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NUCLEOTIDE COMPARISON OF GDF9 GENE IN INDIAN YAK AND GADDI GOAT: HIGH ALTITUDE LIVESTOCK ANIMALS

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ABSTRACT

The present study was undertaken to characterize exon 1 and exon 2 sequence of one of fecundity genes: GDF9 (Growth differentiation factor 9), in high altitude livestock animal (Yak and Gaddi goat). Six nucleotide differences were identified between sheep (AF078545) and goats (EF446168) in exon 1 and exon 2. Sequencing revealed nine novel single nucleotide mutations in exon 1 and exon 2 of Indian yak that compared with *Bos taurus* (GQ922451). These results preliminarily showed that the GDF9 gene might be a major gene that influences prolificacy of Gaddi goats and Indian yak.

Keywords: GDF9 gene, SNP, Indian yak, gaddi goat

INTRODUCTION

In mammals the ovulation rate and the litter size is a result of well regulated interactions of endocrine and paracrine mediators. How precisely the litter size is controlled remains a critical and important question in reproductive biology. Growth differentiation factor 9 (GDF9) belongs to the transforming growth factor, β super family. It plays a critical role as a growth and differentiation factor during early folliculogenesis in female reproduction in mammals (**Elvin *et al.*, 1999**). GDF9 was mapped to ovine chromosome 5 (**Sadighi *et al.*, 2002**).
GDF9 was reported to be expressed exclusively in the ovary, specifically in the oocyte in mouse (**McGrath *et al.*, 1995; Dube *et al.*, 1998; Lan *et al.*, 2003**), rat (**Jaatinen *et al.*, 1999**), sheep (**Juengel *et al.*, 2002; Bodensteiner *et al.*, 2000**), cattle (**Bodensteiner *et al.*, 1999**), and human (**Vitt *et al.*, 2000**). The structure of the GDF9 gene had been reported in mouse (**Incerti *et al.*, 1994**), rat (**Bodensteiner *et al.*, 1999**), and sheep (**Bodensteiner *et al.*, 1999**). Female mice lacking GDF9 were infertile due to a block in folliculogenesis at the primary follicle stage (**Carabatsos *et al.*, 1998**). **Varani *et al.*, 2000** demonstrated that knockout of pentraxin 3, a downstream target of GDF9, caused female mice subfertility.

In India, Yak (Bos grunniens or Poephagus grunniens) is found in Changecgenmo valley in Ladakh and Spiti valleys of Himachal Pradesh, Jammu and Kashmir (J & K) and the North East states, particularly in Arunachal Pradesh, Sikkim, and Nagaland (**Nivsarkar *et al.*, 1997**) and Gaddi goat is found in Chamba, Kangra, Kulu, Bilaspur, Simla, Kinnaur and Lahaul and Spiti in Himachal Pradesh and Dehradun, Nainital, Tehrigarhwal and Chamoli hill districts in Uttrakhand. The objectives of the present study were firstly to seek for the potential variation through sequence alignment and to detect the polymorphisms in Gaddi goat and Indian Yak.

MATERIAL AND METHODS

The blood samples were collected from jugular vein into EDTA containing vacutainer tubes. Genomic DNA was isolated and purified from the blood cells using the standard phenol,chloroform,isoamyl alcohol extraction followed by ethanol precipitation as described by **Sambrook *et al.*, 1989**. After checking the quality and quantity, DNA was diluted to final concentration of 100 ng/ µl in nuclease free water and stored at 4°C. GDF9 gene maps to chromosome 5 and contains 2 exons (**Bodensteiner *et al.*, 1999**) (Figure 1).

Primers already reported by **Chu *et al.*, 2004** {P1 (Exon 1 F: TTGTAGCTAGGACTGCGTTGG R: GCCTTATAGAGCCTCTTCATGT), P2 (Exon 2 F: GCGGGACAGCCTGTTTAACA, R: TGCGGTGACGGGACGATCTTA)} were used in the present study for amplifying the exon 1 and exon 2 of GDF9 gene. Direct DNA sequencing has been done to identify the genetic variations in Gaddi goats and Indian yaks.
PCR amplifications were performed in a 25 µl reaction volume with approximately 100 ng genomic DNA, 1 U Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), 10X buffer, 1.5 mM $MgCl_2$, 200 µM each dNTP and 10 picomole of each primer. PCR was carried out in Eppendorf thermocycler (AG 22331 Hamburg, Germany). PCR conditions were as follows: 2 min at 95°C, 30 cycles of 94°C for 30s, specific annealing temperature for 30s and 72°C for 30s, with final extension at 72°C at 10 min. Amplified product were checked on 2% agarose gel (w/v) in 1X TAE buffer.

The amplified region was sequenced using DNA Sequencer. Prior to sequencing, PCR products were purified by enzymatic method using exonuclease I and antarctic phosphatase. Raw sequence data were edited using Chromas (Ver. 1.45, http://www.technelysium.com. au=chromas.html). Multiple sequence alignments were performed with MegAlign program of LASERGENE software to identify polymorphisms in the exon 1 and exon 2 of GDF9 gene in indigenous goats and phylogenetic analysis was performed with CLC Main Workbench version 5.0.2.

RESULTS AND DISCUSSION

Genomic DNA of 15 yak and gaddi goats were successfully amplified using primer pairs P1 and P2. The results showed that the sizes of the amplification fragments were consistent with the target fragments and had sufficient specificity to sequence and analyze.

Figure 1 Schematic of the GDF9 gene in Sheep

The sequence analysis of the amplified samples of GDF9 gene in gaddi goat revealed nucleotide substitutions within, exon 1 and exon 2. A total six polymorphisms were identified in caprine GDF9 gene as compared with that of exotic Capra hircus (EF446168). Two SNPs were observed in exon 1 at the position 1893C→T and 1962C→A (heterozygous) and one SNP was observed in exon 2 at the position 3629C→T and all the detail given in table 1. Similar results were also reported by **Ahlawat *et al.* (2012)** in Indian goats, where 2 mutations, viz. 1893C→T and 1962C→A and one 3629C→T were analysed by direct DNA sequencing. Gaddi goats tested had only the wild type genotype and same is true for all these loci with animals. On comparing the sequence of indigenous goats with sheep (AF078545), 5 transitions, 1 transversions in exon 1 and exon 2 (Table 1). ***Li et al.* (2003)** identified an A to G mutation at the 152nd base of GDF9 gene in Hu, Dorset and Suffolk sheep by PCR,SSCP, and this single nucleotide mutation caused an amino acid change (asparagine to aspartic acid). Recently, two variants of sheep GDF9 were reported, which were $FecG^{SI}$ (T1034G mutation of CDS region resulting in Phe27Cys change of mature protein) in Brazilian Santa Ines sheep (**Melo *et al.*, 2008**) and FecTT (A1279C mutation of CDS region resulting in Ser109Arg of mature protein) in Icelandic Thoka sheep (**Nicol *et al.*, 2009**). **Chang *et al.*, 2009** identified G2 mutation of GDF9 gene in Small Tail Han, White Suffolk, Texel and Tibetan sheep by PCR,SSCP.

Table 1 SNP in Exon 1 and Exon 2 of Caprine GDF9 gene

Region	Position	Sheep AF078545	Exotic Goat EF446168	Indian Goat (Gaddi)	Genotype frequency
Exon 1	1893	T	C	T	TT, 1.00
	1962	C	A	C/A	CC,0.50 CA, 0.50
	1978	G	A	A	
Exon 2	3387	G	A	A	AA, 1.00
	3629	C	T	C	CC,1.00
	3631	G	A	A	AA,1.00

The sequences of fragments amplified by primer P1 and P2 of Indian yak. The edited DNA sequences were aligned by CLUSTAL W method by using the LASER GENE software and compare with *Bos taurus* nucleotide sequence (Accession no. GQ922451) and results revealed four nucleotide variations in exon 1 at the position 161G→A, 286G→A, 308 A→G and 312T→A and five nucleotide variations were identified in exon 2 at the positions 894C→T, 1019G→A, 1047T→C, 1060G→C and 1062G→A, respectively. These variations are found to be synonymous in nature as these variations do not result in their corresponding amino acids. SNPs 894C→T and 1019G→A found to be polymorphic and the genotype frequencies are given in table 2. **Hanrahan *et al.*, (2004)** discovered eight variants (G1 to G8) of GDF9 gene in Cambridge and Belclare sheep by PCR,SSCP and sequencing. Three nucleotide changes of the eight polymorphisms did not alter amino acids (G2, G3 and G5). Four G?A mutations of eight SNPs resulted in amino acid changes (G1, G4, G6 and G7) which occurred at a position before the furin processing site or unprocessed protein and were unlikely to affect the mature active coding region. However, G8 variant caused serine to phenylalanine at residue 395 which replaced an uncharged polar amino acid with a nonpolar one at residue 77 of the mature coding region and may change the function of GDF9 in sheep.

Table 2 SNPs in Exon 1 and Exon 2 of *Bos grunniens* GDF9 gene

Region	Position	Exotic cattle (GQ922451)	Indian Yak	Genotype frequency
Exon 1	161	G	A	AA,1.00
	286	G	A	AA,1.00
	894	C	C/T	CC, 0.33 TT,0.67
Exon 2	1019	G	A/G	AA,0.33 GG,0.67
	1047	T	C	CC,1.00
	1060	G	C	CC,1.00
	1062	G	A	AA,1.00

BLAST analysis of exon 1 and exon 2 of gaddi goat revealed homology of 100% with *Capra hircus (Jining grey goat)*, 99% with *Ovis aries,* 94% with *Bos grunniens*, 94% with *Bubalus bubalis*, and 93% with *Bos taurus* and in case of Indian yak homology of 99% with *Bos taurus, 98%* with *Bubalus bubalis, 95%* with *ovis aries, 93%* with *Capra hircus and 96%* with *Moschus berezovskii.*

Phylogenetic analysis of GDF9 gene following UPGMA algorithm revealed that Buffalo, Cattle and Indian yak were found in same group and small ruminants were found in one cluster (Fig 2).

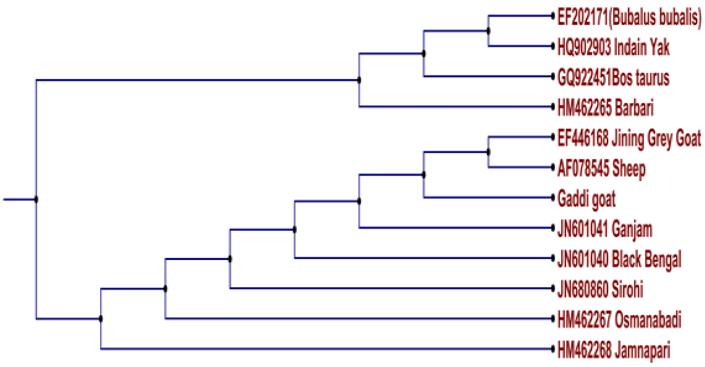

Figure 2 Phylogenetic analysis of GDF9 gene sequence of different species following UPGMA algorithm

CONCLUSION

In conclusion, we can state that in Gaddi goats and Indian yak the GDF9 gene was polymorphic. None of the known polymorphism of fecundity major genes in GDF9 was found in tested Gaddi goats and Indian yak. However, our study tested only a small number of animals presenting this feature and further investigation is required to confirm the link with increased prolificacy in Gaddi goats and Indian yak.

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REFERENCES

AHLAWAT, S., SHARMA, REKHA, MAITRA, A. 2012. Analysis of coding DNA sequence of GDF9 gene in Indian goats for prolificacy associated markers. *Indian Journal Animal Science,* 82 (7), 721–725.

BODENSTEINER, K J., CLAY, C. M., MOELLER, C. L., SAWYER, H. R. 1999. Molecular cloning of the ovine growth differentiation factor 9 gene and expression of growth differentiation factor 9 in ovine and bovine ovaries. *Biology of Reproduction,* 60, 381-386.

BODENSTEINER, K. J., MCNATTY, K. P., CLAY, C. M., MOELLER, C. L., SAWYER, H. R. 2000. Expression of growth and differentiation factor 9 in the ovaries of fetal sheep homozygous or heterozygous for the Inverdale prolificacy gene (FecXI). *Biology of Reproduction,* 62, 1479-1485.

CARABATSOS, M. J., ELVIN, J., MATZUK, M. M., ALBERTINI, D. F. 1998. Characterization of oocyte and follicle development in growth differentiation factor,9,deficient mice. *Devlopmental Biology,* 204(2), 373–384.

CHANG, J. T., LUO, Y. Z., HU, J. 2009. Polymorphism of GDF9 as a candidate gene for fecundity in sheep. *Journal of Gansu Agricultural University,* 44(2), 30–33.

CHU, M. X., LI, B. X., WANG, S. C. YE., FANG, L. 2004. Association between PCR,SSCP of growth differentiation factor9 gene and high prolificacy in small tail Han sheep. *Animal Biotechnology,* 15, 111-120.

DUBE, J. L., WANG, P., ELVIN, J., LYONS, K.M., CELESTE, A. J., MATZUK, M. M. 1998. The bone morphogenetic protein 15 gene is X,linked and expressed in oocytes. *Molecular Endocrinology,*12, 1809–1817.

ELVIN, J.A., CLARK, A. T., WANG, P., WOLFMAN, N. M. MATSUK, M. M. 1999. Paracrine action of growth differentiation factor,9 in mammalian ovary. *Molecular Endocrinology,*13, 1035-1048.

HANRAHAN, J. P., GREGAN, S. M., MULSANT, P., MULLEN, M., DAVIS, G.H., POWELL, R., GALLOWAY, S. M. 2004. Mutations in the genes for oocyte,derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biology of Reproduction,* 70, 900.

INCERTI, B., DONG, J. W., BORSANI, G., MATZUK, M. M. 1994. Structure of the mouse growth differentiation factor 9 gene. In *Biochimica et Biophysica Acta,* 1222(1), 125–128.

JAATINEN, R., LAITINEN, M. P., VUOJOLAINEN, K., AALTONEN, J., LOUHIO, H., HEIKINHEIMO, K., LEHTONEN, E., RITVOS, O. 1999. Localization of growth differentiation factor,9 (GDF,9) mRNA and protein in rat ovaries and cDNA cloning of rat GDF,9 and its novel homolog GDF,9B. *Molecular and Cellular Endocrinology*, 156, 189-193.

JUENGEL, J. L., HUDSON, N. L., HEATH, D. A. SMITH, P., READER, K. L., LAWRENCE, S. B., O'CONNELL, A. R., LAITINEN, M. P., CRANFIELD, M., GROOME, N. P., RITVOS, O., MCNATTY, K. P. 2002. Growth differentiation

factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular development in sheep. *Biology of Reproduction,* 67, 1777-1789.

LAN, Z. J., GU, P., XU, X., JACKSON, K. J., DEMAYO, F. J., O'MALLEY, B. W., COONEY, A. J. 2003. Dependent repression of BMP,15 and GDF,9 mediates gamete regulation of female fertility. *The EMBO Journal,* 22, 4070-4081.

LI, B. X., CHU, M. X., WANG, J. Y. 2003. PCR,SSCP analysis on growth differentiation factor 9 gene in sheep. *Acta Genetica Sinica*, 30(4), 307–310.

MCGRATH, S. A., ESQUELA, A. F., LEE, S. J. 1995. Oocyte specific expression of growth differentiation factor 9. *Molecular Endocrinology,* 9, 131-36.

MELO, E. O., SILVA, B. D., CASTRO, E. A., SILVA, T., PAIVA, S. R., SARTORI, R., FRANCO, M. M., SOUZA, C. J., NEVES, J. P. 2008. A novel mutation in the growth and differentiation factor 9 (GDF9) gene is associated, in homozygosis, with increased ovulation rate in Santa Ines sheep. *Biology of Reproduction,*78, 371.

NICOL, L., BISHOP, S. C., PONG,WONG, R., BENDIXEN, C., HOLM, L. E., RHIND, S. M., MCNEILLY, A. S. 2009. Homozygosity for a single base,pair mutation in the oocyte,specific GDF9 gene results in sterility in Thoka sheep. *Reproduction,* 138, 921–933.

NIVSARKAR, A. N. , GUPTA, S. C. , GUPTA, N. 1997. Yak production. Indian Council of Agricultural Research, New Delhi, p. 382.

SADIGHI, M., BODENSTEINER, K.J., BEATTIE, A. E., GALLOWAY, S. M. 2002. Genetic mapping of ovine growth differentiation factor 9 (GDF9) to sheep chromosome 5. *Animal Genetic*, 33, 244-245.

SAMBROOK, J., FROTSCH, E. F., MANIATIS, T. 1989. Molecular Cloning: A Laboratory Manual, second ed. Cold Spring Harbour, Cold Spring Laboratory Press, NY.

VARANI, S.,ELVIN, J. A., YAN, C., DEMAYO, J., DEMAYO, F. J., HORTON, H. F., BYRNE, M. C., MATZUK, M. M. 2002. Knockout of pentraxin 3, a downstream target of growth differentiation factor 9, causes female subfertility. *Molecular Endocrinology,*16(6), 1154–1167.

VITT, U. A., MCGEE, E. A., HAYASHI, M., HSUEH, A. J. 2000. In vivo treatment with GDF 9 stimulates primordial and primary follicle progression and theca cell marker CYP17 in ovaries of immature rats. *Endocrinology*, 141, 3814-3820.