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Original article

Characterization of *Bovidae* sex-determining gene *SRY*

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Abstract – In mammals, testis determination is under the control of the sex-determining gene *SRY*. This Y-linked gene encodes a protein with a DNA binding domain similar to those found in high-mobility-group proteins. Here we report the cloning and sequences of the *SRY* genes of yak and Chinese native cattle. Our data show that *SRY* genes in *Bovidae* are less divergent, especially in the coding and 3′ regions.

sex determination / *SRY* / evolution / *Bovidae*

1. INTRODUCTION

In mammals, the Y chromosome-linked *SRY* gene is responsible for male sex determination [17]. *SRY* encodes a protein with a central HMG-box present in a wide variety of proteins that bind and bend DNA, suggesting that *SRY* functions as a transcription factor. Mutation in the *SRY* gene can result in male-to-female sex reversal [3,6]. The observation that the *Sry* transgenic XX mouse develops a male phenotype strongly suggests that *SRY* is the dominant gene in sex determination [9]. However, the role of *SRY* as a transcription factor in sex determination in mammals remains elusive and other genes including *SOX9*, *DMRT1*, *WNT1*, *AMH*, *SF1*, *DAX1*, *GATA4*, *LIM1*, *Fra1* and *aromatase* also seem to be involved in the sex-determining pathway [8,13, 16,18]. The evolutionary analysis of the *SRY* coding region among primates

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and rodents suggests that this gene is rapidly evolving [19,20]. In contrast, results from wallaby and domestic ruminants appear to indicate that sequence evolution of the *SRY* gene is less rapid [12,14]. It remains a challenge how the amazingly complex sex determination pathways evolve in various animal systems, especially in the *Bovidae* family. Here, we report the identification of *SRY* gene sequences of another two *Bovidae* species, yak and Chinese native cattle, and the high degree of sequence conservation of the *SRY* genes in the *Bovidae* family.

2. MATERIALS AND METHODS

2.1. Preparation of DNA samples

Yak (*Bos grunniens*) and Chinese native bovine (*Bos taurus*) were collected from the Himalayan Mountains in Qinghai, China and Wuhan, China, respectively. Genomic DNAs were isolated from blood samples of both male and female individuals of the two species using standard molecular biology protocols.

2.2. Cloning and sequencing analysis

PCR primers were designed based on the *SRY* sequence of Japanese bovine (*Bos taurus*) [7]. The sequences of primer sets used in this study are listed in Table I. Primer sets 1, 2, 3 and 6 were used to amplify the *SRY* gene of Chinese native cattle, whereas primer sets 1, 2, 4, and 5 to amplify the yak *SRY* gene, which covers the full length of the *SRY* gene. PCR was carried out as follows: genomic DNA was denatured for 5 min at 94 °C, then 30 s at 94 °C, 40 s at 48 °C and 1 min at 72 °C for 35 cycles. PCR products were subcloned into pUC18 and sequenced with an ABI automatic sequencer. The *SRY* sequences from other species have been reported previously under the accession numbers of Z30265 and AF026566, and reference [15] for sheep, Z30646 for goats, Z30321 for Bison, and AB039748 for Japanese cattle. All sequences obtained were aligned using the Clustalw program. Alignment was optimized manually with aid of DNAsis software. Amino acid identity calculations were as previously described [21].

3. RESULTS AND DISCUSSION

3.1. The 5′ and 3′ regions of the *Bovidae SRY* genes

Three portions (the 5′ region, the coding sequence and the 3′ region) of the *Bovidae SRY* genes are aligned separately for comparison. Figure 1 presents a comparison of the 5′ and the 3′ regions of *SRY* genes from Chinese native cattle,

Table I. Primer sets used in this study.

Primer set No.	Primer sequences	Region amplified (nucleotide number) [a]	Amplification	
			CNB	Yak
1	5′ CAACTTTCAAGTTTGCCTTATGG 3′ 5′ ACAGCCCAATCCTGTTATATA 3′	6–634	+ [b]	+
2	5′ GTATCTGAGATTGCCTGT 3′ 5′ CATCGTCGTTCAATACTC 3′	427–1091	+	+
3	5′ GCTGACTGCCAGGACGTA 3′ 5′ TTGTTACAGGGAAAGTCC 3′	885–1713	+	−
4	5′ TTCATAACCGTAGACAAT 3′ 5′ CATGCATCGGGTTGCATA 3′	673–1511	−	+
5	5′ ACAGTCCAGCTGTGGTAC 3′ 5′ TTGTTACAGGGAAAGTCC 3′	1095–1713	−	+
6	5′ GCATGTAGAGACATTGCA 3′ 5′ TACAGTTTCACCATGGAC 3′	1507–2793	+	+

[a] Based on the nucleotide number of Japanese bovine *SRY*;
[b] The primer set were used (+) or not used (−) to amplify in this species.
Species abbreviations are: Chinese native cattle (CNB), Yak.

yak, Japanese cattle and sheep. In the 5′ region, the alignment shows a high level of sequence conservation among these species except for four variable regions due to insertion/deletion (motif A-D), (Fig. 1a). Sequences in the 3′ region are more conserved with the exception of two insertion/deletion of four bases (Fig. 1b). These insertion/deletion variations are observed between the *Bovinae* and *Caprinae* subfamilies, but not within members of the *Bovinae* subfamily. Thus, the sequence identity appears higher within-subfamily than between-subfamilies. In the 5′ region of the *SRY* gene, sequences that are potentially important for transcriptional regulation of the gene, such as CAAT-box, TATA-box, *SRY*-binding site and *Sp*1-binding site, are conserved, even though the listed sequences are from four species that belong to different subfamilies. These data are consistent with the theory that *SRY* genes are functionally conserved for sex determination and regulation within the *Bovidae* family.

The regions containing insertion/deletions in the *SRY* genes from related species suggest that these regions may not be important for the regulation of *SRY* expression. Interestingly, however, two of these insertion/deletion regions (motif A and D) corresponded to two motifs (E1 and E4) of four conserved regions from *C. elegans* (Fig. 1a). BLAST search revealed another

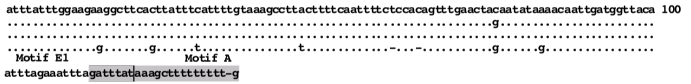

Figure 1. Comparisons of the 5′ region (**a**) and the 3′ region (**b**) of the *SRY* sequences from Chinese native cattle (CNB), Yak, Japanese cattle (JPB) and sheep (SHE). Dots indicate identical nucleotides. The insertion/deletion motifs are shaded. The regions boxed are shared motifs between *Bovidae* and *C. elegans*, and motif E1-E9 are from the cosmids of *C. elegans*: C16E9, T01B11, R10E8, T04C12, M01F1, F53A2,C07E3/F20D12, F52F10 and C03G5 respectively.

five conserved motifs (E5-E9) in the 3′ region of the *SRY* that are also conserved between *Bovidae* and *C. elegans* (Fig. 1b). Appearance of these conserved motifs flanking the *SRY* coding region implies that *SRY* might originate from an ancient gene. Evidences to support this hypothesis include: (1) the Y chromosome-linked *SRY* gene appears to originate from an ancient X-linked *SRY*-box family member, *SOX3* gene [5,10]. *SOX* genes have been found in both vertebrates and invertebrates. (2) Sex chromosomes are thought to have appeared about 240 to 320 Mya (million years ago), shortly after divergence of the mammalian and avian lineages [10]. (3) Our recent finding suggests that the X chromosome occurred much earlier at about 440 Mya when fish appeared (Yi *et al.*, unpublished data). Finally (4) another sex-determining gene *DMRT1* is conserved among humans, chickens, nematodes and flies [16], suggesting that at least some aspects of sexual regulation have a common evolutionary origin.

3.2. The coding region of the *Bovidae SRY* genes

The alignment of the *SRY* coding regions from yak, Chinese native cattle and other species in *Bovidae* shows much higher amino-acid identities along the entire coding region (Fig. 2). The sequence conservation is not only confined to the HMG box as observed in rats and mice [19], but also evident in the C-terminal non-box portion of the *SRY* gene in *Bovidae*, although the N-terminal region among the ruminants investigated was reduced by 11 amino acids in *Bovinae*. We calculated amino acid (aa) identities of the *SRY* gene from different *Bovidae* species. The *Bovidae* family shares 84.7% aa identity, and much higher identities ($> 95\%$) were observed within different subfamilies. In the genus *Bos*, the aa identity reaches to over 99%. Based on the aa identities of the aligned coding regions, an evolutionary tree was constructed, which is consistent with the classic taxonomic relationship (Fig. 3).

Less divergence of *SRY* in *Bovidae* suggests a close relationship between the members of this family. Fertile hybrids were obtained: between Bos $\times$ Bison, goat $\times$ sheep, and yak $\times$ cattle [2,4]. Parents of these fertile hybrids were from members of different genera. Based on sequence comparison, we found that the divergence of the *SRY* gene is related to reproductive isolation in *Bovidae*. Fertile hybrids could be obtained if the aa identity in the coding region of the *SRY* gene is more than 95%. No hybrid between the subfamilies with aa identity of 84.7% was described. If hybrids are obtained between two genus members, male hybrids are always sterile, which supports that the Y chromosome may be mainly, at least partly, responsible for the sterility. Our finding is further supported by the rate of base substitution in the flanking region of the *SRY* genes in the *Bovidae* family (Tab. II): Fertile hybrids are possible when the transition/transversion rate in the 5′ or 3′ regions of *SRY* is less than 1%. A hybrid between cattle $\times$ sheep or goats has not

Figure 2. Alignment of predicted SRY protein sequences from Chinese native cattle, yak, Japanese cattle, bison, sheep and goats. The HMG box is shaded. Dots indicate identical amino acids and dashes denote gaps. Species abbreviations are: Chinese native cattle (CNB), Yak, Japanese cattle (JPB) and sheep (SHE).

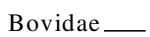

Figure 3. Evolutionary tree based on the amino acid identities among *Bovidae SRY* proteins. The numbers are percentage amino-acid identities.

been obtained, where the transition/transversion rate in the 5′ or 3′ regions between them were around 4%. These observations suggest that the extent of sequence divergence in the *SRY* gene correlates with reproductive isolation in *Bovidae*, although the unbalanced chromosome pairing in hybrids and recessive

Table II. Substitutions per 100 bases for the 5′ and 3′ regions.

	5′ region		3′ region	
	Transition	Transversion	Transition	Transversion
Species (CNB-JPB)	0.2	0	0.9	0.4
Genus (CNB-Yak)	0.8	0.6	0.8	0.5
Family (CNB-SHE)	4.4	4.1	3.9	3.8

Species abbreviations are: Chinese native cattle (CNB), Yak, Japanese cattle (JPB) and sheep (SHE).

X chromosome epistatic interactions with autosomes appears to be a main factor for the isolation.

In natural populations, some head males often have the priority to mate with a majority of females, especially in large animals including cattle and sheep. Since a few males contribute to the next generation, the effective population size for males will be smaller than that of females [11]. Thus unequal effective population size between the sexes would reduce the variation of the Y chromosome, therefore making the genes on the chromosome, such as *SRY*, less divergent. Another reason for the observed lower divergence of *SRY* may be that rapid cladogenesis in the *Bovidae* family offer little time to accumulate mutation [1]. During a series of cladogeneses, however, some key features of ancient genes must be preserved, which may explain the high degree of sequence conservation in the SRY gene in the *Bovidae* family.

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