Genetic typing of mitochondrial DNA in sheep (*Ovis aries*)*

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Abstract Using PCR-RFLP and DNA sequencing, this study confirmed that *Hin*fI polymorphism in the ovine mitochondrial *CO*I gene resulted from the T-C substitution at the nucleotide 234 but this mutation did not encode another amino acid, which was actually a synonymous mutation. This single nucleotide polymorphism can be used as gene typing marker for mitochondrial genome in the research of the interactions between mitochondria and nucleus, extranuclear gene effects, and as molecular discriminating marker for embryo or individual in the research area of gene transfer and animal cloning.

Keywords: sheep, mtDNA typing, single nucleotide polymorphism.

There are two kinds of genomes in animals: one is nuclear genome and the other is mitochondrial genome located extensively within mitochondria. Animal mtDNA encodes 37 genes, including 13 peptides, 22 tRNA and 2 rRNA genes, all of which encode essential components of oxidative phosphorylation in mitochondrial inner membrane, generating cellular energy in the main form of adenosine triphosphate[1]. In recent years, the structure and function of mtDNA become a hot spot in scientific research into molecular evolution, aging, disease diagnosis, apoptosis and quantitative traits loci (QTL)[2~5].

Based on the observation made by geobiological and morphologic methods, the origin of domestic sheep is regarded as derived from urial (*O. Vignei bochariensis*) and argali (*O. Ammon nigrimontana*). Through studying the control region of mtDNA, Wood and Phua discovered two main haplogroups of mtDNA in sheep: the consensus type (type A) and the mutated type (type B)[6]. Hiendleder and his colleagues studied more extensive major breeds distributed over the world by mtDNA polymorphism analysis and sequencing, confirmed the conclusion reached by Wood and Phua, and furthermore, classified modern domestic sheep into two major types. This finding led to a new hypothesis on sheep evolutionary origin[7]. In the experimental analysis, they found that the *Hin*fI polymorphism kept consistent with variations in D-loop, so the *Hin*fI cleavage polymorphism could be used as a discriminating marker for sheep[8], but the change of the sequence remains unknown. In this work, we compare the partial mtDNA *CO*I gene between European and Chinese sheep breeds using PCR-RFLP and DNA sequencing, and elucidate the mechanism of the *Hin*fI polymorphism.

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1 Materials and methods

Blood samples were collected from unrelated sheep within three generations of six different breeds. These breeds included 55 Small-Tail han sheep, 30 Wuzhumuqin sheep, 30 Huyang sheep, 19 Sharolais, 50 Suffolk sheep and 34 Dorset sheep.

DNA template was isolated from the blood samples with a DNA extraction kit (Qiagen). According to sheep mtDNA sequence (GenBank accession number: AF010406), a primer pair was designed as P1F (5′-GCAGAGTTTGAAGCTGCT-3′) and P2R (5′-AGCTGACGTGAAGTAAGC-3′) to amplify a 1053bp DNA fragment including the *Hin*fI polymorphic site. The PCR reaction was performed at 94℃ for 5 min, followed by 30 cycles with denaturation at 95℃ for 30 s, annealing at 58℃ for 30 s and extension at 72℃ for 1 min, and then the reaction was extended at 72℃ for 5 min. PCR products were purified from agarose gels by a Geneclean kit (Biolab LTD), and digested with 21 restriction enzymes (Table 1).

The purified PCR products were ligased to the plasmid vector of PGEM-T (Promega), and transformed to the competent cells of *E. coli* DH5α. The recombined plasmid DNA was isolated and sequenced by an ABI PRISM377 DNA sequencer.

2 Results and discussion

PCR product of mtDNA *CO*1 gene was 1053 bp long. After being digested with 21 restriction enzymes, only *Hin*fI polymorphism was found (Table 1).

All the sheep could be divided into two genotypes according to *Hin*fI polymorphism, type A and B (Fig. 1(a)), and both types could be found in all breeds, but the frequencies of the A or B type were different (Table 2).

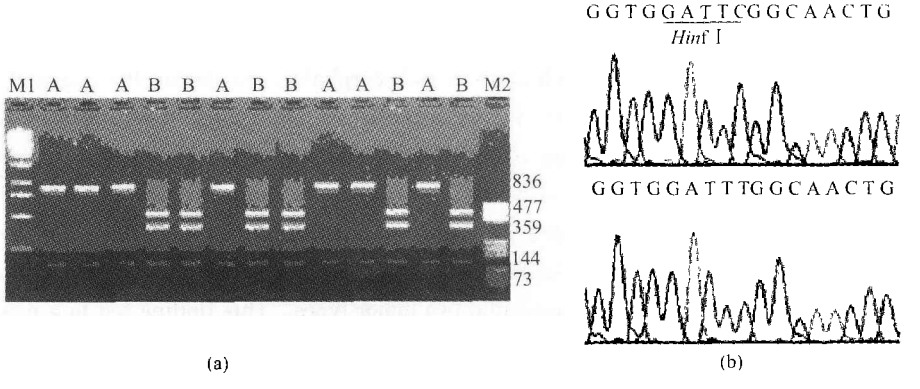

Fig. 1 Single nucleotide substitution detected PCR-RFLP in sheep. (a) M1 and M2, DNA markers; A, consensus individuals; B, mutated individuals. (b) DNA sequence comparison, the upper is the mutated type (B type) showing a HinfI cleavage site, the lower is the conserved type (A type) without *Hin*fI cleavage site, a T to C base substitution is shown.

Table 1 PCR-RFLPs of mtDNA *CO*I in sheep detected by 21 enzymes

Enzymes	Cleavage fragment/bp	Cleavage site	Enzymes	Cleavage fragment/bp	Cleavage site
AccI	511	1	Hap Ⅱ	565, 260, 228	3
*Ava*I	1053	0	*Hin*d Ⅲ	624, 429	1
*Bam*HI	1053	0	*Hin*fI	A: 836, 144, 73	2
*Ban*I	649, 208, 175, 21	3		B: 477, 359, 144, 73,	3
Bgl Ⅱ	558, 495	1	KpnI	1053	0
*Cla*I	1053	0	*Not*I	1053	0
*Dpn*I	686, 559	1	*Pvu* Ⅱ	737, 316	1
*Dra*I	1053	0	*Rsa*I	691, 347, 15	2
*EcoR*I	1053	0	*Sac*I	1053	0
*Eco*R Ⅴ	884, 169	1	*Taq*I	1025, 28	1
Hae Ⅲ	801, 252	1	*Xba*I	1053	0

Table 2 *Hin*fI polymorphism in mtDNA detected in six sheep breeds

Breeds	Cleavage type		Total
	A	B	
Small-Tail han sheep	26	29	55
Wuzhumuqin	23	7	30
Huyang	14	16	30
Sharolais	18	1	19
Suffolk	8	42	50
Dorset	3	31	34

The sequencing data was analyzed by the DNASIS2.5 software, the results indicated that type A of mtDNA was identical to the published sheep sequence[9] and type B showed a T to C substitution at the nucleotide 234 in *CO*I gene, this base variation led to a new *Hin*fI cleavage site (Fig. 1(b)), but the new codon encoded the same amino acid. In type A the three nucleotides TTT encoded phenylalanine, in type B the nucleotide TTC also encoded phenylalanine, so this was a synonymous mutation.

The *Hin*fI cleavage polymorphism in mtDNA *CO*I gene can be used as a molecular marker in typing of populations, maternal lines and even individual identification. The assay is very simple and rapid. We found that the frequencies of type A or B varied in different sheep breeds, but whether it represents some characteristics of different breeds is still to be clarified.

The *Hin*fI polymorphism located in *CO*I gene is actually a single nucleotide polymorphism (SNP), and it is the only nucleotide base variation found in the sequenced mtDNA fragments, confirming further that mtDNA is well conserved in sheep[10]. This *Hin*fI polymorphism not only serves as a gene typing marker for mitochondrial genome but also is useful in the researches of interaction between mitochondrial and nuclear genomes, extranuclear gene effects as well as in the research areas of gene transfer and animal cloning.

References

1 Saraste, M. Oxidative phosphorylation at the fin de siècle. Science, 1999, 283: 1488.

2 Gray, M. W. et al. Mitochondrial evolution. Science, 1999, 283: 1476.
3 Wallace, D. C.. Mitochondrial diseases in man and mouse. Science, 1999, 283: 1482.
4 Green, D. R. et al. Mitochondria and apoptosis. Science, 1998,281:1309.
5 Zhao, X.B. et al. Exnuclear gene effects on milk production traits in dairy cattle. High Technology Letters (in Chinese), 2000, 112(10): 97.
6 Wood, N. J. et al. Variation in the control region sequence of the sheep mitochondrial genome. Anim. Genet., 1996, 27(1): 25.
7 Hiendleder, S. et al. Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: No evidence for contributions from Urial and Argali sheep. J. Hered., 1998, 89(2):113.
8 Hiendleder, S. et al. A diagnostic assay discriminating between two major *Ovis aries* mitochondrial DNA haplogroups. Anim. Genet., 1999, 30(3): 211.
9 Hiendleder, S. et al. The complete mitochondrial DNA sequence of the domestic sheep (*Ovis aries*) and comparison with the other major ovine haplotype. J. Mol. Evol., 1998, 47(4):441.
10 Hiendleder, S. A low rate of replacement substitutions in two major *Ovis aries* mitochondrial genomes. Anim. Genet., 1998, 29(2): 116.