

PORCINE RESEARCH

International Journal of the Bioflux Society
Short communication

Porcine *WAP* c.75T>C substitution present in three different breeds

Oana Rogoz

Zoo Tech Group, Research and Development Department, Cluj-Napoca, Romania.
Corresponding author: O. Rogoz, oana_rogoz@yahoo.com

Abstract. Genetic polymorphisms within the genes encoding the 6 major milk proteins have been intensely investigated in dairy species, to such an extent that, in some species, certain genetic variants are currently being used in marker assisted selection. In domestic pigs, relatively recently (1998), an additional major milk protein has been identified: whey acidic protein (WAP), a protein also encountered in mouse, rat, rabbit and camel milk. Having in mind the lack of information on porcine WAP polymorphisms we sequenced the gene's cDNA in 8 individuals belonging to 3 breeds. Sequence alignments revealed only one mutation (a single nucleotide polymorphism – SNP), namely c.75T>C. The substitution in question, a silent mutation, is the same one differentiating the sequences currently available in GenBank.

Key Words: porcine, whey acidic protein, *WAP*, SNP, silent mutation.

Along with the 4 caseins, the milk of most domestic animals contains 2 whey proteins: lactalbumin and beta-lactoglobulin (Şuteu 2011). Starting from 1987, using different protein-based techniques, researchers were detecting a third whey protein that was named at the time 'novel whey protein' (reviewed by Gallagher et al 1997). This 'novel whey protein' was later isolated and characterized by Simpson et al (1998). It is in fact 'whey acidic protein' (WAP), a protein recognized as the main whey protein in mice, rats, rabbits and camels (Simpson et al 1998; Şuteu 2011). Porcine WAP has a length of 113 AA and a molecular weight considerably higher than 11.7 kDa, estimated based on the AA composition, indicating that the protein is glycosylated (Simpson et al 1998).

At DNA level, porcine *WAP* was cloned and characterized by Rival et al (2001). The gene, having a length of 2087 bp and comprising 4 exons, was mapped using FISH around the telomeric region of SSC18 (Rival et al 2001).

In order to investigate porcine *WAP* polymorphisms, colostrum samples from eight sows (Mangalita – 3; Bazna – 2; Large White – 3) were collected manually in 50 mL sterile tubes and stored at 4°C during transport and at -20°C until further analyses. The Bazna and Mangalita breeds were chosen because they are unimproved breeds (for breed descriptions please refer to Ciobanu et al 2001), while Large White was chosen based on the fact that it is a highly improved breed. RNA extraction from milk somatic cells was performed using the TRIsure (Bioline) reagent. First strand synthesis was performed using the High Capacity RNA-to-cDNA Kit (Applied Biosystems), following the manufacturer's instructions. Subsequently, the cDNA samples were amplified (Figure 1) with the below set of primers (primer design based on GenBank Acc. No. NM_213841.2):

WAP_F (5'–3'): ACCACCATGCCGCTTTCTTAC

WAP_R (5'–3'): GGGATGTCCCTCACTGAAGA

The same primers were used to sequence the PCR amplification products. Aligning the cDNA sequences revealed a single mutation, namely c.75T>C (Figure 2). BLAST analyses showed that sequences containing both T (NM_213841.2), and C (AF034646.1) were already present in GenBank (Figure 3). Theoretic translation of the cDNA sequences (not shown) ascertained that the c.75T>C substitution is in fact a silent mutation, both AAC and AAT codons encoding Asparagine (Asn/N). Construction of theoretic restriction maps revealed that *B*veI endonuclease could be used to distinguish these two variants.

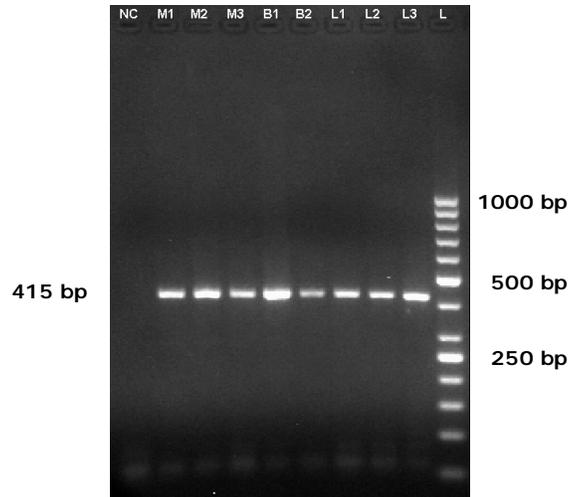


Figure 1. Agarose gel electrophoresis. Migrating profiles of porcine cDNA samples amplified with *WAP*-specific primers. NC: negative control. M1-M3: Mangalita samples. B1-B2: Bazna samples. L1-L3: Large White samples. L: 50 bp DNA Ladder (Fermentas).

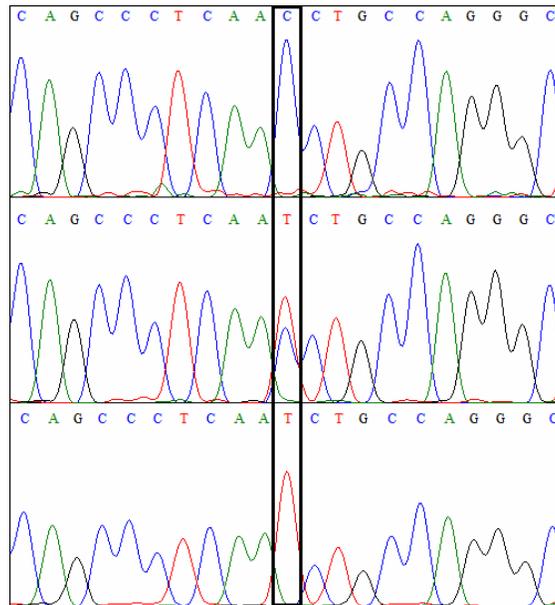


Figure 2. Sequencing chromatograms revealing the porcine *WAP* c.75T>C substitution (marked with a rectangle): cDNA samples amplified with the primers *WAP_F* and *WAP_R* and sequenced with the forward primer. The upper sequencing chromatogram: CC. The lower chromatogram: TT. The sequencing chromatogram in the middle: TC (heterozygous individual – note the overlapping blue and red peaks).

```

CAGCCCTCAACCTGCCAGGGC
|||||
CAGCCCTCAATCTGCCAGGGC

```

Figure 3. Sequence alignment performed using NCBI's Basic Local Alignment Search Tool (BLAST). Upper sequence: GenBank Acc. No. AF034646.1. Lower sequence: GenBank Acc. No. NM_213841.2 (RefSeq). The porcine *WAP* c.75T>C substitution is marked with a rectangle.

Among the investigated individuals, the following genotypes were encountered:

	TT	TC	CC
Mangalita	2	1	0
Bazna	1	0	1
Large White	2	1	0

This reveals that the c.75T>C *WAP* polymorphism is not restricted to local, or highly improved porcine breeds.

References

- Ciobanu D. C., Day A. E., Nagy A., Wales R., Rothschild M. F., Plastow G. S., 2001 Genetic variation in two conserved local Romanian pig breeds using type 1 DNA markers. *Genet Sel Evol* 33:417–432.
- Gallagher D. P., Cotter P. F., Mulvihill D. M., 1997 Porcine milk protein: a review. *Int Dairy J* 7: 99–118.
- NCBI BLAST, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>
- Rival S., Delville-Giraud C., Yerle M., Laffont P., Rogel-Gaillard C., Houdebine L.-M., 2001 Cloning, transcription and chromosomal localization of the porcine whey acidic protein gene and its expression in HC11 cell line. *Gene* 267:37–47.
- Simpson K. J., Bird P., Shaw D., Nicholas K., 1998 Molecular Characterization and Hormone-Dependent Expression of the Porcine Whey Acidic Protein Gene. *J Mol Endocrinol* 20:27–35.
- Şuteu M., 2011 Porcine milk protein polymorphisms. *Porc Res* 1(1): 1–112.

Received: 01 November 2013. Accepted: 09 December 2013. Published online: 30 December 2013.

Authors:

Oana Rogoz, Zoo Tech Group, Research and Development Department, Cluj-Napoca, Romania, e-mail: oana_rogoz@yahoo.com

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

How to cite this article:

Rogoz O., 2013 Porcine *WAP* c.75T>C substitution present in three different breeds. *Porc Res* 3(2):27-29.