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Full Length Research Paper

Special variations within 11.7 kb fragment in goat *polled intersex syndrome*

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The goat *polled intersex syndrome* (*PIS*) is a full association between the absence of horns and intersexuality. Several pairs of primer were designed to detect the complete or partial deletion of 11.7 kb fragment in *PIS* goat. We proved that the 11.7 kb fragment was not deleted completely but partially in *PIS* goat. The special 198 base substitutions were found between normal and *PIS* dairy goats, which formed haplotypes A and B, representing normal and *PIS* dairy goat, respectively. Another special variation was the 18 deletions forming 108 bases deletion that only exist in *PIS* dairy goat. It could be inferred that the special 198 base substitutions and 108 bases deletion might be triggered intersexuality in the dairy goat.

Key words: *Polled intersex syndrome* (*PIS*) dairy goat, special base substitutions, special bases deletion.

INTRODUCTION

The goat polled intersex syndrome (PIS) is a full association between the absence of horns and intersexuality (Asdell, 1944). No recombinant has ever been observed between the two phenotypes (Ricordeau and Lauvergne, 1967; Soller et al., 1969), suggesting that they are either under the control of a single pleiotropic gene or two very closely linked genes. The intersexuality appears as autosomal recessive, whereas the polledness is autosomal dominant, which has been helpful in building resource families (Vaiman et al., 1996). The locus of *PIS* was initially localized to the distal region of goat chromosome 1 (Vaiman et al., 1996). Schibler et al. (2000) localized the goat *PIS* at 1q43 in ~100 kb region, which was the homolog of the human region associated with *Blepharophimosis Ptosis Epicanthus inversus Syndrome* (*BPES*) gene located in 3q23, and suggested that goat *PIS* and human *BPES* could be encoded by a homologous gene. Through a positional cloning approach, Pailhoux et al. (2001) demonstrated that the mutation underlying *PIS* is the deletion of a critical 11.7 kb DNA element containing mainly repetitive sequences, which triggered intersexuality and polledness in goats. Through the bioinformatics analysis of four repeated sequences (1 to 3120, 3988 to 4565, 4989 to 7088 and 7875 to 9135 bp) in goat *PIS* deletion region (AF404302), Li et al. (2008) found that the 7875 to 9135 bp segment belonged to L1M3 subfamily of LINE-1 and the others were bovine dimer-driven family (BDDF), subfamily of RTE. Li et al. (2008) also estimated that the formation time of *PIS* region was at 22.5 to 30 Myr, just the divergence time of Bovidae from Artiodactyla, and the *PIS* region might only exist in Bovidae. However, the variation of 11.7 kb deletion between *PIS* goat and normal goat is not very clear. This study showed that only parts of the 11.7 kb fragment were deleted in *PIS* goat, and there were special variation sites in some regions of 11.7 kb fragment between *PIS* goat and normal goat.

MATERIALS AND METHODS

Samples and DNA extraction

Blood samples were taken from 107 individuals of dairy goat which contained 30 horned female goats, 30 hornless female goats, 4 hornless male goats and 43 *PIS* goats (only show 9 individuals representing different types of *PIS* goats) (Figure 1). DNA extraction was conducted by the phenol extraction method.

Primers design, amplification and sequencing

Several pairs of primer (Table 1 and Figure 2) were designed using Premier 5 based on AF404302, which contained 11.7 kb fragment that were deleted in *PIS* goats (Pailhoux et al., 2001). Primers PIS1, PIS2, PIS3, PIS4 and PIS5 were used for detection of the complete or partial deletion of 11.7 kb fragment. If the 11.7 kb fragment was

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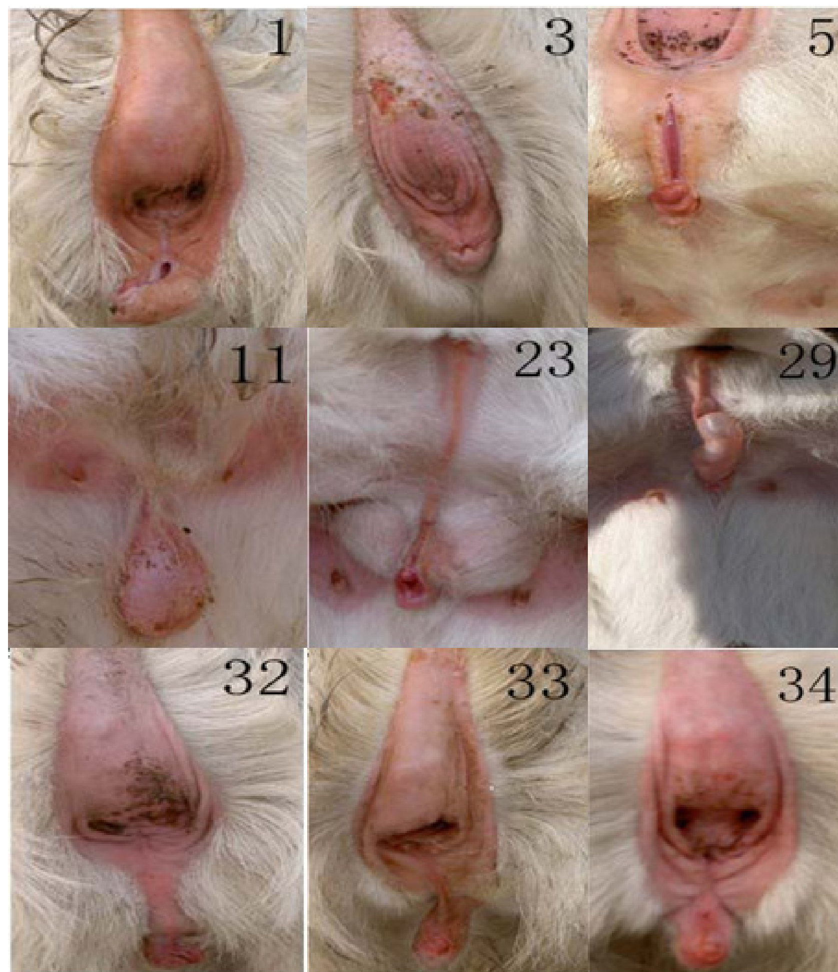

Figure 1. Different types of *PIS* goat.

Table 1. Primers used for variation detection of 11.7 kb fragment.

Primers		Fragment length (bp)	Annealing temperature (℃)
PIS1	F: 5' TCCTGCCTCCTTTCAAAGACAAT 3' R: 5' CAGCAGCAGAAAATTCGACCTAT 3'	739 (26931-27015, 38775-39428) 12498 (26931-39428)	62
PIS2	F: 5' TCCTGCCTCCTTTCAAAGACAAT 3' R: 5' TGGGAAATAGATGGGGATACAGTAG 3'	737 (26931-27667)	68
PIS3	F: 5' CTGTTTCTACTGTATCCCCATCT 3' R: 5' TATGGACTGAGGTTCGTGACAT 3'	1376 (27637-29012)	65
PIS4	F: 5' GGCCTCTTCTTAAAGTCATGT 3' R: 5' CTACACCCTAGATAGAAGGACTAT 3'	897 (38148-39044)	62
PIS5	F: 5' TTTGGCTACTCAGGGTCTTTTGTGT 3' R: 5'TTGCACAGAGAACTCTGCTCAACAT 3'	894 (35532-36425)	62

deleted completely, then the 739 bp fragment will be obtained by PCR amplification of PIS1. Otherwise, no products or 12498 bp would be obtained with PIS1. Meanwhile, the fragments of 737, 1376, 897 and 894 bp by PIS2, PIS3, PIS4 and PIS5 would be obtained if the 11.7 kb fragment was not deleted completely. The primers of PIS2, PIS3, PIS4 and PIS5 were also used for the detection of nucleotide variation within the 11.7 kb fragment between normal and *PIS* goat by sequencing the PCR products.

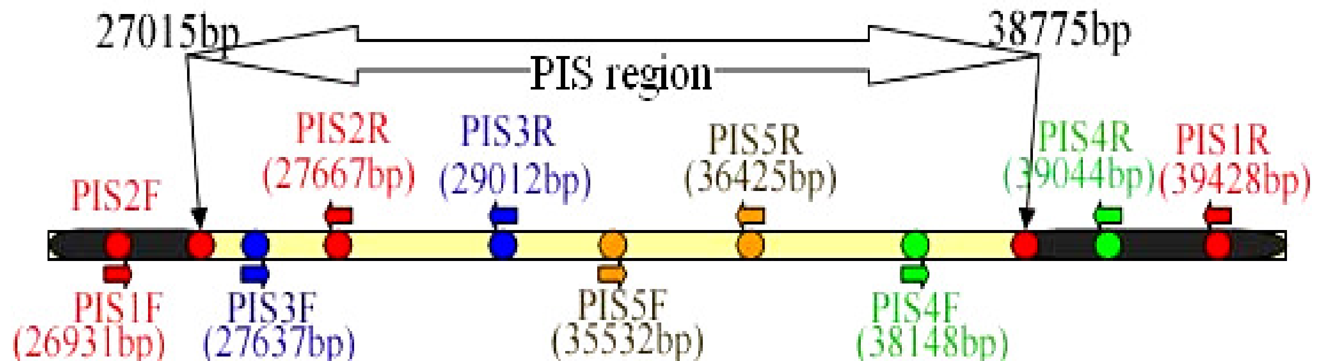

Figure 2. The location of primers to amplify *PIS* region based on AF404302.

PCR was carried out in a Biometra personal PCR instrument with a total volume of 25 μl reaction system containing 1 μl (75 ng/ μl) goat genomic DNA, 1 μl (10 μmol/L) each of forward and reverse primer, 2 μl dNTPs each of deoxynucleotide, 2.5 μl 10×Transtart Taq buffer, 0.3 μl Longtaq polymerase, and 17.2 μl distilled water. The PCR reaction was performed as follows: after predenaturation for 5 min at 94℃, the PCR profile consisted of a denaturation step at 94℃ for 30 s, an annealing step (Table 1) for 30 s, an elongation step at 72℃ for 1 min, followed by an extended elongation at 72℃ for 10 min and 30 cycles. PCR products were detected on 1% agarose gel and were sequenced by Shanghai Sangon Biotechnology Co., Ltd. (China).

RESULTS AND DISCUSSION

Deletion detection of 11.7 kb fragment

Through a positional cloning approach, Pailhoux et al. (2001) demonstrated that the mutation underlying *PIS* is the deletion of a critical 11.7 kb DNA element containing mainly of repetitive sequences, which triggered inter-sexuality and polledness in goats. Our results showed that the 11.7 kb DNA fragment was not deleted completely in *PIS* dairy goat, but was partially deleted within the 11.7 kb fragment. For primer PIS1, no purpose fragment was obtained, indicating that the 11.7 kb fragment was not deleted completely in *PIS* goat. The purpose fragments of 737, 1376 and 894 bp were obtained by PIS2 (Figure 3A), PIS3 (Figure 3B) and PIS5 (Figure 3D), respectively, also indicating that the 11.7 kb fragment was not deleted completely in *PIS* goat. The purpose fragment of 897 bp by PIS4 was not obtained in the *PIS* goats, but appeared in the normal goats (Figure 3C), inferring that this fragment might be deleted or the primer of PIS4 were not homologous with the amplified region. It was also obvious from Figure 3D that the fragment of the normal horned female goat (TN2) was larger than that of the *PIS* goats (individuals 1, 3, 5, 11, 23, 29, 32, 33 and 34), in which 108 bp deletion was proved by sequencing. So, it could be concluded that the 11.7 kb fragment was not deleted completely in the *PIS* goat, but was partially deleted in some regions.

Sequence variation within 11.7 kb fragment

All of the PCR products of PIS2, PIS3 and PIS5 were sequenced to detect variation within 11.7 kb fragment. There was no sequence difference of PIS2 and PIS3 products between the normal dairy goats and the *PIS* dairy goats. For PIS2 products, the *g.A27197T*, *g.A27198C*, *g.T27228C*, *g.C27250T*, *g.A27254T*, and *g.27176_27189del TTCTCCTCTGGCCC* were found in the normal and *PIS* dairy goats when compared with AF404302. For PIS3 products, the *g.27777_27778insC*, *g.A27792G*, *g.G27795T*, *g.G27798T* and *g.C27800T* were detected in the normal and *PIS* dairy goats when compared with AF404302.

The special variations were found for PIS5 within 11.7 kb fragment between the normal and *PIS* dairy goats. First of all, it was very obvious from Figure 4 that there were special 198 base substitutions between the normal and *PIS* dairy goats, forming haplotype A (GTATGAT AACTGTACAGGTCCAACAAGGTCCCTCACAGCGGGT AATCCAAGTTGCATCTAGAAAGAGTGTAACCGAGTGA TGCTATGTACTAGACTTAGTGACAATTAGATGTCGTG TTCTGGTTCCCGGGCGGAGGAGCCTAAATCAGTCTG GAAAAAGGGCACCTTCTTACATCATTACTCTGTGCTT TGTTAAGG) representing normal dairy goats and B (ACTGACCGGAATGTAGTAAATGGTGGTACATTATGA GATATTCTGGTTGGTCATTTCTAGAGTGTTTGTCTTTA ATTCCTAAAGTGAGGTAGAGTAATTCATATGACGACA AGTCAAAGTGTAGGGATTAATCTTATGTATATCCCAT AGACATCTTCTAATTGTTCCTGGTGGGGCGACTCTCC GAAAGGAAACTAT) representing *PIS* dairy goats, respectively. Another special variation between the *PIS* dairy goat and normal goat was the 18 deletions forming 108 bases deletion which only existed in *PIS* dairy goat (Table 2). It could be inferred that the special 198 base substitutions and 18 deletions might be triggered inter-sexuality in the dairy goat.

It could be concluded that the deletion of 11.7 kb fragment in *PIS* dairy goat was not complete but partial. The special 198 base substitutions and 108 bases deletion might be triggered intersexuality in dairy goat.

Figure 3. The amplified result of PIS2 (A), PIS3 (B), PIS4 (C) and PIS5 (D). Individual TN22 and 4 were horned and hornless female goat, respectively. Individual 1, 3, 5, 11, 23, 29, 32, 33 and 34 were *PIS* goats represented in Figure 1.

Figure 4. The mutation sites between normal and *PIS* dairy goats. Individual TN133 are normal hornless buck, TN22 and TN2 are horned and hornless female goats, respectively. Individual 34 represents *PIS* dairy goat.

Table 2. The special deletions existed in *PIS* dairy goat.

S/N	Deletions in *PIS* goat	Deletion number (bp)	Location(bp)
1	ATAAG	5	35617-35621
2	TTTATG	6	35697-35702
3	GA	2	35755-35756
4	CTTCT	5	35774-35778
5	TTCAGTG	7	35786-35792
6	TCTT	4	35823-35826
7	TCTAGTGTAG	10 (The *g.35877_35878insT* and *g.35883_35884insT* existed for normal goat.)	35878-35885
8	AACT	4	35923-35926
9	AATTCT	6	35932-35937
10	TG	2	35981-35982
11	TGTGTGTGT	9	36031-36039
12	AGTGA	5	36056-36060
13	CAGTT	5	36079-36083
14	ACTCAA	6	36101-36106
15	CAA	3 (The *g.36114_36115insC* existed for normal goat.)	36115-36116
16	CCTTTTGCTAT	11	36139-36149
17	TCCATTTTCTT	11	36168-36178
18	TT	2	36184-36185
Total		108	

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