Indian J. Plant Genet. Resour. 35(3): 269–278 (2022)
DOI 10.5958/0976-1926.2022.00081.X

A1/A2 Milk Research in Indian Cattle

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Bovine beta casein A1 and A2 are the most common variants in cattle breeds. Because of the bioactive peptide beta casomorphin-7 (BCM7) produced by raw or processed A1-milk, which has high affinity for opioid receptors and can exert regulatory activities, A1 variant is considered a risk factor for many human diseases, but not by A2-milk, during digestion. The frequency of A1/A2 allele of beta casein is breed dependent and can be very easily checked with simple PCR-RFLP technique. The aim of this article was to review the different studies and assess the status of A1/A2 in Indian native cattle breeds as well as crossbred/taurine populations and breeding bulls being used at different AI centres in India. Analysis of frequency pattern clearly indicated that all Indian native cattle breeds have high frequency of A2 allele and homozygous A2A2 genotype and hence are very good resource for A2 milk. Crossbred, taurine cattle populations and breeding bulls being used at different AI centres have a higher frequency of A2 allele and A1A2 genotype. The percentage of animals with homozygous A2A2 genotype is low. This suggests the need for screening of crossbred/exotic bulls being used in A1 and modify the existing breeding policy so as to drift the herds towards A2.

Introduction

Milk from a variety of livestock species and cow in particular has been included in the diet for infants, children and adults worldwide since it is the most common source of animal proteins and microelements known to be essential for human nutrition. Worldwide, the different sources of milk are cow, buffalo, goat, sheep and camel contributing 85%, 11%, 2%, 1.4% and 0.2% respectively to the total world milk production. With a herd capacity of 264 million cows contribute highest to the total milk production that is 600 million tons every year. Cow milk contains various components like lipids, proteins, amino acids, vitamins, minerals, immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and other bioactive peptides with physiological functionality. It also provides a high quality source of energy, proteins and selected micronutrients such as calcium, magnesium, zinc and phosphorus to most human population. Cow milk generally contains about 3.5 % protein, of which approximately 80 % are caseins and 20 % are whey proteins. More than 95% of the cow milk proteins are constituted by caseins (*αS1-*, *αS2-*, β-, *k-CN*) and whey (*α-LA* and β-*LG*) genes. Milk and milk products are considered as functional foods as digestion of different components of cow milk specially proteins lead to the formation of biologically active molecules that can have a direct and significant effect on health (Marshall 2004) including digestive functions, metabolic responses to absorbed nutrients, growth and development of organs or different diseases.

Beta Casein Variants

Among the caseins, beta casein, the second most abundant protein with an excellent nutritional balance of amino acids is a rich source of such bioactive peptides. It holds special significance as the bioactive peptides may exert regulatory activities in the human beyond nutrition and also act as promoters of different physiological functions (Kostyra *et al*., 2004; Silva and Malcata, 2005). The bovine beta casein (*β-CN*) gene spanning over a region of 8.5 kb, has nine exons and different mutations have led to formation of 15 genetic variants (A1, A2, A3, B, C, D, E, F, G, H1, H2, I, J, K and L). These variants hold importance as digestion of β-casein with different variants not only result in generation of different group of active peptides (Nguyena *et al*., 2015), but also has an influence on milk protein composition and milk-production traits according to its genetic polymorphism (Visker *et al*., 2011).

Among these variants, A1 and A2 are the most common genetic variants, with A2 being the ancestral allele/variant of the gene. These variants differ at amino acid position 67 corresponding to single nucleotide difference cytosine to adenine. β-casein allele A2 has cytosine at position 8101, codon C<u>C</u>T corresponding

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to amino acid proline at position 67. In variant A1, conversion of cytosine to adenine leading to codon change from CCT to CAT replaces proline with histidine at position 67. Based on this A1/A2 variation, milk is generally referred as A1 or A2 milk. On the basis of common variation of Pro67His change, the variants, A1, B, C, F or G having a common amino acid histidine (His) at position 67 (–Tyr60– Pro61–Phe62–Pro63–Gly64–Pro65–Ile66–His67–), but variations at other positions of amino acids are called A1 type. Similarly, variants A2, A3, D, H1, H2 and I alleles having a common amino acid proline (Pro) at position 67 (–Tyr60–Pro61–Phe62–Pro63–Gly64–Pro65–Ile66–Pro67), but variations at other positions are categorized as A2 type.

Bioactive Peptides from Beta Casein A1/A2 Variants

A1 and A2 variants of bovine β-casein, generally called A1/A2 milk differ at amino acid position 67 with histidine in A1variant/A1milk and proline in A2 variant/milk. This polymorphism leads to key conformational changes in the secondary structure of expressed β-casein protein. Because of the structural differences, the bioactive peptides generated upon digestion of A1/A2 beta casein are different. Gastrointestinal proteolytic digestion (leucine aminopeptidase, elastase and carboxypeptidase Y) of A1 β-casein (raw/processed milk) releases a 7 amino acid bioactive peptide 'opioid' called beta-casomorphin 7 (BCM-7) (Fig 1) in small intestine, while proline in A2 β-casein at 67 position prevents the split at this particular site and generates nine amino acid peptide BCM-9 (Kostyra *et al*., 2004; De Noni 2008). Release of BCM-7 from A2 β-casein is minimal under normal gut conditions. In hydrolysed milk with A1 beta-casein variant, BCM-7 level is 4-fold higher than in A2 milk. Further, BCM-7 is released not only from milk but also from almost all milk product including yoghurt and cheese, infant formulas

Beta-casomorphin suggest the peptide from beta casein with opiate properties similar to morphine, that includes affinity to opioid receptors, especially the MOP (μ-opioid receptor). The beta-casomorphine-7 has been identified as the "atypical" opioid peptide and exerts its influence on nervous, digestive, and immune functions via the MOR. In addition to BCM7/9, digestion of beta casein results in the release of other encrypted casomorphins including the non-opioid peptides (BCM5, BCM11, Immunopeptides). The BCM-9 is also an opioid agonist but with lesser affinity for μ-opioid receptor. The BCM-5, which is the more potent than BCM-7 and BCM-9,

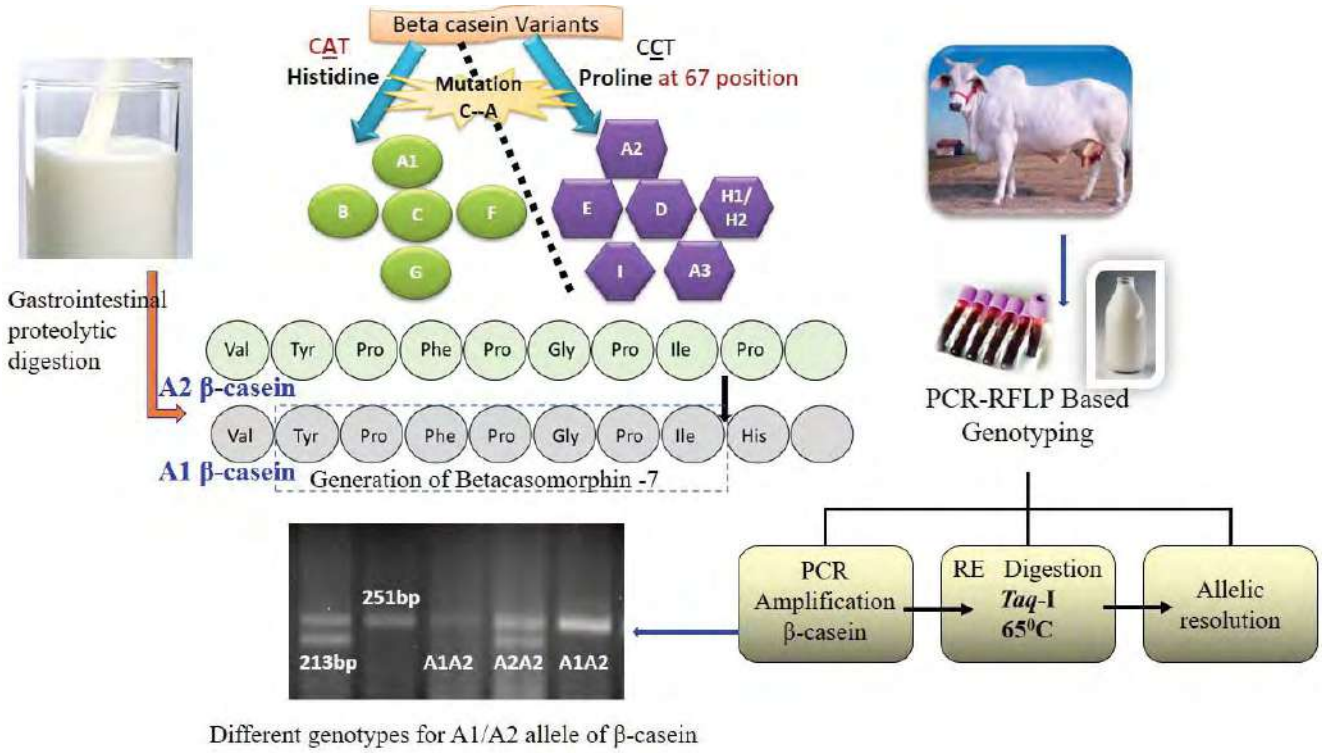

Fig. 1. Different variants of Beta-casein, their digestion and genotyping protocol

is primarily released from further proteolytic digestion of BCM-7 and BCM-9 by brush border peptidases. Various epidemiological, biochemical data or animal trials suggest the association of A1 milk (cow milk with A1 β-casein) as a risk factor for digestive discomforts, type I diabetes, coronary heart disease, arteriosclerosis, sudden infant death syndrome etc. (Kuellenberg *et al*., 2022; Sodhi *et al*., 2022; Yadav *et al*., 2020; Brooke-Taylor *et al*., 2017; Laugesen and Elliott, 2003; Tailford *et al*., 2003)

Status of β-casein Variants across Taurine Breeds

Although a clear link between A1 β-casein and a disease state has not yet been confirmed, the importance of monitoring the status of A1/A2 alleles in dairy animals as a precautionary measure has been realized. The distinguishing amino-acid sequence that characterizes A1 beta–casein is essentially unique to some European cattle breeds. Asian and African cattle; goats, sheep, yak and camel; all produce A2 milk. Human beta-casein is also of the A2 type as defined by the relevant amino-acid sequence (proline) fixation of A2 allele in buffalo breeds have also been reported in different studies (Mishra *et al*., 2009; Ramesha *et al*., 2016). Animals belonging to Genus Bos, which includes *Bos Indicus* (Indian humped Zebu), *Bos taurus* (Exotic) and Yak were initially of A2A2 type only but later due to point mutation A2 allele was replaced with A1 some 5000-10,000 years ago and some animals got A1A2 or A1A1 genotype (Ng-Kwai

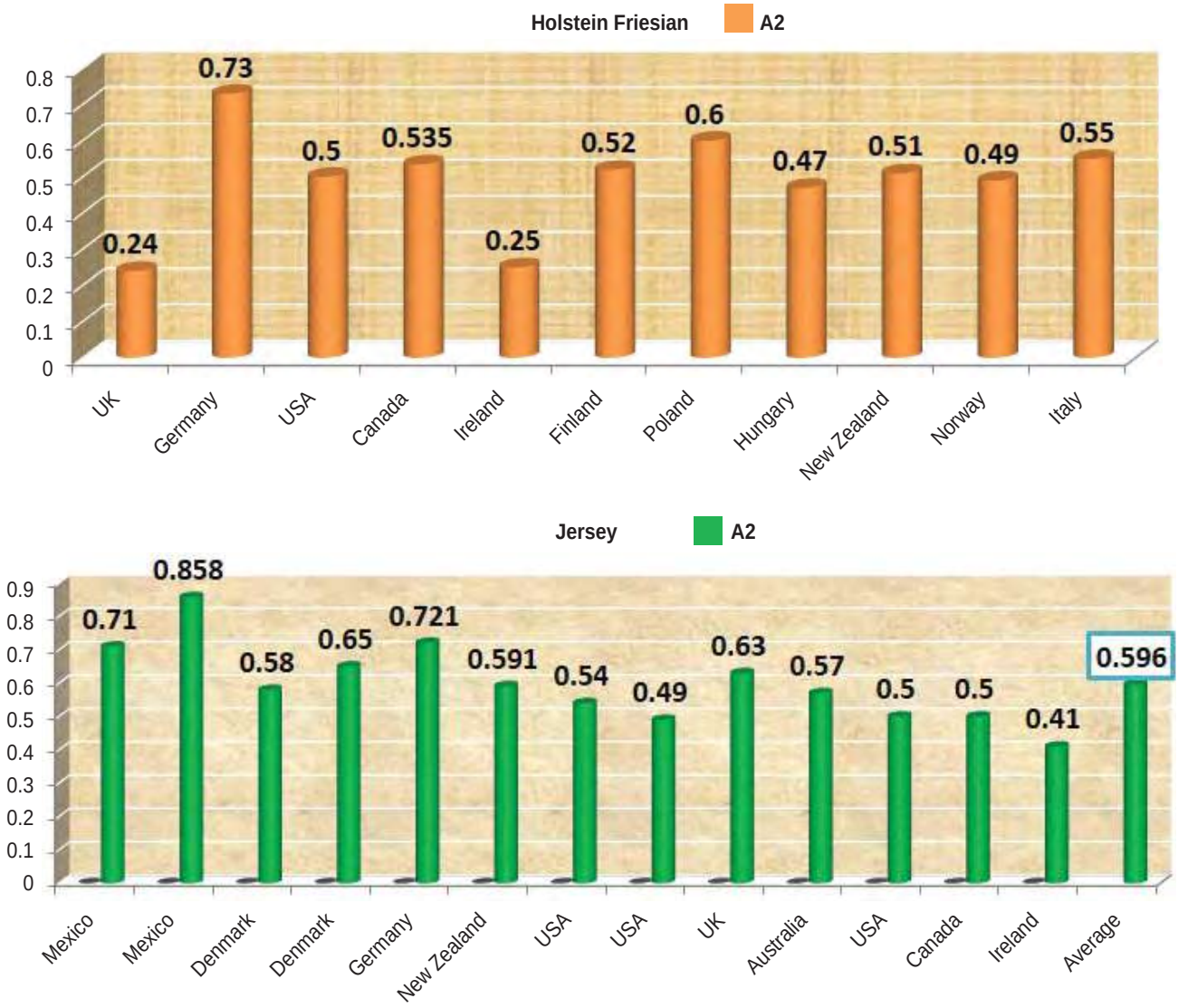

Fig. 2. Status of A1/A2 variant in *Bos taurus* breeds

and Grosclaude, 2002). With the advent of selective breeding for high production, better fertility and protein quality, unconsciously, genetically superior bulls carrying A1 allele were used in breeding programs. Hence, A1 variant became prominent in European cattle some thousands of years ago while making selection for high milk production. The frequency of A1 beta-casein with A1 variant varies breed by breed. A1 β-casein is a major variant of β -casein in the milk of the common dairy cows of north European origin: Friesian, Ayrshire, British Shorthorn, and Holstein. Also, the frequency of A1 has increased over the last century as the *Holstein Friesian* (*HF*) has become predominant in many countries for its high milk production character. Artificial insemination of large number of cows with semen of *HF* bulls multiplied A1 gene frequency over time. Several reports indicate that A1/A2 frequency is breed as well as area specific. Frequency of A1 allele of beta casein is high in Holstein Friesen (HF) breed in North America but low in German HF. Overall, across countries frequency of A1allele in HF ranges between (40–65%). Compared to HF, frequency of A2 allele is higher in Jersey. Breeds like Guernsey, Kerry, Channel Island cows; Southern French breeds; Charolais and Limousin also have higher frequency of A2 allele compared to HF.

Indian Native Cattle Breeds: A Resource for A2 Allele

Cattle genetic resources are the backbone of the farmer's economy since centuries producing milk as well as draught power. With 190.90 million cattle that is around 12.5% of world cattle population and producing 165.4 million tons of milk, India ranks first in the total milk produced annually (Livestock animal-husbandry-statistics, 2018). India is home to some of the best zebu (*Bos indicus*) breeds represented by 50 well-defined breeds. Besides these, many lesser-known cattle populations are also spread out in the length and breadth of the country each having its own special features contributing towards milk pool. The status of A1/A2 alleles of β-casein gene was delineated systematically first time at ICAR-NBAGR, Karnal in 2009, under the DBT funded project and later in National fund project. In a comprehensive analysis, nearly 4000 animals representing 27 breeds of Indian cattle and 231 animals from eight buffalo breeds have been genotyped to understand the distribution of A1/A2 variants. Blood samples were collected from the random, true to the breed animals by visiting the specific breeding tract of the breed. DNA was isolated and PCR-RFLP genotyping protocol was followed to ascertain the allelic status. The process included amplification of 251bp region of β-casein exon 7 harbouring the mutant site using specific primer pair restriction digestion of the PCR products with *Taq-I* restriction enzyme followed by visualization on 3% Ethidium bromide stained agarose gel. Three restriction fragments corresponding to different genotypes: A1A2 (251 & 213bp); A2A2 (251bp) and A1A1 (213bp) were observed for the analysed samples (Fig. 1).

The data on 15 Indian native cattle breeds (Mishra *et al*., 2009) from different agroclimatic regions and of different utility (milch, dual and draft purpose) revealed that majority of the Indian native cattle (97.4%) have A2A2, the desirable genotype, followed by heterozygous A1A2 (2.6%) genotype. None of the animal showed homozygous A1A1 genotype. Out of 861animals screened, only few animals of Malnad Gidda and Kherigarh cattle showed heterozygous A1A2 genotype with frequency of 0.191 and 0.218, respectively. The study was further extended to 3400 animals representing 27 Indian native cattle, crossbred (Karan Fries and Frieswal) and exotic (Holstein Frisian and Jersey) cattle (Mukesh *et al*., 2022). Across the Indian native cattle, highest frequency was observed for A2A2 genotype (0.905), followed by heterozygous A1A2 (0.091) and homozygous A1A1 (0.004) genotype. The overall frequency of favourable A2 allele across all the 2500 animals genotyped was 0.95 and its distribution across the different utility types was 1.0, 0.95 and 0.92 for milch, dual and draft purpose breeds, respectively. As observed by Mishra *et al*. (2009), all the animals belonging to dairy/milch breeds (Gir, Tharparkar, Rathi, Red Sindhi, and Sahiwal) showed only homozygous A2A2 genotype that is fixation of A2 allele. The other cattle breeds showing complete absence of A1A1/A1A2 genotype were Belahi, Konkan Kapila, Kangyam, Nimari, Red Kandhari, Malvi, Amritmahal, Kankrej, Hariana and Mewati (Table 1). The percent of heterozygous genotype was higher in draft purpose breeds compared to dual purpose breeds. Across the different climatic regions, breeds from arid and semiarid region showed higher frequency of A2 allele that is 0.98 and 1.00, respectively. Frequency of A2 allele in breeds from humid subtropical region mainly consisting of mainly draft and dual-purpose breeds was 0.90.

Table 1: Frequency profile of A1/A2 allele of beta casein and corresponding genotypes across Indian native, crossbred and taurine cattle in India

S No	Breed/ Population	Allelic Frequency		Genotypic Frequency			Sample No	References
		A1	A2	A1A1	A1A2	A2A2		
1	Gir	0.000	1.000	0.000	0.000	1.000		Mukesh *et al.*, 2022
		0.146	0.854	-	-	0.833	24	Patel *et al.*, 2020
		0.172	0.828	0.006	0.331	0.663	172	Patel *et al.*, 2019
		0.000	1.000	0.000	0.000	1.000	45	Mishra *et al.*, 2009
	Gir Bulls	0.222	0.778	0.037	0.370	0.593	54	Patel *et al.*, 2019
2	Rathi	0.000	1.000	0.000	0.000	1.000		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	30	Saran *et al.*,2019
		0.000	1.000	0.000	0.000	1.000	46	Mishra *et al.*, 2009
3	Red Sindhi	0.00	1.00	0.00	0.00	1.00		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	33	Mishra *et al.*, 2009
4	Sahiwal	0.00	1.00	0.00	0.00	1.00		Mukesh *et al.*, 2022
		0.15	0.85	0.000	0.30	0.70		Pandey *et al.*, 2019
		0.067	0.933	0.005	0.124	0.871	26	Saran *et al.*, 2019
		0.37	0.63	0	0.75	0.25	12	Srinivas *et al.*, 2019
		0.06	0.94	0	0.13	0.87	306	Kumar *et al.*,2018
		0.00	1	0	0	1.00	15	Haq *et al.*, 2012
		0.000	1.000	0.000	0.000	1.000	47	Mishra *et al.*, 2009
5	Tharparkar	0.00	1.00	0.00	0.00	1.00		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	30	Saran *et al.*, 2019
		0.000	1.000	0.000	0.000	1.000	14	Haq *et al.*, 2012
		0.000	1.000	0.000	0.000	1.000	44	Mishra *et al.*, 2009
6	Badri	0.09	0.91	-	-	-		Mukesh *et al.*, 2022
		0.12	0.88	0	0.24	0.76	90	Hussain *et al.*, 2019
7	Belahi	0.000	1.000	0.000	0.000	1.000		Mukesh *et al.*, 2022
8	Deoni	0.08	0.92	-	-	-		Mukesh *et al.*, 2022
		0.29	0.71	0	0.58	0.42	12	Srinivas *et al.*, 2019
		0.000	1.000	0.000	0.000	1.000	40	Ramesha *et al.*, 2016
9	Gaolao	0.08	0.92	-	-	-		Mukesh *et al.*, 2022
10	Hariana	0.000	1.000	0.000	0.000	1.000		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	48	Mishra *et al.*, 2009
11	Kankrej	0.000	1.000	0.000	0.000	1.000		Mukesh *et al.*, 2022
		0.083	0.917	-	-	-	24	Patel *et al.*, 2020
		0.107	0.893	0.000	0.214	0.786	28	Patel *et al.*, 2019
		0.000	1.000	0.000	0.000	1.000	30	Saran *et al.*, 2019
		0.03	0.97					Rangel *et al.*, 2017
		0.000	1.000	0.000	0.000	1.000	32	Mishra *et al.*, 2009
	Kankrej Bulls	0.067	0.933	0.000	0.154	0.867	60	Patel *et al.*, 2019
12	Konkan Kapila	0.00	1.00	0.00	0.00	1.00		Mukesh *et al.*, 2022
13	Ladakhi	0.08	0.92	-	-	-		Mukesh *et al.*, 2022
		0.100	0.900	0.000	0.210	0.790	82	Sodhi *et al.*, 2018
14	Malnad Gidda	0.07	0.93	-	-	-		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	6	Sridharan *et al.*, 2022
		0.20	0.80	0	0.40	0.60	10	Srinivas *et al.*, 2019
		0.014	0.986	0.000	0.029	0.971	104	Ramesha *et al.*, 2016
		0.096	0.904	0	0.191	0.809		Malarmathi *et al.*, 2014
		0.21	0.79	0.13	0.18	0.69		Navyashree, 2014
		0.096	0.904	0	0.191	0.809	47	Mishra *et al.*, 2009

S No	Breed/ Population	Allelic Frequency		Genotypic Frequency			Sample No	References
		A1	A2	A1A1	A1A2	A2A2		
15	Mewati	0.000	1.000	0.000	0.000	1.000		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	40	Mishra *et al.*, 2009
16	Ongole	0.020	0.980	0.000	0.019	0.981		Mukesh *et al.*, 2022
		0.040	0.96	0	0.080	0.92	12	Srinivas *et al.*, 2019
		0.06	0.94	0	0.11	0.89	38	Ganguly *et al.*, 2013
Draft Cattle Breeds								
17	Amritmahal	0.000	1.000	0.000	0.000	1.000		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	5	Sridharan *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	50	Inamdar *et al.*, 2019
		0.000	1.000	0.000	0.000	1.000	37	Mishra *et al.*, 2009
18	Bargur	0.063	0.937	0.00	0.125	0.875	48	Raja *et al.*, 2021
19	Dangi	0.14	0.86	-	-	-		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	31	Jawane *et al.*, 2018
20	Kangayam	0.000	1.000	0.000	0.000	1.000		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000		Kathiravan *et al.*, 2021
		0.000	1.000	0.000	0.000	1.000	22	Malamathi *et al.*, 2014
		0.000	1.000	0.000	0.000	1.000	48	Mishra *et al.*, 2009
21	Kherigarh	0.00	0.89	-	-	-		Mukesh *et al.*, 2022
		0.109	0.891	0	0.218	0.783	23	Mishra *et al.*, 2009
22	Khillar	0.14	0.86	-	-	-		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	12	Ramesha *et al.*, 2016
23	Malvi	0.00	1.00	0.00	0.00	1.00		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000	44	Mishra *et al.*, 2009
24	Nagori	0.06	0.94	-	-	-		Mukesh *et al.*, 2022
25	Nimari	0.00	1.00	0.00	0.00	1.00		Mukesh *et al.*, 2022
		0.000	1.000	0.000	0.000	1.000		Mishra *et al.*, 2009
26	Punganur	0.000	1.000	0.000	0.000	1.000	3	Sridharan *et al.*, 2022
		0.080	0.92	0	0.17	0.83	12	Srinivas *et al.*, 2019
27	Ponwar	0.13	0.87	-	-	-		Mukesh *et al.*, 2022
28	Red Kandhari	0.00	1.00 1.000	0.00 0.000	0.00 0.000	1.00 1.000	39	Mukesh *et al.*, 2022 Mishra *et al.*, 2009
		0.000						
29	Umblachery	0.20	0.80	-	-	-		Mukesh *et al.*, 2022
		0.02	0.98	0.000	0.050	0.950	42	Raja *et al.*, 2021
30	Vechur	0.20	0.80	-	-	-		Muhammed and Stephen, 2012
	Kasargod local	0.39	0.61	-	-	-		
		0.042	0.958	0.000	0.083	0.917	48	Ramesha *et al.*, 2016
Crossbred Cattle								
31	Karan fries	0.29	0.71	0.09	0.40	0.51	460	Mukesh *et al.*, 2022
		0.169	0.831	0.000	0.338	0.662	59	Ramesha *et al.*, 2016
		17.5	82.5	0.08	0.19	0.73	73	Jaiswal K *et al.*, 2013
		0.208	0.792	0.125	0.166	0.709	24	Haq *et al.*, 2012
32	Frieswal							
	Heifers	0.32	0.67	0.12	0.40	0.48	124	Ganguly *et al.*, 2013
	Bulls	0.44	0.56	0.23	0.42	0.35	48	
	Mean	0.37	0.63	0.17	0.39	0.44	100	
33	Vrindvani	0.35	0.65	0.11	0.47	0.42	354	Kumar *et al.*, 2018

S No	Breed/ Population	Allelic Frequency		Genotypic Frequency			Sample No	References
		A1	A2	A1A1	A1A2	A2A2		
34	Hardhenu	0.66	0.34	0.32	0.68	0.00	50	Ramkaran *et al.*, 2017
35	HF cross	0.64	0.36	0.29	0.71	0.00	14	Kathiravan *et al*., 2021
		0.479	0.521	-	-	-	24	Patel *et al*., 2020
		0.375	0.625	0.091	0.567	0.342	263	Patel *et al*., 2019
		0.50	0.50	0	1.0	0	12	Srinivas *et al.*, 2019
		0.638	0.362	0.28	0.72	0.00	47	Shende *et al.*, 2017
		0.294	0.706	0.000	0.588	0.412	17	Ramesha *et al.*, 2016
	Rathi X HF	0.000	1.000	0.000	0.000	1.000	10	Saran *et al.*, 2019
	Sahiwal X HF	0.32	0.68	0.00	0.64	0.36	50	Pandey *et al.*, 2019
	Dangi X HF							
	HF 75 %	0.13	0.86	0.06	0.13	0.81	15	Jawane *et al.*, 2018
	HF 62.5 %	0.03	0.97	0.000	0.06	0.94	17	
	Overall	0.08	0.92	0.03	0.09	0.88	32	
	Kangayem X HF	0.405	0.595	0.17	0.46	0.37	63	Malamathi *et al.*,, 2014
36	Jersey Cross	0.69	0.31	0.380	0.620	0.000	29	Kathiravan *et al*., 2021
37	**Breeding Bulls**							Sodhi *et al.*, 2012
	Holstein	0.441	0.559	0.216	0.451	0.333	51	
	Jersey	0.325	0.675	0.025	0.600	0.375	40	
	Crossbred	0.298	0.702	0.101	0.393	0.506	89	
	Mean	0.355	0.645	0.114	0.481	0.405		
Taurine breeds								
38	Holstein Friesian							
		0.500	0.500	0.000	1.000	0.000	2	Kathiravan *et al.*, 2021
		0.565	0.435				23	Patel *et al*., 2020
		0.169	0.831	0.000	0.338	0.662	59	Ramesha *et al.*, 2016
39	Jersey	0.077						Ramesha *et al.*, 2016
		0.25	0.75	0.000	0.50	0.50		Kathiravan *et al.*, 2021
40	Karan Swiss	0.107	0.893	0.00	0.214	0.786	14	Haq *et al.*, 2012

In line with data generated at ICAR-NBAGR, other researchers have also reported the complete absence of A1 allele in Kangayam (Malarmathi *et al*., 2014, Kathiravan *et al*., 2021), Amritmahal (Inamdar *et al*., 2019); Gir (Paradkar *et al*., 2021); Malvi & Nimari (Pandey *et al*., 2021) cattle; and predominance (≥ 0.90) of the A2 allele in Indian native cattle breeds including Kankrej-0.97; Malnad Gidda-0.98; Ongole-0.94; Umblachery-0.98; Kasargod-0.958; Bargur-0.937, Umblachery-0.98 and Ladakhi-0.90 (Table 1). The variations in the frequency of A2 allele was mainly due to the small sample size or collection of samples from the organized herds/farms, for instance frequency of A1 allele in Sahiwal cattle maintained in organized farm was 0.15 (Pandey *et al*., 2021). Complete absence of A1 allele has also been reported for Deoni, Khillar (Ramesha *et al*., 2016) and Dangi (Jawane *et al*., 2018) cattle. Overall, the findings indicate the preponderance of A2 β-casein variant and also pointed towards the indicine origin of A2 allele.

Status of β-casein Variants in Crossbred Cattle

India continues to be the largest milk producing country in the world. Population statistics indicate approximately 51% of the Indian cattle population consist of registered/ recognized indicus breeds, crossbred cattle constitute only 13%, while others are nondescript cattle. However, in terms of milk production, crossbred cattle and Indicus breeds contribute 24.4 and 20.7 percent respectively to the national milk pool. Considering the contribution of crossbred cattle towards milk produced, it is important to genotype the crossbred population in the country for the status of A1/A2 allele of beta casein. Karan Fries, one

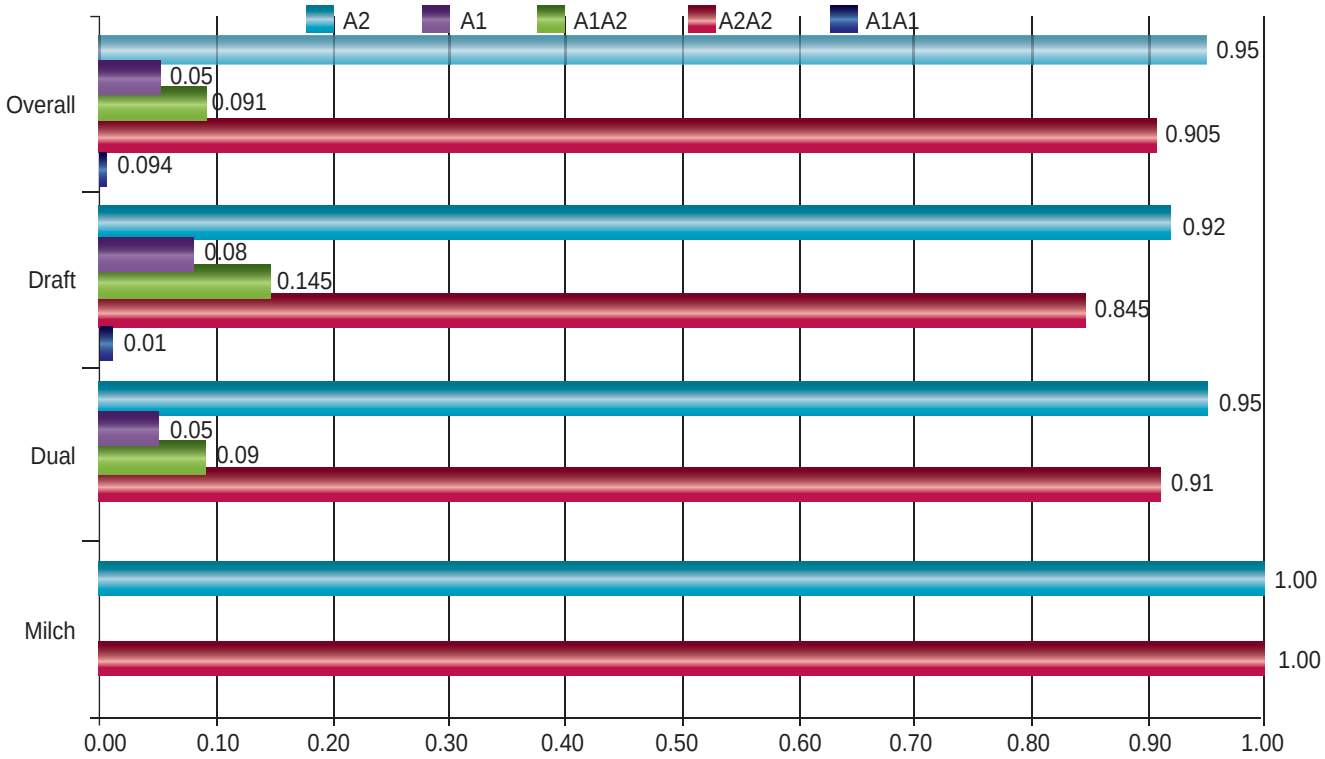

Fig. 3. Overall and utility wise status of β-casein A1/A2 alleles and genotypes in Indian cattle

of the crossbred populations developed using Holstein Friesian (exotic breed) and Tharparkar (indigenous breed) with the aim to enhance milk production coupled with high disease resistance and adaptability to local conditions; Frieswal (cross of Holstein Friesian and Sahiwal); Hardhenu and other crossbred cattle available across the country have been genotyped by different researchers (Table 1). Genotyping of 686 crossbred animals (Karan Fries and Frieswal) by Mukesh *et al.* (2022) revealed the predominance of A2 allele with a frequency of 0.71. Though the observed frequency of A1 allele (0.29) was much higher compared to Indian native cattle, the contribution towards A1 allele was majorly from the heterozygous A1A2 genotype rather than homozygous A1A1. Frequencies of the three genotypes A1A1, A1A2 and A2A2 was 0.09, 0.40 and 0.51 respectively. Similar allelic and genotypic profile (A1-0.32; A2-0.67; A1A1-0.12; A1A2-0.40; A2A2-0.48) with high frequency of A1A2 genotype was observed by Ganguly *et al*. (2019) while genotyping 124 Frieswal heifers. Frequency for A2 allele in HF crossbred cow was observed to be 0.595 (Malarmathi *et al*., 2014) while in Vrindavani (crossbred cattle) frequency of 0.65 has been recorded for A2 alleles. Most of the studies pointed towards higher frequency of A2 allele even in Indian crossbred populations except for HF crossbred (A2-0.31) as well as Jersey crossbred (A2-0.36) in Tamil Nadu (Kathiravan *et al*., 2021), Hardhenu crossbred cattle of Haryana region (0.34: Ramkaran *et al.*, 2017), and HF crossbred of Maharashtra (0.36: Shende *et al*., 2017).

Status of A1/A2 β-casein Variants in Breeding Bulls

In India, Holstein Friesian (HF) and Jersey cattle have been extensively used since 1960 for crossbreeding and genetic improvement programme. Considering the widespread use of taurine germplasm in our country's cross-breeding program and fact that these cattle could be the potential source for undesirable A1 allele, it is essential to ensure the status of A1/A2 alleles in breeding bulls being used at different AI centres. Frequency profiling of A1/A allele and corresponding genotypes in crossbred, taurine (Holstein Frisian and Jersey) and Indicus bulls being used in different AI centres was reported by Sodhi *et al.* (2012). As per the report, frequency of A2 allele was 0.88 in Indicus bulls, 0.702 in cross-bred bulls, while in Jersey and Holstein Frisian bulls, it was 0.675 and 0.559 respectively. Among the frequencies for three genotypes across taurine and crossbred bulls, least observed frequency was of

homozygous A1A1 genotype (0.114) followed by A2A2 (0.405) while maximum frequency of 0.481was observed for heterozygous A1A2 genotype. Congruently, the mean frequencies of A1 and A2 alleles was 0.355 and 0.645 respectively (Fig. 3). Similar values of highest mean frequency of A1A2 genotype (0.475) and least mean frequency for A1A1 genotype (0.095) for semen samples from different breeding bulls have also been reported by Mukesh *et al.* (2022). Across different categories of breeding bulls, the frequency of A1A2 genotype was higher in Jersey (0.64) and HF bulls (0.449) in comparison to crossbred (0.39) and indigenous bulls (0.24) while trend of frequency of A2 allele was highest in indicus (0.88) followed by crossbred (0.70), Jersey (0.64) and HF bulls (0.56).

Kathiravan *et al.* (2021) also reported higher percentage of heterozygous genotypes for Jersey (0.50), HF (1.0), Jersey crossbred (0.62) and HF crossbred (0.71) bulls. Though, the data indicated predominance of the desirable A2 allele across all studied breeding bulls, still there is a need for careful screening of sire lines being used in the breeding programmes as 65-70% of exotic (JSC and HF) or crossbred bulls being currently used in AI program in India are source of A1 allele.

The presence of large crossbred cattle populations and higher proportions of the A1 allele in exotic animals necessitates careful screening of animals and fine-tuning of existing breeding programmes. Such an approach would be an effective measure to prevent the dissemination of the undesirable A1 allele in our existing A2-predominant indigenous cattle populations. Initially, information regarding the screening of bulls for the A1A2 genotype in government semen stations was not available. But in recent years, many of the AI centres have availed the genotype testing facility at ICAR-NBAGR and got the bulls (semen straws) certified as A2 bulls. Specific herds can be converted to A2 type by selective breeding within 4 years using

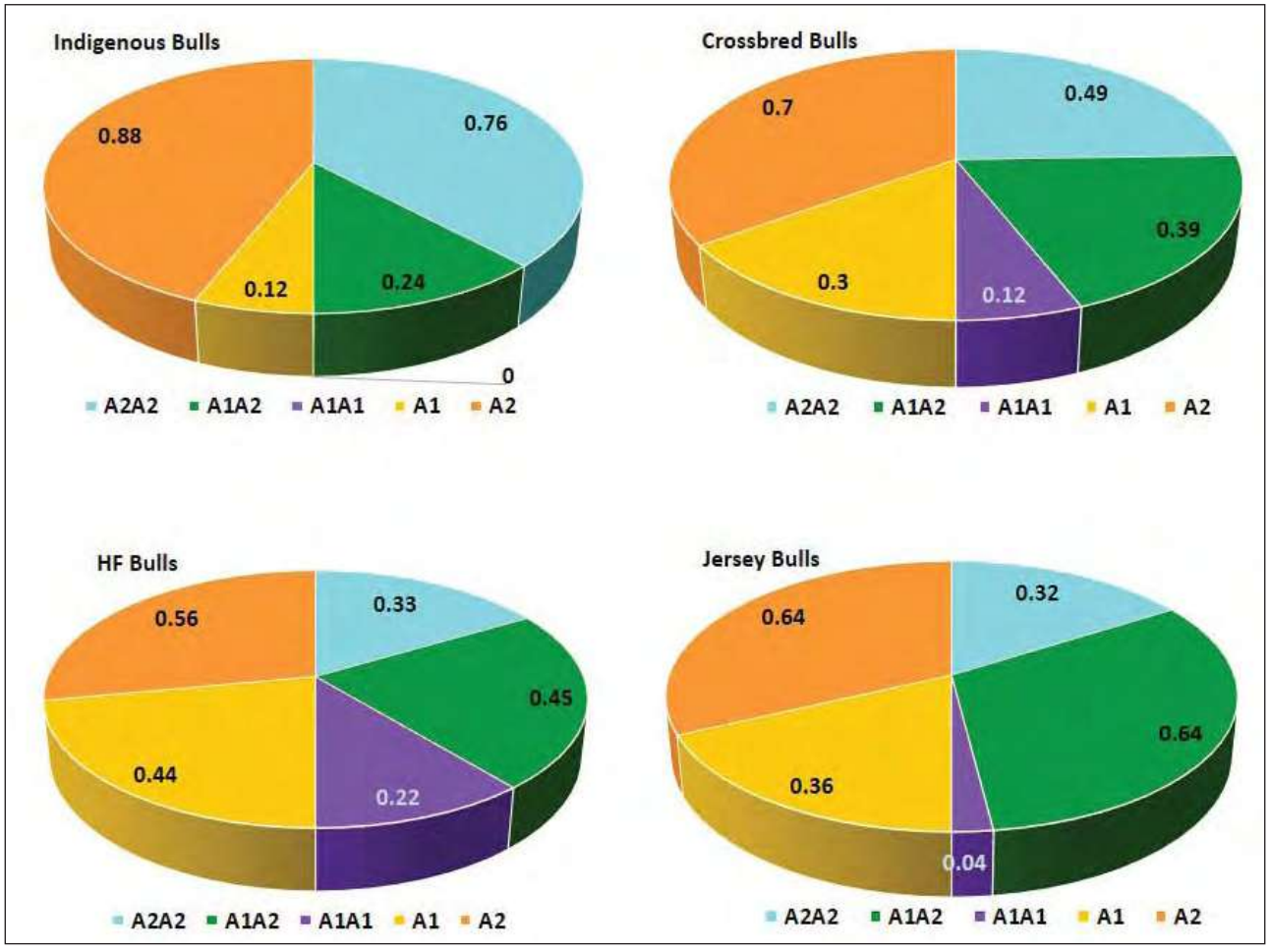

Fig. 4. Overall Status of A1/A2 variants in breeding bulls

intensive methods of animal selection that incorporate the use of certified A2 semen to eliminate all A1 beta-casein from the milk (Pal *et al*., 2015).

Conclusion

The A1/A2 hypothesis is both intriguing and potentially very important for public health, if it is proved correct. The BCM-7 released from A1 type has been the central theme for hypothesis, whereas the BCM-9 released from A2 type milk need be investigated for the claimed health ailments. The epidemiological data and animal trials point towards potential health hazards of consuming A1 type milk or derived BCM-7 while A2 milk is considered safe for drinking. Work is in progress in different labs across globe to authenticate the fact. Till that time, it is essentially important to monitoring the status of A1/A2 alleles in dairy animals as a precautionary measure. The studies conducted on Indian native cattle strongly demonstrate that Indian cattle naturally harbor the preferred A2 allele and thus are a good resource for A2 milk. Further, though, the frequency of A1 allele is high in crossbred cattle and breeding bulls, its A1A2 genotype mainly contributing for this high A1 allele frequency. The overall frequency of homozygous A1A1 genotype is very low across all the categories of animals that is Indian breeds, taurine or crossbred populations, as well as breeding bulls. Hence, allelic profiling for A1/A2 beta casein in breeding bulls and bringing in changes in the breeding program accordingly can very easily help to drift the herds towards A2 and minimize the risk of disseminating the A1 allele in Indian cattle.

Suggested Readings

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