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## Impact of milk protein on human health: A1 verses A2

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### Abstract

Milk is often regarded as “nature’s most perfect food” because it contains essential nutrients needed for growth and development of calf as well as humans. Milk protein is two types- casein and whey protein. The most abundant  $\beta$  casein protein exist in A1 and A2 form which is determined by the presence of histidine and proline amino acid respectively. A1 milk is reported to have a negative impact on human health due to BCM-7, hypothesized to be released during digestion and a risk factor for certain diseases like DM-1, IHD, SIDS, autism etc. Majority of exotic breeds produce A1 type of milk whereas Indigenous cattle breeds have a higher frequency of A2 type of allele in their milk. Thus the issue of A1/A2 milk has remained controversial so far and there is no conclusion and final scientific opinion that consumption of A1 type of milk possesses high risk for above-mentioned diseases

**Keywords:** Allele, Cattle, Milk, Protein

### Introduction

Milk is the highly evolved mammary gland’s secretion of mammals and a perfect food for infants. Milk has about 86% water, 4.6% lactose sugar, 3.7% triglycerides, 2.8% milk protein, 0.54% minerals and 3.36% other constituents. Milk protein constitutes 36%  $\alpha$ -Casein, 27%  $\beta$ -Casein, 9%  $\kappa$ -casein, 27% peptides and amino acids. It also contains immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and several others bioactive peptides. The lipids in milk are emulsified in globules coated with membranes and proteins are in colloidal dispersions as micelles. The casein micelles are seen and observed as colloidal complexes of protein and salts, especially calcium<sup>[15]</sup> whereas lactose and most other minerals are in solution form. Specific proteins from milk are involved in the early development of immune response and other milk proteins get involved in the non-immunological defence (e.g. lactoferrin). Different fatty acids are present in the milk<sup>[13]</sup> and all these fatty acids constitute nutrient-rich milk food.

The protein fraction composition of  $\beta$ -casein has become of special interest recently because of a possible relationship between  $\beta$ -casein genotype and the health of consumers. Milk that contains A1  $\beta$ -Casein and A2  $\beta$ -Casein are known as A1 milk and A2 milk, respectively. A1 protein variant is commonly found in milk from crossbred and European breeds of cattle. A2 milk is found mostly in indigenous cows and buffaloes of India (in Asia also).

Milk composition varies with stage of lactation, age, breed, nutrition, energy balance and health status of the udder. Colostrum also differs considerably from milk to milk and the proteins are higher in colostrum than in the later locational milk<sup>[26]</sup>. The milk composition changes with the change in the nutrients given to the growing infants during the entire lactation period.

### Casein

The bovine milk contains a huge array of proteins from antimicrobials to hormones and enzymes to antibodies<sup>[5]</sup>. Milk proteins have been put into three categories so far according to their solubility potential casein, whey proteins and milk fat globule membrane (MFGM) proteins, among which milk casein constitutes around 80%<sup>[24]</sup> and whey proteins about 14%<sup>[18]</sup>. The biological function of caseins is to carry calcium and phosphate and to form a clot in the stomach for efficient digestion. Casein is a group of proteins coded by four tightly-linked autosomal genes (CSN1S1, CSN1S2, CSN2 and CSN3). They are sub-divided into four families:  $\alpha$ s1-,  $\alpha$ s2-,  $\beta$ - and  $\kappa$ -CN. Casein molecular mass is about 18-25 kDa and is quite

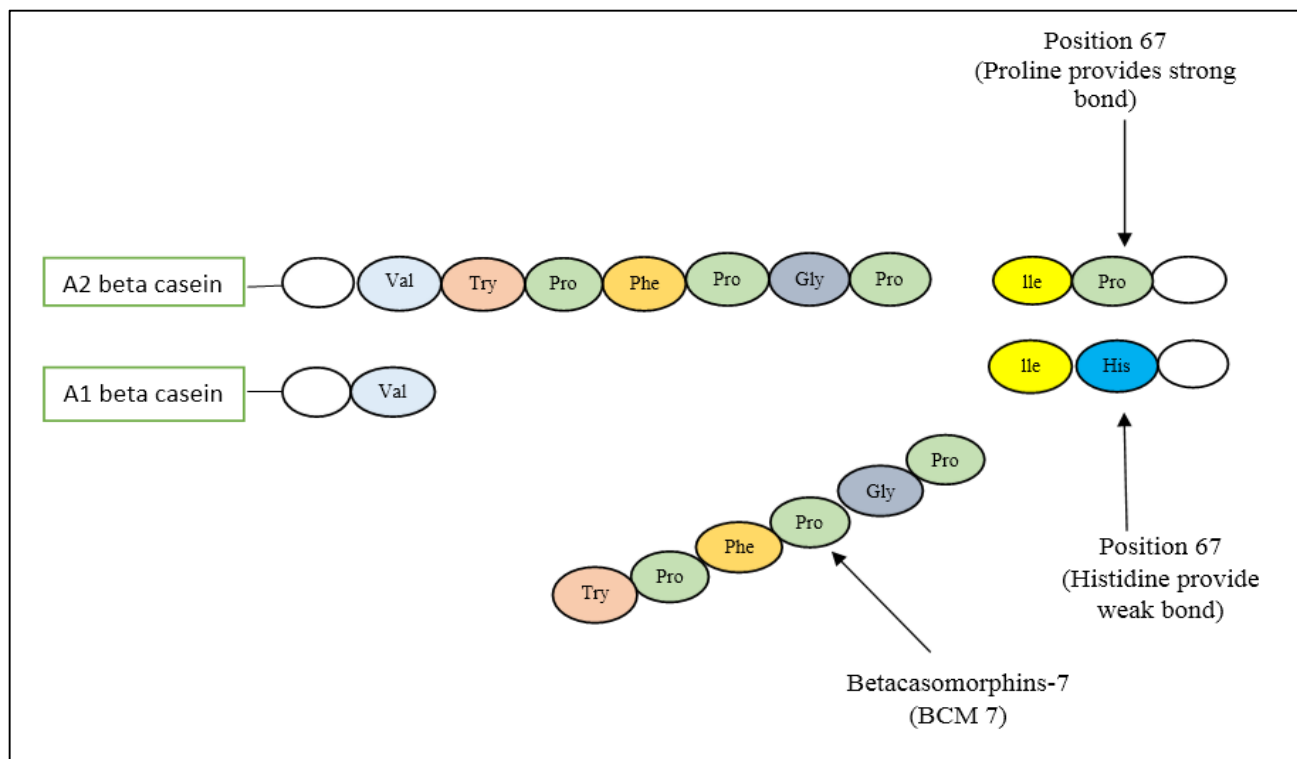
heterogeneous in nature as it develops through posttranslational modifications and alternative splicing of the gene product and genetic polymorphisms<sup>[4, 17]</sup>. Caseins show phosphorylation as they are basically phosphoproteins. Caseins do not have stable secondary and tertiary structures and due to which they become susceptible to proteolysis.

### $\beta$ -casein polymorphism

There are 12 genetic variants of  $\beta$ -casein: A1, A2, A3, B, C, D, E, F, H1, H2, I, G<sup>[14]</sup>. Another A4 allele is found in

Korean native cattle but nucleotide substitution is not yet recognized. Various forms of  $\beta$ -casein in dairy cattle breeds are A1, A2, B, A3 and C<sup>[8]</sup> among these A1 and A2 forms are most common. In position 67 of the  $\beta$ -casein chain, proline in A2 is substituted by histidine in A1<sup>[30]</sup> (Fig 1).

Beta casomorphin-77 (BCM7) is produced from A1  $\beta$ -casein during gastrointestinal digestion<sup>[6]</sup>. BCM-7 may get absorbed in the infants GIT as compared to adults. In A1 milk, the BCM-7 level is 4 fold higher than in A2 milk.



**Fig 1:** Schematic representation of formation of beta casomorphin-7 with the breakdown of A1 milk chain of amino acid due to the presence of histidine at position 67. However, amino acid chain remains intact in A2 milk amino acid chain due to the absence of histidine at position 67.

The A1 allele is more frequent in exotic cattle (A1 milk) while Indian native dairy cows and buffaloes have only A2<sup>[38]</sup> and hence is a source of safe milk i.e A2 milk<sup>[20]</sup>. The A2 allele gene in Indian milch breeds of cows and buffaloes are 100% (Red Sindhi, Sahiwal, Tharparkar, Gir and Rathi), other Indian breeds used for farming is around 94% and while in exotic breeds (HF and Jersey), it is around 60% (NBAGR, 2011). A1  $\beta$ -casein is absent in the milk of pure Asian and African Cattle<sup>[23]</sup>.

The A1/A2 milk is determined by a pair of genes on the sixth chromosome<sup>[29]</sup>. There are two major alleles of the gene i.e. A1 and A2  $\beta$ -casein alleles. Neither allele is dominant over the other rather; they are co-dominant i.e. additive in their effect. Therefore, an A1A2 cow will produce A1 and A2  $\beta$ -casein in equal amounts. An A2A2 cow will only produce A2  $\beta$ -casein and an A1A1 cow will only produce A1  $\beta$ -casein.

### Beta-casomorphins and its role

The A1 and A2 variants of bovine  $\beta$ -casein differ at amino acid position 67 with histidine in A1 and proline in A2 milk. This polymorphism leads to key conformational changes in the secondary structure of expressed  $\beta$ -casein protein<sup>[6, 18]</sup>. Due to presence of histidine at amino acid 67 position, digestion of A1  $\beta$ -casein milk releases a 7 amino acid bioactive peptide called beta-casomorphin-7 (BCM-7) in

small intestine, while proline in A2 milk at 67 positions prevents the breakdown and generates peptide BCM-9<sup>[30, 45]</sup>. It is believed that generation of BCM-7 is the major causative factor associated with A1 milk related health disorders. However, A2  $\beta$ -casein has not been linked to any of such health issues<sup>[14]</sup>.

BCM7 causes human health hazards as it can potentially affect numerous opioid receptors in the nervous, endocrine and immune system. It is known to oxidize low dietary lipoproteins (LDL) and which forms arterial plaque. Few epidemiological studies demonstrated that consumption of  $\beta$ -casein A1 milk associated as a risk factor for type 1 diabetes, coronary heart disease, arteriosclerosis and sudden infant death syndrome<sup>[18, 16, 6, 40, 42, 21, 39, 1, 35, 34, 36]</sup>. At the same time, other diseases such as autism, schizophrenia<sup>[16, 39, 44]</sup> have also been shown to have associated with consumption of  $\beta$ -casein A1 milk.

BCM-7 has been documented as an opioid and bio-modulatory product<sup>[46]</sup>. Synthetic BCM-7 can also inhibit responses of lymphocytes to stimulants *in vitro*. BCM-7 is a bioactive<sup>[31]</sup> compound which considered exorphins<sup>[19]</sup> and has been shown to increase with more milk proteins in the diet. A1-derived BCM-7 has been shown to have a longer half-life<sup>[27]</sup>, and also detected in urine<sup>[3]</sup> of mice fed with A1 milk. These particular exorphins have also been encoded in

the human breast milk proteins but they are less active as compared to the bovine milk proteins [10]. BCM-7 Peptides derived from caseins have also been shown to produce immune reactivity [37] and detected in human plasma. There are several reports that claim certain precursors of BCM-7 are also present in the plasma of newborn calves having consumed A1 milk from cows [41] but the same thing did not happen in the case of adults particularly dogs [32]. BCM-7 gets transmitted into circulation following digestion, notably in new-born or young mammals and easily detected the urine [3] of patients with neurological conditions relying on casein-free diet. Studies have also demonstrated the transmission of BCM-7 through blood-brain barrier [36]. Few studies have also shown traces of BCM-7 may cross the breast parenchyma-blood barrier into plasma and subsequently penetrate the blood-brain barrier to reach the central nervous system of the developing foetus [25]. It is alarming to note that it also causes apnea in infants due to the consumption of bovine milk of A1 origin which lead to sudden death in new-borns [36].

McLachlan (2001) conducted a study on Masai (East African) and Samburu (Northern Kenyan) tribes and found that they had virtually no heart diseases despite consuming a diet rich in cattle milk. It may be probably due to the tribe's dependency on zebu cattle's milk, which is a breed that carries the A2 allele exclusively. Besides this, epidemiological analyses concerning the two alleles of  $\beta$ -casein and the incidence of cardiovascular diseases indicate the apparent relationship between the risk of chronic disease and milk protein variant intake [16]. The population in the countries which were predominantly depended on Holstein, Jersey, Brown Swiss and other breed's milk had a greater incidence of CVD than nations with low milk consumption. After digestion of  $\beta$ -casein, may release a bioactive peptide, betacasomorphin-7, which is responsible for many human disorders like Type 1 diabetes, autism, schizophrenia and heart diseases but A2 milk does not cause such type of illnesses (Woodford, 2006; [20, 33]). Infants may absorb BCM-7 due to an immature gastrointestinal tract which might lead to sudden infant death syndrome. Another relevant component needs to be addressed is the calcium content of A1 milk. An average person is able to get only about 700 mg of calcium per day, which comes primarily from dairy products [43, 7]. This amount is the recommended amount of 1,000-1,500 mg (NIH Consensus Development Conference, 1994). Calcium content of milk may reduce the risk of osteoporosis and colon cancer [9, 2] and be including milk in the diet may promote weight loss [28]. The A1 milk's calcium to magnesium ratio is 10:1, which is far higher than the ideal ratio (A2 milk's) i.e., 2:1. It indicates that relying on milk containing A1 for calcium will lead to magnesium deficiency and Ca: Mg imbalance, but it doesn't occur in case of A2 milk. A1 milk may also cause acne, eczema, upper respiratory infections, asthma, ear infections, tonsillitis and allergies. The inflammation from A1 milk casein causes lymphatic congestion and metabolic suppression. Massive histamine release from casomorphin may provoke the digestive problems.

#### **Assessment methodologies of presence of betacasomorphins in milk**

Reliable methodologies are needed to correctly quantify them. Reversed-phase high performance liquid chromatography (RP-HPLC) is used in the separation of peptides and amino acids. RPHPLC-UV [22] and ion-exchange chromatography [11] are used for the separation and quantification of BCM7

present in cheese while HPLC-UV has been also used for determining BCM7 content in human milk [12]. Recently, HPLC along with mass spectrometry (MS) regarded as the best method for identification and quantification of peptides in any dairy products. Various mass spectrometry techniques such as tandem mass spectrometry (MS/MS), quadrupole ion-trap mass spectrometry (QIT-MS), and time of flight mass spectrometry (TOF-MS) also helps in the identification of BCM's in the several dairy products. Tandem mass spectrometry allows accurate quantification of BCM5 and BCM7 at low levels (low mg/g) and hence considered very sensitive for investigation purposes. Similarly, QIT-MS has also been observed to quantify BCM5 and BCM7 in single reaction stroke.

#### **Conclusion**

The A2 milk should only be recommended as it prevents the human beings from milk related health complications, which are due to A1 milk. In India, the population of the exotic/crossbred milch cattle increased from 14.4 million to 19.42 million i.e. an increase of 34.78% (19th Livestock Census- 2012). Bovine  $\beta$ casein gene has given rise to 12 different genetic variants, among them A1 and A2 variants have been observed to be very common. The A1 and A2 variant differs at amino acid position 67 with histidine (CAT) in A1 and proline (CCT) in A2 (SNP difference). This single nucleotide polymorphism has caused a conformational change in the secondary structure of expressed  $\beta$ -casein protein. Intestinal degradation of A1  $\beta$ -casein (raw/processed milk) produces bioactive peptides i.e. beta casomorphin 7 (BCM7) which gets digested soon in the infants and affects them more. Production of BCM-7 from A1 consumption is four fold higher in infants compared to adults. A1 allelic frequency has been found to be more in exotic cows compared to the native dairy cows with a hump. Moreover, it has been seen and observed that the population which consumed milk containing high levels of  $\beta$ -casein A2 variant have a lower incidence of cardiovascular disease and type-1 diabetes. However, the detailed investigation needs to be done in this direction to address the issue precisely.

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