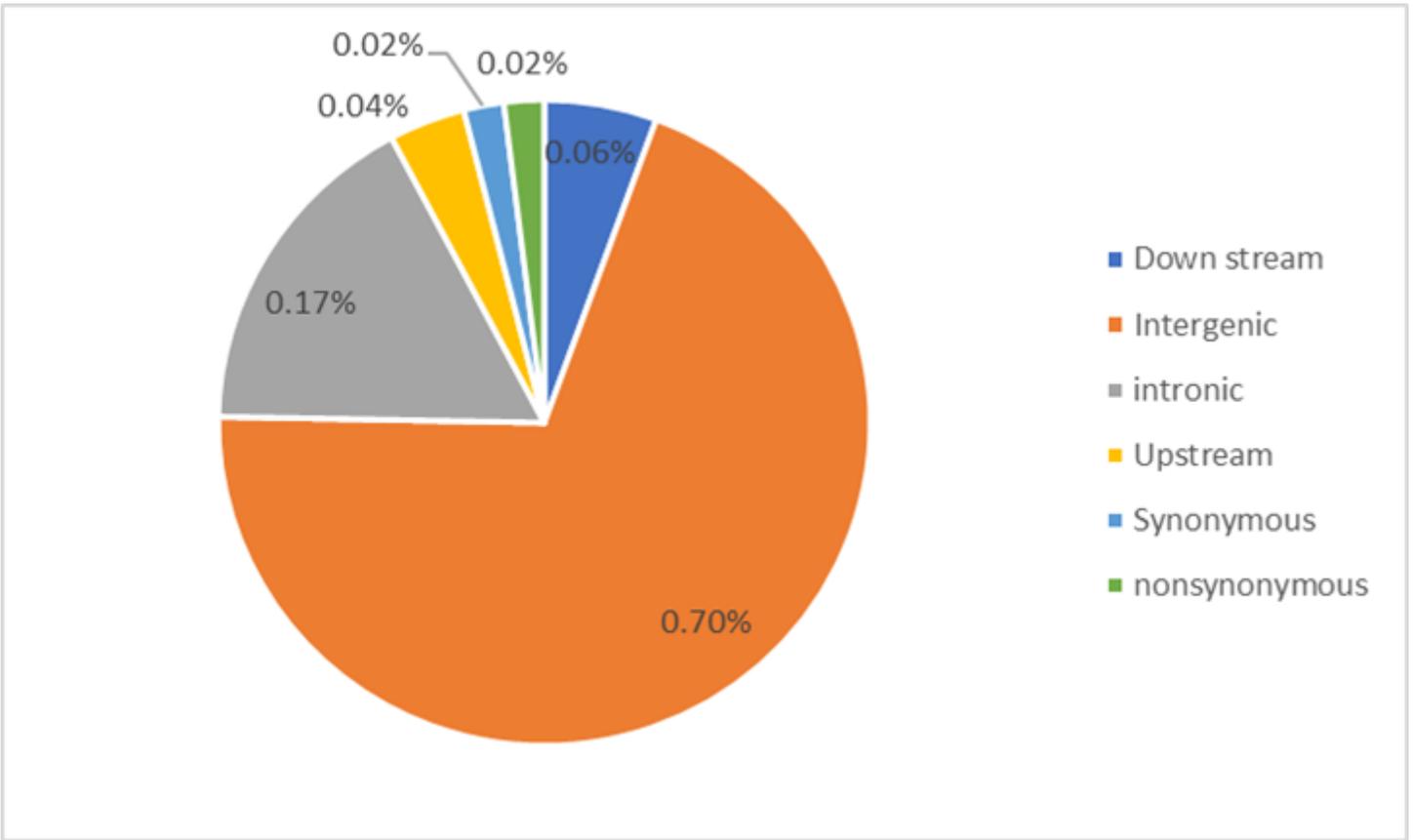


Figure 4

European beef cattle specific SNPs corresponding gene proportion across a genome.