

Characterization of porcine β -casein G allele (*CSN2G*)

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Abstract. Following the sequencing of porcine (*Sus scrofa*) β -casein cDNA we have identified a new allele at this locus (*CSN2*). Our sequence (GenBank accession number GU827390) differs from the sequence reported by Alexander and Beattie (X54974) in the following positions: 294, 443, 468, 647, four SNPs being observed. Because of the guanine in position 294 differentiating the newly found allele from all other previous sequences, we have chosen to name this allele "G". This is in fact a silent mutation, both the codons CCT and CCG respectively (position 83 in the mature protein) encoding the same amino acid (Pro). Taking in consideration all the differences found at DNA level, the protein derived from the nucleotide sequence of G allele is unique (there are no 100% similarities when compared to all other known protein sequences). For future references we propose, for this allele, the nomenclature: *CSN2G*.

Key words: β -casein, *CSN2G*, G allele, porcine milk proteins, polymorphism.

Introduction. The milk of almost all livestock species contains six major proteins. Four of them belong to the casein fraction (α S₁-, α S₂-, β - and κ -casein) and two are whey proteins (β -lactoglobulin and α -lactalbumin). In porcine milk, a third major whey protein was discovered (Simpson et al 1998), named whey acidic protein (WAP).

The casein genes have been mapped on SSC8 and the whey protein genes α -lactalbumin and whey acidic protein on chromosomes SSC5 and SSC18, respectively. β -lactoglobulin has been assigned to chromosome SSC1 (Ballester et al 2005). The WAP gene has been localized on the subtelomeric region of the chromosome 18 (Rival et al 2001).

Genetic polymorphisms of milk proteins were intensely studied in dairy species but little work has been done in the case of pigs. Alexander & Beattie (1992) were the first to sequence porcine β -casein cDNA (GenBank accession number X54974).

The aim of our study was to characterize, at cDNA level, a previously described polymorphism (Șuteu et al 2009), that occurs within the β -casein gene (*CSN2*). Alignment of obtained β -casein cDNA sequences and BLAST analyses concurred to the identification of a new allele at this locus, in a local Romanian breed – Mangalita. The present paper characterizes porcine β -casein G allele, both at cDNA and protein level.

Material and Method. Mangalita (Figure 1) is one of the old type porcine breeds, originating several centuries ago as a result of crossing between European and Asian primitive pigs. Mangalita was introduced in Romania from Serbia in the 19th century. Red Mangalita is one of the varieties, established by crosses between the Blond Mangalita and the Salonta pig. The main features of these pigs are curly hair, thick backfat, palatable meat and disease resistance (Ciobanu et al 2001; Zăhan et al 2009).

Colostrum sample collection was performed manually, in 50ml sterile tubes. No preservatives were added. Samples were stored during transport at 4°C and then frozen at -20°C. Total RNA was isolated using the PureZOL reagent (Bio-Rad Laboratories, Hercules, CA) from milk somatic cells, following the protocol of Balteanu et al (2010).

First strand synthesis was performed using 8µl of total RNA, oligo(dT)₁₈ primer and RevertAid H Minus reverse transcriptase (Fermantas, Vilnius Lithuania) according to the protocol recommended by the producer.



Figure 1. Mangalita breed individual (red variety).

The 693bp PCR product sequenced was obtained after amplification of the total cDNA samples with the primers BCZP-F and BCZP-R (Şuteu et al 2011). The two primers were designed based on the porcine β -casein reference mRNA (GenBank Acc. No. X54974).

BCZP-F: 5' GCA AGA GCG AAG GAA GAA CT 3'.

BCZP-R: 5' CAA AAC TGA GGA GAG GGG AT 3'.

DNA sequencing was performed at MacroGen Europe (Amsterdam, The Netherlands). The sequencing reaction was performed according to the chain-terminator method, using dideoxynucleotides, starting from 30µl of PCR product. The 693bp amplicon was sequenced with the forward and reverse primers (the same primers used for the amplification of the cDNA, BCZP-F and BCZP-R respectively).

Results and Discussion. Beta-casein, like α S1 and α S2, belongs to the family of calcium-sensitive proteins in milk (Das et al 2000). The porcine beta-casein cDNA is 1100 bp in length, excluding the poly(A) tail, and encodes a preprotein of 232 amino acids (Alexander & Beattie 1992).

As a result of sequencing the porcine β -casein cDNA we have discovered a new allele at this locus. The sequence of this allele has been deposited in GenBank and received the following accession number: GU827390. The SNP differentiating the newly found allele from all the previously described variants is in fact a silent mutation, both the codons CCT and CCG respectively encoding the same amino acid (Pro). Because of the guanine found in the position 294 of the cDNA (Figure 2) we have chosen to name this allele "G".

At DNA level this point mutation (T>G) is located in exon 6, the largest exon of CNS2 gene (519nt). Our studies revealed that this exon is highly polymorphic within and between breeds and populations (data not shown).

Our sequence differs from the sequence reported by Alexander and Beattie (GenBank accession number X54947) in the following positions: 294, 443, 468, 647, four SNPs being observed. These are depicted in Table 1, where a more comprehensive comparison between all the known porcine beta-casein sequences is shown.

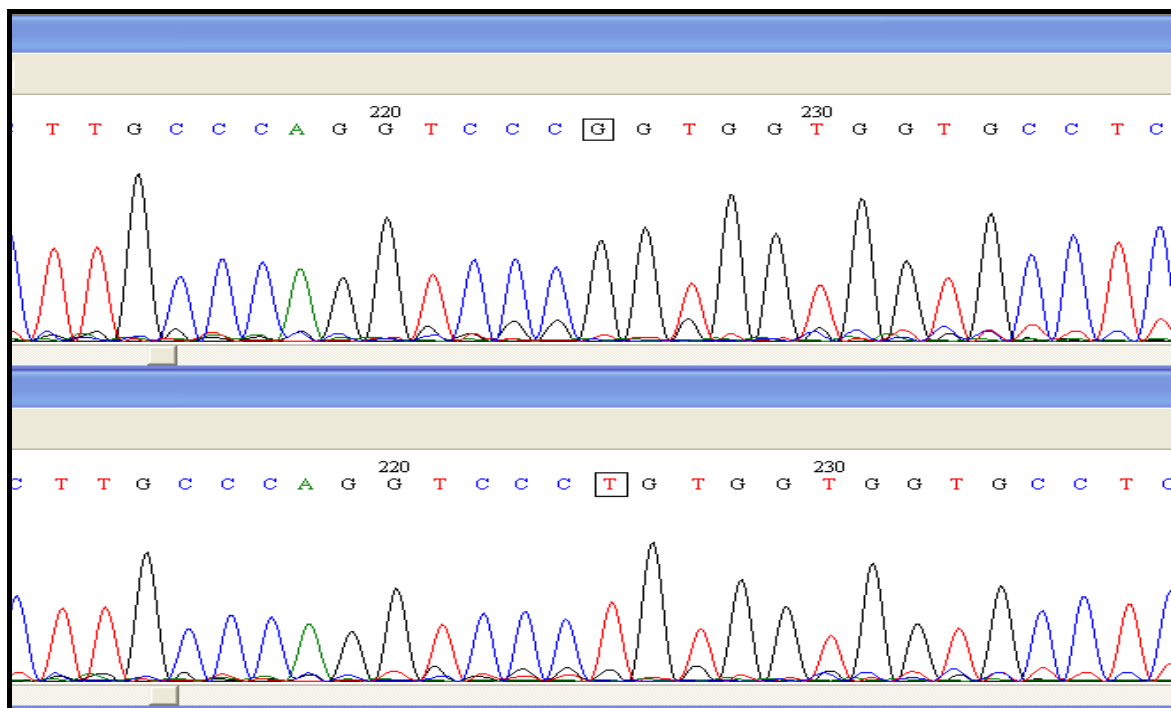


Figure 2. The sequencing chromatogram of G allele (upper part) compared an usual allele (lower part). The mutational event (SNP) differentiating G allele from all other porcine β -casein sequences is shown in a square.

Table 1

Comparison between allele G (GenBank accession number GU827390) and other known porcine β -casein sequences

Sequence (GenBank Acc. No.)	Position within the cDNA								
	147	175	294	433	435	443	468	590	647
GU827390	T	A	G	G	T	G	C	G	G
NM_214434	T	A	T	G	T	T	T	G	A
X54974	T	A	T	G	T	T	T	G	A
EU242520	A	G	T	A	T	G	C	G	G
EU025876	T	A	T	G	T	T	T	G	A
EU213063	T	A	T	G	C	T	T	A	A

The mutation in position 294 is a silent mutation, but when we take into consideration all the differences at DNA level (Table 1), the protein derived from the nucleotide sequence of G allele is unique (there are no 100% similarities when compared to all other known protein sequences). Although the mutational event distinguishing allele G is not so spectacular, this allele encodes a new β -casein variant, never encountered before, ADE60010 (NCBI Protein, EBI), D5LG68 (UniProt).

Based on the theoretical translation of G allele, we were able to compare it with the sequence of Alexander & Beattie (1992). The amino acid comparison is presented in Table 2.

G variant contains supplementary Gly and Arg amino acid residues as compared to the composition of the protein described by Alexander & Beattie (1992). Similarly, the amino acids Cys and Trp are missing from the composition of G variant. From the amino acid composition of porcine β -casein G we computed a molecular weight of 24363.30 Da and an isoelectric point (pI) of 5.99 for the mature protein (217 aa), in the non-phosphorylated form.

Table 2

Amino acid composition of porcine β -casein encoded by *CSN2G*
(adapted after Gallagher et al 1997)

<i>Amino acid</i>	<i>Allele G</i>	<i>Porcine β-casein</i>	<i>Bovine β-casein</i>
Asx	9	9	9
Thr	8	8	9
Ser	13	13	16
Glx	40	41	39
Pro	37	37	35
Gly	6	5	5
Ala	10	10	5
Val	17	18	19
Cys	—	—	—
Met	5	5	6
Ile	9	9	10
Leu	26	26	22
Tyr	5	5	4
Phe	8	8	9
His	5	5	5
Lys	13	13	11
Arg	6	5	4
Trp	—	—	1
TOTAL	217	217	209

Based on the fact that the guanine in position 294 of the cDNA creates a recognition site for the enzyme Hpa II, a genotyping protocol that distinguishes this new allele has been developed (for more details see Şuteu et al 2010). Figure 3 shows the migrating profiles of the three genotypes. Large scale genotyping (data not shown) proved that porcine β -casein G allele is not limited to the Mangalita breed (where it was identified for the first time).

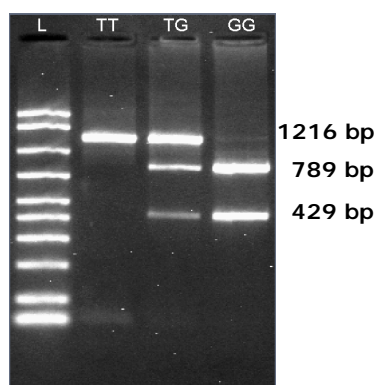


Figure 3. Observed β -casein genotypes following Hpa II restriction. L: AmpliSize Molecular Ruler50-2,000 bp Ladder (BioRad).

Conclusions. Through sequencing and RFLP analysis we have identified and confirmed the presence of a new porcine β -casein (*CSN2*) allele, allele G. Sequence alignments combined with the theoretical translation of the nucleotide sequence allowed us to characterize it and conclude that it is translated into a variant of β -casein never before described in the field literature.

Lack of a standard nomenclature of *CSN2* alleles, makes it difficult to interpret and discuss the results of similar studies. Therefore, for future references, the authors propose for this allele (GenBank accession number GU827390) the name: *CSN2G*.

The discovery of a new allele in such an endangered breed (only 18 sows left at Turda Research Station) shows the importance of genetic resources preservation. This proves once again that local unimproved breeds are a reservoir of genetic diversity. At the same time, the fact that a high degree of polymorphism is encountered (within the population) proves that little/no inbreeding has occurred and that the mating schemes have been designed and respected properly.

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