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Comparative analysis the variability of a complete mitochondrial genome of polledness intersexual goat (PIS- -) from China

GuangXin E, Yong-Fu Huang*, Yong-Ju Zhao, Ri-Su Na, Zhong-Quan Zhao, Ya-Wang Sun, Li-Peng Chen, and Xiao-Yu Qiu

College of Animal Science and Technology, Chongqing Key Laboratory of Forage & Herbivore, Chongqing Engineering Research Centre for Herbivores Resource Protection and Utilization, Southwest University, Chongqing 400716, China.
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ABSTRACT

The polledness intersexual goat (PIS- -) (*Capra hircus*), deformed individuals, could have malformed reproductive organs and loss of reproductive function. Here, we first determined the complete mitochondrial genome of Chinese native polledness intersexual goat (PIS- -) is 16,640 nt in length, consisting of 13 protein-coding genes, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes and a non-coding control region. As in other mammals and intrastrains, most mitochondrial genes are encoded on the heavy strand, except for ND6 and eight tRNA genes, which are encoded on the light strand. Its overall base composition is A: 33.5%, T: 27.3%, C: 26.1% and G: 13.1%. The complete mitogenome of the Chinese indigenous breed of goat could provide a basic data for further understanding the contribution of mitochondria in the sex-developmental mechanism, tissue and organ cell deformity of polledness intersexual goat *via* genomic compare.

Key words: Goat (Capra hircus), Mitochondrial genome, Polledness intersexual goat (PIS- -), Protein-coding genes.

INTRODUCTION

In goats, the PIS (polled intersex syndrome) mutation is responsible for both the absence of horns in males and females and sex-reversal affecting exclusively XX individuals (Pailhoux *et al*., 2001). The mode of inheritance is dominant for the polled trait and recessive for sex-reversal, as well as the linkage between hornlessness and intersexuality (Asdell, 1944; Pailhoux *et al*., 2005). In XX PIS"/"mutants, the expression of testis-specific genes is observed very precociously during gonad development (Pannetier *et al*., 2003; Boulanger *et al*., 2008; Boulanger *et al*., 2014). But final conclusion of intersexual development is unclear at this moment. The proportion of intersexual goat was from 4% to 15% among different countries in early reports (Bosu *et al*., 1984; Hamerton *et al*., 1969). Therefore, the intersexual goat is key influence of the development of goat husbandry. Up to date, no complete mitochondrial genome of intersexual goat has been reported. In current study, we first determined the mitochondrial genome of a native individual which belong to the indigenous and ecotype population in southwest of China. Then, we compared it with the mitochondrial genome of others to insight the possible variant which contribute to intersexual development of goat.

MATERIALS AND METHODS

Twenty-three primer sets (Zhang *et al*., 2014) were used to amplify overlapping segments of a Wutong black goat (polledness intersexual goat (PIS- -)) mitochondrial genome. PCR amplification was carried out in a PTC-100™ PCR instrument (MJ Research, Inc., Massachusetts, USA) with a total reaction volume of 50 ìL containing 150 ng DNA, 5μL 10× PCR standard reaction buffer, 10 pmol/L dNTPs, 50 mmol/L $MgCl_2$, 20 pmol/L each forward and reverse primer, 2.5 U Taq DNA polymerase from Promega (China).

Following an initial denaturation at 95°C for 3 min, 30 cycles were performed with 94°C for 45 s, annealing temperature of each primer for 45 s, 72°C for 1 min. The final cycle was followed by an extension at 72 for 10°C min. PCR products were detected on 1.5% agurose gel including 0.5 μg/mL of ethidium bromide, photographed under UV light, and sequenced by Bioasia Biological and Technology Co. Ltd. (Beijing, China).

Complete mitochondrial genome was edited and aligned using BioEdit (Version 5.0.9) and MEGA6 (Tamura *et al*., 2013), and the genomic map of complete mitochondrial genome was drawn by OrganellarGenomeDRAW (Lohse *et al*., 2013).

Thehe possible private variability of mitochondrial coding gene region in intersexual goat was identified in comparison to others 35 individuals which published (the GenBank number of Ref sequences see Supplemental material I).

RESULTS AND DISCUSSION

In current study, we firstly determined the mitochondrial genome of these deformed individuals (GenBank accession number: KP662714) from Wutong black goat which belongs to the native and ecotype population in southwest of China.

*Corresponding author's e-mail: H67738337@swu.edu.cn.

The complete mitogenome of this individual is 16,640 bp in length, and consists of 13 protein-coding genes, 2 ribosomal RNA genes (rRNA), 22 transfer RNA (tRNA) genes and a control region (CR) (Table 1). The overall base composition of the mitogenome is A: 33.5%, T: 27.3%, C: 26.1% and G: 13.1%. As in other mammals, most mitochondrial genes are encoded on the heavy strand, except for ND6 and eight tRNA genes which are encoded on the light strand. All 13 mitochondrial protein-coding genes share the start codon ATG, except for ND2, ND3 and ND5 which initiate with start codon ATA. Three protein-coding genes are inferred to terminate with an incomplete termination codon T- - (COXIII, ND3 and ND4), while Cytb and ND2 use AGA and TAG instead of TAA as the stop codon, respectively. The remaining eight protein-coding genes end with the typical termination codon TAA. The putative origin of light-strand replication (OL; 36 bp in length), as in most vertebrates, is located in a cluster of the WANCY region (Table 1 and Figure 1).

Via the alignment of complete mitochondrial genome across the intersexual individual and that of previous reported, no private variability was identified.

A variety of disorders and diseases can be caused by nuclear mutations of mitochondrial genome, include Kearns-Sayre syndrome, MELAS syndrome and Leber's

TABLE 1. Characteristics of the mitochondrial genome of Chinese native polledness intersexual goat (PIS- -)

Genes	Position		Sizes Nucleotide	Code		Strand
	From	To	(bp)	Start	Stop	
D-loop	1	1212	1212			H
tRNA-Phe	1213	1280	68			H
12S rRNA	1281	2234	954			H
tRNA-Val	2237	2303	67			H
16S rRNA	2304	3869	1566			H
tRNA-Leu	3876	3950	75			H
NADH dehydrogenase subunit 1 (ND1)	3953	4908	956	ATG	TAA	H
tRNA-Ile	4909	4977	69			H
tRNA-Gln	4975	5046	72			L
tRNA-Met	5049	5117	69			H
NADH dehydrogenase subunit 2 (ND2)	5118	6159	1042	ATA	TAG	H
tRNA-Trp	6160	6226	67			H
tRNA-Ala	6228	6296	69			L
tRNA-Asn	6298	6370	73			L
OL	6373	6408	36			L
tRNA-Cys	6403	6470	68			L
tRNA-Tyr	6471	6538	68			L
Cytochrome c oxidase subunit I (COI)	6540	8084	1545	ATG	TAA	H
tRNA-Ser	8082	8150	69			L
tRNA-Asp	8158	8225	68			H
Cytochrome c oxidase subunit II (COII)	8227	8910	684	ATG	TAA	H
tRNA-Lys	8914	8980	67			H
ATPase subunit 8 (ATPase8)	8982	9179	198	ATG	TAA	H
ATPase subunit 6 (ATPase6)	9140	9820	681	ATG	TAA	H
Cytochrome c oxidase subunit III (COIII)	9820	10603	784	ATG	T—	H
tRNA-Gly	10604	10672	69			H
NADH dehydrogenase subunit 3 (ND3)	10673	11018	346	ATA	T—	H
tRNA-Arg	11020	11088	69			H
NADH dehydrogenase subunit 4L (ND4L)	11089	11385	297	ATG	TAA	H
NADH dehydrogenase subunit 4 (ND4)	11379	12756	1378	ATG	T—	H
tRNA-His	12757	12826	70			H
tRNA-Ser (AGY)	12827	12886	60			H
tRNA-Leu (CUN)	12888	12957	69			H
NADH dehydrogenase subunit 5 (ND5)	12958	14778	1821	ATA	TAA	H
NADH dehydrogenase subunit 6 (ND6)	14762	15289	528	ATG	TAA	L
tRNA-Glu	15290	15358	69			L
Cytochrome b (Cytb)	15363	16502	1140	ATG	AGA	H
tRNA-Thr	16506	16575	70			H
tRNA-Pro	16575	16640	66			L

* H and L denote heavy and light strands, respectively.

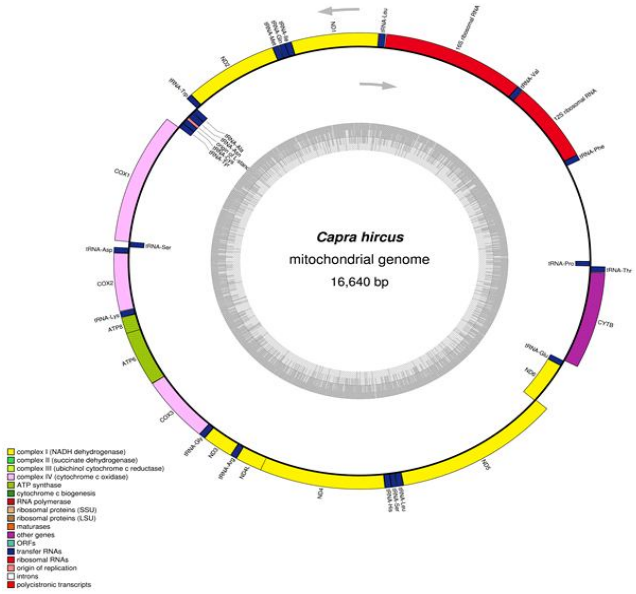


FIG 1: Physical maps of complete mitochondrial genomes in a polledness intersexual goat

hereditary optic neuropathy (Zeviani *et al*., 2004; Taylor *et al*., 2004), Wilson's disease (Chinnery *et al*., 2003). In addition, other pathologies with etiology involving mitochondrial dysfunction include retinitis pigmentosa, and diabetes mellitus (Schapira *et al*., 2006; Pieczenik *et al*., 2007). In current study, we tried to discover the specific site which possibly contribute to the developmental disorder. However, there was no private variability in the mitochondrial coding gene of intersexual individual. In fact, the critical deletion of 11.7-kb DNA element could the responsible for the development of intersex in at chromosome band 1q43, shown to be homologous to human chromosome band 3q23 was reported (Pailhoux *et al*., 2001). Thus, no site in mitochondrial coding gene region related to intersex phenotype in this study.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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