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BETA-CASEIN GENETIC VARIANTS AND PROTEIN PROFILE IN DAIRY CATTLE

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Abstract

Milk and dairy products are highly recommended in humans as health-promoting foods, based on the well-known benefic effects. Beta-casein is one of the milk proteins, encoded by the CSN2 gene showing controversy related to its genetic variants impact on human health. Even if A1 and A2 milk is still debating, A1 allele shows a proved negative association with various human diseases, opposite to A2 allele. For such considerations beta-casein SNPs should be considered in planning of dairy cattle breeding programmes accordingly. This paper aims to overview the interference of beta-caseins from cow`s milk in terms of genetics advances up to the present, related to the actual recommended dairy cattle breeding strategies and to human health impact.

Key words: milk protein, beta-casein, genetic variants, dairy cattle, milk quality, human health.

INTRODUCTION

The growing trend of globalization side effects result in directing major concerns for reducing mortality and improving global life expectancy. On this line, milk and dairy products are highly recommended in dietary guidelines as health-promoting foods (Oberritter et al., 2013).

Even if milk and milk products intake is known be inverse associated with some diseases development, including cancer, milk proteins quality based on their encoding genetic variants is still under debate related various diseases occurrence and progression (Ali et al., 2019; Bermejo et al., 2019). Moreover, milk proteins genetic variants vary along cattle breed, including beta-caseins (Park et al., 2021).

The present paper aims to overview the interference of beta-caseins from cow`s milk in terms of genetics advances up to the present, related to the actual recommended dairy cattle breeding strategies and to human health impact.

BETA-CASEIN GENETICS

Beta-casein, also known as β-casein or CSN2 is one of the 4 caseins found in milk next to: alpha s1 (CSN1S1), alpha s2 (CSN1S2), kappa (CSN3), and gamma, known for influencing milk protein content and milk

composition (Threadgill and Womack, 1990), being located on chromosome 6 in cattle.

From all caseins, beta-casein is encoded by the CSN2 gene and represents 36% of milk total protein content, data related CSN2 gene positional info in *Bos taurus* being detailed in figure 1 and the encoded amino acid sequence in figure 2.

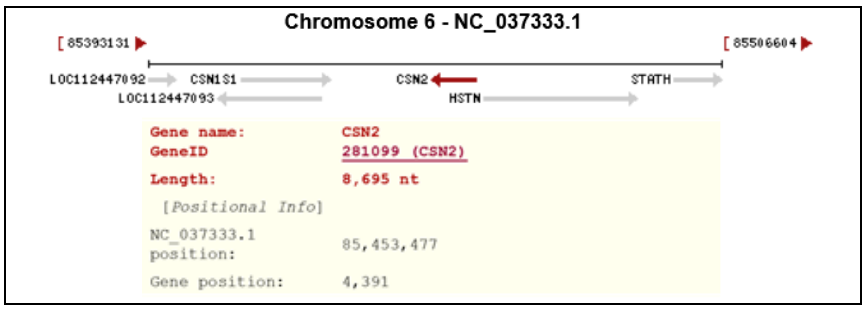

Fig. 1 CSN2 positional info in *Bos taurus* (selected data retrieved from NCBI Gene)

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>sp|P02666|CASB_BOVIN Beta-casein OS=Bos taurus OX=9913 GN=CSN2 PE=1 224aa
linear   02-JUN-2021

MKVLILACLVALALARELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYP
FPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEA
MAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKA
VPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
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Fig. 2 Protein sequence of CSN2 in *Bos taurus* (selected data retrieved from NCBI Protein)

All caseins are located in the casein gene cluster known as BTA6 (BTA position 6:87181619) in a certain order, spanning cca 250 kb and showing the highest SNP density in the upstream, intron and exon regions, missense and synonymous mutations being also referred (table 1) (Boettcher et al., 2004; Meier et al., 2019).

Table 1

CSN2 SNP density/10kb (data adapted from Meier et al., 2019)

Gene	Upstream	Intron	Exon	Missense	Synonymous
CSN2	10.0	15.6	8.7	6.1	2.6

CSN2 gene shows many different genetic variants identified in dairy cattle: A1, A2, A3, B, C, D, E, F, G, H1, H2, I, J, K, L, of which the most common are A1 and A2, B variant is less common, and A3 and C variant are rare (table 2) (Kamiński et al., 2007; Gallinat et al., 2013; Meier et al., 2019). The A2 variant is considered the oldest variant, from which the other variants originated via mutation processes (Farrel et al., 2004).

Table 2

Polymorphic profile of β-caseins (data adapted from Kamiński et al., 2007 and Meier et al., 2019)

β-casein genetic variants	**Polymorphic sites in amino acid sequence**													
	18	25	35	36	37	**67**	72	88	93	106	117	122	137	138
A2*	Ser-P	Arg	Ser-P	Glu	Glu	Pro	Glu	Leu	Gln	His	Gln	Ser	Leu	Pro
A1*						His								
A3*										Gln				
A4	change not yet recognized													
B*						His						Arg		
C*			Ser		Lys	His								
D	Lys													
E*				Lys										
F						His								Leu
G						His						Leu		
H1		Cys						Ile						
H2							Glu		Leu					Glu
I*									Leu					

** detected in European cattle breeds*

CSN2 gene has a common SNP in exon 7 which is believed to be associated with human health also known as rs43703011 or A1A2 SNP. A1 and A2 variation consists in a substitution mutation changing a cytosine (C) to adenine (A) nucleotide, substitution changing histidine (present in A1 type) with proline (present in A2) at 67th position of β-casein polypeptide (table 3) (Bonsing et al., 1988; Meier et al., 2019).

Table 3

Description of A1 and A2 SNPs of CSN2 in cattle missense variants (data adapted from Meier et al., 2019)

Casein gene variant	BTA position	Allele	Amino acid	Protein seq. Position	SNP ID
CSN2*A1	6:87181619	T/G	His/Pro	82 (67)	rs43703011
CSN2*A2	6:87181619	T/G	His/Pro	82 (67)	rs43703011

Based on the amino acid in position 67 these variants can be classified into 2 groups: A1 group (H67) including A1, B, C, F, G and A2 group (P67) harboring A2, A3, H1, H2, I, J, K, L (VGL, 2021).

A1 and A2 allele variants have a particular influence on milk's technological characteristics and also implications for human health (EFSA,

2009; Brooke-Taylor et al., 2017). The A1 variant improves curd consistency, milk coagulation, and micelle size, but results in lower milk digestibility compared with the A2 variant (Pearse et al, 1986 Ng-KwayHang, 2006).

CSN2 VARIANTS IMPACT ON HUMAN HEALTH

CSN2 variants may be involved in milk intolerance and some human diseases, due to the production of a bioactive peptide with opioid activity during digestion named β-casomorphin-7 (BCM-7) (Jianqin et al., 2016).

A1 milk lead to 4-fold higher levels of BCM-7 resulted from beta-casein metabolism during gastrointestinal digestion, than A2 milk (Jarmolowska et al., 1999).

BCM7 shows negative effects for human health, being considered the primary causative factor for health and digestive disorders associated with A1 milk, due to its strong opioid activity; BMC-7 has a role in stimulating human lymphocyte T proliferation in vitro, cytomodulatory properties, etc. (EFSA, 2009; Deth et al., 2016).

BCM-7 may be a risk factor for different human diseases (figure 3). It seems that BCM-7 presence is associated in humans with ischemic heart disease, atherosclerosis, type 1 diabetes, sudden infant death syndrome (Sun et al. 2003; Tailford et al. 2003), autism, schizophrenia (Cade et al. 2000, Priyadarshini et al., 2018).

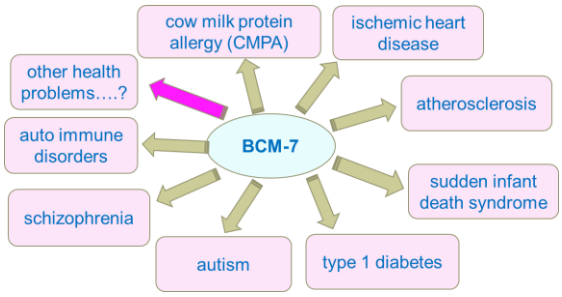

Fig. 3 BCM-7 interference in various human diseases

On the other side, A2 milk generates another peptide called BCM-9 with no such effects (Jianqin et al., 2016) and there is no relationship reported between the BCM-9 resulted from A2 milk and cow milk protein allergy (CMPA) or other health problems (Park and Haenlein, 2021), genotypes harboring A2 allele being highly recommended in dairy cattle breeding.

BETA-CASEIN ALLELES AND GENOTYPES IN VARIOUS CATTLE BREEDS

Various studies assessed the casein gene cluster in many dairy cattle breeds by means of sequence variation in coding regions, promoter region and microsatellites, proving casein haplotypes correlation with milk yield, fat, and protein percentage (Formaggioni et al., 1999; Boettcher et al., 2004; Gallinat et al., 2013 Ahmed et al., 2017).

CSN2 variants can be detected at lower or higher frequencies in various cattle breeds (figure 4) (Hohmann et al., 2018; Meier et al, 2019).

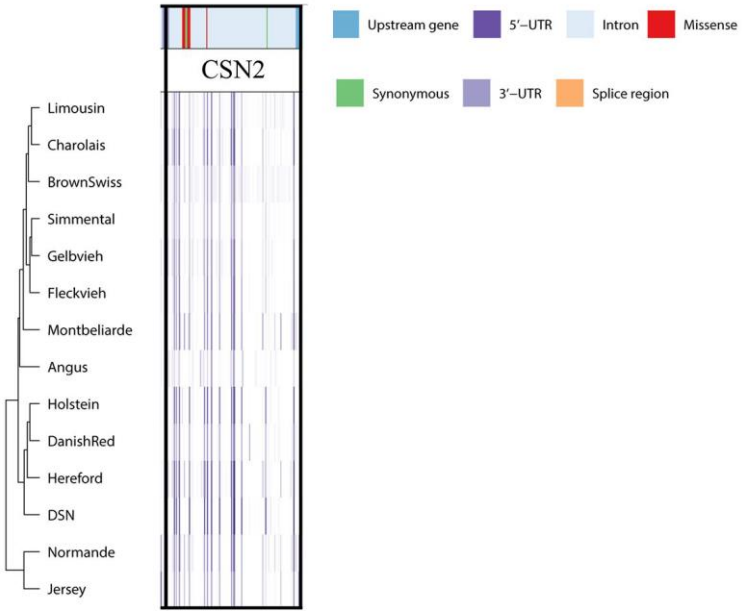

Fig. 4 Clustering of per-breed alternative allele frequency for the identified sequence variants in CSN2 gene including the 1,000-bp upstream region. The alternative allele frequencies are presented in various breeds. The clustering was mainly based on intron variants (light blue areas) as they make up 87.3% of all detected variants. (data adapted from Meier et al., 2019)

In general, the northern European cattle breeds such as: Holstein, Friesian, Ayrshire and British Shorthorn are known to produce milk showing a high A1 β-casein composition, meanwhile cattle breed as Guernsey, Jersey, Simmental, Charolais and Limousin from Channel Islands and southern France produce A2 β-casein milk (Kaminski et al., 2007).

Indigenous cattle and buffalo from India and various countries from Asia, mostly produce a milk with an A2 β-casein high content (Priyadarshini et al., 2018, Park and Haenlein, 2021).

A1 and A2 alleles of beta-casein can be assessed in milk at peptide level or in animals at DNA level, so that the homozygous or the heterozygous state of genotypes can be determined. In this way, milk produced from A2 homozygous genotype will have only the A2 variant of beta-casein in its composition, meanwhile heterozygous genotypes will generate a milk having both A1 and A2 variant of beta- casein.

Animal selection based on genotypes will ensure an adequate management of animal couplings acting to increase A2 favorable allele frequency and to decrease undesirable alleles frequency, so that, at the end will result cattle herds showing the A2A2 homozygous genotype from the initial dairy cattle population, oriented to A2 milk production.

However, ``A1 and A2 Milk`` continue to be a global debate related to milk quality and its risk for various disease occurrence and progression (Ali et al., 2019).

On the other side A2 cow's milk, or milk without the A1 variant is commercially available in countries such as: Australia, the United Kingdom, the United States, New Zealand, and the Netherlands is widely recommended for people who are milk-intolerant. Formula for newborns containing A2 variant is sold in China and Australia and is promoted commercially as being more gentle on infant's digestive system (Brooke-Taylor et al, 2017).

CONCLUSIONS

Advances technologies as microarray, systematic genotyping or sequencing can be useful molecular tools that can be used for identifying and monitoring milk alleles frequencies in cattle, and also for selection of specific genotypes, i.e. A2 genotypes, including selective commercialization of bull semen targeting the obtaining of cattle herds producing A2 milk.

Milk resulted from the homozygous A2 genotype shows a higher digestibility related to the gastrointestinal transit, compared to that obtained from other beta-casein genotypes.

A1 variant association with the etiology of various diseases requires further investigations, scientific reports related to the side effects of A1 and A2 milk on human health are still contradictory.

Dairy cattle farmers should consider the genotype based selection for producing milk with human health benefits, implementing a selection against the A1 allele of beta-casein for obtaining an A1-free milk. The potential negative impact of A1 variant of beta-casein on human health enhanced the planning of dairy cattle breeding programmes based on beta-casein SNPs. Dairy cattle breeding programmes in Romania should stand for such policies based on genetic screening of beta-casein variants and marker-assisted selection (MAS), in order to align Romanian cattle breeds

with those of other countries, which have already promoted A2 milk production and commercialized A2 milk as a product with beneficial properties.

Acknowledgments

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