Unique variations of SRY gene result in distinct patrilineal phylogeny of Capra hircus and other domestic Bovidae*

Xin CAI1, **, Hairong ZHANG2, Tserang Donko MIPAM3

1 School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, Sichuan 621010, China.
2 Department of Agronomy, Dezhou University, Dezhou, Shandong 253023, China
3 College of Life Science and Technology, Southwest University for Nationalities, Chengdu 610041, China

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Patrilineal phylogeny of Beichuan White goat and other domestic Bovidae was inferred from 5’-UTR and coding region of SRY gene. Variation analysis revealed 208 variable sites, meanwhile, a 50-bp fragment inserted downstream of the initiation codon (ATG) of SRY genes modified the translational initiation process in Bos and Bubalus, while the mechanism of what should be explained in a further study. Amino acid sequence alignments of HMG-box region indicated a high degree of conservation among goats and other Bovidae. All the sequences of Bovidae clustered into Bos, Bubalus and Capra. Bos indicus, Bos taurus, Bos javanicus, Bos frontalis, Bos grunniens and Bison bonasus were comprised in genus Bos, while Bubalus bubalis and Syncerus caffer belonged to genus Bubalus. Beichuan white goats and other Capra hircus specimens were clustered into genus Capra. Patrilineal phylogeny of Bovidae exhibited a discrepancy from the earlier matrilineal analysis.

KEY WORDS: Bovidae / Capra hircus / SRY gene / Patrilineal phylogeny

The family Bovidae (suborder Pecora, order Artiodactyla) includes 128 extant species in 45 recent genera, which comprise domesticated forms (goats, sheep, and cattle), the large herding antelopes of the African plains, and the small solitary,

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**Corresponding author: caixin2323@126.com
territorial forms usually found in more forested areas [Allard et al. 1992]. Matrilineal phylogeny of Bovidae was well established based on mtDNA sequences. Allard et al. [1992] suggested that Bovidae family was monophyletic and included two clades: one including the tribes Boselaphini, Bovini, and Tragelaphini, and another for an Antilopini/Neotragini grouping. Further study based on mtDNA cyt b and 12S rRNA genes and two nuclear genes indicated that Bovidae consist of two major lineages, i.e. Bovinae which contain the tribes Bovini, Boselaphini and Tragelaphini, and Antilopinae which encompasses all other bovids. Within Bovinae, the tribe Bovini is divided into buffalo Bovini (Bubalus and Syncerus) and cattle Bovini (Bos and Bison) and Tragelaphini are possibly related to Boselaphini [Hassanin and Douzery 1999]. However, matrilineal analysis based on mtDNA is not adequate to depict the phylogenetic picture of Bovidae. Patrilineal investigation based on Y chromosome haplotypes should be another powerful tool to describe the veil of Bovidae phylogeny.

In mammals, SRY gene is located near the pseudoautosomal boundary of Y chromosome and encodes a nuclear factor-like protein harboring a DNA-binding domain known as the HMG box [Panyen and Cotinot 1993, Nagai 2001]. This gene is one of more conserved Y specific genes during evolution in a number of mammals due to its immunity to recombination with X chromosome in meiotic XY bivalent [Panyen and Cotinot 1993]. Therefore, SRY gene could be employed as one of the optimum molecular markers to investigate patrilineal phylogeny of Bovidae and other mammals. Cheng et al. [2001] cloned and sequenced the SRY genes of yak and Chinese native cattle. Their results showed that SRY genes in Bovidae were less divergent, especially in the coding and 3´regions. Nevertheless, the phylogeny of Bovidae based on sequence variation of SRY genes was poorly understood up to now. Here, we sequenced the 5’-UTR and coding region of SRY genes of Beichuan White goat from Sichuan province of China, and analysed the patrilineal phylogeny of the breed and other domestic bovids on the basis of the of SRY genes variation.

Material and methods

Sample preparation and genomic DNA extraction

Blood samples of seven male Beichuan White (BW) goats (BWG01-BWG07) from Beichuan county, Sichuan province, were withdrawn and genomic DNA was extracted according to standard protocols.

Gene cloning

The sequence of 5’-UTR and coding region of SRY gene of BW goats was amplified by using the primers G-SRY-F (5’-TAAGTGGAGAAGCGGGGATAGT-3’) and G-SRY-R (5’-AGCGTGCTTTGTTAGCGAGAG-3’) designed according to the SRY gene sequence of Capra hircus (acc. no. EU581862). PCR was performed in a 50 μL reaction mixture containing 200 ng of genomic DNA, 1.5 mM MgCl₂,
10 mM Tris-aHCl (pH 8.3), 50 mM KCl, 0.6 units of Taq polymerase (TaKaRa), 10 μM of each primer and 0.2 mM of each dNTP. For thermal cycling a PTC-200 thermocycler was used (MJ Research Inc.) under the following conditions: 4 min denaturation at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 52°C, 30 s at 72°C, and final 7 min extension at 72°C, before cooling to 4°C for 10 min. PCR products were purified using a Qiagen QIAquick PCR purification kit and ligated to pMD™ 18-T Vector (TaKaRa). The subsequent transformation and clone screening were performed according to standard protocols. The positive clones identified were sequenced on an ABI 3730 automated sequencer at Shanghai Major BioTech Co. Ltd, Shanghai, China.

Polymorphic and phylogenetic analysis

The sequence of 5´-UTR and coding region of SRY gene of BW goats, and those of Bos javanicus, Bos taurus, Bos indicus, Bos frontalis, Bison bonasus, Bos grunniens, Bubalus bubalis and Syncerus caffer were retrieved from GenBank (Tab. 1). All sequences were aligned and edited in Clustal X [Thompson et al. 1997] with parameters set to default. Amino acid sequences of the coding region of SRY

<table>
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<th>Animal</th>
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<th>GenBank accession number</th>
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| Table 1. GenBank accession numbers of SRY genes for Beichuan White goats (BW), other Bovidae and Sus scrofa used for phylogenetic analysis |
gene were explored by NCBI software ORF Finder (http://www.ncbi.nlm.nih.gov). Polymorphic sites of SRY protein sequences were analysed using MEGA 4 [Kumar et al. 2008]. The NJ tree [Saitou and Nei 1987] based on 5’-UTR and coding region of SRY gene sequences was reconstructed in MEGA using the corresponding sequences of Sus scrofa as outgroup, with the reliability of the tree topology assessed by 1000 bootstrap replications [Felsenstein 1988].

**Results and discussion**

Each sequence of 5’-UTR and coding region of SRY gene of seven BW goats amplified was 1184 bp in length (Fig. 1). After removing the primer sequences and editing by multiple alignments, we have obtained seven sequences with a length of 1098 bp which covered 5’-UTR (371 bp) and coding region of SRY gene (723 bp). Each ORF sequence of SRY gene for BW goat was 723 bp in length and the predicted SRY protein was composed of 240 amino acids (GenBank accession numbers: JN561342-JN561348). However, the coding regions of SRY gene for 14 Bos and Bubalus animals, including Bos javanicus, Bos taurus, Bos indicus, Bos frontalis, Bison bonasus, Bos grunniens, Bubalus bubalis and Syncerus caffer, were 690 bp in length and the predicted SRY proteins were composed of 229 amino acids (aa). These results are in accordance with the study of Cheng et al. [2001], who reported the SRY protein sequences from Chinese native cattle, yak, Japanese cattle and bison to be 229 aa and those from sheep and goats of 240 aa in length.

![Fig. 1. PCR amplification of 5’-UTR and coding region of SRY genes from seven Beichuan White goats. M stands for DL2000 DNA Marker and the numbers 1-7 indicate different individuals of Beichuan White goats.](image)

Variation analysis of 5’-UTR and coding region of SRY gene from nine Capra animals (including seven BW males) together with those from 14 Bos and Bubalus representatives revealed 208 variable sites. Meanwhile, a 50-bp fragment insertions were examined downstream the initiation codon (ATG) of SRY genes from Bos and
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Fig. 2. The specific inserted sequences approximating to the initiation codon of SRY genes from Bos and Bubalus animals compared to those from Capra. Bos grunniens and Bos javanicus shared the identical sequences showed in shade. The underlined sequences are specific to Bos grunniens and the boxed specific to Bubalus bubalis. The shaded and boxed sequence is specific to Syncerus caffer. The initiation codons are bolded.

- DQ336528-Bos javanicus
- DQ336526-Bos taurus
- DQ336527-Bos indicus
- DQ336530-Bos frontalis
- DQ336535-Bison bonasus
- DQ336532-Bison bonasus
- DQ336531-Bos grunniens
- AF149463-Bos grunniens
- AF149462-Bos taurus
- FJ373272-Bos grunniens
- EU547257-Bos grunniens
- GQ259332-Bubalus bubalis
- DQ336535-Bubalus bubalis
- DQ336534-Syncerus caffer
- EU581862-Capra hircus
- DQ29163-Capra hircus

- BW03
- BW05
- BW06
- BW07
- BW01
- BW02
- BW04
Bubalus groups compared to those from Capra group (Fig. 2). We suggest that it was these 50-bp insertion fragments between the initiation codon (ATG) and the immediately following codon (ATG) that modified the translational initiation process in Bos and Bubalus, while the mechanism of what should be explained in a further study. On the other hand, these 50-bp insertion fragments exhibited some specific characteristics. Bos javanicus, Bos taurus, Bos indicus, Bos frontalis and Bison bonasus groups shared the identical insertion fragment, as shown on Figure 2. The insertion fragments shared by Bos grunniens, Bubalus bubalis and Syncerus caffer occurred special enough to distinguish them from another (Fig. 2). Therefore, we could conclude that 5′-UTR of SRY genes were more divergent in Bovidae, what was in contrast with the conclusion that SRY genes in Bovidae were less divergent, especially in the 3′-UTR regions [Cheng et al. 2001].

The high-mobility-group protein HMG of Bovidae is composed of 77-residues termed HMG box (Fig. 3), which is conserved motif representing a functional protein

![Fig. 3. Amino acid sequence alignments of HMG-box region from Beichuan White goats and other Bovidae. Mutations are scored relative to the reference sequence (acc. no. DQ336527). Sequence identity is indicated by points and the differences are noted. Numbers at the top of the Figure indicate the amino acid sequence position.](image-url)
domain necessary for DNA binding activity of SRY [Wright and Dixon 1988]. The mutations in the SRY gene associated with sex inversion have been located within the HMG box [Hawkins et al. 1992, McElreavey et al. 1992]. Amino acid sequence alignments of HMG-box region indicated a very high degree of conservation among BW goats and the other representatives of Bovidae (Fig. 3). Among the genus Bos (including Bos javanicus, Bos taurus, Bos indicus, Bos frontalis and Bison bonasus) only two variable sites were observed and more than 97% of homology was exhibited, while the sequences from the genus Bubalus showed seven variable sites and more than 90% of homology (Fig. 3). All representatives of BW goat together with two “extra” sequences (GenBank accession no. D82963, EU581862 of the genus Capra also displayed higher degree of conservation, with five variable sites and more than 93% homology.

Neighbour-joining tree was constructed from 5’-UTR and coding region of SRY genes on the basis of Kimura two-parameter distances, with Sus scrofa as the outgroup. All the sequences were reasonably clustered into phylogenetic clades representing different genus with more than 93% bootstrap values, namely Bos, Bubalus, Capra and Sus (Fig. 4). Therefore, the Bovidae family compromised Bos, Bubalus and

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Capra examined in the present study. The sequences of *Bos indicus*, *Bos taurus*, *Bos javanicus*, *Bos frontalis*, *Bos grunniens* and *Bison bonasus* were clustered into genus *Bos*, while *Bubalus bubalis* and *Syncerus caffer* to genus *Bubalus*. This result is in accordance with the view of Hassanin and Douzery [1999] who claim that *Bovini* should be divided into buffalo *Bovini* (*Bubalus* and *Syncerus*) and cattle *Bovini* (*Bos* and *Bison*). All individuals of BW goats together with two sequences of *Capra hircus* were clustered into genus *Capra*. Within the phylogeny of *Bos*, *Bos taurus* and *Bos indicus* were the most closely related species commonly known as cattle. *Bos javanicus* and *Bos frontalis* were more closely related to *Bos taurus* and *Bos indicus* than to *Bos grunniens*, while more divergent from *Bison bonasus*. This was not in accordance with the results we obtained from the phylogeny of *Bovidae* inferred from mtDNA cyt *b* gene that *Bos javanicus* and *Bos grunniens* were found to be more divergent from *Bos taurus* and *Bos indicus* than the European bison was from the two lineages [Cai et al. 2007]. In this case, we conclude that using of different molecular markers may lead to inconsistent results of phylogenetic inferences. Therefore, comprehensive markers, matrilineal, patrilineal and nuclear should be employed to more accurately investigate the phylogeny of *Bovidae*.

Herein, we concluded that the sequences of 5′-UTR of *SRY* genes from BW goats and other *Bovidae* exhibited abundant variations. The 50-bp fragment inserted downstream of the initiation codon (ATG) of *SRY* genes modified the translational initiation process in *Bos* and *Bubalus* groups while the mechanism of what should be explained in a further study. Amino acid sequence alignments of HMG-box region indicated a high degree of conservation among goats and other *Bovidae*. All the sequences were reasonably clustered into phylogenetic clades representing different genus with more than 93% bootstrap values, namely *Bos*, *Bubalus*, *Capra* and *Sus*. However, patrilineal phylogeny of *Bovidae* exhibited a discrepancy from the previous matrilineal analysis, for different molecular markers may lead to inconsistent inference of phylogeny.

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