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Characterization of CSN1S2 locus by PCR-RFLP in Indian goats

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Casein complex in goat displays polymorphism at various loci and is well characterized (Alexander *et al.* 1988, Grosclaude and Martin 1997, Ramunno *et al.*2005). The 3 calcium sensitive caseins α_{s1}-CN,ß - CN and α_{s2}-CN are codded by CSN1S1, CSN2 and CNS1S2 genes, respectively, and K-CN, coded by CSN3, plays a role for casein micelle stabilization (Alexander *et al.* 1988). The two-allele polymorphism (A and B allele) of α_{s2} -casein in goat milk was widely distributed in different goat populations (Russo *et al.* 1986, Boulanger *et al.* 1984). Subsequently allele C was identified by Bouniol *et al.* (1994) and E allele by Lagonigro *et al* (2001) and Veltri *et al.* (2000); F and D alleles by Ramunno *et al.* (2001a). CSNIS2A is the ancestral variant and can lead to B, C F alleles due to intragenic substitutions (Sachhi *et al.* 2005,Caroli *et al.* 2006).The $CSN1S2^A$, $CSN1S2^B$ and $CSN1S2^C$ was associated with about 2.5-g/1 protein content per allele of α_{s2}casein (Boulanger *et al.* 1984,grosclaude *et al.* 1987, Bouniol *et al.* 1994) and $CNSIS2^D$ was associated with lower and $CANIS2^o$ was associated with a null content of such protein (Ramunno *et al.* 2001b). The variability of α_{s2} casein locus has been analysed in 5 different Indian goat breeds by SDS-PAGE (Prakash *et al.* 2002, Rout *et al.* 2004b). However the distribution of allele D and null allele (CSN12°) has not been reported in Indian breeds. Therefore the present study was designed to analyse the variability/presence of D and null allele in Indian goats.

Blood samples (365) were collected from natural habitat belonging to 4 major geographical agro-climatic zones of India, including at least 1 breed from each major geographical region. The sampling was carried out from Jamunapari, the best Indian dairy goat (Rout *et al.* 2004a) and classified as endangered breed (http://dad.fao.org; Rout *et al.* 2000), in their natural habitat at Chakaranagar area of Etawah district of Uttar Pradesh; Jakhrana, high milk yielder from Alwar area of Rajasthan; Barbari, medium size breed of semi-arid zone and known for its adaptability over a wide range agro-climatic situation; Black Bengal, the typical dwarf breed of eastern Indian and known for high prolificacy and meat, quality; Beetal, one of the largest breeds of goat and reared mainly for milk and meat, found in Punjab along the Indo-Pakistani border; Sirohi, a medium to large size breed, best known for meat, milk production and resistant to number of diseases, found in Ajmer, Bhilwara, Tonk, and Jaipur in Rajasthan; Marwari, a medium size breed with compact body and strong legs, found in Bikaner area of Rajasthan; Osmanabadi, medium size breed with comparatively long body and long legs, found in Ahmednagar and Solapur area of Maharashtra;Gaddi, adapted to hilly environment of Himachal Pradesh; Chegu, known for Pashmina production, distributed in high altitude of Lahaul and Spiti valley of Himachal Pradesh, Uttarkashi Chamoli and Pithoragarh districts of Uttrakhand; local goats found in the area adjacent to CIRG, Makhdoom and Hathras area of Uttar Pradesh designated as local UP and from adjacent region of Gwalior and Morena area of Madhya Pradesh designated at local MP. An effort was made to collect samples from unrelated individuals based on the information provided by farmers. DNA was isolated from the samples using the atandard protocol of Thangaraj *et al.* (2002).

Genotyping: The variation at DNA level was analyzed in 12 genetic groups (Table 1). Exon 11 of α_{s2} -casein was amplified using a set of forward and reverse primer (F 5' - GACACATAGAGAAGATTC-3' R 5'- CGTTGGGACATTTTATCT-3 (Ramunno *et al.* 2001a). PCR was carried out in a final volume of 50 µl using following components: 200 µM dNTP, 20 pmol of each primers, 5.0 µl of 10 ×PCR assay buffer, 3mM $MgCl_2$, 0.04% BSA, 1unit to *Taq* DNA polymerase and 100 ng of genomic DNA. Amplification was carried out with the following conditions: initial cycle 97°C for 2 min, 50.6°C for 45 sec and 72 °C for 2.30 min followed by 31 cycles of denaturation at 95°C for 45 sec and 72°C for 2.30 min followed by 31 cycles of denaturation at 95°C for 45 sec, annealing at 50.6°C for 45 sec and extension at 72°C for 2.30 min, and the final extension

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Table 1. Observed genotypic frequencies at CSN1S2 locus in different Indian goat population

Breed/genetic group	No of animals	$CSN1S2^{N*/N*}$
Jamunapari	35	1.000
Barbari	35	0.771
Marwari	35	1.000
Sirohi	17	0.823
Jakhrana	42	0.809
Beetal	20	0.650
Chegu	26	0.880
Local MP	20	0.050
Local. UP	20	1.000
Black Bengal	36	0.583
Gaddi	21	0.714
Osmanabadi	58	0.810

$CSN1S2^{N*/N*}$ N* = A, B, C or E.

at 72°C for 10 min. A 2.0% w/v agarose gel was used to check the amplified product. About 30 µl of PCR product was digested with 10 Units of *NcoI* restriction enzyme overnight at 37°C, in a water-bath.

Genetic variation at CSN1 S2 locus: PCR amplification was carried out in the DNA region spanning from exon 11 of the CNS1S2 gene followed by digestion with *NcoI* enzyme.

PCR amplified product was observed 301 bp: Further digesting PCR product with *Nco*I enzyme exhibited 168 bp+133 bp in all the analysed samples. We did not obtain undigested 301 bp or 133 bp+62 bp in our analyzed samples indicating that neither null allele (CSN1S°) nor D allele (CSN $1S2^{D}$) was present in Indian goats. The molecular analysis at DNA level revealed the absence of D and null allele (O) in Indian population. Genotypic frequency at CSN1S2 locus is presented in Table 1. The absence of amplication of exon 11 in the individuals varies from 17.6% to 95% over the population indicating mutation in the primer annealing region. About 95% of individual of local MP goat did not exhibit the amplication of exon 11of the α_{s2}casein locus. The absence of amplification of exon 11 was observed in significant proportion in Beetal, Black Bengal and Local MP population.

Amplification of exon 11 and digestion with *Nco*I did not show the presence of null (CSN1S2°) allele in the analyzed population. The presence of stop codon at nucleotide 60 of the 11th exon (transition G to A characterizes the null allele and is responsible for the absence of α_{s2}-casein in goat milk. Again complete absence of amplification of exon 11 may be due to insertion or deletion at DNA sequence. It has been also proved that large insertion and deletion are responsible for the absence of α_{s1} casein in goat milk and intragenic recombinatin event possibly responsible for the generation of new variants (Martin *et al.* Bevieacqua *et al.* 2002). The reasons for lack of PCR amplification of the studied fragment in significant proportion of individuals may be due to mutation, which needs further characterization by sequencing of other fragments representing exon 11 and/or intro 10. The high incidence of absence of α_{s2}-casein in local MP goats can be exploited for producing milk with specific nutritional properties (for producing most humanized milk from goats). Such milk could be useful for specific nutritional or dietary purposes or for specific technological processes (Ramunno *et al.* 2001a, Meignanalakshmi 2006). The analysis indicated that Indian goats are carrying ancestral variant at CSN1S2 locus and allels are associated with normal α_{s2}CN synthesis level (Sachhi *et al.* 2005, Caroli *et al.* 2006). This analysis indicated that there was comparatively less substitution at CSN1S2 locus in Indian goats as compared to goats from Italy (Caroli *et al.* 2006).

SUMMARY

An investigation was carried out in 365 DNA samples of 12 Indian goat breeds/genetic groups to characterize the CSN1S2 locus and to observe the frequency of D allele and null (O) at this locus. The molecular analysis at DNA level revealed the absence of D and null allele in Indian goat population. About 95% of individuals of local MP goats did not exhibit the amplification of exon 11 of the α_{s2} -CN locus. The analysis indicated that Indian goats are carrying ancestral variant at CSN1S2 locus and alleles are associated with normal α_{s2}-CN synthesis level.

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