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Identification of β Casein Genotypes in Indian Gir and Crossbred Exotic Cows from Mumbai Dairy Farms

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ABSTRACT

Background: Collective evidence of polymorphic β-casein and associated health problems has led to the concern about milk consumption and cow breeding policies worldwide. This association has also engrossed the interest of dairy scientist and industry in evaluation of β casein genotype distribution. With increasing proportion of exotic and crossbred cows in India it is worth while to screen cattle for A1A2 β casein and enhance indigenous cow breeds.

Methods: The present study intended to identify β casein genotypes in pure Indian Gir cows and crossbred Holstein and jersey cows from three local dairy farms. We analysed β casein genotypes by PCR-RFLP method in total 95 cows during the period of 2017-2019.

Result: All the indigenous Gir cows had fixed A2 allele whereas crossbred Jersey and Holstein Frisian both had A1A2 as the most common genotype (frequency: 0.473 and 0.6 respectively) followed by A2A2 (Frequency 0.368 and 0.333 respectively) and A1A1 (Frequency 0.158 and 0.066 respectively). The results show that in this study group Gir, a native Indian breed has fixed A2 β casein variant whereas crossbred Jersey and Holstein Frisian have A1A2 as a most common genotype. Screening of cattle for β casein genotypes is vital to monitor the frequency of A1 beta casein in native Indian cow breeds.

Key words: β casein, BCM-7, Gir, Genotyping, PCR-RFLP.

INTRODUCTION

Milk is a natural food that provides complete nutrition for the growth and care of human body. It is a rich source of first class proteins as well as other nutritional elements for vegetarians and non-vegetarians. Presence of immunoglobulins, cytokines, growth factors, hormones, multiple vitamins and trace elements makes it an essential part of human nourishment (Godse *et al*. 2017). In last few years reports on adverse effects of milk such as milk allergy, lactose intolerance and risk of diabetes and cardiovascular diseases have generated confusion about milk consumption even for babies (Lamb *et al*. 2015). Cow milk containing A1 β casein and its association with various health complications has increased the confusion even more.

In last two decades, β-casein polymorphism has acknowledged substantial research interest in animal breeding and dairy industry. A1 and B variants of β-casein are sources of exogenous bioactive peptides, considered to be a risk factor for a spectrum of diseases (Elliot *et al.* 1999, McLachlan 2001). This association has engrossed the interest of dairy scientist and industry in evaluation of β casein genotype distribution.

β-casein is one of the vital proteins present in bovine milk. The β casein gene is known as a highly polymorphic gene. Up till now 12 β casein variants have been identified *viz*. A1, A2, A3, B, C, D, E, F, H1, H2, I and G (Kaminiski *et al.* 2007). Among these variants, A1 and A2 forms are most common in dairy cattle followed by B (Farrell *et al.* 2004). A1 β casein was derived from single point mutation that occurred in A2-β casein gene in European-origin cattle (Pal *et al*. 2015). This mutation changes β-casein reading frame, from CCT to CAT which causes replacement of proline with histidine at 67th position of amino acid chain (Ng-Kwai-Hang and Grosclaude 2002). Isoleucine (at 66th position) and histidine peptide bond (A1 and B β casein) is more susceptible to proteolytic enzymes as compared to isoleucine and proline bond (A2 β casein). Due to this secondary conformational change, proteolytic digestion of A1 β casein releases β-casomorphin (BCM-7) in the intestine (Kaminski *et al.* 2007).

BCM-7 is a heptapeptide (H-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-OH) which is the most studied β-casomorphin. Presence of three proline residues makes it highly resistant to enzymatic degradation. These proline residues provide a distinctive low energy conformation, structurally similar to morphines (Trivedi *et al*. 2014, Kumar *et al*. 2020). Also N-terminal tyrosine residue of BCM-7 increases its affinity to opioid receptors. Activation of opioid receptors of immune, nervous and endocrine system causes various health

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implications such as ischemic heart disease, sudden infant death syndrome, schizophrenia, autism, type 1 diabetes and milk intolerance (Kamiński *et al*. 2006, Caroli *et al*. 2009).

These considerations have evolved an argument about A1 and A2 milk types in the dairy industry for over two decades. Various methods have been developed to evaluate β casein polymorphism in cows *viz*. DNA amplification (PCR-RFLP, allele specific-PCR; multiplex-PCR), microarray based methods, sequencing *etc.* Although the hypothesis of correlation between consumption of A1 milk and risk of several diseases requires further research, many European countries have started screening and accordingly crossbreeding of cows.

All Indian zebu cattle harbor A2 β casein, however crossbreeding with exotic breeds for genetic improvement has introduced A1 allele in Indian cows. As per the 19th livestock census report, (2012) Indian milch cattle population was 67.54 million, where indigenous milch cattle are 48.12 million and exotic milch cattle are 19.42 million. There was an increase of 0.17% in the population of indigenous milch cattle over the previous census (2007) whereas the exotic/ crossbred milch cattle are increased by 34.78%. Further in 2019 exotic milch cattle have become 26.08 million with an increase of 34.3% and indigenous milch cattle are 46.57 million with a marginal increase of 0.8%. In this scenario preferring A2 herds and shifting the frequency of breeds towards A2 becomes difficult.

In this study we have analyzed cows for β casein polymorphism from three local dairy farms in Mumbai. These farms are located in the middle of the city allowing limited free movement and tracking of cows. This also facilitated to study their feeding and milking routine in the farm. This is the first report of the status of β casein polymorphism in cow from a metropolitan city of India. We are also first to report the β casein genotype of a pure Gir herd in India.

MATERIALS AND METHODS

We collected samples from 95 cows from 3 dairy farms during the period of 2017 and 2019. These farms are maintained by Bombay Gowrakshak Trust. The milk of these farms is supplied in dairies as well as donated in the orphanages. In these farms cows are well taken care for their health. There were total 56 pure Gir, 20 crossbred Holstein Friesian and 19 crossbred Jersey cows. Only milking cows were randomly selected for blood collection and their details were recorded. Pedigree details of these animals were provided by the farm management. Blood samples were collected from 95 cows and 10 hair samples and 10 milks samples were also collected from these cows for method standardization and comparison.

About 5 ml of blood was collected aseptically from each animal from jugular vein in an EDTA tube. The blood samples were immediately transported under cold condition to our laboratory at Kasturba Health Society and Medical Research Centre, Vile Parle. The samples were stored at 4°C and processed within 24 hrs. Bovine genomic DNA was isolated from cow blood by proteinase K enzymatic digestion and phenol chloroform precipitation (Sambrook *et al*. 1989). DNA extraction from hair samples was carried out using the protocol described previously (Kumar *et al*. 2005). Genomic DNA extraction method described by De *et al*. (2000) was employed for raw milk samples. The DNA samples were analyzed for purity and stored at -20°C till further processing. The samples were genotyped for β casein gene using PCR-RFLP method.

The 251 base pair fragment of exon 7 at CSN2 gene was amplified by the primers reported previously (Lien *et al*. 1992). Polymerase chain reaction was performed using a total reaction volume of 25 µl containing 50ng of genomic DNA, 100 µM dNTPs, 10 pmol of each primer, 2.5 mM $MgCl_2$ and 1.5 U *Taq* DNA polymerase. Thermal cycling conditions were programmed at 95°C for 5 min, followed by 30 cycles at 94°C for 30 sec, 63°C for 40 sec and 72°C for 20 seconds. Final extension was performed at 72°C for 3 min. The amplified product of 251 base pairs (bp) was digested with 0.5U *Taq* I restriction enzyme at 65°C for 4 hours. The digested fragments were resolved on 3% agarose gels in 1× TAE buffer. The genotypes of samples were recorded according to the size of digested fragment.

Primers used for PCR

CASB67R

5′CCTGCAGAATTCTAGTCTATCCCTTCCCTGGGCCCATCG′

CASB122L

5′GAGTCGACTGCAGATTTTCAACATCAGTGAGAGTCAGGCCCTG3

RESULTS AND DISCUSSION

Beta casein genotype analysis in 3 cattle populations was investigated. 95 cows from 3 farms were included in this analysis. They were kept in the same herd and fed with a standard diet. None of the selected cows showed any clinical symptoms of mastitis or any other illness.

Genetic characterization of exon 7 of CSN2 gene was carried out by PCR-RFLP method. Different genotypes showing representative banding patterns observed on gel are shown in Fig 1. The heterozygous A1A2 pattern had 251 bp, 213 bp and 38 bp and A2A2 pattern had fragments of 251 bp.

All pure Gir cows had A2A2 β casein genotype. In crossbred Jersey cows A2 (frequency: 0.605) was the most common allelic variant of β casein followed by A1 (frequency: 0.394). The heterozygous β casein genotype A1A2 was the most frequent in this group (frequency: 0.473), followed by A2A2 (frequency: 0.368) and A1A1 (frequency: 0.158). Crossbred Holstein Frisian cows also had A2 as the most common allelic variant (frequency: 0.633) followed by A1 (frequency: 0.366). Maximum cows had heterozygous genotype A1A2 (frequency: 0.6), followed by A2A2 (frequency: 0.333) and A1A1 (frequency: 0.066) genotypes (Table 1).

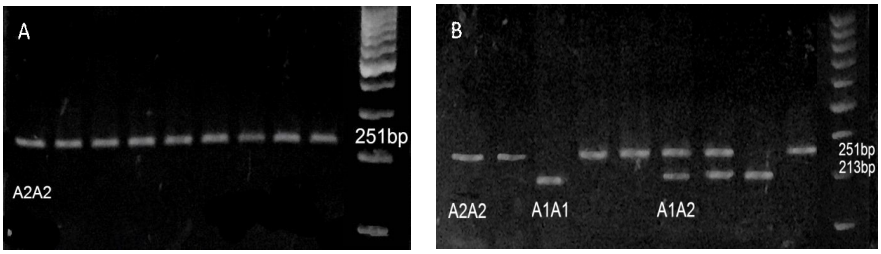

Fig 1: β casein genotypes in representative samples.
(A) Gir cows, (B) Crossbred Jersey and Holstein Frisian cows.

Table 1: Allelic and genotypic frequencies of β casein in Gir, Crossbred Jersey and crossbred Holstein Frisian cows.

	Allelic frequency		Genotypic frequency		
	A1	A2	A1A1	A1A2	A2A2
Gir	0	1	0	0	1
Crossbred Jersey	0.394	0.605	0.158	0.473	0.368
Crossbred HF	0.366	0.633	0.066	0.6	0.333

The data indicates that pure indigenous cows have A2A2 as a fixed β casein genotype. Whereas greater proportion of A2 allele in crossbred Jersey and crossbred Holstein cows was observed; although among these crossbred cows 93% cows had 50% inheritance from indigenous breed and 7% cows had 38% inheritance from indigenous breed.

The present study concurs with the results of Mishra *et al*. (2009) where they have reported the status of β casein genotype in 15 indigenous cow breeds from all over the India. According to their report, the entire indigenous cow breeds lack A1 β casein allele except two breeds with low frequency. Further, the same group has reported β casein genotype in Holstein Frisian, Jersey and crossbred cows (Sodhi *et al*. 2012). The mean allelic distribution of A2 and A1 β casein was 0.645 and 0.355 respectively. This data also corresponds to our results of crossbred Jersey and Holstein Frisian cows' genotype analysis. Rupasinghe *et al.* (2020) also reported higher frequency of A1 beta casein (0.47) in Holstein Friesian cattle in Sri Lanka as compared to their native cattle breed.

In another report by Ganguly *et al*. (2013) one more native cow breed Ongole was analyzed for β casein genotype. In these cows allelic frequency of A2 and A1 β casein were 0.94 and 0.06 respectively. Ramesha *et al.* (2016) have analyzed β casein genotypes of indigenous breeds Khillar, Deoni, Malnad Gidda and Kasargod cattles. Among these Khillar and Deoni breeds had fixed A2 allele whereas Malnad Gidda and Kasargod cattles had A1 allelic frequency as 0.014 and 0.042 respectively.

Evidently the indigenous cow breeds are gradually drifting towards A1 β casein type due to crossbreeding with exotic cows (Raja *et al.* 2021, Kumar *et al.* 2019). The study with native cow breeds was reported a decade ago where there were two native cow breeds had A1A2 variant of β casein gene (Mishra *et al.* 2009). It is possible that in this decade drastically increasing population of crossbred and exotic cows may have incorporated A1 allele in other native breeds as well.

In India, existence of large populations of exotic and crossbred cattle with higher frequencies of the A1 β casein allele, make it necessary to take effective measures to preserve existing A2-predominant gene pool of native cattle. Standard procedures for genotype screening and certification methods for A2 milk are essential to disseminate possible undesirable effects of A1 β casein (Cielinska *et al*. 2019). Systemic monitoring for β casein genotype along with milk production enhancement would be the effective approach to be on the safer side until the A1/A2 beta casein controversy is resolved. Further if the adverse effects of A1 β casein are validated, India will have a great opportunity in global A2 milk industry; wherein A2 allele is predominant in native cattle.

Our study demonstrated, Gir which is a native Indian breed has fixed A2A2 β casein variant whereas crossbred Jersey and Holstein Frisian have A1A2 as a most common genotype. Although the link between A1 β casein and associated health risks is not well established it is better to take precautionary actions in crossbreeding of cattle. According to the research carried out at National Dairy Research Institute, Karnal, screening of cattle for β casein genotypes is vital to monitor the frequency of A1 beta casein in native Indian cow breeds (Shashank *et al.,* 2018). Finally resolution of the A1/A2 β casein problem is crucial for human health as well as preservation of native Indian cows.

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REFERENCES

18th Livestock Census, (2007). All India Report, Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries Krishi Bhawan, New Delhi.

19th Livestock Census. (2012). All India Report, Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries Krishi Bhawan, New Delhi, 2014.

20th Livestock Census. (2019). All India Report, Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries Krishi Bhawan, New Delhi..

Caroli, A.M., Chessa, S. and Erhardt, G.J. (2009). Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. Journal of Dairy Science. 92(11): 5335-52.

Cieślińska, A., Fiedorowicz, E., Zwierzchowski, G., Kordulewska, N., Jarmołowska, B. and Kostyra, E. (2019). Genetic polymorphism of β-casein gene in polish red cattle-Preliminary study of A1 and A2 frequency in genetic conservation herd. Animals. 9(6): 377.

De, S., Singh, R.K., Gupta, P.K., Palia, S. and Butchaiah, G. (2000). Genotyping of dairy animals using DNA from milk somatic cells. Indian Journal Animal Sciences. 70: 944-946.

Elliott, R.B., Harris, D.P., Hill, J.P., Bibby, N.J. and Wasmuth, H.E. (1999). Type I (insulin dependent) diabetes mellitus and cow milk: Casein variant consumption. Diabetologia. 42: 292-96.

Farrell, H.M., Jimenez-Flores, R., Bleck, G.T., Brown, E.M., Butler, J.E., Creamer, L.K., Hicks, C.L., Hollar, C. M., Ng-Kwai-Hang, K.F. and Swaisgood, H.E. (2004). Nomenclature of the proteins of cows' milk-sixth revision. Journal of Dairy Science. 87(6): 1641-74.

Ganguly, I., Gaur, G.K., Singh, U., Kumar, S. and Mann, S. (2013). Beta-casein (CSN2) polymorphism in Ongole (Indian zebu) and Frieswal (HF × Sahiwal crossbred) cattle. Indian Journal of Biotechnology. 12: 195-98.

Godse, C.S., Paradkar, P.H., Loke, V.M., Udipi, S.A., Vaidya, R.A. and Vaidya, A.D.B. (2017). Cow's Milk: Nutritional relevance beyond its intolerance and allergies. The Indian Practitioner. 70(4): 29-34.

Kaminski, S., Cieslinska, A. and Kostyra, E. (2007). Polymorphism of bovine β-casein and its potential effect on human health. Journal of Applied Genetics. 48(3): 189-98.

Kaminski, S., Ruść, A. and Cieślińska, A. (2006). A note on frequency of A1 and A2 variants of bovine beta-casein locus in Polish Holstein bulls. Journal of Animal Feed Science 15: 195-98.

Kumar, P., Choudhary, V., Bhattacharya, T.K., Bhushan, B. and Sharma, A. (2005). PCR-RFLP based genotyping of cattle using DNA extracted from hair samples. Indian Journal of Biotechnology. 4: 287-89.

Kumar, S., Singh, R.V., Chauhan, A., Kumar, A. and Yadav, J.S. (2020). Analysis of beta-casein gene (CSN2) polymorphism in Tharparkar and Frieswal cattle. Indian Journal of Animal Research. 54(1): 1-5.

Kumar, S., Singh, R.V. and Chauhan, A. (2019). Molecular characterization of A1/A2 Beta-casein Alleles in Vrindavani crossbred and Sahiwal cattle. Indian Journal of Animal Research. 53(2): 151-155.

Lamb, M.M., Miller, M., Seifert, J.A., Frederiksen, B., Kroehl, M., Rewers, M. and Norris, J.M. (2015). The effect of childhood cow's milk intake and HLA-DR genotype on risk of islet autoimmunity and Type 1 Diabetes: The diabetes autoimmunity study in the young (DAISY). Pediatric Diabetes. 16(1): 31-38.

Lien, S., Aleström, P., Klungland, H. and Rogne, S. (1992). Detection of multiple β-casein (CASB) alleles by amplification created restriction sites (ACRS). Animal Genetics. 23: 333-38.

McLachlan, C.N. (2001). B-casein A1, ischaemic heart disease mortality and other illnesses. Medical Hypotheses. 56: 262-72.

Mishra, B.P., Mukesh, M., Prakash, B., Sodhi, M., Kapila, R., Kishore, A., Kataria, R.R., Joshi, B. K., Bhasin, V., Rasool, T.J. and Bujarbaruah, K.M. (2009). Status of milk protein, b-casein variants among Indian milch animals. Indian Journal of Animal Sciences. 79(7): 722-25.

Ng-Kwai-Hang, K.F. and Grosclaude, F. (2002). Genetic Polymorphism of Milk Proteins. Advanced Dairy Chemistry, Chapter 16, [(Eds) Fox, P.F. and McSweeney, P.L.H.] Kluwer Academic/ Plenum Publishers, New York. 737-814.

Pal, S., Woodford, K., Kukuljan, S. and Ho, S. (2015). Milk intolerance, B-casein and lactose. Nutrients. 7(9): 7285-97.

Raja, A., Rajendran, R. and Ganapathi, P. (2021). Detection of A1 and A2 alleles at beta-casein locus in bargur and umblachery (Indian Zebu) cattle breeds by allelespecific PCR. Indian Journal of Animal Research. DOI: 10.18805/ IJAR.B-4273.

Ramesha, K.P., Rao, A., Basavaraju, M., Alex, R., Kataktalware, M.A., Jeyakumar, S. and Varalakshmi, S. (2016). Genetic variants of beta-casein in cattle and buffalo breeding bulls in Karnataka state of India. Indian Journal of Biotechnology. 15: 178-81.

Rupasinghe, R.K., Shanjayan, N., Lokugalappatti, L.G.S. and Wickramasinghe, S. (2020). Genetic variants of β-casein gene in indigenous and exotic dairy cattle breeds in Sri Lanka. Asian Journal of Dairy and Food Research. 39(3): 217-220.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Shashank, C.G., Puri, R.K., Gandhi, G., Kaur, T. and Kushwaha, M.K. (2018). A1 and A2 beta casein: Twin faces of milk. Journal of Pharmacognosy and Phytochemistry. 7(4): 221-224.

Sodhi, M., Mukesh, M., Kataria, R.S., Mishra, B.P. and Joshi, B.K. (2012). Milk proteins and human health: A1/A2 milk hypothesis. Indian Journal of Endocrinology and Metabolism. 6(5): 856.

Trivedi, M.S., Shaha, J.S., Al-Mughairya, S., Hodgsona, N.W., Simmsa, B., Trooskensb, G.A., Criekingeb, W.V. and Detha, R.C. (2014). Food-derived opioid peptides inhibit cysteine uptake with redox and epigenetic consequences. Journal of Nutritional Biochemistry. 25(10): 1011-18.