Journal of Biochemistry International

Volume 10, Issue 1, Page 105-116, 2023; Article no.JOBI.12122
ISSN: 2454-4760

Identification of Breed-Specific SNPs in Ethiopian Indigenous and European Beef Cattle Breeds and how they May Influence Adaptation and Selection Signatures

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DM, ZE, TST, and HD full write up and designed the study. Author TD, read and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/JOBI/2023/v10i18694

Original Research Article

Received: 26/10/2023
Accepted: 30/12/2023
Published: 31/12/2023

ABSTRACT

Background: Understanding the genetic foundation of locally adapted indigenous cattle breeds is critical information for developing appropriate genetic improvement and conservation initiatives. To investigate breed-specific SNPs, and minor allele frequency of three Ethiopian cattle breeds Begait, (n = 40), Boran (n = 40), and Fogera (n = 43) were genotyped with 80K SNP array. Three European beef cattle breeds (Angus, n = 42), Hereford (n = 35), and Charolais (n = 37) were also used for comparison.

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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 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 94% and 62%, respectively. A total of 7759 and 48 SNPs were identified as breed-specific in Ethiopian cattle breeds and European beef cattle breeds respectively. These SNPs resided with 3364 genes for Ethiopian cattle breeds and 17 genes for European beef cattle breeds. Interestingly important biological processes and pathways related to tropical adaptation in Ethiopian cattle populations were identified through gene Ontology analysis.

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.

Keywords: Breed-specific SNPs; minor allele frequency.

1. INTRODUCTION

Ethiopia's smallholder farming system is supported by indigenous cattle. Farmers prefer indigenous cattle because they are more adaptable and reproducible under low-input management approaches [1]. The country is strategically located near the Horn and East Coast, which serve as cattle entry points into Africa and microsatellite data analysis showed Ethiopian cattle to be hybrid populations [2]. The livestock species have evolved distinct breeds as a result of natural and artificial selection, as well as genetic drift due to limited population sizes. Most species are represented by distinctive breeds with specific phenotypic and thus also genotypic characteristics. By relating individual DNA sequence variation to the one universal template, we encounter a danger to miss population specific variation, since all polymorphism detections are “biased” towards the sequence structure represented by the reference genome [3]. That is why breed-specific reference genomes are essential for providing more accurate genomic inferences, which account for DNA sequence variation among particular breeds. Such diversity occurs due to breed formation history, differences in selection pressure as well as migration barriers [4]. 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 regulating qualitative and complex traits [5]. 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 [6].In indigenous cattle, gene mapping and identification of candidate genes associated with agro-economically significant traits help to attain rapid genetic gain [7]. Single nucleotide polymorphism (SNP) markers analysis has become the standard approach in recent years for genome-wide studies [8]. The present study investigates minor allele frequency (MAF) and breed-specific SNPs of Ethiopian indigenous cattle and European beef cattle breeds using bovine GGP-80K bead chip.

2. METHODS

2.1 Study Breeds, Sample Collection

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 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 from the database (*http: //gong_lab. hzau.edu. cn/Animal _SNPAtlas/*).

2.2 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 ≥ 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.

2.3 Data Analysis

To examine within-breed genetic variability minor allele frequencies were estimated using PLINK [9]. Breed-specific SNP, an SNP has been declared to be breed-specific if it has an allele that is present in only one breed [10]. 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 DISCUSSION

3.1 Minor Allele Frequency

Minor allele frequency (MAF) was estimated from genotypic data of 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 [11-16,]. 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 Zwane et al. [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].

SNP variation in Ethiopian and European cattle breeds were 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).

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 showed 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 ≥ 1% in both breeds.

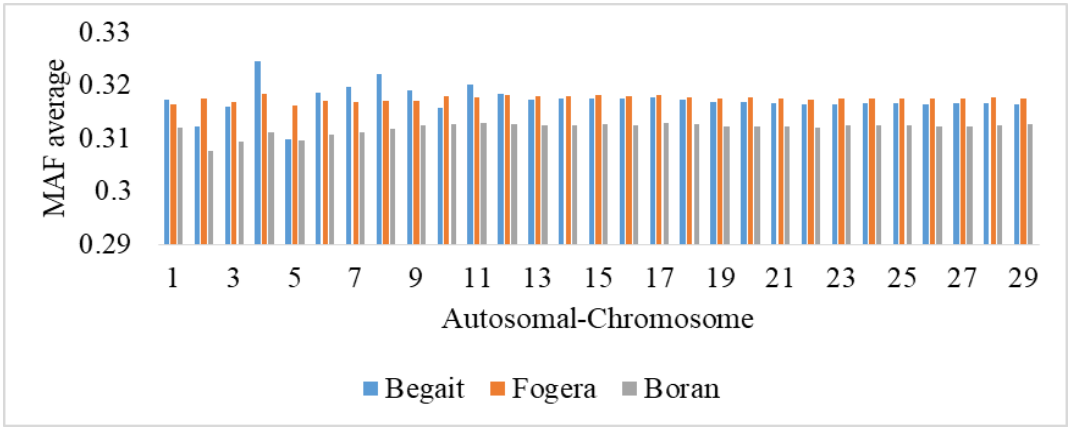

Fig. 1. MAF distribution of Ethiopian cattle breeds across the autosomal chromosome, single nucleotide polymorphism

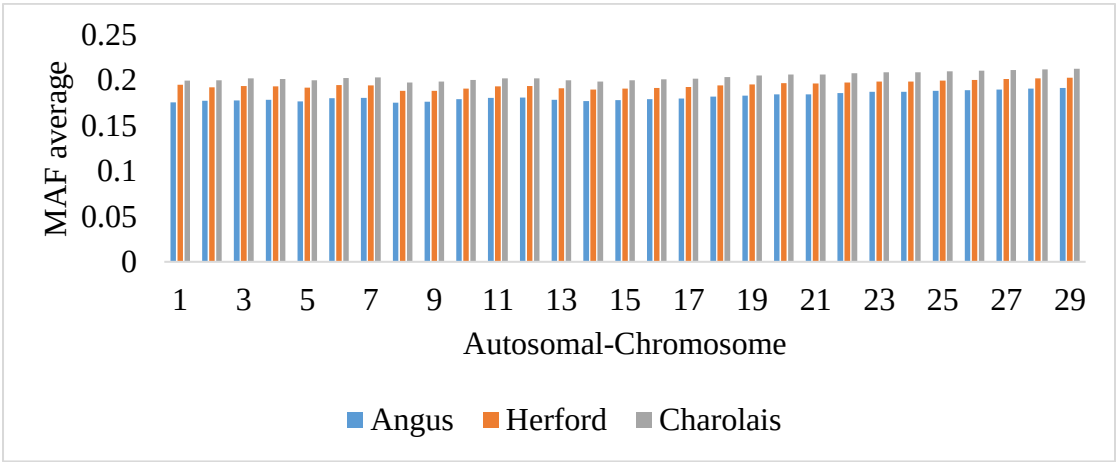

Fig. 2. MAF distributions in European cattle breeds across the autosomal-chromosomes

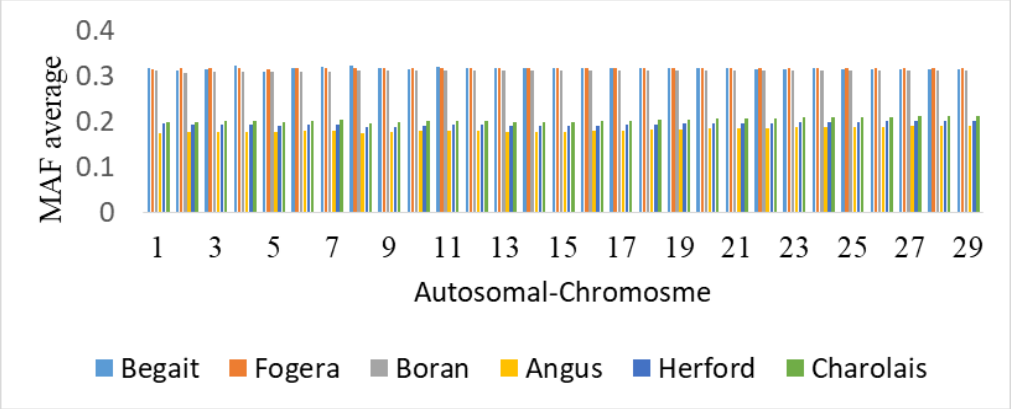

Fig. 3. MAF distribution in Ethiopian and European breeds across the autosomal chromosomes

Table 1. Average MAF in Ethiopian and European cattle breeds

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

Table 2. MAF distribution of 80K SNP bead chip in Ethiopian and European cattle breeds

Breed	n	Fixed (0)		Rare (>0nd<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	62292	0.93
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

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; 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).

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). Molecular functions of genes corresponding to European beef cattle breed-specific SNPs were identified as linked to a particular trait.

The identification of functional variants in European beef cattle, such as missense variants and variants inside upstream and downstream genic regions, would allow these variations to be tested for their impacts on complex characteristics. Some of the European cattle-specific SNPs corresponding genes were *LOC618554* gene which it is associated with the olfactory transduction pathway. As previously mentioned, these genes have been identified as significant genetic factors affecting mammalian evolution and adaptation. Sensing changes in the environment is vital for survival. Animals from invertebrates to vertebrate's employ both visual and olfactory inputs to direct survival behaviors including detection of food sources, finding mates, and predator avoidance [20]. The *C1H21orf62* gene is a poll locus mutant that is a candidate for longhorn growth. The poll locus is responsible for the lack of horns [21]. At both taurine breeds and taurine–indicine crosses, the POLL locus has been localized to the centromeric region of bovine chromosome 1 (*BTA1*) in an interval of around 1 Mb. 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* is linked to host defenses against pathogens. The regulation of apoptosis, phagocytosis, as well as the development of reactive oxygen species and inflammatory mediators [25]. *MGRN1* is an essential component of the homeostasis control system in neurons, acting on two levels: in the cytoplasm, directly challenging protein aggregates and toxic stress, and in the nucleus, achieved at the expense of its ‘‘cytoplasmic" feature, through localization inactive chromatin regions to potentiate the cellular response to proteotoxicity [26]. *MIR34A* 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*, which are linked to ciliopathies, which are developmental and degenerative disorders [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, and its levels of activation are regulated in response to genotoxic stresses [30] (Table 6).

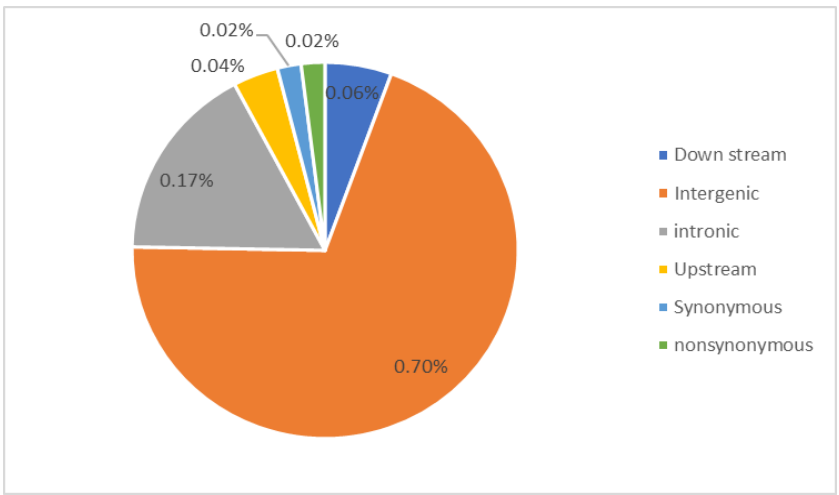

Fig. 4. European beef cattle specific SNPs corresponding gene proportion across a genome

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 breed, 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 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

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	*C1H21orf62*	rs110875985	poll locus mutant	(21)
1	42749580	*DCBLD2*	rs109175475	Angiogenesis	(23)
2	31080979	*SCN2A*	rs110334343	Voltage-gated sodium channel regulation	(29)
8	4270697	*GALNTL6*	rs29015318	Growth and feed consumption	(24)
8	41527418	*RFX3*	rs42495334	Ciliaogenesis	(28)
9	45784946	*HACE1*	rs137743222	Host defenses against pathogens	(25)
14	72255136	*RAD54B*	rs109689318	DNA damage and repair	(30)
25	39154079	*LOC618554*	rs110798174	Olfactory transduction pathway	(20)
25	3768108	*MGRN1*	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 some of the SNPs identified as a cellular response to heat (GO:0034605), any process that causes a change in the state or activity of a cell as a result of a heat stimulus above the ideal temperature [31]. Effects of heat stress in tropical cattle include reduced production, decreased animal welfare, decreased fertility, increased susceptibility to disease, and increased mortality and affect all domesticated animals [32] Ethiopian cattle breeds such as Boran and Begait are evolved under arid and semi-arid environments (high ambient temperature, recurrent drought). Ethiopian cattle-specific SNPs were localized with genes known to be associated with heat stress. Accordingly, the candidate genes (*ST8SIA1, ANO1, C27H8orf4, FGF1, HSF1, MYOF,* and *SCARA5*) have a biological process of cellular response to heat. *HSF1* gene selected for adaptation in subtropical climate [33]. SNPs also associated

with cellular response to forskolin (GO:1904322) were identified, as a result of a forskolin stimulation any process that causes a change in the state or activity of a cell (in terms of motility, secretion, enzyme synthesis, gene expression, and so on). Genes involved in the forskolin response in cells were *EPHA5, GNAI1, ADCY1, ADCY3*. 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 through its interaction between the nervous and endocrine systems. The pituitary gland is an endocrine gland that secretes hormones that regulate many other glands. Genes involved in pituitary gland development were *GATA2, GLI2, TBX19, BMPR1A, KDM1A, PAX6.* Regulation of p^H (GO:0006885), within an organism or cell, any activity involving the preservation of internal equilibrium of hydrogen ions, hence regulating the internal p^H and the candidate genes involved in the regulation of p^H were *ATP12A, EDNRB, SLC9A1, SLC9A9*. Cell morphogenesis (GO:0000902), the process through which a cell's size or form is determined and organized during development. Due to a shortage of feed, the amount of energy required for the maintenance of body tissues and production traits in tropical cattle exceeds the amount of energy gained from dietary sources. As a result, the cattle must rely on body fat as an energy source. The amount of fatty acid that can be oxidized to completion by the liver's tricarboxylic acid cycle or exported from the liver as very-low-density lipoprotein is limited. Genes involved in cell morphogenesis were *CAP2, FRY, NOX4, SOX6, SS18, TBCCD1, CAPZB, COL4A3BP, DMRT1, EGFR, IL7R, MAEL, NRG1, STK4*. Energy homeostasis (GO:0097009), any procedure that involves balancing food intake (energy input) and energy expenditure. Tropical cattle highly control the energy demanding process by making off and on, due to shortage of feed and water [34]. Genes identified as involved in energy homeostasis were *ACACB, AMPD2, LEPR, MRAP2, NR4A3*. Positive regulation of Ras protein signal transduction (GO:0046579) is any procedure that triggers or increases the frequency, rate, or scope of Ras protein signaling. Ras proteins were discovered because of their link to cell transformation. Since then, they've been proven to influence processes including cell migration and neural activity, as well as regulate cell proliferation, differentiation, and apoptosis. Ras controls the activation of a variety of signaling molecules by translocating them to the plasma membrane [35]. The identified genes involved in the positive regulation of Ras protein signal transduction were *KITLG, RASGRP1, SHOC2, IGF1, MMD2, NRG1, NOTCH2*. Cellular potassium ion homeostasis (GO:0030007) is any procedure for maintaining an internal steady state of potassium ions at the cellular level. 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 [36]. Genes involved in cellular potassium ion homeostasis were *ATP12A, ATP1A1, ATP1B1, KCNMA1*.

3.6 Pathway Analyses of Candidate Genes Corresponding with Ethiopian Cattle Specific SNPs

Two hundred eleven (211) genes were identified as involved in various pathways (Table 7). One of the identified pathways was melanogenesis which is the complex 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 [37]. 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 [38]. 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 [39]. 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* identified as involved in melanogenesis pathway. *KITLG* gene selected for roan hear pigment [40].

Insulin is a metabolically active hormone that contributes to growth, development, energy balance, and nutritional homeostasis through carbohydrate, fat, and protein synthesis in a variety of anabolic mechanisms. Food restriction in tropical cattle is often accompanied by a decrease in basal insulin concentrations, followed by a rise in systemic insulin concentrations following re-feeding and

compensatory growth, reflecting the availability of dietary substrate and hepatic gluconeogenesis [41]. The candidate genes, (*ADCYAP1R1, ATP1A1, ATP1B1, GNAQ, RAPGEF4, ADCY1, ADCY3, ADCY8, CREB3L2, CREB3L4, CREB3, CREB5, CACNA1D, CAMK2A, CAMK2B, CAMK2D, GLP1R, PLCB1, PLCB2, PLCB4, KCNMA1, KCNMB1, KCNN1, KCNN3, KCNU1, and SNAP25)* were detected as involved in insulin secretion pathway.

Toll-like receptor signaling pathway (TLR), is an important part of the body's innate immune system. This route is characterized by highly conserved proteins, indicating that they play a key role in host survival through identification of pathogen-associated molecular patterns bacteria, viruses, protozoa, and fungus in mammals. Using a radiation hybrid panel, cattle TLR genes were recently localized to chromosomes. The nucleotide sequences of the TLR2, TLR4, and TLR6 genes in cattle were searched for novel SNPs that could be exploited in disease resistance research [42]. Genes, *AKT3, FOS, RELA, TBK1, TRAF6, CTSK, IKBKB, IL12A, LBP, MAPK1, MAPK10, MAPK9, MAP2K6, MAP3K8, PIK3CB, PIK3R2, RIPK1, SPP1* were detected as novel SNPs involved in the Toll-like receptor signaling pathway. Platelet activation signaling is important for platelet function in hemostasis. 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, the increased demand for calcium in periparturient cattle could negatively affect immune cell intracellular calcium reserves. This decrease in intracellular calcium reserves in immune cells may reduce intracellular calcium release in response to an activating stimulation, contributing to the immunological suppression [43]. 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. Rap1 is a tiny cytosolic protein that functions as a signal transduction switch. Rap1 inhibits Ras signaling by including an effector domain that is similar to that of Ras. Rap1 has been reported in various animal species to fulfill different roles as an evolutionarily conserved protein [44]. 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.* Endocytosis is a cell's method for removing ligands, nutrients, plasma membrane proteins, and lipids from the cell surface and transporting them into the cell interior. *RF1, ARFGEF1, ARAP2, CBLB, CBL, GRK5, GRK7, HRAS, RAB10, RAB11FIP3, RAB22A, SH3GL2, TRAF6, AMPH, BIN1, CAPZA2, CAPZB, CHMP4B, CCDC53, DNM3, EGFR, FGFR2, FGFR3, HSPA2, IL2RA, KIF5A, KIF5B, LDLRAP1, PARD3, PIP5K1B, SNX6, TFRC, TGFBR1, VPS36, VTA1* genes were detected as involved in Endocytosis pathway.

Table 7. 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
Endocytosis	35	2.4E-2

4. CONCLUSION

The levels of genetic variation for SNPs on the Bovines GGP-80K assays identified in this study indicates 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.

DISCLAIMER

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ETHICAL APPROVAL

All authors declare that animal samples were obtained in compliance with local/national laws in force at the time of sampling. The animals used in this study were owned by governmental institutions (ranches) established for research. Data exchange was by national and international regulations and approved by the owners. The procedure involving sample collection followed the recommendation of directive 2010/63/EU. In addition, the Scientific Ethical Review Board of Addis Ababa University approved the experimental design and animal data collection for the present study (certificate reference number RECSoANS/BIO/05/2021). All methods were carried out following relevant guidelines and regulations.

ACKNOWLEDGEMENTS

This study was supported by the grants from National Research Foundation (Nos.NRF-2017R1A2B1008883 and NRF-2017K1A3A1A09085855) in Korea. We express our appreciation to the International Livestock Research Center (ILRI), Addis Ababa, Ethiopia, for providing logistic support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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