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Akhilesh Pandey
Assistant Prof. Dept. of AGB, Veterinary College, Jabalpur, Madhya Pradesh India

MS Thakur
Assistant Prof. Dept. of AGB Veterinary College, Jabalpur, Madhya Pradesh India

Correspondence
Akhilesh Pandey
Assistant Prof. Dept. of AGB, Veterinary College, Jabalpur, Madhya Pradesh India

Alpha-S1 casein gene polymorphism and association with milk production traits in Malvi and Nimari cattle of Madhya Pradesh

Akhilesh Pandey and MS Thakur

Abstract
The present study was carried out in the Department of Animal Genetics and Breeding of College of Veterinary Science and A H Jabalpur. According to the research finding populations of 50 Malvi and 50 Nimari cattle were found to be monomorphic after PCR-RFLP profile at the αs1-casein gene (CSN1S1) locus. Its monomorphic properties revealed that only one type of uncut banding pattern (AA genotype); which was of 310 bp. No any animal observed with AB and BB genotypes in above two breeds of cattle. The genotypic frequency AA was 100 per cent in screened animals for present investigation in above mentioned both breeds of cattle. The allelic frequency for allele A in the screened animal of Malvi and Nimari cattle was 1.00 and for allele B was 0.00 in investigation. Chi-square values were found to be non- significant in both Malvi and Nimari population, indicating that the populations of these two cattle breeds under were in Hardy-Weinberg equilibrium (HWE) at this locus. Association of αs1-Casein (CSN1S1) gene polymorphic variants with milk production traits revealed that there is no significant difference in the milk production per lactation by the AA genotype of Malvi and Nimari but Nimari showed significantly higher Daily milk yield in AA genotype than Malvi.

Keywords: αs1-casein, Gene, Malvi, Nimari, variants

Introduction
Milk protein is an important and functional nutrient in bovine milk. Casein (Cn) and whey proteins are two major protein groups present in the milk. Casein (Cn) is a family of milk proteins that exists in several molecular forms and is the main protein present in the bovine milk [1]. The Cn genes are linked and inherited as a cluster value and can play an important role in marker assisted selection for milk traits. The caseins constitute approximately 80 per cent of the protein content of bovine milk, out of which 40 per cent αs1 (CSN1S1).The αS1-casein (CSN1S1) is localized in bovine chromosome 6. First demonstration of the genetic polymorphism of Alpha S1 CSN done by starch gel electrophoresis. The two electrophoretic bands were named A and B in order of their decreasing mobility; variant B is diffused in all the species. Mutation in Alpha S1 CSN gene noticed and this mutation gives rise to the same Alpha S1 'A' variant with the deletion of exon 6 [2]. The Alpha S1 CSN gene is present in the region of intron 8, exon 9 and intron 9. They are found to be divergent sequences, with a large number of point mutations [3].
Molecular markers opined for the use to define the genetic makeup (genotype) and predict the performance of animal is a powerful aid in animal breeding [4]. The selection based on these markers is known as Marker Assisted Selection (MAS). Several candidate genes for milk qualities have been studied by various authors. α- S1 casein gene is one such candidate gene found to be associated with milk quality.

Objective
To study the Alpha-s1 (αs1) casein gene polymorphism and their association with milk production traits in Malvi and Nimari cattle of Madhya Pradesh.

Materials and Methods
The present research work was conducted on 100 lactating cows comprising 50 each of Malvi and Nimari cattle from 2016-2017. The data was collected from the animals maintained at the following cattle breeding farms or from home tract of respective breeds of cattle.

Table 1: Details of experimental samples collection

S. No	Breed	No. of Animals	Cattle breeding farms
01	Malvi	50	Government Cattle Breeding Farm, Agar, (MP)
02	Nimari	50	Government Cattle Breeding Farm, Rodia, Khargone (MP)

Identification number, Parity, Lactation length and Lactation yield of each animal under study, were recorded. About 100ml milk sample from each cow were collected in the sterilized tube and mixed with 0.8% formalin and then 5 ml blood sample was collected from same cow in EDTA coated test tube. Collected samples were maintained in cold chain during transportation and in laboratory. In first phase of research the milk samples were processed for Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L) analysis and they were analyzed by Milk analyzer of the Department of Veterinary Medicine, College of Veterinary Science & A.H., Jabalpur.

In second phase of research blood samples were processed and genomic DNA was extracted by modified John *et al.* (1991) method [5] and then Quality of DNA was assessed through 0.80% horizontal submarine agarose gel electrophoresis. The concentration, purity of DNA was checked by Nanodrop Spectrophotometer. The Optical density (OD) value at 260 nm and 280 nm was measured using Nanodrop Spectrophotometer (Nanodrop 1000, Thermo Scientific). DNA samples with an OD 260/280 ratio of 1.70 to 1.90 were considered further subjected to agarose gel electrophoresis for quality check. The DNA concentration was determined and samples were diluted up to approximate 30 ng/µl for final concentration with sterile nuclease free water (MiliQ) for further use. The casein gene primers were used for the amplification of PCR product as described in following table02.

Table 2

1	αs1- casein	(F): 5-TGCATGTTCTCATAATAACC-3 (R): 5-GAAGAAGCAGCAAGCTGG-3	310 bp	Mir *et al.* (2014)

A master mix for desired number of samples was prepared and liquated 22 µl in each PCR tube. 3 µl genomic DNA (30 ng/pl) was added in each tube to make the final volume 25 µl. The PCR tubes were kept in a preprogrammed thermo cycler (Mastercycler gradient, Eppendorf) and set at the standardized reaction programme.

Table 3

1. Initial denaturation	94	05 min
2. Final denaturation	94	01 min
3. Annealing	54.5	01 min
4. Extension	72	01 min
Repeat cycle 2 to 4 for 35 times		
5. Final Extension	72	10 min

To confirm the targeted PCR amplification the PCR products were analyzed on 2.00 % agarose gel (5 µl of PCR product mixed with 1 µl of gel loading dye). The electrophoresis at constant voltage of 90 volt for 50 minutes at 37˚C using 0.5X TBE buffer was conducted. The mass ruler DNA ladder (100 bp- 1000 bp) as a molecular size marker was used for sizing of the DNA bands. In PCR-RFLP profile the following enzyme was used.

Table 4

S. No.	casein Gene	Restriction Enzyme	Restriction Site	Base Pair	RFLP product
01	αs1	*HindIII*	5'….A AGCTT…..3' 3'….TTCGA A……5'	310bp	310bp

Digested PCR products were analyzed on 2.50 % agarose gel (5 µl of PCR product mixed with 1 µl of gel loading dye). The electrophoresis at constant voltage of 90 volt for 50 minutes at 37˚C using 0.5X TBE buffer was conducted. The mass ruler DNA ladder (100 bp- 1000 bp) as a molecular size marker was used for sizing of the DNA bands.

Gene and genotype frequencies for different casein genes under study were estimated using Popgene 32 (version1.32), microsoft Windows-based freeware for population genetic analysis [6]. Association study of various polymorphic variants of milk protein genes for Milk yield (MY), Daily milk yield (DMY), Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L) data were subjected to least squares analysis of variance employing linear model:

The chi-square test (x^2) was employed to test the status of Hardy-Weinberg equilibrium (HWE) in the different population of four breeds of cattle [7].

Results and Discussion

Gene and Genotypic frequencies at αs1-casein (CSN1S1) gene locus in different breeds of cattle

The genotypic and allelic frequencies of αs1-casein gene (CSN1S1) in Malvi and Nimari cattle are presented in table 05. In the present study, all the screened populations of Malvi (50) and Nimari (50) revealed that the genotypic frequency AA was 100 per cent. The allelic frequency for allele A in the screened animals of Malvi and Nimari cattle was 1.00 and for allele B was 0.00(Plate 01 and 02)

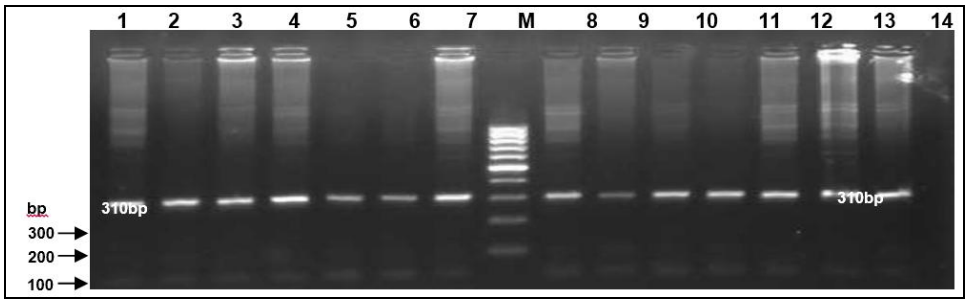

Plate 1: RFLP product of αS1 gene of Malvi cow, electrophoresed on 2.5% agarose gel, M: 100bp DNA ladder, Lanes: 1-14

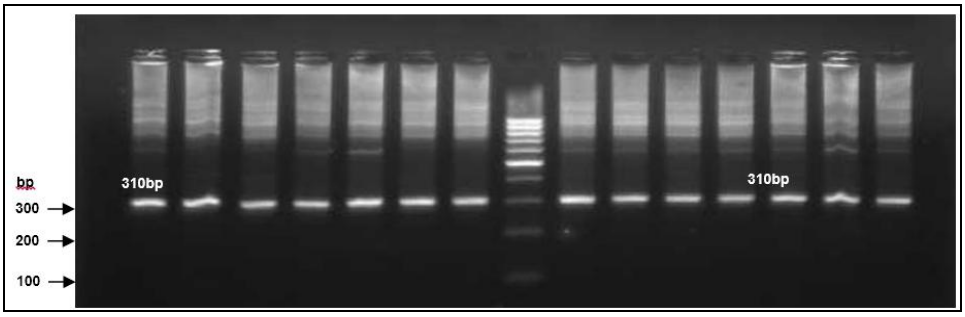

Plate 2: RFLP product of αS1 gene of Nimari cow, electrophoresed on 2.5% agarose gel, M: 100bp DNA ladder, Lanes: 1-14

Table 5: Distribution of gene and genotypic frequency of αs1-casein (CSN1S1) variants in different breeds of cattle

Breeds	Genotype Frequencies			Chi – square (χ^2) value	Gene Frequencies	
	AA	AB	BB		A	B
Malvi	1.00 (50)	0.00 (0)	0.00 (0)	0.00^{NS}	1.00	0.00
Nimari	1.00 (50)	0.00 (0)	0.00 (0)	0.00^{NS}	1.00	0.00

NS-Non-significant

Chi-square values for testing correspondence between observed and expected genotypic frequencies at this locus were found to be non- significant in Malvi, and Nimari cattle, indicating that the populations of these two cattle breeds under study were in Hardy-Weinberg equilibrium at this locus (Table 5).

Szymanowsky *et al.* (2004) [8], Judu *et al.* (2009) [9] and McLean *et al.* (1987) [10] also reported the higher frequency of AA genotype in Polish Red (PR) and Black-and-White (BW) cattle. Contrary to above findings Caroli *et al.* (2008) [11] reported predominance of B allele over C allele in HF, Brazilian Zebu cattle and Carora cattle, respectively.

Non-significant Chi-square values revealed that the populations of Malvi and Nimari breeds of cattle with respect to various genotypes were in HWE at this locus. In accordance to above findings Golijow *et al.* (1999) [12] reported that the populations of Argentine Creole and Argentine Holstein were found in HWE for αs1-casein gene (CSN1S1) loci. This fact indicates that the artificial selection is not disturbing the equilibrium of gene frequencies in the milk production related loci in the four breeds of cattle.

Association of αs1-Casein (CSN1S1) gene polymorphic variants with Milk yield

Milk Yield (MY) in different breeds of cattle

The source of variation, mean sum of squares along with F-value for above mentioned breeds was noticed significant. The effect of breed was found significant (P<0.01) for MY trait. The mean MY per lactation in Malvi and Nimari cattle has been presented in table 6.

Only AA genotype was observed in all the animals of Malvi and Nimari. The mean MY per lactation (L) of AA genotyped animals were found be 1035.10±36.40 and 952.50±24.70 respectively in Malvi and Nimari cattle. Among the both breeds of cattle, significantly higher mean MY was recorded in Malvi and comparatively lower in Nimari breed of cattle (Table 06). Contrary to these findings Hristov *et al.* (2014) [13] showed that the BB genotype determines higher milk production.

Table 6: Mean of Milk Yield per lactation (L) in different breeds of cattle at αs1-Casein (CSN1S1) gene locus.

Genotypes	Malvi	Nimari
AA	1035.10^{c}±36.40 (50)	952.50^{c} ±24.70 (50)
AB	0.00±0.00 (0)	0.00±0.00 (0)
BB	0.00±0.00 (0)	0.00±0.00 (0)
Overall	1035.10^{c}±36.40 (50)	952.50^{c} ±24.70 (50)

Means bearing the different superscript differ significantly (p<0.01), Numbers in the parentheses denotes number of animals

Daily Milk Yield (DMY) in different breeds of cattle

The effect of breed was found highly significant (p<0.01) for DMY trait. The mean DMY (L) in Malvi, Nimari, Sahiwal and HF crossbred cattle has been presented in table 07.

Table 7: Mean of Daily Milk Yield (L) in different Breeds at αs1-Casein (CSN1S1) gene locus.

Genotypes	Malvi	Nimari
AA	3.40^{d}±0.78 (50)	4.77^{c}±0.14(50)
AB	0.00±00 (00)	0.00±00 (00)
BB	0.00±00 (00)	0.00±00 (00)
Overall	3.40^{d}±0.78 (50)	4.77^{c}±0.14 (50)

Means bearing the different superscript differ significantly ($p<0.05$), Values in parentheses are number of animals.

The mean DMY (L) for Malvi and Nimari cattle were found to be 3.40±0.78 and 4.77^{c}±0.14 liters, respectively. The significantly higher DMY was noticed in Nimari cattle compared to Malvi breed of cattle (Table 07). The results revealed in the present study are in accordance as reported by Szymanowska *et al.* (2004) [8] in Polish Black and White cattle.

Conclusion

PCR-RFLP profile of 50 Malvi and 50 Nimari cattle at the αs1-casein gene (CSN1S1) locus revealed that only one type of uncut banding pattern (AA genotype) which was of 310 bp. No any animal observed with AB and BB genotypes in above two breeds of cattle. The genotypic frequency of AA was 100 per cent in screened animals for present investigation. The allelic frequency for allele A in the screened animal of Malvi and Nimari cattle was 1.00 and for allele B was 0.00 in the investigation. Chi-square values were found to be non-significant in both Malvi and Nimari population, indicating that the populations of these two cattle breeds under were in Hardy-Weinberg equilibrium (HWE) at this locus. Association of αs1-Casein (CSN1S1) gene polymorphic variants with milk production traits revealed that there is no significant difference in the milk production per lactation by the AA genotype of Malvi and Nimari but Nimari showed significantly higher Daily milk yield in AA genotype than Malvi breed of cow.

References

1. Alipanah M, Alexandrovna K**,** Veladimirovich RG. Kappa casein and PRL-RSAI genotypic frequencies in two Russian cattle breeds. Archivos De Zootecnia. 2005; 57:131-138.
2. Wilkins RJ, Xie Tao. Two distinct gene mutations - one milk proteins polymorphisms. The example of the alpha s1 casein A variant. International Dairy Federation. 1997; 9702:330-333.
3. Ferranti P, Scaloni A, Caira S, Chianese L, Malorni A, Addeo F. The primary structure of water buffalo alpha (s1)- and beta- casein identification of phosphorylation sites and characterisation of a novel beta- casein variant. Journal of Protein Chemistry. 1998; 17:835-844.
4. Beuzen ND, Stear MJ, Chang KC. Molecular markers and their use in animal breeding. The Veterinary Journal. 2000; 160:42-52.
5. John SW, Weitzner G, Rozen R, Scriver CR. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acid Research. 1991; 19(2):408.
6. Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. Popgene 32 version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada. 1999, 1-28.
7. Snedecor GW, Cochran WG. Statistical method 8th edn. The lowa State College Press, Inc. Amer. lowa USA. 1994, 950.
8. Szymanowsky M, Eulalia S, Marek L, Lech Z. Association of nucleotide-sequence polymorphism in the 5'-flanking regions of bovine casein genes with casein content in cow's milk. INRAEDP Sciences. 2004; 84:579-590.
9. Judu I, Merike H, Sirje V, Haldja V, Pssa T, Kaart T *et al*. The effect of milk proteins on milk coagulation properties in estonian dairy breeds. Veterinarija Medicine Zootechnika. 1991; 46(68):14-19.
10. Mclean DM, Graham ERB, Ponzoni RQ, Mckenzie HA. Effects of milk protein genetic variants and composition on heat stability of milk. Journal of Dairy Research. 1987; 54:219-235.
11. Caroli S, Chessa F, Chiatti D, Rignanese B, Melendez R, Rizzi GC. Carora cattle show high variability in *α*s1-casein. Journal of Dairy Science. 2008; 91(1):354-359.
12. Golijow CD, Giovambattista G, Rípoli MV, Dulout FN, Lojo MM. Genetic variability and population structure in loci related to milk production traits in native Argentine Creole and commercial Argentine Holstein cattle. Genetics and Molecular Biology. 1999; 22(3):395-398.
13. Hristov P, Neov B, Sbirkova H, Teofanova D, Radoslavov G, Shivachev B. Genetic polymorphism of kappa casein and casein micelle size in the bulgarian rhodopean cattle breed. Biotechnology in Animal Husbandry. 2014; 30(4):561-570.