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Status of milk protein, ß-casein variants among Indian milch animals

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ABSTRACT

The role of A1 and A2 beta casein milk variants and human health is a matter of concern for scientific investigations. The status of A1/A2 beta casein variants in *Bos taurus* cattle breeds from different countries have shown presence of A1 variant in European cattle, which has been linked to range of illness. However, no data on beta casein A1/A2 frequency is available on diverse Indian cattle (*Bos indicus*) breeds. Also no report is available on river buffalo breeds which contribute more than 55% of total milk produced in the country. In this study we report the frequency of beta casein variants among 618 animals of 15 zebu cattle breeds and 231 buffaloes of 8 river buffalo breeds. The beta casein A1/A2 frequency data indicated the predominance of A2 variant (0.987) in zebu cattle breeds while the river buffalo indicated only A2 milk type. The results point towards the origin of A2 variant in *Bos indicus* cattle. This is the first report of A1/A2 milk variant from majority of Indian zebu cattle and riverine buffaso breeds.

Key words: ß-casein variants, Milk protein, Milch animals

Cows' milk contains two major protein groups: caseins and whey proteins and out of which caseins account for 80% of milk proteins (Niki *et al.* 1994, Marten *et al.* 1994). ß-casein, a major protein is the second most abundant protein in cow's milk that contains 209 amino acids. Bovine beta casein (CASB) gene belongs to the cluster of 4 casein genes: alpha s1, alpha s2, beta and kappa, located on chromosome 6 (Rijnkels 2002).

There are 12 genetic variants of beta-casein: A1, A2, A3, B, C, D, E, F, H1, H2, I, and G out of which A1, A2 and B are the most common genetic variants (Farell *et al.* 2004).

The A1 and A2 variants differ only at amino acid position 67, which is histidine in A1 or proline in A2 milk. Another variant B also has histidine at position 67 but is less frequent than A1 or A2 genetic variants. The natural mutation that gave rise to this difference is a result of a single nucleotide polymorphism at codon 67 of the beta casein gene: CCT (A2, proline)- CAT (A1, histidine). This difference in amino acid sequence suggests a conformational difference in the secondary structure of the expressed protein (Eliott 1999, Mc Lachlan 2001). A bioactive seven-amino-acid peptide called ß-casomorphin-7 (BCM-7) is released in the small intestine by digestion of A1 ß- casein milk while proline in A2 milk at position 67 prevents a split at this site (Roginski 2003, & Kostya *et al.* 2004).

The bioactive peptide beta-casomorphin-7 (BCM-7), a strong opioid is released *in-vitro* by the successive gastrointestinal protelytic digestion of beta-casein A1 and B but not by *A2* (Hartwig *et al.* 1997, Elliot *et al.* 1999). A1ß-casein cow milk has been associated as a risk factor for type I diabetes (DM-I), coronary heart disease (CHD), arteriosclerosis, and sudden infant death syndrome (Langersen and Elliot 2003, Sun *et al.* 2003, Tailford *et al.* 2003, Truswell 2005).

The hypothesis that high consumption of A1 ß-casein increases the risk of several disease syndromes is very intriguing and interesting for basic as well as application studies and potentially very important for population health. The A2 Corporation was setup in New Zealand in the late 1990s to test cows and market A2 milk at premium price in several countries which appeared not to have the disadvantage and health issues associated with A1 milk.

Genetic characterization of ß-casein variants for the majority of *Bos taurus* breeds indicated carrier of A1 casein variants in most of the dairy breeds except few taurine breeds like Jersey, Gurensy and othekrs having A2 milk variant (Kaminski *et al.* 2007). It was hypothesized that the A2 ß-casein variant is most probably of *Bos indicus* origin. There is no comprehensive genetic data on A1/A2 ß-casein variants of zebu cattle and river buffalo breeds. In the present study

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we report the first ever comprehensive analysis of wide diversity of indigenous zebu cattle and river buffalo breeds from various agroclimatic regions of India to delineate the frequency of A1/A2 ß-casein milk variants.

MATERIALS AND METHODS

A total of 618 animals representing 15 different breeds of Indian zebu cattle from different agro-climatic regions of India along with 231 animals representing 8 different river buffalo breeds were genotyped in beta-casein (CSN2) locus by PCR-RFLP method using primers reported by Lien *et al.* (1992). Blood samples were collected from unrelated animals of each breed from their respective breeding tracts. Genomic DNA was isolated using the standard protocol of SDS-proteinase K digestion followed by phenol: chloroform extraction (Sambrook *et al.* 1989).

The PCR-RELP genotyping protocol for distinguishing A1 and A2 ß-casein variants is similar to that reported by Lien *et al.* (1992) and Kaminski *et al.* (2006). The primer pair reported by Lien *et al.* (1992); CASB 122 L and CASB 67 R were used to amplify the 251 bp fragment of exon 7 of CSN2 gene. PCR was performed in a reaction volume of 25µl with 150-200ng of genomic DNA, 1 unit of Taq DNA polymerase, 1.5 mM $MgCl_2$, 200 µg each dNTPs and 5 pmol of each primer. PCR was carried out in cycler machine with annealing temperature of 63°C and products were evaluated after electrophoresis in ethidium bromide stained 1.5% agarose. PCR products were purified by ethanol precipitation and then digested with 5units of *Taq*-I restriction enzyme at 65°C for 3 h. The digested products were separated by electrophoresis on 3.5% agarose gel in 1X TAE buffer.

RESULTS AND DISCUSSION

Amongst the 618 animals of 15 different Indian cattle breeds screened, two genotypes (A2A2 and A1 A2) were observed and not a single animals had A1A1 genotype. The genotype and allele frequencies of CASB (A1A2)/*Taq*-I variants in each of the 15 Indian cattle breeds analyzed are presented in Table 1. The CASB (A1A2 variants/ *Taq*-I polymorphism in 231 river buffalo individuals, only one allele (A2) and one genotype (A2A2) were observed (Table 2).

Two DNA restriction fragments corresponding to A1A2 genotype (251 and 213 bp) were observed while one single band of 251 bp was observed for A2A2 genotype (Fig. 1). The reported A1A1 genotype with 2 bands (213 and 38 bp) was absent in all the Indian cattle screened for this locus.

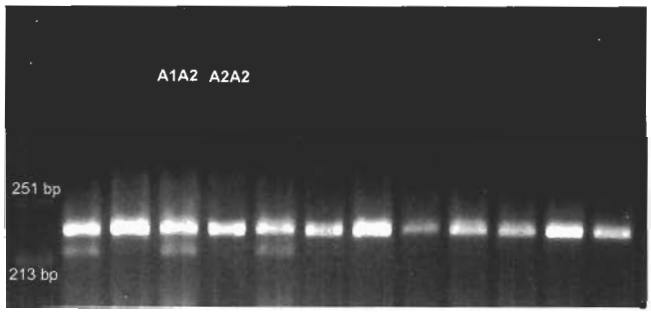

Fig. 1. PCR-RELP of ß-casein (CASB) *Taq* 1 showing A2A2 and A1A2 genotypes in *Bos indicus* cattle samples.

Out of 618 animals belonging to 15 cattle breeds screened for ß-casein variants only few animals from 2 breeds namely, Malnad Gidda and Kherigarh showed the presence of A1A2 genotype with genotypic frequency of 0.191 and 0.218

Table 1. Allelic and genotypic frequency at ß-casein (A1A2 variant/*Taq*-I} across the Indian cattle breeds

Agro-climatic zone	Cattle breed	Utility type	Sample no. (N)	Allelic frequency		Genotype frequency		
				A1	A2	A1A1	A1A2	A2A2
Tropical wet	Kangayam	Draught	48	0	1	0	0	1
Tropical wet and dry	Nimari	Draught	45	0	1	0	0	1
	Red Kandhari	Draught	39	0	1	0	0	1
Humid sub tropical	Malnad Gidda	Draught	47	0.096	0.904	0	0.191	0.809
	Kherigarh	Draught	23	0.109	0.891	0	0.218	0.783
Semi arid or steppe	Malvi	Draught	44	0	1	0	0	1
	Amritmahal	Draught	37	0	1	0	0	1
	Kankrej	Milch	32	0	1	0	0	1
	Gir	Milch	45	0	1	0	0	1
	Sahiwal	Milch	47	0	1	0	0	1
	Hariana	Dual	48	0	1	0	0	1
Desert or arid	Tharparkar	Dual	44	0	1	0	0	1
	Rathi	Milch	46	0	1	0	0	1
	Mewati	Dual	40	0	1	0	0	1
	Red Sindhi	Milch	33	0	1	0	0	1
	Mean		618	0.013	0.987	0	0.026	0.974

Table 2. Allelic and genotypic frequency across Indian buffalo breeds

Buffalo breed	Geographical	Animal No. (N)	CASB A1/A2 Variants				
			Allelic frequency		Goegraphical frequency		
			A1	A2	A1A1	A1A2	A2A2
Murrah	North	22	0	1	0	0	1
Mehsana	West	49	0	1	0	0	1
Marathwada	Central	40	0	1	0	0	1
South Kanara	South	10	0	1	0	0	1
Mainpur	North east	40	0	1	0	0	1
Assamese swamp	North east	40	0	1	0	0	1
Nilli Ravi	North west	22	0	1	0	0	1
Pandharpuri	West	8	0	1	0	0	1
Total		231	–	1	–	–	1

respectively. The mean frequency of A2A2 and A1A2 genotypes for all *Bos indicus* cattle breeds was 0.974 and 0.026 respectively while the mean A1 and A2 allele frequency was 0.013 and 0.987 indicating preponderance or near fixation of A2 ß-casein variant in zebu cattle (Table 1).

Amongst the 231 river buffaloes belonging to 8 breeds/ populations, only one genotype A2A2 with a gene and genotypic frequency of 1 was observed (Table 2). Thus our results for the first time report the absence of undesirable A1 allele and fixation of desirable ß-casein A2 allele in Indian river buffalo.

Till date, no data has been published on the ß-casein allele frequency in *Bos indicus* cattle breeds. The frequency of the CSN2 alleles in various *Bos taurus* cattle breeds of different

Table 3. Occurrence of ß-casein gene variants in various cattle breeds and countries (Kaminski et al. 2007)

Breed	Country	No. of animals	Frequency of beta-casein alleles			References
			B	A1	A2	
Guernesy	USA	400		0.010		Swaissgood 1992
	USA	3861	0.010–0.020	0.010–0.060	0.880–0.970	Enennam *et al.* 1991
Jersey	Germany	43	0.186	0.093	0.721	Ehrmann *et al.* 1997
	Denmark	157	0.350	0.070	0.580–0.650	Bech *et al.* 1990
	New Zealand	1328	–	0.123	0.591	Winkelman and Wickham 1997
	USA	387	0.290–0.370	0.090–0.220	0.490–0.540	Eenennam *et al.* 1991
Brown	Germany	232	0.170	0.108	0.705	Ehrmann *et al.* 197
Swedish	USA	282	0.100–0.180	0.140–0.150	0.660–0.720	Swaissgood 1992
	USA	259	0.100–0.180	0.140–0.180	0.660–0.720	Eenennam *et al.* 1991
Simmental	Croatia	621	0.150	0.190	0.630	Curik *et al.* 1997
	Germany	229	–	0.343	0.566	Ehramann *et al.* 1997
HF	USA	526	0.010–0.060	0.310–0.660	0.240–0.620	Swaissgood 1992
	USA	6000	0.010–0.040	0.310–0.490	0.490–0.620	Eenennam *et al.* 1991
	Hungary	768	0.107	0.418	0.470	Baranyi *et al.* 1997
	Germany	229	0.026	0.472	0.496	Ehrmann *et al.* 1997
	Poland	143	–	0.402	0.598	Kaminski *et al.* 2006a
	New Zealand	3761	–	0.465	0.510	Winkelman *et al.* 1997
	Norway	306	–	0.400	0.490	Lien *et al.* 1993
Black-and-White	Denmark	223	0.030–0.080	0.550	0.390	Bech *et al.* 1990
Red-and-White	Sweden	394	0.008	0.460	0.531	Lunden *et al.* 1997
	Germany	179	0.020	0.573	0.366	Ehrmann *et al.* 1997
Ayrshire	New Zealand	37	–	0.432	0.527	Winkelman and Wickham 1997
	Finland	686	0.001	0.509	0.490	Ikonen 1997
	United Kingdom	29	0–0.003	0.600	0.400	Swaissgood 1992
	USA	45	0	0.720	0.280	Swaissgood 1992
	Red Denmark	169	0.044–0.060	0.710	0.230	Bech *et al.* 1990

countries worldwide is depicted in Table 3.

This is the first report of ß-casein A1/A1 allele frequency data on Indian zebu cattle breeds which indicates the absence of A1/A1 genotype among investigated breeds and also near fixation of A2 ß-casein variant in Indian cattle breeds except for very low frequency of A1/A2 heterozygotes in Malnad Gidda and Kherigarh cattle population. As these two cattle populations are lesser known and crossbreeding with taurine blood could be the possible reason for appearance of A1A2 genotype in few animals. The data on allelic frequency of A1/A2 genotype in Indian cattle breeds *(Bos indicus)* is in contrast to the data on allelic frequency of undesirable A1 allele, ranging from 0.01 to 0.72 throughout the world (Kaminski *et al.* 2007).

Interestingly, the Indian native milch breeds investigated in the present study, viz. Gir, Tharparkar, Rathi, Red Sindhi, Sahiwal and dual purpose breeds like Kankrej and Hariana indicated presence of only A2 variant which has been the favoured ß-casein variant in cow milk.

There is considerable uncertainty associated with the issue of A1 versus A2 milk. The A1/A2 hypothesis is both intriguing and potentially very important for human health if it is proved correct. Although, the clinical implications of A1 milk on human health is still under discussion, but it should be taken seriously and further confirmation of earlier reports is required. It will also be essential to screen the crossbred and purebred taurine (exotic) cattle in India for A1 variant, which are being used in large scale crossbreeding programme for milk productivity enhancement. The presence of A2 variant in Indian cattle and river buffalo breeds will have an edge over the crossbred/taurine milk having A1 variant. Since crossbred milk constitutes a major part of total milk produced in India, the screening of AI bulls amongst crossbred and exotic taurine cattle populations is an immediate task to draw a sound breeding policy to minimize the risk of A1 milk.

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