J. Agr. Sci. Tech. (2014) Vol. 16: 79-90

Genetic Characterization, Polymorphism and Evolution Analysis in Part of the *EDNRB* Gene in Cattle and Goat

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ABSTRACT

The aim of this study was to analyze the polymorphisms existing in the 5´ flanking region, exonic, and some parts of the intronic regions as well as methylation analysis of the *EDNRB* gene in cattle and goat. These regions were sequenced in three different breeds of cattle including Sistani, Golpayegani, and Holstein and were compared with that of Marghoz goat. The results identified that this gene encodes 441 amino acid residues in cattle and goat with highly conserved domains between them. A total of 55 transition and 43 transversion mutations were observed in the 5´ and 3´ flanking and exonic regions. Among the six coding mutations in goat *EDNRB* gene, a missense mutation of C236>T resulted in substitution of Alanine-79 to Valine. The frequency of the T allele in the goat population was 0.77. In addition, an insertion of $TG_{(29)}$ repeat was identified at the -773 upstream of the gene in goat samples. The promoter region of the gene harbors two GC rich conserved motifs and transcription factor binding sites for GATA and SP1 in both species, which may modulate the expression of the gene. The methylation analysis of a 182 bp fragment of the 5´ region of the *EDNRB* gene, located at the fringe of the CpG island, was found to be completely methylated in both cattle and goat. Evolutionary analysis revealed that the cattle and goat EDNRB proteins were sisters in their own clade and d_N / d_S ratio demonstrated that the *EDNRB* gene underwent purifying selection during evolution.

Keywords: Methylation, Mutation, Promoter, Selection.

INTRODUCTION

Endothelins are a family of 21 amino acid peptides that act on G protein-coupled heptahelical receptors and are thought to function as local hormones (Baynash *et al.,* 1994; Fuchs *et al.,* 2001). These proteins are involved in many diverse physiological and developmental processes (Shah, 2007). They act as signal transducers, transmitting the signal in the cell, and modulating different cellular regulators via two distinct high affinity endothelin receptor (EDNR) subtypes, endothelin receptor A (EDNRA) and endothelin receptor B (EDNRB). In mammals, activation of the EDNRA is associated with pronounced vasoconstriction whereas EDNRB vocation is linked to vasodilation (Pollack *et al.,* 1995). EDNRB accepts all three isopeptides of endothelines (ET-1, ET-2, and ET-3) equally (Baynash *et al.,* 1994). Subsequent analysis has identified roles for EDNBR signalling in the proliferation and differentiation of neural crest–derived cells including neuroendocrine, melanocytes and their precursors and in promoting the dendricity of fully differentiated melanocytes (Parichy *et al*., 2000). The main role in the application of the coat color pattern, formed by neural crest–derived pigment cells, in parentage test, trademark and economical purpose in different breeds, motivated scientists to find molecular marker recognized by coat color. Therefore, many genetic studies have been started to

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[DOR: 20.1001.1.16807073.2014.16.1.19.3]

characterize and identify genes required for the pigment cell development and pigment pattern formation in mammals. Currently, as many as eleven loci have been postulated to be involved in coat color variation in domestic horse breeds (Bellone, 2010). In Holstein cattle, polymorphisms of melanocyte-stimulating hormone receptor (*MSHR*) gene were identified to be related to red coat color (Joerg *et al.,* 1996). The *EDNBR* and *Kit* genes have been excluded as candidate genes for white spotting in Border collies (Metallinos and Rine, 2000). The *EDNBR* gene was considered as a candidate for coat color in some other species (e.g. mice, rats) (Steingrimsson *et al.,* 2006). Tyrosine-related protein 1 (TYRP1) and melanocortin 1 receptor (*MC1R*) genes excluded as taupe brown color of Brown Swiss (Berryere *et al.,* 2003) and as brown or red coat color in Japanese and Korean cattle (Sasazaki *et al.,* 2005) or Holstein (Dreger and Schmutz, 2009), respectively. Accordingly, survey of the identified genes associated with coat color development indicates that three loci have been determined molecularly that are essential for pigment synthesis, melanocyte survival, migration and proliferation (Van Raamsdonk *et al.,* 2004). Among them, the mutations of *EDNRB* affect the development of two neural crest-derived cell types, melanocytes and enteric neurons and resulted in produce congenital patches of white hair and skin (Bennett and Lamoreux, 2003). Therefore, it might be a potential candidate gene associated with coat color pattern in mammals.

On the other hand, the promoter region of the *EDNRB* gene has an important conserved sequence to act as transcriptional binding sites in which the transcriptional activity of the gene is regulated and modification of these sites may abolish the expression of the gene (Zhu *et al.,* 2004). For instance, Sox10 regulates the expression of the *EDNRB* gene in human melanocyte-linage cells in coordination with a transcription factor Sp1 which enhances the degree of its transactivation (Yokoyama *et al.,* 2006), but no report could be found on possible transcriptional sites of the *EDNRB* in animals and likely involvement in the regulation of the gene.

Furthermore, DNA methylation is required for normal development of animals. DNA methylation has been implicated in transcriptional repression and generally occurs at CpG dinucleotides (Pao *et al.,* 2001). However, CpG islands of the promoters are usually not methylated, but contradictory reports could be observed on this hypothesis. Some authors have reported that the change of methylation intensity of CpG islands at promoter site is negatively associated with gene expression level (Matsushima *et al.,* 2002; Avencia *et al.,* 2010), whereas others have observed no obvious correlation between CpG islands methylation and gene expression (Fan and Zhang, 2009). Therefore, to understand the involvement and function of CpG islands, as an important element in modulating the expression of the gene, methylation analysis of these sites in the promoter regions of the *EDNRB* gene should to be considered. In spite of some reports on structural study of the *EDNRB* gene in animals or human, its promoter region, which regulates the expression of the gene in cattle and overall structure of the gene in goat, remain to be elucidated. The current study was conducted to analyze the genetic structure and polymorphism of the *EDNRB* gene in some breeds of cattle, with different coat pattern, including *Bos taurus* (Golpayegani and Holstein), *Bos indicus* (Sistani) and compare with that of in *Capra hircus* (Marghoz goat). Dominant coat pattern of Holstein, Golpayegani, and Sistani breeds of cattle are black and white, brown, and black, respectively, and Marghoz goat shows a typical white coat color (Tavakolian, 1999). The information provides an insight into understanding of the structure of this gene and its likely association with different coat color patterning in these breeds of animals in addition to application in evolutionary analysis of the *EDNRB* gene among them.

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MATERIALS AND METHODS

DNA Extraction and Primer Design

Genomic DNA of the animals was extracted using standard phenol-chloroform method. In order to design *the* gene specific primers in cattle and goat, *in silico* search for *EDNRB* gene sequences revealed genomic sequence of the gene (NC_007310) in cattle and some parts in goat (EU644500). According to these available DNA fragments, ten sets of primers were designed to amplify *EDNRB* gene in cattle and goat (Figure 1). Some important parameters and efficiencies of the primers were evaluated by Oligo software version 5.0. Nucleotide sequences of the primers and PCR conditions are outlined in Table 1.

PCR Amplification and Genomic DNA Sequence Analysis of the *EDNRB* Gene in Cattle and Goat

PCR amplification was carried out in a 10 µL reaction mixture containing 20 ng DNA, 0.2 µM each oligonucleotide primers, 0.2 mM each of dNTPs, 1.5 mM $MgCl_2$, 1X PCR buffer and 0.25 unit of *Taq* polymerase (Amersham Bioscience). After the initial denaturation at 94°C for 5 minutes, 35 cycles of amplification were performed each consisting of denaturation for 30 seconds at 94°C, annealing for 30 s at 55–60°C, extension for 60 seconds at 72°C followed by a final elongation step of 7 minutes at 72°C. The resulting PCR products were resolved by gel electrophoresis, bands were purified using phenol- chloroform, subcloned into pGEM®-T easy vector (promega) and transformed into *E. coli* (Jm109). From every sample, 4 clones were eluted and sequenced in both directions employing the dideoxy chain termination method using a BigDye terminator cycle sequencing kit. The sequencing analysis was performed on an automated DNA sequencer (ABI PRISM ™ Genetic Analyzer model 310). The nucleotide sequences were confirmed using their PCR products as template for direct sequencing. The sequences and deduced amino acid sequence were analyzed using MEGA software version 4.0 (Tamura *et al.,* 2007).

Promoter Sequence Analysis of the *EDNRB* Gene

Identification of the putative transcription factor binding sites of the gene was performed using TESS software (Schung and Overton, 1997). About 1 kb upstream of the transcription start site (TSS) of the *EDNRB* gene was used for *in silico* promoter analysis. CpG islands on the sequence of the gene was detected by EMBOSS CpGPlot (http://www.ebi.ac.uk/Tools/emboss/cpgplot/index.html).

Bisulfite Treatment of the DNAs and Methylation Specific Polymerase Chain Reaction (MSP) Analysis

Sodium bisulfite treatment of 1 µg of DNAs of skin tissues was performed as described previously (Olek *et al.,* 1996) with some modifications. In brief, DNA was denatured with 3M NaOH for 30 min at 37°C. Then, the DNA was treated with a

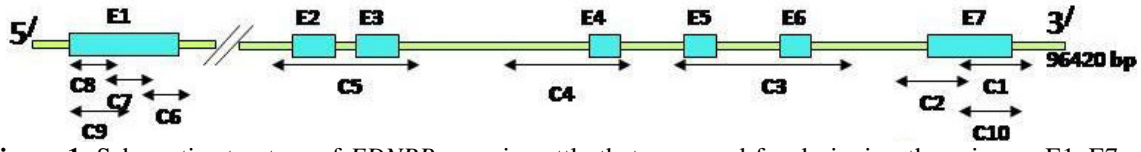

Figure 1. Schematic structure of *EDNRB* gene in cattle that was used for designing the primers. E1–E7 represent the exonic regions, C1–C10 show the sequenced fragments and

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Table 1. Characteristics and sequences of the primers.

Primers	Primer sequence (5' - 3')	Tm [a] (°C)	PCR product length (bp [b])
Primers for cloning and sequencing			
C1F	*TTG GAG GAA AAG CAG TCG TGC*	59	634
C1R	*AGT CTG TCT GAT TCT CCC TCC*		
C2F	*GGG TCT GTA GCA TCT CTT TG*	59	597
C2R	*ATT ACT GGA ACG GAA GTT GTC*		
C3F	*AGT CTG ATT GTG GTT TTA TTT*	65	1365
C3R	*ATG GAA GCA GGC AAA TGA GG*		
C4F	*CGA ACC CGC ATC TCT TAT GTC*	63	844
C4R	*AAA ATA TTT CTA TTT CCT TAC*		
C5F	*CAC AAG TTC ATT CAC CTC CAC*	59	945
C5R	*TCT ACC AGT CTG TCC ATA CGA*		
C6F	*TTC AGG ATA GCG GCT TGC AGG*	63	633
C6R	*GAT CCT AGC AGG CAC CCC TTA*		
C7F	*GGA AGG GGT CTG AAA GTC CAG*	69	470
C7R	*ACC AGC AAG GGA AGG AAG ACA*		
C8F	*ACA CTG TCA GGC ATT CCC TCG*	69	512
C8R	*CTC TGC TTT CGA CTC CAC AGC*		
C9F [c]	*TTT CGT CAG AGC CAG ACC CTC*	68	542
C9R	*GGG CAA ATC AAG GCA GGT TAG*		
C10F [c]	*TCA TGT TTA TGC TGC TGG TGC*	60	428
C10R	*AAG TGG TGT ATG AAT AGA CCG*		
Primers for MSP analysis			
F-MSP	*ATTTTGTTTTATTGTGA*-3	45	182
R-MSP	*AACACAACAACACCCAC*-3		
Primers for SNP and repeated nucleotide analysis			
F-Fau1	*ATGATGGAGACCCCGACTGAG*	60	318
R-Fau1	*GATAATGTGCAGCAGGTCTCC*		
Fms	*TTATCCTGGGGTTTCAGTTTG*	61	149-161
Rms	*ACTTCAAACGCTCAAGCCCTC*		

[a] Melting temperature; [b] Base pair, [c] These primers were only used to amplify the gene fragments in goat.

solution of 3M sodium bisulfate and 0.5 mM hydroquinone (sigma) at pH 5.0 by overnight incubation at 50°C in the dark. The modified DNA was desalted using the DNA Clean-Up column (Promega) according to the manufacturer's instructions. Primers for amplification of the putative DMR region of *the EDNRB gene* were designed by the MethPrimer (http://www.urogene.org/methprimer/) (Table 1). PCR amplification of the bisulfate modified DNA was carried out in a final volume of 25 µL PCR mix as stated above.

Identification of SNPs and Evolutionary Analysis

Identification of SNPs and insertion-deletion (Indels) were carried out by sequencing of three individuals of each breed and comparing the data with BLAST search against the gene sequences at NCBI. The frequency of the alleles at di nucleotide repeat $TG_{(29)}$-773 and C236> T SNP loci were evaluated by the application of suitable primers (Table 1) and amplification of these loci in Marghoz goat (n= 106) followed by silver staining visualization of the bands or

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digestion by *FauI* restriction enzyme, respectively.

The amino acid sequences of the EDNRB for animals used in evolutionary analysis were obtained from NCBI. The accession numbers of the sequences for animals were as follows: Mouse NP_031930.1, Human NP_000106.1, Dog NP_001010943.2, Horse NP_001075306.1, Pig NP_001033091.1, Chicken NP_001001127.1, Rat NP_059029.1, Quail BAF42697.1, Fish NP_001092915 and Rabbit CBL55500. Alignment of the *EDNRB* amino acid orthologs from a variety of species was accomplished using MEGA software and employed to generate a phylogenetic analysis. Phylogenetic and molecular evolutionary analyses were also conducted using MEGA 4.0 software. Polygenic trees were obtained using Neighbor-Joining method (Saitou and Nei, 1987). Bootstrap values were obtained that are the average of 100 randomized re-samplings. A maximum likelihood approach (PAML) was used to examine d_N/d_S ratios to the mammalian lineages. The codon-based likelihood method of Yang allows for analysis of linage specific d_N/d_S ratio (Goldman and Yang, 1994).

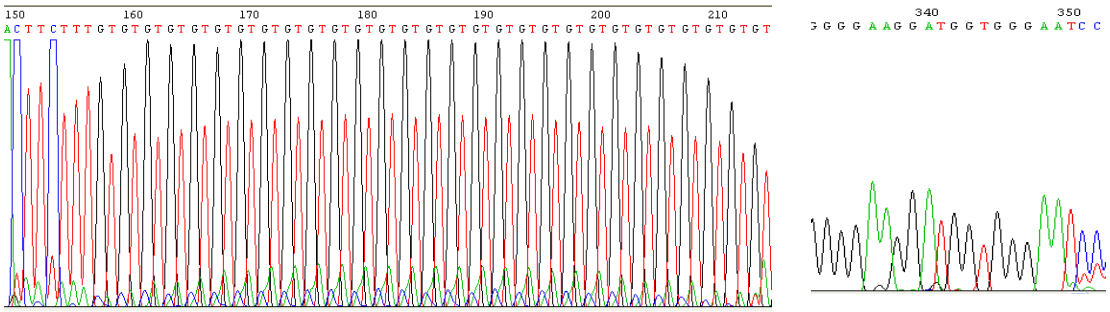
RESULTS

In the present study, more than 5 kb of the *EDNRB* gene was cloned and sequenced as described in the previous section. These nucleotides cover the entire coding regions and some parts of the 5´ and 3´-UTRs (untranslated regions) of the gene. The nucleotide sequence data reported in this paper appears in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB609187.

The coding region of the sequences was translated and the deduced amino acid was determined. The *EDNRB* gene consisted of seven exons, six introns, and 441 amino acid residues which could constitute a protein of 49,371 Da. Comparison of the *EDNRB* completed sequence revealed no differences between the cattle breeds and showed high similarity between the cattle breeds and goat (84.2%). Coding region sequences similarity in both species showed high homology with 100% identity in cattle breeds and 98.86% identity in goat. Sequence alignment of goat and cattle *EDNRB* gene revealed a lot of Indel and single nucleotide polymorphisms (SNP) in the coding or non-coding regions. A total of 55 transitions with 21 C to T and 34 G to A mutations, and 43 transvertions with 29 G to T or C and 14 A to T or C mutations was observed in the 5' and 3' flanking and exonic regions.

Analysis of the $TG_{(29)}$ dinucleotide repeats (Figure 2-a) indicated that the alleles ranged from 149 to 161 bp, observed and expected heterozygosity were 0.05 and 0.74, PIC and observed and effective alleles were 0.69, 6 and 3.8, respectively. The frequency of the dominant (T) allele at C236> T locus (Figure 2-b) was 0.77 in goat population.

A search for consensus sequences of



(a) (b)

Figure 2. (a) Chromatograph of (TG_{29}) repeated in the promoter and (b) Chromatograph of transition of GCG236> GTG locus in Goat *EDNRB* gene.

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TATA and/or CAAT box at the promoter region of the gene revealed no such sequences for *EDNRB* gene in cattle and goat. EMBOSS CpGPlot detected two 250-330 bp-long CpG islands in *EDNRB* gene of the species. The MSP analysis of a 182 bp fragment of the 5´ region of the *EDNRB* gene, located at the fringe of the CpG island, was found to be completely methylated in both cattle and goat (Figure 4).

Figure 4. An example of 182 bp methylation specific polymerase chain reaction for the EDNRB promoter region using skin tissue in cattle and goat. 1 and 3 correspond to methylated, 2 and 4 correspond to unmethylated reactions in cattle and goat, respectively.

Following a search for possible transcription factor binding domains, several putative sites were identified in the promoter region of the gene, but only SRY, GATA and SP1 are shown (Figure 3). All the detected sites showed minimum 75% core similarity match and more than 90% matrix similarity match. The SRY transcription factor sites were observed just upstream of the first CpG island region of the cattle *EDNRB* promoter (Figure 3) while none could be found in goat gene. Two GATA transcription sites were detected downstream of the gene in cattle and goat and three TF were identified upstream of TSS while one of those could not be seen in cattle gene. The SP1 transcription factors were generally observed upstream of the gene among the CpG island regions in cattle and goat promoter region.

Multi alignment of EDNRB amino acid sequences from cattle (Sistani, Golpayegani and Holstein), goat, human, mouse, horse, pig, rabbit, chicken, dog, rat, zebrafish and quail (data not shown) revealed several regions of highly conservative transmembrane domains between mammalian species. Almost all transmembrane domains structure were conserved among the species, so that the third and seventh transmembrane domains structure were entirely conserved in all animals. In an effort to determine the evolutionary relationship between members of EDNRB amino acid, a phylogenetic tree was constructed using MEGA 4.0. Fourteen different EDNRB proteins were used for phylogenetic analysis. Neighbor-Joining analysis was then used to generate a phylogenetic tree (Figure 5). High bootstrap values, shown on the branch points in Figure 5, indicate that the tree is robust. As could be seen in the evolutionary tree of the amino acid sequences, EDNRB proteins separate into the different clades reflecting their evolution. The cattle and goat EDNRB proteins are sisters in their own clade. The rabbit EDNRB protein appears as a sister–clade to the cattle EDNRB protein. Similarly, the two rodent EDNRB proteins, dog, and human EDNRB proteins form separate clades.

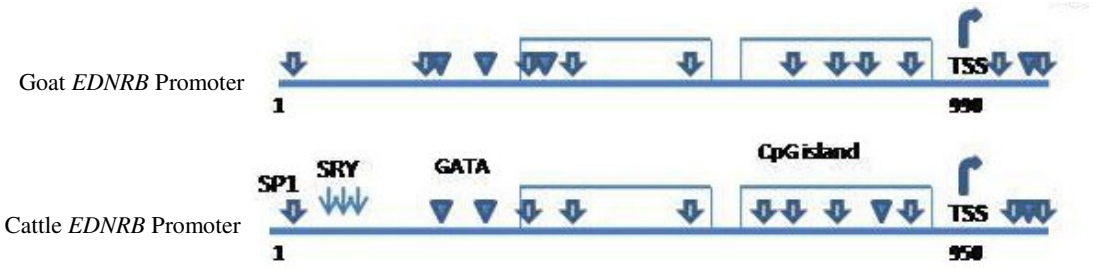

Figure 3. Schematic representation of potential transcription factors in the promoter region of *EDNRB* gene.

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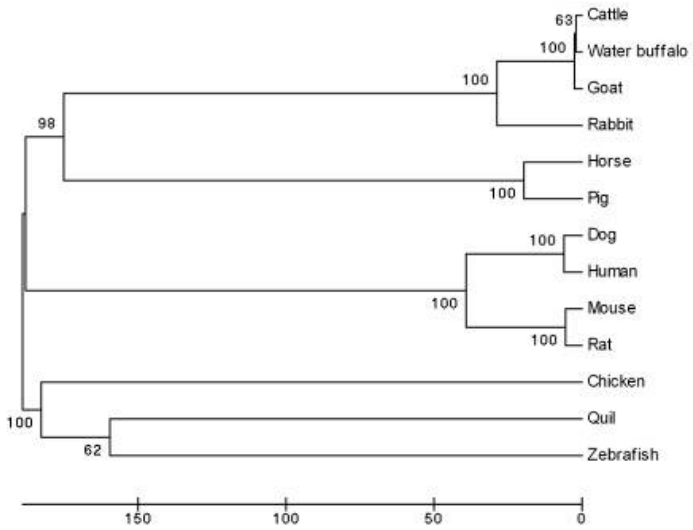

Figure 5. Phylogenetic tree of the EDNRB proteins. The tree was generated by neighbor-Joining analysis of the EDNRB amino acid similarities using MEGA 4.0. Bootstrap values above the branches indicate the degree of support for that linage branch. Values over 60 are generally indicative of good support. The units at the bottom of the tree indicate the number of substitution events.

DISCUSSION

The present study describes the structure of the main parts of the *EDNRB* gene in cattle and goat. Screening of the gene structure in animals with different coat color may be particularly useful in the identification of genetic elements concerning the gene function and expression in development of coat pattern in animals. The sequence of the *EDNRB* gene shows high conservation between two species, but the promoter region of the gene seems to be variable. However, two conserved CpG islands (Figure 3) were detected upstream of the gene in both species. The conservation of these islands is in concordance with the major role of the CpGs in the regulation of gene expression and its stability during the molecular evolution (Fan and Zhang, 2009).

DNA methylation is the major modification of mammalian genomes and is known to have an intense effect on gene expression. When a promoter is methylated, its expression will depend mainly on transcriptional factor abundance and the ability to bind to methylated recognition elements (Curradi *et al.,* 2002). As indicated in Figure 3, numerous sites of GATA and SP1 transcription factors located in the CpG island region and or downstream of the start site may modify the expression of the gene.

Occurrence of the nucleotide transitions in gene sequence in both species was higher than the nucleotide transvertions. Cytosine methylation might be the result of these alterations in most of the mammalian genomic sequences especially in the promoter regions (Vignal *et al.,* 2002).

Many Indels were observed across the sequenced fragments of the gene in cattle and goat, but four Indel were only observed at the 5' site of the sequence in goat, among them an insertion of $TG_{(29)}$ repeat was identified at the -773 nucleotide. To our knowledge, this dinucleotide repeated microsatellite marker has not been reported in other animals. Therefore, this polymorphism could be a potential breed specific marker in goat *EDNRB* gene and could be used for mapping studies or association analysis in goat populations.

To uncover whether there is a breed specific polymorphism related to coat color among the species under study, we couldn't

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find any discrepancy among the different cattle breeds at the *EDNRB* sequences level, in which, the entire coding region of the gene have been sequenced. The high identity of the protein alignment shows the conservation of the gene structure during the evolution. However, many coding variations were observed between goat and cattle breeds (Table 2), but only six mutations resulted in alteration of the amino acid residues. These residues are mostly not conserved between animal species or biochemical characteristics of the most substituted amino acids have not been changed, therefore, protein structure and function may not be affected. Only a missense mutation of C236 to T in the first exon of the gene was observed in goat *EDNRB* gene, in which introduced the Alanine-79 to the Valine at this site. The Alanine-79 residue is conserved in human, mouse, cattle, and buffalo. Polymorphism analysis of this site in goat population indicated a high frequency for the Valine-79 residue (Figure 6). The proteolytic cleavage site of the EDNRB peptide was determined

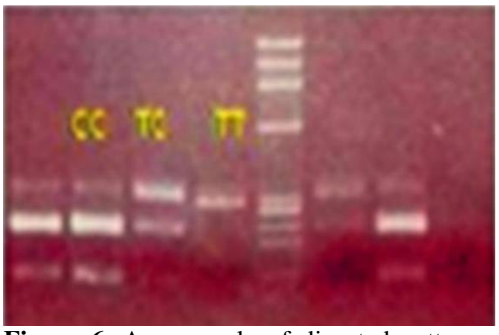

Figure 6. An example of digested pattern of EDNRB C236> T in goat population. Ladder; ΦX174-HaeII.

to be between Alanine-79 and Glycine-80 (Kozuk *et al.,* 1991), therefore, protein purification analysis in goat requires validation of the effect of this proteolytic cleavage site mutation on distribution of the dominant 52 KDa domain of the protein in population.

The protein alignments revealed that EDNRB peptide consists of seven hydrophobic domains, a typical feature of the G protein–coupled receptor family (GPCR). Occurrence of many mutations in

Table 2. Differences in the coding sequence of *EDNRB* gene in cattle and goat.

Amino acid number	Cattle sequence	Goat sequence	Result	
			Cattle	Goat
22	GCG	GCA	Silent mutation	
41	CCG	CTG	P	L
44	GGA	AGA	G	R
45	GAG	GAA	Silent mutation	
63	GAC	GAG	D	E
64	CCG	CCT	Silent mutation	
71	CAG	CAA	Silent mutation	
72	ATT	GTT	I	V
79	GCG	GTG	A	V/A
92	CCC	CCT	Silent mutation	
121	CTA	CTG	Silent mutation	
162	CTT	CTC	Silent mutation	
217	ACA	ACG	Silent mutation	
236	GCT	GCC	Silent mutation	
243	ACC	ACG	Silent mutation	
250	AAA	AAT	K	N
284	CCG	CCA	Silent mutation	
285	CTG	TTG	Silent mutation	
345	AAG	AAA	Silent mutation	
360	TTA	TTG	Silent mutation	
410	AAA	AAG	Silent mutation	

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the first transmembrane is associated with Lethal white foal syndrome (Bellone, 2010), while in the third, fifth, and seventh transmembrane they cause Hirschsprung's disease in human or piebald in mouse (Puffenberger *et al.,* 1994; Shin *et al.,* 1997). This demonstrates the importance of the conserved domains of the EDNRB protein and could be studied further.

As indicated in Figure 3, the transcription factor (TF) sites of the SRY are less conserved in the promoter of the *EDNRB* gene because none is present simultaneously in cattle and goat *EDNRB* promoter. Since, the expression of the *EDNRB* gene in human melanocyte-lineage cells is regulated by SRY (Yokoyama *et al.,* 2006) and conservation of the binding sites of the SRY seems to be crucial for the gene function (Zhu *et al.,* 2004), evaluation of the expression pattern of the *EDNRB* gene in embryonic developmental stages would be worthwhile in various tissues of the cattle and goat. This will shed a light on understanding the effective involvement of the transcription factors with the expression pattern of the gene in other animals. However, it should be noted that the transcription factors of the current study identified by *in silico* differ from others (Zhu *et al.,* 2004), which may be due to the evolution of the transcription factor concept and involvement of a simple common regulation of the gene.

To determine whether this gene has been under positive or negative selection during evolution, the d_N/d_S ratio was determined for the mammalian lineages. This ratio is defined as the number of non-synonymous nucleotide changes (those that change amino acid sequence) per non-synonymous site (d_N) divided by the number of synonymous nucleotide change (those that do not change amino acid sequence) per synonymous site (d_S). A d_N/d_S ratio equal to 1.0 provides evidence of neutral evolution, while d_N/d_S ratio> 1.0 indicates positive Darwinian selection and d_N/d_S ratio< 1.0 is indicative of purifying selection (Li, 1997).

The analysis revealed that among all mammalian lineages, the d_N/d_S ratio averages 0.435. This is evidence for purifying selection against amino acid changes. The high degree of similarity of major parts of the protein in mammals, fowl, rodents, human, and canis reveals that the structure of EDNRB has changed little during decades of evolution (Soufir *et al.,* 2005). Phylogenetic analysis of the EDNRB proteins identified three clades for linage of these sequences between animals. The cattle and goat sequences, in close relationship with that of rabbit, is located in one branch and due to important function of the protein show a conservation of the main parts of the sequence during evolution. However, the other mammals' sequences of the EDNRB protein are arranged in separate clades but, mainly because of the highly similarity between sequences, the distance of the sequences is close together.

CONCLUSIONS

Our study on sequence analysis of the *EDNRB* gene in different cattle breeds and goat offers wide molecular information of the gene. Furthermore, this research paves the way for considering the promoter region as a variable region of the gene and applying the identified SNPs in future breeding strategies and animal identification, especially in goat populations.

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بررسی خصوصیات ژنتیکی، چند شکلی و تکاملی بخشی از ژن *EDNRB* در گاو و بز

ع. ا. مسعودی

چکیده

هدف از تحقیق حاضر بررسی چند شکلی در نواحی بالادست (′۵)، اگزونی و بخشی از ناحیه اینترونی و همچنین آنالیز متیلاسیون ژن *EDNRB* در گاو و بز بود. این نواحی در سه نژاد گاو شامل سیستانی، گلپایگانی و هلشتاین و بز مرخز تعیین توالی شدند. نتایج نشان داد که این ژن کد کننده ۴۴۱ اسید آمینه در گاو و بز بوده که دارای بخشهای حفاظت شده بالای پروتئینی هستند. در مجموع ۵۵ تغییر جابجائی نوکلئوتیدی و ۴۳ تغییر جایگزینی نوکلئوتیدی در نواحی بالادست، پائین دست و اگزونی ژن مشاهده شد. در میان ۶ تغییر نوکلئوتیدی در ژن *EDNRB* بز یک تغییر جابجائی نوکلئوتیدی T<236C باعث جایگزینی اسید آمینه آلانین-۷۹ به والین می شود، آنالیز جمعیتی نشان داد که فراوانی این جایگزینی ۷۷٪ می باشد. بعلاوه اضافه شدن یک تکرار (TG_{29}) فقط در ناحیه ۷۷۳- بالادست ژن در نمونه های بز مشاهده شد. ناحیه پروموتر ژن در بر گیرنده دو ناحیه سرشار از گوانین و سیتوزین و نیز جایگاه اتصال فاکتورهای نسخه برداری GATA و SP1 در هر دو گونه بود که می تواند برای القاء بیان ژن موثر باشند. آنالیز متیلاسیون ۱۸۲ جفت بازی ناحیه بالا دست ژن و در محدوده مناطق سرشار از گوانین و سیتوزین، نشان دهنده متیله شدن این نواحی در هر دو گونه گاو و بز می باشد. بررسیهای فیلوژنتیکی نشان داد که

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پروتئینهای ژن فوق در گاو و گوسفند در یک شاخه قرار گرفته و نسبت d_N/d_S نشان می دهد که ژن EDNRB در طی تکامل تحت تاثیر انتخاب خالص شده قرار گرفته است.

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