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Polymorphism analysis of kisspeptin (KISS1) gene and its association with litter size in Ethiopian indigenous goat populations

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Polymorphism analysis of the Kisspeptin (KISS1) gene and its association with litter size were conducted in two (Gondar and Woyto Guji) Ethiopian indigenous goat populations. Three new pairs of primers were designed for touchdown PCR condition. A total of 173 and 242 Sanger sequences were employed for further analysis of exon1 (1210 bp) and exon2 (325 bp) regions of the KISS1 gene, respectively. In addition, the identified polymorphic sites were associated with litter size of the animals included in the study. General linear model was carried out for the association analysis. The study revealed that five complete substitutions and 15 polymorphic sites were detected in both exon regions with 2:1 transition and transversion ratio. Most of the polymorphic sites were observed in more than 1% of the sequences qualifying the single nucleotide polymorphisms (SNPs) definition. The overall average codon bias index was 0.301 with 64.09 and 62.47% GC contents in exon1 and exon2, respectively. The overall average H_E was 0.18863±0.21 for exon1 and 0.03155±0.01. Only four loci, which constitute 10 genotypes, from the polymorphic sites showed significant contribution of litter size of the goat populations studied. Heterozygous genotypes (TC and GC at g.950T>C and g.3416G>C, respectively) had performed highest estimates of litter size in three of the loci which contributed for litter size. However, the homozygous genotype observed at locus g.3811C>T showed highest contribution for fecundity trait. 18 to 31% performance differences of litter size were observed due to the influence of the genotypes. In conclusion, the SNP detected in those target regions of the gene confirm the contribution of the KISS1 gene for fecundity trait suggesting importance of the gene for marker assisted selection in goat breeding programs.

Key words: Goat, litter size, KISS1 gene, polymorphism.

INTRODUCTION

Goats, with their diverse genetic resources, play fundamental role for the national economy of Ethiopia. They contribute about 12% of the total annual meat production and together with sheep; they contribute about 90% of the live animal/meat and 92% of the total skin export trade value (FAO, 2004). Goats in Ethiopia are reared by resource poor farmers. Particularly, in rural livelihood, goats are potential livestock species to meet the nutritional needs of the people, and considered as the "Future Animal" not only for rural and but also urban prosperity (Ahlawat et al., 2016).

Recent genomic researched have focused on screening of genes that contribute for expression of economic traits. Reproduction traits are among crucial economic traits in animal husbandry, and are coordinated during normal puberty and the adulthood (Zhang et al., 2011). Among reproduction traits, genetic studies have indicated that litter size and ovulation rate can be genetically determined by the action of genes (Deldar-Tajangookeh et al., 2009). However, it is stated that, not only for fitness traits (for example litter size), the identification of candidate genes that are responsible for variation in continuous traits (for example growth traits) has been a challenge in modern genetics (An et al., 2013). As effect, to date, little has been divulged on the major genes associated with, for instance, litter size in goats. It has been previously shown that KISS1 gene highly contributes for multiple births in goat (An et al., 2013; Othman et al., 2015). KISS1 gene is a gene that encodes Kisspeptin (formerly known as metastin) protein (Gottsch et al., 2009), and is located on the long arm of chromosome 1 (1q32) (Messager et al., 2005). It is expressed in the hypothalamus region of the forebrain (Gottsch et al., 2009) and is a G-protein coupled receptor ligand (GPR54) (Messager et al., 2005). Kisspeptin-GPR54 signaling has an important role in initiating secretion of gonadotropin-releasing hormone (GnRH) (Dungan et al., 2006; Smith et al., 2006). Signaling between kisspeptin and its receptor, GPR54, is now recognized as being essential for normal fertility by regulating the reproductive system (De Roux et al., 2003; Funes et al., 2003; Kirilov et al., 2013). In this line, beside to the *pulse mode*, that is effected by the secretion of the reproductive neuropeptide gonadotropin releasing hormone (GnRH) which is essential for reproductive events in both sexes (like spermatogenesis, follicular development, and sex steroid synthesis), the *surge mode* of GnRH induces ovulation in females (Okamura et al., 2013).

It has also been noted that the central or peripheral administration of kisspeptin stimulates GnRH-dependent luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion in various mammalian species from rodents to humans and it also administers reproductive functions of animals (Gottsch et al., 2004; Navarro et al., 2005; Shahab et al., 2005). Gottsch et al. (2009) also indicated that neurons that express KISS1 play a crucial role in the regulation of pituitary luteinizing hormone secretion and reproduction, and these neurons are the direct targets for the action of estradiol-17β (E_2), which acts via the estrogen receptor α isoform (ERα) to regulate KISS1 expression. Kisspeptin/neurokinin B/dynorphin (KNDy) neurons located in the hypothalamic arcuate nucleus might play a central role in the generation of GnRH pulses in goats, and perhaps in other mammalian species (Okamura et al., 2013). In the arcuate nucleus (Arc) where the dynorphin gene (Dyn) is expressed in KISS1 bearing neurons, E_2 inhibits the expression of KISS1 mRNA; however, E_2 induces the expression of KISS1 in the anteroventral periventricular nucleus (AVPV) (Gottsch et al., 2009). ERα signals through multiple pathways, which can be categorized as either classical involving the estrogen response element (ERE), or non-classical, involving ERE-independent mechanisms. However with these all regulatory functions of the KISS1 gene and its receptor, failure or loss of function or deletion of, for instance, GPR54 causes to hypogonadotropic hypogonadism (De Roux et al., 2003; Chu et al., 2012). It is a deficiency of the pituitary secretion of follicle-stimulating hormone and luteinizing hormone which cause impairment of pubertal maturation and reproductive function.

In general, despite the limited efforts done in small ruminant livestock so far, the expression and regulation of KISS1 gene plays magnificent role in multiple births in goat. This study might serve as an additional input for further evaluation and utilization of the gene in marker assisted breeding program. Therefore, this study was initiated to evaluate the polymorphic nature and role of KISS1 gene on litter size in two Ethiopian indigenous goat populations.

MATERIALS AND METHODS

The study goat populations

Two indigenous goat populations of Ethiopia, Gondar and Woyto Guji, were selected for the study. The two populations are found in

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the extreme north and south corners of the country (Supplementary Figure S1). The two populations are kept under different production systems and agro-ecology. Woyto Guji goat population is found in arid environment. The production system is categorized predominantly by pastoral production system with some crop production (Workneh, 1992; Netsanet, 2014); whereas, Gondar goat population is found in mid to high altitude (1000 to 3000 m.a.s.l.) areas. The area is characterized by mixed crop livestock production system. Gondar goat has 13.58(± 6.44) months of age at first kidding, 8.58±2.02 months kidding interval and 72.18% and 6.38% of frequency of twine and triple births, respectively (Alubel, 2015); whereas Woyto Guji goat has 21.97±0.41months of age at 1st kidding, 6.79 ±0.10 months kidding interval and 1.33 litter size: 1.33 (Netsanet, 2014).

The blood samples were drawn out from the jugular vein with a volume of nine milliliter under aseptic conditions using ethylenediaminetetraacetic acid (EDTA) anticoagulant. The collected samples were brought to the laboratory with ice box and were stored at -20°C until it was subjected to DNA extraction. Salting out protocol was used for the DNA extraction (Shinde et al., 2008). The DNA quality and concentration were tested by nanodrop, and 1.0 to 1.5% agarose gel electrophoresis was used to evaluate the degradation.

Primers employed, target regions and PCR conditions

To amplify the two exon regions of KISS1 gene, three pairs of primers were newly designed from sequences of *Ovis aris (*Acc:HGNC:6341*)* and C*apra hircus (Acc.* GU142847.1) KISS1 genes obtained in the data base, DNA Data Bank of Japan (DDBJ) and were evaluated by Oligo Analyzer 3.1: Integrated DNA technology package (available at: https://eu.idtdna.com/Analyzer/Applications/OligoAnalyzer/default.aspx?err=true) (Supplementary Table S1). The first exon (exon1) has a length of 1,210 bases and exon2 has 325 bases. For both exons, touchdown PCR program was employed with the respective PCR conditions indicated at Supplementary file1 Figure S2. The PCR products, in both directions, were sequenced at Bioscience for eastern and central Africa-International Livestock Research Institute (BecA-ILRI) hub, Nairobi, Kenya. Sample gel image is indicated at Supplementary Figure S3. Sanger sequence products were aligned with complete *cds* of Jining Grey goat population kisspeptin (KiSS-1) gene available in the GenBank (acc. GU142847, ver. GU142847.1).

A total of 173 animals (DNA sequences), 58 of which were from Gondar goat population and 115 from Woyto Guji goat population, were used to analyze exon1; whereas, 250 DNA sequences (Gondar 117 and Woyto Guji 133) were used for exon2, and 242 samples used for the final analysis for the latter exon. The different number of animals which were used for the two exon regions is because some of sequences of exon1 showed poor or low chromatogram peaks and excluded from the analysis. The phenotype data were extracted from the field monitoring activity of the community based breeding program which was started in 2013. The phenotype data (breed type, parity and birth type) were associated with information of the polymorphic regions.

Data management, statistical analysis and packages employed for analysis

CLC main workbench 7.0.4 (CLC Bio-Qiagen) and SeqTrace ver 9.0 (Stucky, 2012) packages were used to generate trace files and visualize the chromatograms. Bioedit and MEGA 6 (Tamura et al., 2013) were employed to correct possible base calling errors and to align the sequences together with the reference sequence of Jining Grey goat sequenced by Cao et al. (2010). Multiple sequence alignments were done in CLC Workbench with ClustalW algorithm (Thompson et al., 1994). Segregating sites (SNPs) were used to analyze the association study using SAS ver.9.1.

General linear models were used to analyze the association of genotype with phenotype performances of the populations studied. (i) Exon1: $Y_{ijklmn} = \mu+P_i+B_j+G1_k+G2_l+G3_m+e_{ijklmn}$; where, P = Parity at i^{th} (i= 1, 2, 3, 4, 5) parity of the doe gave birth; B=Population at j^{th} population (j = 1, 2); G_1= $Site_1$ at k^{th} genotype (k = GG, GC); G_2 = $Site_2$ at l^{th} genotype (l = CC, CT, TT); G_3 = $Site_3$ at m^{th} genotype (m = CC, TC, TT). (ii) Exon2: $Y_{ijkl} = \mu+P_i + B_j +G_k+ e_{ijkl}$. Parities after the fifth parity were merged as the fifth parity because of fewer observations and non-significant influence of parties after the fifth parity. Genotypes other than indicated in the model were excluded from the model since they showed non-significant effects and did not contribute for fitness (coefficient of determination-R^2) of the model.

RESULTS

Detection of single nucleotide and codon usage

In this study, complete substitutions with respect to the reference sequence (Gene bank: acc. GU142847) were observed at five sites (at g.3436 T >C, g.3592C>A, g.3688A>C, g.3878 A >C and g.4023A>C), of which four of them were *transversions*. In addition to the compete substitutions, 12 polymorphic sites in exon1 and three in exon2 were detected (Table 1) in which ten of them (at 945, 950, 3354, 3533, 3649, 3696, 3808, 3811, 3963 and 3989 loci) were *transition* mutations and the remaining five were *transversion* mutations. These polymorphic regions generated 31 haplotypes in which four them (linkage combination at 3649, 3808, 3963 and 3989 loci) showed highest linkage disequilibrium accumulations.

Twelve polymorphic sites were observed in more than 1% of the sequences. Moreover, from the usable nucleotides, in both Gondar and Woyto Guji goat populations, the GC contents were 64.09% for exon1 and 62.47% for exon2. In line with this, the codon bias index was 0.301. Codon observed in five polymorphic sites (g.3354A>G, g.3696C>T, g.3783T>A, g.3927C>G and g.4023A>C) were degenerative codons and codons at five polymorphic sites have small number of observations and excluded from the association analysis though they qualified the SNP definition. Hence, only four loci (g.950T>C; g.3416, g.3811 C>T and g.3963T>C) were remained for the association analysis. From these four screened SNPs 10 genotypes were obtained (Table 2). These are TT and TC genotypes at g.950T>C, CC and GC at g.3416G>C, CC, TC and CC genotypes g.3811C>T and TT, TC and CC genotypes at g.3963T>C.

Amino acid substitutions and their association to litter size

In exon1, from the five sites where complete nucleotide

Table 1. Detected SNPs and IUPAC symbols (N).

Locus (>)	Nucleotide in the reference sequence	IUPAC symbol (N)	Nucleotide substituted (N)	Nucleotide similar with the reference (N)	Total
g.0895G>C	G	-	C(239)	G(3)-	242
g.0945C>T	C	-	T(3)	C(239)	242
g.0950T>C	T	Y(6)	-	C(236)	242
g.3354A>G	A	R(1)	G(172)	-	173
g.3416G>C	G	S(8)	C	G(165)	173
g.3436T>C	T	-	C(173) [†]	-	173
g.3533A>G	A	R(1)	-	A(172)	173
g.3592C>A	C	M(173)	A[†]	-	173
g.3649G>A	G	R(51)	A(41)	G(81)	173
g.3688A>C	A	M(173)	C[†]	-	173
g.3696C>T	C	Y(1)	-	C(172)	173
g.3770G>C	G	S(1)	C	G(172)	173
g.3783T>A	T	W(2)	A	T(171)	173
g.3808G>A	G	R(53)	A(58)	G(62)	173
g.3811C>T	C	Y(22)	T(3)	C(148)	173
g.3878A>C	A	M(173)	C[†]	-	173
g.3927C>G	C	S(2)	G	C(171)	173
g.3963T>C	T	Y(38)	C(4)	T(131)	173
g.3989G>A	G	R(54)	A(39)	G(60)	173
g.4023A>C	A	M(173)	C[†]	-	173

[†]Complete substitution in all sequences of the study populations; IUPAC = International Unit for Pure and Applied Chemistry.

Table 2. Amino acid changes observed in polymorphic sites.

Locus	Amino acids and codons in the reference sequence		Amino acids and codons in sequences studied		Amino acid position	N	Remark
	Amino acid	Codon	Amino acid	Codon			
g.0895G>C	Arginine	CGG	Glycine	GGG	R0298G	3	
g.0945C>T	Tryptophan	UGG	Cysteine	UGU	W0315C	3	
g.0950T>C	Stop codon	UAG	Tryptophan	UGG	*0317W	6	**
g.3354A>G	Cysteine	UGC	Cysteine	UGU	C1118C	1	
g.3416G>C	Alanine	GCC	Glycine	GGC	A1139G	8	**
g.3436T>C	Serine	AGC	Glycine	GGC	S1146G	173	
g.3533A>G	Methionine	AUG	Threonine	ACG	M1179T	1	
g.3592C>A	Alanine	GCC	Serine	UCC	A1198S	173	
g.3649G>A	Cysteine	UGU	Arginine	CGU	C1217R	94	
g.3688A>C	Stop codon	UAG	Glutamic acid	GAG	*1230E	173	
g.3696C>T	Threonine	ACA	Threonine	ACG	T1232T	1	
g.3770G>C	Serine	UCG	Tryptophan	UGG	S1257W	1	
g.3783T>A	Proline	CCA	Proline	CCU	P1261P	2	
g.3808G>A	Histidine	CAC	Tyrosine	UAC	H1270Y	111	
g.3811C>T	Glutamic acid	GAA	Lysine	AAA	E1271K	25	**
g.3878A>C	Valine	GUG	Glycine	GGG	V1293G	173	
g.3927C>G	Threonine	ACG	Threonine	ACC	T1309T	2	
g.3963T>C	Isoleucine	AUA	Methionine	AUG	I1321M	42	**
g.3989G>A	Serine	UCA	Leucine	UUA	S1330L	93	
g.4023A>C	Valine	GUU	Valine	GUG	V1341V	173	

N = number of individuals the mutations observed at the respective locus; **= contributed for litter size.

Table 3. Least square mean standard error (LSM±SE) estimation of fecundity trait.

Exon1			Exon2		
Factors	**N**	**LSM±SE**	**Factors**	**N**	**LSM±SE**
Overall mean	177	1.403±0.12	**Overall mean**	242	1.435±0.09
Parity		***	**Parity**		***
1	40	1.307± 0.14[c]	1	50	1.219±0.10[e]
2	34	1.328± 0.15[c]	2	47	1.300±0.11[d]
3	39	1.614±0.14[a]	3	47	1.696±0.11[a]
4	32	1.448±0.14[b]	4	49	1.537±0.11[b]
5	32	1.319±0.14[c]	5	49	1.425±0.11[c]
Population		***	**Population**		***
Gondar	115	1.681±0.13[a]	Gondar	111	1.688±0.10[a]
Woyto Guji	62	1.126±0.13[b]	Woyto Guji	131	1.182±0.09[b]
Genotype at g.3416G>C		***	**Genotype at g.950T>C**		***
GG	169	1.284±0.09[b]	TT	236	1.349±0.03[b]
GC	8	1.523±0.19[a]	TC	6	1.520±0.18[a]
Genotype at g.3811C>T		***			
CC	151	1.539±0.12[a]			
CT	22	1.355±0.17[b]			
TT	4	1.316±0.25[c]			
Genotype at g.3963T>C		***			
TT	134	1.350±0.14[b]			
TC	38	1.581±0.14[a]			
CC	5	1.279±0.23[c]			

substitution detected, the following amino acid changes were observed on sequences of the populations studied with respect to the reference sequence: Serine to proline at locus g.3436T>C, isoleucine to leucine at locus g.3688A>C, histidine to proline at locus g.3878A>C and glutamine to histidine (Table 2). However, no amino acid changes were observed at loci g.945C>T, g.3354A>G, g.3592C>A, g.3696C>T, g.3783T>A and g.3963T>C because of the degenerative codons (Table 2).

Similarly, the remaining mutations at g.895G>C, g.3533A>G, g.3649G>A, g.3770G>C, g.3808G>A, g.3927C>G and g.3989G>A did not contribute for the fitness of the model suggesting these mutations do not directly influence the litter size though they cause amino acid changes. Therefore, mutations only at g.950T>C, g.3416G>C, g.3811C>T and g.3963T>C were considered for the association study (Table 3). Hence, the analysis of least square mean and standard error (LSM±SE) revealed that there was a 24% increment of litter size in GC genotype individual animals than GG genotypes at locus g.3416G>C in the goat population studied.

Similarly, 18% (among TT and TC genotypes), 22% (among CC and TT genotypes) and 31% (among TC and CC genotypes) litter size differences were observed at loci g.950T>C, g.3811C>T and g.3963T>C, respectively on the animals studied. Genotype TC of locus g.3963T>C is the highest contributor; whereas genotype CC of the same locus is the least contributor.

On the same analysis, highest (LSM±SE =1.614±0.4) and lowest (LSM±SE =1.307±0.14) litter size were obtained in the 3rd and 1st parties in both exons studied, respectively. Similarly, higher estimate of litter size was observed in Gondar goat (LSM±SE=1.681±0.13) than Woyto Guji goat population (LSM±SE =1.126±0.13).

DISCUSSION

The *transition* to *tranversion* ratio, in both exon1 and exon2 of the present study, was 2:1. In former report, G to C *transversion* at site 296, G to T *transversion* at site 454, T to A *transversion* at site 505, G to A *transition* at site 3433 and C to A *transversion* at site 3688 in goats were reported (Cao et al., 2010). Similarly, 2.44:1 SNPs *transition* to *transversion* ratio was detected in the genome wide sequences analysis of three Moroccan goat populations (Benjelloun et al., 2015). *Transition* type substitutions may occur more frequently than *transversion* type substitutions (Kimura, 1980). Contrary to the current study, Feng et al. (2009) and Cao et al. (2010) could not find mutations/base variations in exon2.

Most of the identified SNPs in the current study

qualified the SNP definition. According to Brookes (1999), the bi-allelic form of variation at a specific location in the genome could be a SNP if it is found in more than 1% of the population. Brookes (*ibid*) also stated that SNPs are abundant forms of genome variation, distinguished from rare variation by a requirement for the least abundant allele to have a frequency of 1% or more population. However, in rare cases, there are tri- or tetra-allelic forms for SNPs at a specific location (Kim and Misra, 2007) though this case was not observed in both target regions of the KISS1 gene in the current study.

In the current study, only three polymorphic sites from the total twenty mutations were observed at exon2. However, Feng et al. (2009) and Cao et al. (2010) did not detect any mutation at exon2 in goat. Cao et al. (2010) reported six polymorphisms in the KISS1 gene (G296C, G454T and T505A in intron1; G3433A and C3688A in exon 3 and a 18 bp deletion/insertion in 1960 to 1977 site in intron2) in five Chinese goat breeds. In another study, ten polymorphisms were reported in KISS1 gene of three Chinese goat populations (g.1147T>C, g.1417G>A, g.1428_1429delG, g.2124C>T, g.2270C>T, g.2489T>C, g.2510G>A, g.2540C>T, g.3864_3865delCA and g.3885_3886insACCCC) (An et al., 2013).

In the current study, highest percentages of GC contents in both exon regions (64.09% for exon1 and 62.47% for exon2) were obtained indicating important influence of the codons in the target gene expression (Bernardi et al., 1985; Ikemura, 1985). The CBI (CBI=0.301) estimate shows presence of considerable natural selection pressure in the populations studied (Sharp and Li, 1987). Natural selection favors higher expression and enhanced codon usage optimization in short genes (Fox and Erill, 2010), like KISS1 gene.

In the current study, fours SNPs amongst the detected polymorphic loci in both exons of the KISS1 gene affected litter size of the goats studied (Table 3). However, in the analysis of molecular variance, the relative lower estimate of the fitness model (R^2 ~35%) (Supplementary Table S2) might be because of the fact that multiple birth can be influenced by other genes, like GDF9, BMPR1B, BMP15 genes and also controlled by growth hormones. Growth hormone (GH) of mammals plays an important role in involving cell division, ovarian folliculogenesis, oogenesis and secretory activity (Hull and Harvey, 2002; Ola et al., 2008). By acting through specific receptors within the ovary, GH is expedient in controlling proliferation and apoptosis, oocyte maturation, and the expression and synthesis of receptors to hormones and related substances (Hull and Harvey, 2000; Sirotkin et al., 2003). Silva et al. (2009) also stated that the effect of GH on ovarian function is mainly through inducing the development of small antral follicles in the gonadotrophin-dependent stages and stimulating oocyte maturation. On the other hand, in the absence of the genotypes indicated in the model, it (R^2) was fitted only to 20%. A 15% increment of the fitness model, only by the genotypes observed in the KISS1 gene, implies the significant contribution of the candidate gene on multiple births compared to other genes, growth hormones and other phenotypic fixed effects. This is due to the fact that kisspeptin together with its receptor (GPR54) stimulates the release of LH and FSH in female goats, it is a key regulator and catalyst for the puberty onset, and it is a fundamental gatekeeper of sexual maturation in mammals (Hashizume et al., 2010; Cao et al., 2010 and 2011; Chu et al., 2012).

As a result, the genotypes identified on the coding regions of the KISS1 gene have shown remarkably significant ($P<0.001$) contribution (18 to 31% increment of litter size) on fecundity trait. However, this result is far lower than the finding reported for the CC genotype of Jining Grey goat does (litter size difference estimated to be 0.80 at locus 296) (Cao et al., 2010). In addition, the authors reported that G3433A caused one amino acid change (Ala, A, GCC) to (Thr, T, ACC) at residue 86 (A86T) though non-significant influence of the genotypes (CC, CA, AA) was observed at locus 3688. Complete substitution of A>C (monomorphism) was detected at the latter locus in the current study. In other study, significant ($P < 0.05$) effect of litter size was reported at T2643C locus in goats (Hou et al., 2011). In sheep, KISS1 mRNA expressing cells are found in the arcuate nucleus (ARC) and dorsallateral preoptic area and both appear to mediate the positive feedback effect of estradiol to generate the preovulatory GnRH/LH surge (Smith et al., 2011). The luteinizing hormone (LH) surge has been associated with an increase in the LH response to kisspeptin in humans and sheep (Dhillo et al., 2007; Smith et al., 2009), indicating the surge may be generated by increased kisspeptin output and sensitivity. These all findings indicate that KISS1 gene is an excellent candidate gene for reproductive traits in human and livestock (An et al., 2013).

It is also possible to deduce that, in the ANOVA table (Supplementary Table S2), population as source of variation took the highest share of variation among the fitness model suggesting the genotype component plays a vital role in regulating the reproductive cycles of female animals. Of course, joint consideration of multiple traits can provide additional information compared to information contained in individual traits as suggested by Pei et al. (2009).

Conclusion

In this study, there were 20 mutations, 15 of them were polymorphic, which detected at the two exon regions of the KISS1 gene. Among the polymorphic sites which caused amino acid changes, four of them have significant contribution to litter size of the goat populations studied

confirming relevance of the KISS1 gene for fecundity trait.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Supplementary Table S1. Primers designed for analysis of KISS1 gene.

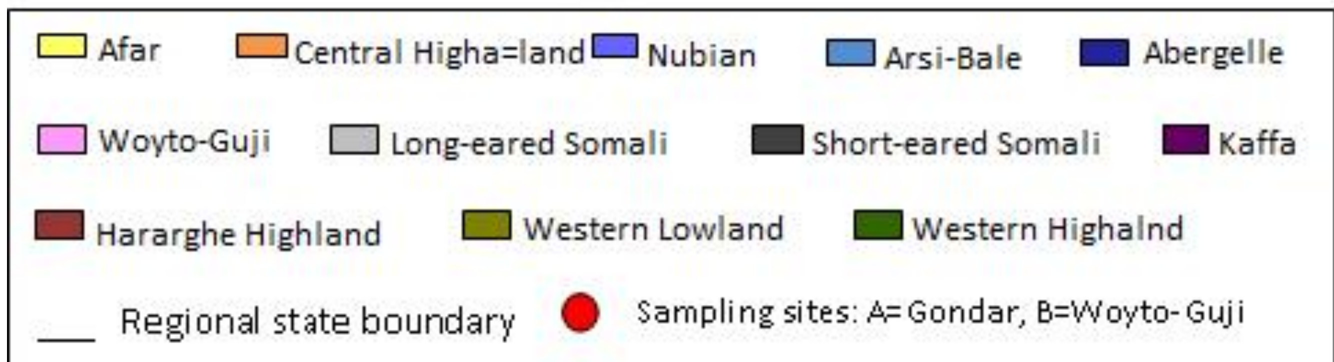
Region	Name given	Sequence	L	GC%	T_m
Exon1-Region1	CH_KISS1_Exon1_F2	5'-TTATGTCACTGCAGCTGG-3'	18	50.0	52.3
	CH_KISS1_Exon1_R1	5'- CTTGCTACTCACTGGCTG -3'	18	55.6	52.9
Exon1-Region2	CH_KISS1_Exon1_F1	5'- AGCGCTGAGCTTCCTAG -3'	17	58.8	54.3
	CH_KISS1_Exon1_R3	5'-GGCAATGGTCAGCATCATC-3'	19	52.6	54.1
Exon2	Chi_KISS1_Exon2_F	5'-CACTGTCCCACTGCATCTC-3'	19	57.9	55.5
	Chi_KISS1_Exon2_R	5'-GTAACGGCAGAAGAGCCTC-3'	19	57.9	55.5

L = length (bp).

Supplementary Table S2. Analysis of variance of exon1 and exon2 regions of KISS1 gene.

Exon1					Exon2				
Source	**Df**	**Type III SS**	**M. Square**	**F Val.**	**Source**	**Df**	**Type III SS**	**M.Square**	**F Val.**
Parity	4	2.46961285	0.61740321	2.93**	Parity	4	6.83085662	1.70771415	8.85***
Population	1	10.60811916	10.60811916	50.34***	Population	1	14.80968402	14.80968402	76.79***
Genotype at G3416C	1	0.41633801	0.41633801	1.98**	Genotype at T950C	1	0.16954025	0.16954025	0.88**
Genotype at C3811T	2	0.38815068	0.19407534	0.92 *					
Genotype at T3963C	2	0.78901259	0.39450630	1.87**					
Error	166	34.98043349	0.21072550			235	45.32326482	0.19286496	
Total	176	52.28248588				241	69.55371901		

R2 = 0.331; CV= 34.87%; ** = P<0.01; ***=P<0.001; R2 =35%; CV = 33.01%.



Supplementary Figure S1. Geographical distribution of goat breeds of Ethiopia. Source: Based on FARM-Africa (1996).

a. Exon2

PCR program:

- $95^o{}_C$ 5 minutes
- $94^o{}_C$ 15 seconds ⎤
- $64^o{}_C$ - $60^o{}_C$ 30 seconds ⎬ 5 cycle
- $72\,^o{}_C$ 45 seconds ⎦
- $94^o{}_C$ 15 seconds ⎤
- $58^o{}_C$ 45 seconds ⎬ 35 cycle
- $72^o{}_C$ 1 minute ⎦
- $72^o{}_C$ 10 minutes
- $4^o{}_C$ ∞

PCR reaction:

Primer (10pM/ µl) F	0.4µl
Primer (10pM/ µl) R	0.4µl
BSA	1.0µl
Hi-Di	0.5µl
H_2O (Nuclease free)	17.3µl
Template	0.4µl

b. Exon1_R1(exon1 region1)

PCR program:

- $95^O{}_C$ 5 minutes
- $94^O{}_C$ 15 minutes ⎤
- $62\text{-}58^O{}_C$ 30 seconds ⎬ 5 cycles
- $72\,^O{}_C$ 45 seconds ⎦
- $94^O{}_C$ 15 seconds ⎤
- $56^O{}_C$ 45 seconds ⎬ 5 cycles
- $72^O{}_C$ 1 minute ⎦
- $72^O{}_C$ 10 minutes
- $4^O{}_C$ ∞

PCR reaction:

•Primer (10pM/µl)F	0.4µl
•Primer (10pM/µl)R	0.4µl
•BSA (100X:10mg/ml)	2.0µl
•Hi-Di	0.5µl
•H_2O (Nuclease free)	16.3µl
•Template DNA	0.4µl

c. Exon1_R2 (exon1 region2)

PCR program:

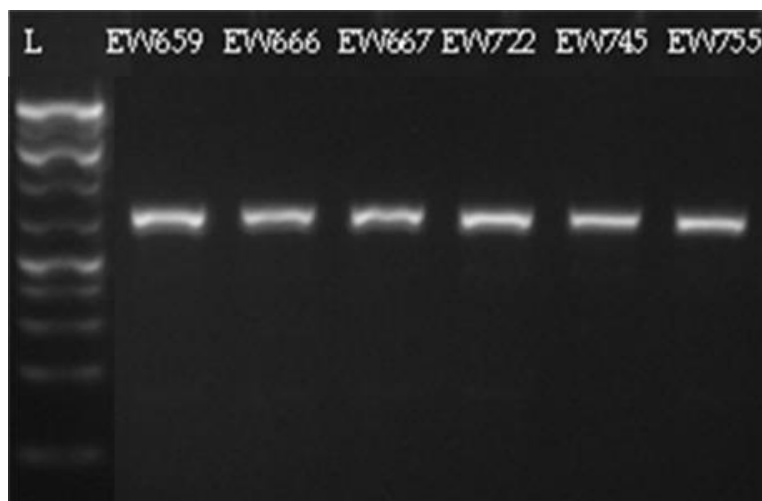
- $95^O{}_C$ 5 minutes
- $94^O{}_C$ 15 minutes ⎤
- $62\text{-}58^O{}_C$ 30 seconds ⎬ 5 cycles
- $72\,^O{}_C$ 1.0 minute ⎦
- $94^O{}_C$ 15 seconds ⎤
- $56^O{}_C$ 1.0 minute ⎬ 5 cycles
- $72^O{}_C$ 1.0 minute ⎦
- $72^O{}_C$ 10 minutes
- $4^O{}_C$ ∞

PCR reaction:

•Primer (10pM/µl)F	0.4µl
•Primer (10pM/µl)R	0.4µl
•BSA (100X:10mg/ml)	2.0µl
•Hi-Di	0.5µl
•H_2O (Nuclease free)	16.1µl
•Template DNA	0.6µl

Exon1_R2

Supplementary Figure S2. PCR conditions for exon1 and 2 regions of KISS1 gene amplification.



Supplementary Figure S3. Non purified PCR product gel image of exon2 of KISS1 gene.