Review Article

iMedPub Journals
www.imedpub.com

Journal of Animal Research and Nutrition
ISSN 2572-5459

2021
Vol.6 No.7:101

DOI: 10.36648/2572-5459.6.7.101

Human Health and Milk Proteins: The A1/A2 Milk Hypothesis

Ayushi Chaudhary, Harjeet Singh, Gyanendra S Senger and Umesh Singh

Molecular Genetics Laboratory, C.G. & B. Division, ICAR-Central Institute for Research on Cattle, Meerut-250 001, Uttar Pradesh, India

***Corresponding author:**
Ayushi Chaudhary

ayushichaudhary9502@gmail.com

Molecular Genetics Laboratory, C.G. & B. Division, ICAR-Central Institute for Research on Cattle, Meerut-250001, Uttar Pradesh

Abstract

Indigenous breeds of cows and buffaloes are of A2 milk type and hence are a source for safe milk. The A1/A2 status of cattle is determined by the two alleles for beta-casein gene i.e. A1 and A2 cattle may be homozygous for one type of protein (A1A1 or A2A2), or heterozygous (A1A2). A1A1 cattle would produce only A1 milk, A2A2 would produce only A2 milk and A1A2 cattle would produce milk with both A1 and A2 beta - caseins. NBAGR also reported moderate to high frequency of A2 allele among the breeding bulls, further supporting the belief that milk being sold in India is safe for human consumption. Considering the lack of concrete scientific evidence for A1/BCM-7 ill-effects on human health, and predominance of A2 milk in Indian cattle there is no need to move to A2 only.

Keywords: Guanidine; Histidine; BCM-7; A1/A2; Immunoglobins; Amino acids.

Received: June 19, 2021; **Accepted:** July 12, 2021; **Published:** July 19, 2021

Citation: Chaudhary A, Singh H, Senger GS, Singh U (2021) Human Health and Milk Proteins: The A1/A2 Milk Hypothesis. J Anim Res Nutr Vol.6 No.7:101.

Preamble

Milk is enormously evolved secretion of mammary glands produced by mammals being perfect food for novice. Globally, India is the highest milk producer (176.4 million tonnes) as well as consumer of milk, neither exports nor imports milk. Since long back milk is considered as complete source of nutrients (both micro and macronutrients) providing a range of proteins, carbohydrates and other important components of human food [1].

Around world cow, buffalo, sheep, camel and goat are known as the main producers of milk out of which cow being the major producer of milk i.e. 83% (about 600 million tonnes) of the total milk produced annually. Also, India is the leading producer of milk (176.3 million tonnes approx.) which is uppermost in the world. In relation to India alone, 46.9% milk is yielded by buffalo while 49% of milk is produced by cattle [2].

The major fraction of milk contains water which constitute about 85% of milk while remaining 15% of milk is composed of sugars such as lactose (4.6%), milk proteins (2.8%), fats (3.7%), minerals (3.36%) and other component like hormones, immunoglobulin's, growth factors, cytokines, nucleotides, peptides, polyamines and enzymes [1-3].

Major Polymorphs of Cow's Milk

Milk protein is divided into two major classes i.e. casein and whey protein. The casein may be further sub-divided as alpha, beta and kappa casein **(Figure 1)** out of which beta-casein is more ubiquitous containing balanced proportions of essential amino acids [4]. The overall protein makeup of milk is 36% α-Casein, 27% β-Casein, 9% κ-casein whereas other peptides and amino acids comprise about 27% of total milk proteins [3]. Out of all casein proteins in milk β-Casein are of foremost importance as it is the reason for generation of various genetic variants of milk. So far about 12 genetic variants are known namely A1, A2, A3, B, C, D, E, F, H1, H2, I and G out of which 'A1' and 'A2' are often found. 'C' is the rarest of rare and not so often found in milk proteins [5]. β-Casein of Cows' milk contains 209 amino acids [6]. A fluctuation of histidine (A1 type) and proline (A2 type) at position 67 is profoundly observed which give rise to two polymorph of same protein of β-Casein viz. variants A1 and A2 [3].

A1 Milk: A Valid Risk?

β-Casein of cow milk is made up of 209 amino acids. It accounts 24-28% of total milk protein and its molecular weight is about 24 kD [7]. It is position 67 where A1 and A2 variants of cows' milk differ from each other. While A1 contains histidine at this position, A2 have proline instead of histidine. Beta casein releases a range of opioids known as beta casomorphins (BCMs). β-casomorphin-7 (BCM-7), a seven amino acids long bioactive peptide, released in small intestine during digestion of A1 β-Casein which have

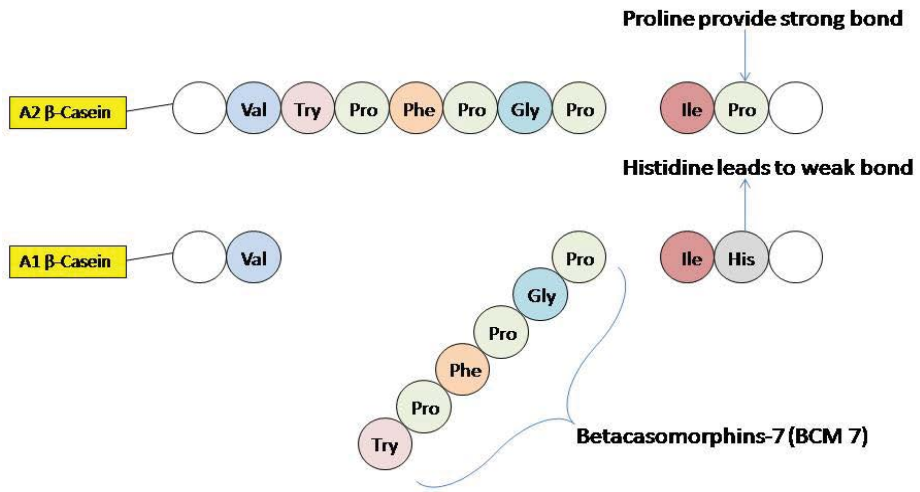

Figure 1 Depiction of BCM 7 (Betacasomorphins-7) breakdown at 67th position of peptide due histidine amino acid in A1 beta-casein variant.

been found associated as a factor of risk while human health is concerned [1,6].

Health Problems Associated to A1 β-Casein Variant

A number of workers had worked upon the problems related to A1 variant of β-Casein of cow milk. It is believed that β-casomorphin-7 (BCM-7), an opioid, have ability to oxidize low dietary lipoproteins which could lead to the formation of arterial plaques. Digestive enzymes lack ability to break A2 protein due to the presence of proline instead of histidine [1,6].

Also some of the studies carried out in various European countries such as United Kingdom, Iceland, Norway, Austria as well as in American subcontinent revealed a strong association between β-casein A1 and diabetes mellitus (DM1) **(Table 1)** [8].

There are three classes of receptors for opioid in brain viz. μ (MOP), κ (KOP) and δ (DOP) [9]. These receptors are distributed in immune cells, endocrine glands, brain and in intestine. Some of the studies carried out by Brantl [10] and Henschen [11] presume that bovine milk has opioid effect and MOP are the receptors through which they interact mostly [12]. It was also found that BCM-7 of bovine milk has stronger affinity as compared to human milk for MOP receptors [13]. Pol [14] reported that genes producing inflammatory enzymes like myeloperoxidases are enhanced by BCM-7 that could lead to the expression of MOP receptors in intestine. Though, no report has been encountered related to human brain.

Methodologies Available for Evaluation of Beta-casomorphins in milk

Number of workers in recent past have been worked or working on to identify and quantify the presence of betacasomorphins in milk. Some of the methodologies which are in use are summarized in **Table 2** given below. Above mentioned table illustrated various methods which are more or less in use to identify or quantify betacasomorphine in milk proteins. Apart from these techniques there are several methods which are in use for genotyping various polymorphs of milk proteins in cattle such as PCR–SSR (simple sequence repeat polymorphism or microsatellite markers), PCR-SSCP (Single Strand Conformation Polymorphism), Restriction fragment length polymorphism (PCR–RFLP analysis), Single nucleotide polymorphism (SNP) assay followed by direct sequencing, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). All these methods are PCR-based methods which can be used to discriminate different variants of milk proteins. Also, there are some techniques viz. isoelectric focussing and RT-HPLC which are not dependent of PCR. Some of the major advantages and disadvantages of these methods are summarized in **Table 3**.

Significance of β-Casein Milk Variants Concerning India

All across the world A1 and A2 alleles of β-Casein gene distributed variably in different cattle breeds. There is a serious debate going on weather A1 milk is not suif for human health when compared to A2 milk. Mishra et al [23] were in support of the opinion that consumption of A1 type milk may be hazardous for human health. Also, some other reports have been encountered [3] which recommends that A2 milk is better for human health and crossbreeding should be avoided for overcoming serious consequences on human health. According to Jawale [3] exotic or crossbreed cattle variants have been elevated in number from 14.4 million to 19.42 million (approximately 34.78%), which is a serious concern as per their opinion. But, according to other reports published by Truswell [6] and EFSA [24] 98% out of 618 cattle which were screened were belonging to A2 type. They also stated that out of 180 bulls from different regions and randomly selected were genotyped out of which only 11% bulls limited to A1A1 genotype.

Genotype Project

Studies by the National Bureau of Animal Genetic Resources (NBAGR), Karnal covering 22 desi breeds have established that

Table 1: Some of the major health related issues related to A1 β-Casein milk.

Sr. No.	Health Problem(s)	Target Organ(s)	Reference(s)
1	Type I diabetes	Pancreas	Laugesen and Elliott, [8]
2	CVD (Cardiovascular disease)	Heart	Laugesen and Elliott, [8]
3	Arteriosclerosis	Blood vessels and Heart	Tailford et al. [15]
4	Schizophrenia	Brain	Woodford, [16]
5	Autism	Brain	Woodford, [16]
6	SIDS (Sudden Infant Death Syndrome)	Unexplained	
7	Autistic Spectral Disorder	Brain	Jaiswal et al. [17]
8	Digestive Health Lactose intolerance	Digestive system	Swinburn and Boyd [18]
9	Magnesium Deficiency and Imbalance	Electrolyte imbalance	Pal et al. [19]
10	Endometriosis	Uterus	Pal et al. [19]

Table 2: Methodologies Available for Evaluation of Beta-Casomorphins in Milk.

S. No.	Method/Technique	Theory	Reference
1	Reversed-phase high performance liquid chromatography (RP-HPLC)	It is exercised for separation of peptides and amino acids	Muehlenkamp, MR , Warthesen J.J [20]
2	RPHPLC- UV	Employed for the separation and quantification of BCM7 present in cheese	Muehlenkamp, MR , Warthesen J.J [20]
3	Ion-exchange chromatography	Employed for the separation and quantification of BCM7 present in cheese	Jarmolowska et al. [21]
4	HPLC-UV	Has been used for determination of BCM7 amount in human milk	Jarmolowska et al. [21]
5	Tandem mass spectrometry	It helps precise quantification of BCM5 and BCM7 at minor levels	Priyadarshini et al. [22]
6	Quadruple ion trap mass spectrometry (QIT-MS)	Measure BCM5 and BCM7 in single reaction.	Priyadarshini et al. [22]

Table 3: Major Advantages and Disadvantages of these Methods.

S. No.	Method	Advantages	Disadvantages
1	IEF	High resolution, resulting in greater separation of solutes Easier, because the placement of sample application is not important The sample and ampholytes can be mixed before application	Carrier ampholyte batch-tobatch reproducibility of the ampholytes Phenomenon of cathodic drift owing to electroendosmosis in the gel causes an eventual breakdown of the basic end of the pH gradient with time
2	SDS-PAGE	Highly reproducible Inexpensive, fast, sensitive, expressive Economic (no sophisticated equipment required) Easy (clear, one-dimensional separation principle)	Limited pI range (4-8) Proteins >150 kD not seen in 2D gels Difficult to see membrane proteins (>30% of all proteins) Only detects high abundance proteins (top 30% typically) Time consuming
3	RT-HPLC	Speed (minutes) High resolution Sensitivity (ng to fg) Reproducibility of +/- 1% Accuracy Automation	Cost Complexity Low sensitivity for somecompounds Irreversibly adsorbed compounds not detected Coelution difficult to detect
4	PCR-SSR	Require very little and not necessarily high quality DNA Highly polymorphic evenly distributed throughout the genome Simple interpretation of results Easily automated, allowing multiplexing Good analytical resolution and high reproducibility	Complex discovery procedure Expensive

predominant genotype in India's native cattle is A2A2, confirming that our indigenous cows and buffaloes produce A2 milk.

The frequency of A2 allele was Almost 100 per cent in the five high-yielding milk breeds — Red Sindhi, Gir, Rathi, Sahiwal and Tharparkar, meaning that these breeds do not have A1 allele or A1A1/A1A2 genotype. In the remaining breeds, the availability of A2 allele was 94 per cent.

References

1. Jois S, Bindu Shree C, Chethan AR, Kiran Naik CP, Sachin S (2018) Classification of A1 milk and A2 milk by observational and scientific methods. IRJET 5: 39–41.
2. NAAS (2019). Harnessing Full Potential of A1 and A2 Milk in India: An Update. Strategy Paper No. 12, National Academy of Agricultural Sciences, New Delhi: 20.
3. Jawale B, Kaluskar A, Garde J, Sabnis S (2017) "The Reality of the White" A1 vs. A2 Milk- A Critical Review. IJSR ISSN (Online): 2319–7064.
4. Banerjee S (2018) "A2 Milk: The Unknown Story about a Milk Protein". Acta Sci Nut Health 2: 28–31.
5. Farrell HM, Jimenez-Flores, Bleck R, Brown GT, Butler EM, et al. (2004) Nomenclature of the proteins of cows' Milk—Sixth revision. J Dairy Sci 87: 1641–1674.
6. Truswell AS (2005) The A2 milk case: a critical review. Eur J Clin Nutr 59: 623–631.
7. Parasher A, RK Saini (2015). A1 milk and its controversy-a review. Int J Bioassays 12: 4611–4619
8. Laugesen M, Elliott R (2003) Ischaemic heart disease, type 1 diabetes, and cow milk A1 beta-casein. N Z Med J. 116: 1168.
9. IUPHAR (2008). "The IUPHAR Database on Receptor Nomenclature and Drug Classification."
10. Brantl V, Teschemacher H, Henschen A, Lottspeich F (1979) Novel opioid peptides derived from casein (beta-casomorphins). I. Isolation from bovine casein peptone. Hoppe-Seyler's Z Physiol Chem 360: 1211–1216
11. Henschen A, Lottspeich F, Brantl V, Teschemacher H (1979) Novel opioid peptides derived from casein (betacasomorphins). II. Structure of active components from bovine casein peptone." Hoppe-Seyler's Z Physiol Chem 360: 1217–1224.
12. Barnett MP, McNabb WC, Roy NC, Woodford KB, Clarke AJ (2014) Dietary A1 beta-casein affects gastrointestinal transit time, dipeptidyl peptidase-4 activity, and inflammatory status relative to A2 beta-casein in Wistar rats. Int J Food Sci Nutr 65: 720–727.
13. Koch G, Wiedemann K, Teschemacher H (1985) "Opioid activities of human betacasomorphins. Naunyn Schmiedeberg's Arch Pharmacol 331: 351–354.
14. Pol O, Sasaki M, Jimenez N, Dawson VL, Dawson TM, et al. (2005) The involvement of nitric oxide in the enhanced expression of μ-opioid receptors during intestinal inflammation in mice. Br J Pharmacol 145: 758–766.
15. Tailford KA, Berry CL, Thomas AC, Campbell JH (2003) A casein variant in cow's milk is atherogenic." Atheroscler 170: 13–19.
16. Woodford KB (2006) A critique of Truswell's A2 milk review. Eur J Clin Nutr 60: 437–439.
17. Jaiswal KP, De S, Sarsavan A (2014) Review on bovine beta-casein (A1, A2) gene polymorphism and their potentially hazardous on human health. Int J Environ Anim Conserv 3: 1–12.
18. Swinburn B (2004) Beta casein A1 and A2 in milk and human health. Report to New Zealand Food Safety Authority.
19. Pal S, Woodford K, Kukuljan S, Ho S (2015) Milk Intolerance, Beta-Casein and Lactose. Nutrients 7: 7285–7297.
20. Muehlenkamp MR, Warthesen JJ (1996) [beta]- Casomorphins: Analysis in cheese and susceptibility to proteolytic enzymes from Lactococcus lactis ssp. cremoris. J Dairy Sci 79: 20–26.
21. Jarmolowska B, Kostyra E, Krawczuk S, Kostyra H (1999) Betacasomorphin- 7 isolated from Brie cheese. J Sci Food Agr 79: 1788.
22. Priyadarshini P, Mishra C, Mishra B, Swain K, Rout M, et al. (2018) Impact of milk protein on human health: A1 verses A2. Int J Chem Stud 6: 531–535.
23. Mishra BP, Mukesh M, Prakash B, Sodhi M, Kapila R, et al. (2009) Status of milk protein, b-casein variants among Indian milch animals. Indian J Anim Sci 79: 722–725.
24. European Food Safety Authority (2009). "Review of the potential health impact of β- casomorphins and related peptides". EFSA J. 7: 231.