

POLYMORPHISM OF THE B-CASEIN GENE IN THE HUNGARIAN RACKA SHEEP

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Abstract

Milk composition of mammalian species varies widely with reference to genetic, nutritional factors and environmental conditions. The aim of this study was to determine allele and genotype frequencies at the β - casein gene in a Hungarian sheep breed. Racka sheep is multi-purpose breed used for milking, wool and meat, with two color variants black and white. The study was carried out on 50 sheep: 25 white and 25 black variant by means of Taqman probe analysis. Molecular analysis of the β - casein, CSN2 locus showed that the genetic variant A were dominant, had higher frequency in both color variations. The frequency of the G variant were low in the racka population.

Key words: Racka-sheep, CSN2, Taqman probe

INTRODUCTION

The primary functions of milk is to protect the health of a newborn mammal. The milk composition – carbohydrate (lactose), fat, protein, vitamins, minerals and enzymes – are the same in every species, but the quality and the quantity of the contents are different. Higher fat and protein level are in sheep milk than cow or goat milk; only a few species as buffalo and yak milk contain more fat. Sheep milk also generally has a higher lactose content than milk from cows, buffaloes and goats. The high fragments of sheep milk make it particularly appropriate for cheese and yoghurt making. The animal productivity and agricultural traits, such as composition, or protein and fat quantity are inherited from generation to generation. However information and functional features are determined by genes. (Sabahelkhier et al, 2012)

Nowadays, detection of milk protein genetic variants gave rise to the analysis of milk protein polymorphisms in many animal species, such as cattle, goat or sheep. In the first stage of analysis cattle milk proteins were

in the focus of attention; the aim was to show the relationship between milk protein variants and the genetic background.

Milk proteins were classified in two groups. Caseins, the 80% of the protein content are: α S1-, α S2-, β -, κ - és γ - caseines. About 20% of milk protein content consist of whey proteins beta-lactoglobulin alpha-lactalbumin serum albumin and immunoglobulins. Casein polymorphisms are important, because of they have significant influence on the qualitative and quantitative traits and technological properties of milk.

The complete sequence of the β -casein (CNS2) was described by Provot et al. (1995). The 9149-bp transcription unit encoding ovine β -casein and 4636 bp of 5' flanking region were completely sequenced, that composed of nine exons.

A new study was to develop a protocol for simple and rapid genotyping of A and G variants at the CSN2 by LightCycler analysis. (Sztankóová et al, 2011)

MATERIAL AND METHOD

The aim of this study are the ancient-looking and rare racka sheep is a typically Hungarian animal breed. Racka breed originate from Hungary, and there are two version of the colors in racka sheep: black and white. The racka has glossy hair, and has the horns are corkscrew-twisted and V shaped.

There was collected 25 – 25 samples of the two colors, and investigated to determine of the CSN2 gene allele variants by Taqman probe according to the LightCycler analysis of Sztankóová, 2014.

The LightCycler combines rapid amplification of nucleic acids in capillaries with melting curve analysis based on fluorescence resonance energy transfer and based on the sensitive detection of point mutations with 2 different, specific oligonucleotide probes. Each assay has a reference negative control, containing water instead of sample DNA. (Loeffler et al, 2000, Sztankóová et al, 2008).

TaqMan Gene Expression Assays use a pair of unlabeled PCR primers and a TaqMan probe with a FAM or VIC dye label on the 5' end, minor groove binder, and nonfluorescent quencher on the 3' end. The negative control are helpful for measuring any false positive signal. An allelic discrimination experiment there are three main steps: a pre-read, amplification run and post-read run. Samples were visually verified by analysing the results, the PCR curves. (Manga and Dvořák, 2010)

Hair samples were taken by picking up and collected in paper bags. Genomic DNAs were extracted as previously described (Zsolnai and Orbán,

1999, FAO/IAEA 2004). ABI 7900 Fast Sequence Detection Instruments are available for TaqMan probe-based work.

RESULTS AND DISSCUSIONS

At locus CSN2, allelic variants differ by nucleotide substitution A → G, at position 183. The G variant were described in italian sheep breeds. (Chessa et al, 2010) Many studies, similar results showed that the variant A had greater frequency (50 to 80%) compared with the G variant. The most common genotype alleles were in the two Czech sheep breed Sumava and Valachian: AA frequencies are 0.624 and 0.705. Followed by AG frequencies are 0.308 and 0.259 and GG frequencies are 0.068 and 0.036. (Sztankóová et al, 2011)

50 samples were collected from Hungarian racka sheep, 25-25 white and black variants. The frequencies of the variants are in Table 1-3.

Table 1

Frequencies of the CSN2 variants in white racka sheep

Gene	Genotype	Number of samples	Frequencies	Alleles	Frequencies
CSN2	AA	21	0.81	A	0.92
	GG	0	0	G	0.08
	AG	4	0.19		
Sum		25	1		1

Table 2

Frequencies of the CSN2 variants in black racka sheep

Gene	Genotype	Number of samples	Frequencies	Alleles	Frequencies
CSN2	AA	19	0.76	A	0.88
	GG	0	0	G	0.12
	AG	6	0.24		
Sum		25	1		1

Table 3

Frequencies of the CSN2 variants in the whole racka herd					
Gene	Genotype	Number of samples	Frequencies	Alleles	Frequencies
CSN2	AA	40	0.80	A	0.90
	GG	0	0	G	0.10
	AG	10	0.20		
Sum		50	1		1

In this study the main genotype was the AA in the herd, and there were not found any GG variant animal. The presence of variant A allele was 90 % in the whole population. The frequency of the G allele was very low in the herd, only 10% of the whole samples. There were no significant different among the white and the balck variant animals.

CONCLUSIONS

The ACRS-PCR was one of the most widely used methods for genotyping the CSN2 gene, but requires on average two working days for complete analysis. A new way for the genotyping could be the TaqMan system that seems to be rapid, maximally sensitive and suitable enough for the detection of the point mutations. (Manga and Dvořák, 2010)

Casein polymorphisms are important to their effect on qualitative and quantitative traits of milk. The caseines in the milk are the α S1-, α S2-, β -, κ - és γ - caseines. This study described the β -casein gene variants, in the Hungarian Racka sheep breed. The caseines have significant fluence on the milking traits. The effect of CSN2 was described in a study, G allele on a higher milk yield and the association of the A allele with an increased fat content. The frequencies of the alleles A and G were 0.7644 and 0.2356 in the Merino population. (Corral et al, 2010)

The number of animals with genotype GG was probably too low to reveal significant associations, in 403 milk samples of East Friesian Dairy and Lacaune sheep (Giambra et al, 2014)

In two Czech national sheep populations (Sumava and Valachian), molecular analysis showed that genotype AA had the higher frequency, followed by the AG, and GG genotypes. (Stankóová et al, 2011)

According to the other results, in our study the commonly genoty was AA in the herd. There were no mean differencies in the two color

variant animals in the frequencies. Sheep carrying the GG genotype was not presented in the work. Such information could be the first step, to investigate other Hungarian sheep breeds in point of the β -casein polymorphism.

The different genotypes could be used for influence the milking traits of the herds in the future.

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