

## Bovine beta casein A1 variant as risk factor for human health

Martina Miluchová\*, Michal Gábor, Anna Trakovická, Jana Hanusová

*Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*

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Milk and dairy products such as cheese, yoghurt, butter and many others are for their rich source of protein and minerals important part of human nutrition. Milk protein is a source of peptides that are bioactive including: antibacterial activity, antihypertensive activity, angiotensin-converting enzyme inhibitory (ACE-I) activity and opioid activity. The term "opioid" refers to chemical substances that have a morphine-like activity in the body. Some of them are known to play an important role in the response to stress and pain, and the control of food intake. The first pharmacological descriptions of milk-derived peptides were  $\beta$ -casomorphins. Many studies show that  $\beta$ -casomorphin-7 and other peptides have possible effects on the central nervous system, a certain relationship with the child sudden death syndrome, atherosclerosis, and cardiovascular disease, insulin - dependent diabetes mellitus (DM-1), auto-immune conditions, autism, schizophrenia and postpartum psychosis.

**Keywords:** beta-casein, beta-casomorphin 7, milk, cattle

### 1 Introduction

#### 1.1 Milk and its nutritional benefits

Milk and dairy products such as cheese, yoghurt, butter and many others are for its rich source of proteins and minerals important part of human nutrition. For this reason, many breeds of cattle, sheep and goats are used just for milk production (Miluchová, 2014b). Milk is a highly specialized, complex nutrient system developed by mammalian evolution to promote post-natal growth (Melnik et al., 2013). Bovine milk also contains bioactive peptides that are linked to positive health effects on the cardiovascular, nervous and gastrointestinal systems (Choi et al., 2012). However, many studies show that consumption of milk with the beta-casein A1 in humans is associated with higher incidence of some diseases (Elliott et al., 1999; McLachlan, 2001; Sun et al., 2003; Teschemacher, 2003; Woodford, 2008; Clarke and Trivedi, 2014).

#### 1.2 Beta-casein

Polymorphism of beta-casein gene was analyzed in many breeds including Holstein cattle (Hanusová et al., 2010, Miluchová et al., 2014a), Pinzgau cattle (Bláhová et al., 2004; Miluchová et al., 2014b), Slovak Spotted breed (Miluchová et al., 2013), Czech Spotted and Czech Holstein breed (Manga et al., 2006), Frieswal cattle (Ganguly et al., 2013), Holstein-Friesian, Irish-Friesian, Dutch-Friesian (Keating et al., 2008), Latvian brown dairy cattle (Smiltiņa et al., 2010), Ayrshire breed (Ikonen et al., 1997), Reggiana breed (Caroli et al., 2004), Creole breed (Rincon et al., 2006) and Carora cattle (Caroli et al., 2008). Detailed genotype and allele frequencies of CSN2 gene in different breeds of cow kept in Slovak Republic are presented in Table 1.

The most common forms of beta-casein in dairy cattle breeds are A1 and A2, while B is less common (Kamiński et al., 2007; Keating et al., 2008). The original beta-casein protein in bovine milk was A2. A2 is more comparable to the human beta casein than A1 in terms of digestive breakdown (Clarke and Trivedi, 2014).

Many studies show that the consumption of the beta-casein A1 in humans is associated with a higher incidence of certain diseases such as diabetes mellitus type I (Elliott et al., 1999), and ischemic heart disease (McLachlan, 2001). It is assumed that this is due to  $\beta$ -casomorphin 7 (BCM-7) release during

\* **Corresponding Author:** Martina Miluchová, Slovak University of Agriculture in Nitra, Faculty of Agrobiological and Food Resources, Department of Genetics and Breeding Biology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic. E-mail: [martina.miluchova@centrum.sk](mailto:martina.miluchova@centrum.sk)

the gastrointestinal digestion of the beta- casein A1 . Proteolysis during milk fermentation and cheese ripening (induced by microbial enzymes) also leads to the formation of various bioactive peptides (Gobbetti et al., 2002).

Table 1 Genotype and allele frequencies of the CSN2 gene in different breeds of cow (Miluchová et al., 2013, 2014a, b)

Breed	Genotype frequencies			Allele frequencies	
	A1A1	A1A2	A2A2	A1	A2
Slovak Spotted breed	0.1261	0.3333	0.5406	0.2928	0.7072
Pinzgau breed	0.3023	0.5233	0.1744	0.5640	0.4360
Holstein breed	0.1379	0.4598	0.4023	0.3678	0.6322

### 1.3 *Beta-casomorphins*

The first pharmacological descriptions of milk-derived peptides that were later called  $\beta$ -casomorphins date back to the late 1970's (Brantl et al., 1979). Beta-casomorphins (BCMs), the peptides originating from beta-casein, are a group with a chain length of 4 – 11 amino acids (aa), all starting with tyrosine residue in position 60 (Kostyra et al., 2004) and have been identified in bovine milk and isolated from bovine milk (Kamiński et al., 2007). These peptides are released from  $\beta$ -casein at position 60 which is the tyrosine residue and the other residues are released from other positions under appropriate conditions (Nguyen et al., 2015).

### 1.4 *Beta-casomorphin 7 (BCM-7)*

The BCM7 peptide has been identified in raw milk (Cieślińska et al., 2012) and some cheeses, but was not found in commercial yoghurt (De Noni and Cattaneo, 2010). Whether BCMs are formed and the amount of formed BCM as a result of different processing steps needs further investigation. It possibly will depend on the heat treatment and fermentation process, but remains an intriguing unknown (Nguyen et al., 2015).

The sequence of BCM-7 corresponds to positions 60-66 of the bovine beta-casein amino acid sequence. This peptide has 7 amino acids with the sequence Tyr-Pro-Phe- at the N-terminus and has opiate properties within or close to the BCM-7 sequence (Kamiński et al., 2007).

*In vitro*, the bioactive peptide BCM-7 is yielded by the successive gastrointestinal proteolytic digestion of beta-casein A1 and B (but not A2) by pepsin, pancreatic elastase, and leucine aminopeptidase. Elastase cleaves the peptide bond between Ile and His, releasing the carboxyl terminus of BCM-7. Pepsin and leucine aminopeptidase are required to release the amino terminus of this peptide (Elliott et al., 1999). Release of beta-casomorphin 7 from beta-casein variant A1 but not from variant A2 is shown in Figure 1.

Many studies point to opioid nature of  $\beta$ -casomorphin-7 and other peptides and the possible effects on the central nervous system, a certain relationship with the child sudden death syndrome, atherosclerosis, and cardiovascular disease, insulin-dependent diabetes mellitus (DM-1), autism, schizophrenia and postpartum psychosis (McLachlan, 2001; Elliot et al., 1999; Sun et al., 2003; Teschemacher, 2003). It may also be implicated in an additional range of auto-immune conditions. This protein is also linked to milk intolerance in some people (Woodford, 2008).

## 4 Conclusions

The presence or absence of the A1 allele of the CSN2 gene is associated with the ratio of saturated and unsaturated fatty acids in the milk. It is known that a high intake of saturated fatty acid in the diet is a major risk factor for heart disease in humans. Connection of fat and milk protein containing A1 beta-casein variant is a risk factor for health. Many studies show that the consumption of the beta-casein A1 in humans is associated with a higher incidence of certain diseases such as diabetes mellitus type I, and ischemic heart disease. It is assumed that this is due to the fact that during the gastrointestinal digestion of the beta-casein A1  $\beta$ -casomorphin 7 (BCM-7) can be released. Therefore, generation of suitable genetic basis of farm animals can ensure production of qualitatively and nutritionally valuable food of animal origin.

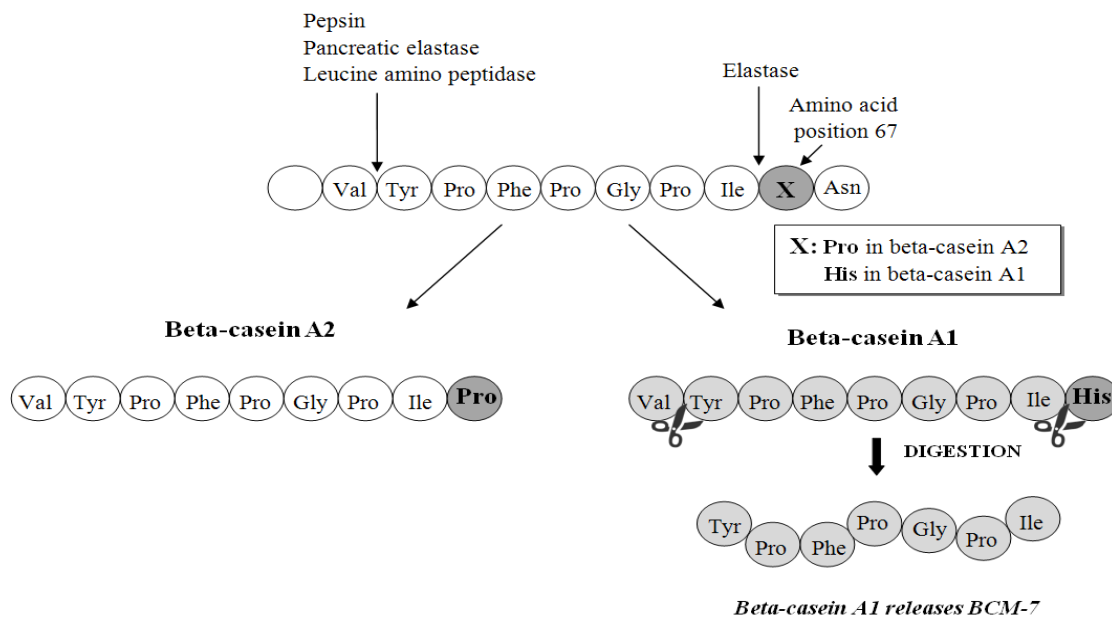


Figure 1 Release of beta-casomorphin-7 from beta-casein variant A1 but not from A2 (modified from Kamiński et al., 2007)

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