

# A1 AND A2 MILK – A REVIEW

G. Prakash and K. Anilkumar

Department of Animal Breeding and Genetics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala - 680 651

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## ABSTRACT

Caseins and whey proteins are two major protein groups present in the milk. Casein makes up around 80 % of the milk protein. *Casein* genes are situated on bovine chromosome 6. There are 4 casein protein genes in cattle namely,  $\alpha_{s1}$ -casein (*CSN1S1*),  $\alpha_{s2}$ -casein (*CSN1S2*),  $\beta$ -casein (*CSN2*) and  $\kappa$ -casein (*CSN3*). Alleles in the *CSN2* gene are A1, A2, A3, B and C. Among these, A1 and A2 variants are reported to be the most common allelic variants of  $\beta$ -casein in cattle and others (B, A3, and C) are rare. Most of the European breeds of cattle possess 50 per cent of A1 allele which has evolved as an outcome of natural mutation from A2 allele. Indian milk breeds of cattle and buffaloes carry almost 100 percent of A2 allele. The A1 and A2 variants differ only at position 67, which is histidine (His) in A1 where as it is proline (Pro) in A2. It is reported that a bioactive  $\beta$ -casomorphin-7 (BCM-7) will be released on digestion of A1  $\beta$ -casein, which can potentially affect numerous opioid receptors in the nervous, endocrine, and immune systems. The evidence is still too weak for any strong conclusions to be made. Many studies establish that there is no convincing or probable evidence that A1 allele of  $\beta$ -casein of cow milk is having any adverse effect on humans. More

detailed studies are needed for arriving at a final conclusion.

**Keywords:**  $\beta$ -casein, A1, A2, BCM-7

## INTRODUCTION

Milk is a wholesome, fresh, clean, lacteal secretion obtained by complete milking of healthy milch animals, excluding that obtained within 15 days before and 5 days after calving. It is the most common food for infants and adults. It is the most perfect source of energy, proteins and micronutrients such as calcium, phosphorus and others to most of the normal body metabolism. Cow milk is composed of 86.6 % of water, 4.9 % lactose (milk sugar), 4.6 % triacylglycerol (milk fat), 3.4 % milk protein (whey and casein) and 0.7 % ash (minerals and vitamins). Bovine milk contains 3-5 % protein which is composed of 80 % casein and 20 % whey (Threadgill and Womack, 1990). Some of the reports state that whey proteins make about 16 % of the total milk protein (D'Alessandro *et al.*, 2011). Components of bovine casein protein are  $\alpha_{s1}$ -casein 39-46 %,  $\alpha_{s2}$ -casein 8-11%,  $\beta$ -casein 25-35%, and  $\kappa$ -casein 8-15% (Rijnkels, 2002). In India, milk pricing system is based on milk fat percentage and SNF content of milk. Now-a-days  $\beta$ -casein protein had much

importance because of the controversy attached to the association of  $\beta$ -casein protein and health related issues.

#### Evolution of A1 from A2 Allele

Current phylogenetic studies indicate that initial form of  $\beta$ -casein is A2  $\beta$ -casein. Around one lakh year ago, both *Bos taurus* and *Bos indicus* species evolved from ancient *Bos* genus. During that period, all the animals carried only A2 allele. Ten thousand years before, A1 allele evolved from A2 allele through natural mutation in *Bos taurus* species. African and Indian *Bos indicus* primarily carry A2 allele. In European *Bos taurus*, all the breeds carry A1 and A2 allele at different proportions. (Mir *et al.*, 2014; Jaiswal *et al.*, 2014)

#### Basic Genetics

Four types of casein protein genes in cattle are  $\alpha_{s1}$ -casein (*CSN1S1*),  $\alpha_{s2}$ -casein (*CSN1S2*),  $\beta$ -casein (*CSN2*) and  $\kappa$ -casein (*CSN3*). Many allelic variants have been identified in  $\beta$ -casein like A1, A2, A3, B and C variants (likewise upto 22  $\beta$ -casein variants are reported in bovine). Among these A1 and A2 allele variance is most commonly reported in dairy cattle and other alleles (e.g. B, A3, and C) are very rare. In bovine, sixth chromosome determines the A1/A2 status of a cow (Formaggioni *et al.*, 1999; Cui *et al.*, 2011; Caroli *et al.*, 2009; Kaminski *et al.*, 2007; Nilsen *et al.*, 2009; Ng-Kwai-Hang, 1998; Ikonen *et al.*, 1999). Each cow carries two copies of the  $\beta$ -casein allele. Those animals which carry A2A2 alleles produce A2  $\beta$ -casein - A2 milk, A1A2 heterozygous animal produce both A1 and A2  $\beta$ -casein in their milk because of co-dominant gene action and A1A1 homozygous animal produce only A1  $\beta$ -casein - A1 milk. (Farrell *et al.*, 2005).

#### Allele Frequency

Researches conducted on indigenous cows, buffaloes and exotic cows revealed that A1 allele is more frequent in exotic cattle while Indian native dairy cows and buffaloes have predominantly A2 allele. The allele frequency varies between breeds, country and population. For example, European breed Holstein Friesian (HF) carry equal proportion of A1 and A2 allele and Southern European breeds like Jersey carry 35 % of A1 allele and 65 % of A2. An exception to this is Guernsey breed that carry about 90% of A2 allele. Indian milk breeds of cows and buffaloes carry 100% of A2 allele whereas it comes around 98% in other Indian breeds.

According to Ng-Kwai-Hang and Grosclaude (2002), there is no A1  $\beta$ -casein in the milk of pure Asian and African cattle. But some Indian breeds carry A1 allele and examples are Malnad Gidda 9.6 %, Kherigarh 10.9 % (Mishra *et al.*, 2009). Kangayam carry only A2 allele and cross bred HF carry 59.5 % A2 allele (Malarmathi *et al.*, 2014). Some other Indian breeds like Ongole, Vechur, Gaolao, Dangi, Khillar, Umbalacheri, Ponwar and Red Khadari carry low frequency of A1 allele. Frieswal carry A2 and A1 allele at a frequency of 0.35 and 0.65 (Ganguly *et al.*, 2013), where as it is 0.825 and 0.175, respectively in Karan Fries (Jaiswal and Sarsavan, 2013). A1 allelic frequency in HF, Jersey and crossbred are 0.441, 0.325 and 0.298, respectively (Sodhi *et al.*, 2012). Frequency of A1 and A2 alleles in Holstein bulls are 0.402 and 0.598, respectively (Kaminski *et al.*, 2006), whereas the corresponding values are 0.177 and 0.809, respectively in Czech Fleckvieh bulls (Kucerova *et al.*, 2006).

### Marker for selection

The polymorphic nature of the  $\beta$ -casein is reported to be correlated with breeding value of total milk production and milk constituents. A1A1 genotype of cross bred cattle of Kerala is reported to have significantly high peak yield ( $14.64 \pm 3.181$  kg) compared with other genotypes, A1A2 and A2A2, which were  $8.54 \pm 0.194$  kg and  $9.09 \pm 0.125$  kg, respectively. The breeding values of animals with genotype A2A2 are negative for yield parameters but positive for content parameters and vice versa in A1A1 genotype. It indicates that A1 genotype is associated with increased milk yield. Hence this is a potential dairy trait marker (Muhammed and Stephen, 2012).

### Is A1 milk harmful?

The controversy started after a hypothesis developed by Elliott (1992) and McLachlan (2001), that a protein in the milk of some cows is an important risk factor for type I diabetes (DM-I) and coronary heart disease (HD) (possibly also schizophrenia and autism). The implicated protein is the A1 form of  $\beta$ -casein, the second most abundant protein in cow's milk: its commonest genetic variants are A1, A2 and B  $\beta$ -casein.

Cow milk  $\beta$ -casein is the second most abundant protein which contains 209 amino acids. Only at position 67 A1 differ from A2 variant, which is histidine (His) in A1 and proline (Pro) in A2 milk. This variation is due to point mutation, the nucleotide CCT switched into CAT. In allele, A3 106<sup>th</sup> position histidine changed into glutamine, whereas in allele B 67<sup>th</sup> position proline changes to histidine and serine to arginine at position 122. In A2  $\beta$ -casein, in 67<sup>th</sup> amino acid proline had strong affinity between the adjacent amino acids where

as in A1  $\beta$ -casein, histidine doesn't have such affinity. During the digestion of A1  $\beta$ -casein a bioactive 7 amino acids compound is released in small intestine, known as BCM-7 (beta-casomorphin-7). But A2  $\beta$ -casein digestion do not release BCM-7 (Woodford, 2011).

BCM-7 is a heptapeptide and has strong affinity towards the  $\mu$ -opioid receptors. Many studies prove that BCM-7 cause many adverse effects on human health. It is reported to cause type-I diabetes mellitus (DM-I), cardiovascular disease and some neurological disorders (Woodford, 2011).

Occurrence of DM-I in non-obese diabetic (NOD) mouse fed with A1  $\beta$ -casein was significantly higher when compare to mice fed with A2  $\beta$ -casein (Elliott *et al.*, 1997), but Yin *et al.* (2002) found that milk caseins are unlikely to be exclusive promoters of Type I diabetes, but could enhance the outcome of diabetes in some cases. Other diet components such as wheat could be more important promoters of Type I diabetes. Yin *et al.* (2010) reported that beta-casomorphin-7 can protect rats from hyperglycemia and free radical-mediated oxidative stress in diabetic rats.

McLachlan (2001) reported that there is no heart disease in some population such as the Masai (East African) and Samburu (Northern Kenyan) who are consuming milk came from Zebu cattle, which is a breed exclusively with A2 allele, but subsequently It was reported that there is no evidence of A1 milk supplementation having any effect on cardiovascular health (Chin-Dusting *et al.*, 2006) and on plasma cholesterol concentrations (Venn *et al.*, 2005) in humans over consumption of casein A2.

Truswell (2005) has pointed out that

for the incidence of both DM-I and Coronary Heart Disease (CHD), the between-country correlation method is shown to be unreliable and negated by recalculation with more countries and by prospective studies in individuals. The animal experiments with diabetes-prone rodents that supported the hypothesis about diabetes were not confirmed by larger, better standardized multicentre experiments. The single animal experiment supporting an A1  $\beta$ -casein and CHD link was small, short, in an unsuitable animal model and had other design weaknesses.

Brooke-Taylor *et al.* (2017) concluded that in animals and at least in some human population groups, the A1 derivative peptide BCM-7 is pro-inflammatory. The balance between the extent to which these effects are direct inflammatory responses to BCM-7 or indirect consequences of delayed transit influencing other biological processes, is yet to be elucidated. Although the current gastrointestinal evidence is linked to BCM-7 and m-opioid pathways, the possibility that some gastrointestinal effects involve non opioid pathways is relevant. There is now a need for further clinical studies of A1 effects in a broad range of population groups (ages, ethnicities and different genetic haplotypes) and dietary conditions.

In India, almost all the indigenous dairy and buffaloes A2 allele is fixed and crossbreds also predominantly carry A2 allele (Ramesh *et al.*, 2016). Compared to other Western countries India produce less amount of A1  $\beta$ -casein and per day A1  $\beta$ -casein consumption is also very less. But till date, even a single correlated study is not reported in India to prove A1 milk's adverse effect on human health. In

2015, ICAR approved a project entitled "Delineating  $\beta$ -casein Variants in Indian Cows and Potential Health Implications of A1A2 Milk" in the period of July 2015 to June 2018. National Bureau of Animal Genetic Resources (NBAGR), National Dairy Research Institute (NDRI) and Punjab University (PU) are working on this project. Objectives are to establish experimental evidence for the cause and effect relationship of BCM-7 peptide, A1, A2 and A1A2 milk with disease progression in mice model.

## CONCLUSION

One of the important components of milk protein namely  $\beta$ -casein protein had much importance than other constituents of milk because of the possible association with health related issues. The data available and conclusion derived by researchers on A1/A2  $\beta$ -casein polymorphism and its association with human health are contradictory. The A1/A2 hypothesis is both intriguing and potentially very important for public health, if it is proved correct. In depth research is needed to verify the range and nature of BCM-7 interactions with the human gastrointestinal tract and whole organism. This requires more of animal trials and generation of data on human subjects having the problems related to A1/A2  $\beta$ -casein milk consumption.

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