

## Promoter Characterization and Expression Pattern Analysis of Porcine *TCAP* Gene

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**Abstract:** *TCAP* is one of the titin interacting Z-disc proteins involved in the regulation and development of normal sarcomeric structure. In this study, researchers cloned the 5' upstream region of porcine *TCAP* gene. Bioinformatic analysis of the 5' regulatory region has revealed that in addition to several ubiquitous transcription factors binding sites (SP1, AP1 and C/EBP) were found, several putative muscle-specific transcription factor binding sites (MyoD, MyoG and MEF2) were present in this region. Tissue expression analysis showed that the porcine *TCAP* gene was expressed abundantly in skeletal muscle. The study suggested that *TCAP* gene might be a prospective candidate gene affecting muscle mass and meat quality traits in the pig and would lay the groundwork for the further investigations on the regulation and physiological function of porcine *TCAP* gene.

**Key words:** Porcine, *TCAP*, promoter, bioinformatics, China

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### INTRODUCTION

*TCAP* is one of the titin interacting Z-disc proteins involved in the regulation and development of normal sarcomeric structure (Mason *et al.*, 1999). Mutations in the *TCAP* were associated with the seventh form of autosomal recessive limb-girdle muscular dystrophy, termed Limb-Girdle Muscular Dystrophy type 2G (LGMD2G) (Moreira *et al.*, 2000). Patients with *TCAP* mutations develop a marked weakness in the distal muscles of the legs with proximal involvement and most patients lose the ability to walk by the third or fourth decade of life (Moreira *et al.*, 2000).

*TCAP* has also been shown to interact with and regulate the secretion of Myostatin (MSTN), a negative regulator of muscle growth that inhibits both cell proliferation and differentiation (Nicholas *et al.*, 2002). Knockdown of *TCAP* by RNA interference in C<sub>2</sub>C<sub>12</sub> myoblast cells inhibits myoblast differentiation and impairs muscle cell growth (Markert *et al.*, 2008). Given the important role of *TCAP* in myoblast proliferation and differentiation, it has been proposed that *TCAP* might offer a new therapeutic target for muscular dystrophies. Hitherto, a lot of studies about *TCAP* are carried out in human and mice, relatively little is known concerning the porcine *TCAP* gene. In this study, researchers isolated partial sequences of porcine *TCAP* gene promoter and did

some correlated bioinformation analysis. Researchers also, analyzed its tissue expression pattern. These would lay the foundation for further investigations on the regulation and physiological function of porcine *TCAP* gene.

### MATERIALS AND METHODS

**Animals and sample preparation:** Three adult Yorkshire pigs were slaughtered. Blood samples were collected immediately after the slaughter of pigs for genomic DNA extraction using phenol/chloroform method (Qiao *et al.*, 2010). Ten different tissues including heart, liver, spleen, lung, kidney, stomach, skeletal muscle, uterus, ovary and adipose tissues were collected then immediately frozen in liquid nitrogen and stored at -80°C for spatial expression analysis. Total RNA was extracted from different tissues with a Trizol reagent (Invitrogen). In case the samples were seriously contaminated with genomic DNA, DNase I (Takara) treatment on the total RNA was carried out before first-strand cDNA synthesis. Reverse transcription was performed as described before (Qiao *et al.*, 2010).

**Isolation of the porcine *TCAP* promoter:** According to contrast the genome sequence of porcine *TCAP* gene (GenBank Accession No.: CU640399.2) and the porcine genome, researchers design the primers with primer 5.0.

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The primers were forward: 5'-CCTGGGAGGGAAGGTCAA-3'; reverse: 5'-CTGGGCTTTGTGCTCTGTC-3'. PCR reactions were carried out in a total volume of 25  $\mu$ L containing 1  $\mu$ L (50 ng) of DNA as template, 0.5  $\mu$ L of each primer (5  $\mu$ M), 2.0  $\mu$ L of each dNTP (2 mM), 2.5  $\mu$ L of 10 $\times$ PCR buffer, 2.0  $\mu$ L of (25 mmol L<sup>-1</sup>) Mg<sup>2+</sup> and 1  $\mu$ L (1 U  $\mu$ L<sup>-1</sup>) of Taq DNA polymerase and 15.5  $\mu$ L sterile water. The PCR amplification profiles were as follows: 94°C initial denaturation for 4 min, 35 cycles of 94°C denaturation for 45 sec, 62°C annealing for 40 sec and 72°C extension for 90 sec followed by a final extension at 72°C for 10 min.

#### Expression pattern analysis of the porcine TCAP gene:

To check the relative expression level of TCAP mRNA in various porcine tissues, semi-quantitative Reverse Transcription (RT)-PCR was performed as described before (Qiao *et al.*, 2010). The house-keeping gene *GAPDH* was used as the internal control for determination of target mRNA profiles. The control primers were forward: 5'-ACCACAGTCCATGCCATCAC-3'; reverse: 5'-TCCACCACCTGTTGCTGTA-3'. The TCAP specific primer pair were forward: 5'-GCTGGTGATGCGAATGGG-3'; reverse: 5'-AGACAGGGAGCGACGAAGG-3'. The PCR reaction was carried out with the following cycling parameters: 95°C initial denaturation for 4 min, 28 cycles of 95°C denaturation for 40 sec, 58°C annealing for 40 sec and 72°C extension for 25 sec. A final extension was performed at 72°C for 7 min.

**Bioinformatic analysis:** Predictions of putative promoter regions and transcriptional start site were performed with [http://www.fruitfly.org/seq\\_tools/promoter.html](http://www.fruitfly.org/seq_tools/promoter.html) (BDGP). The putative transcription factor binding sites were predicted with <http://www.cbrc.jp/research/db/TFSEARCH.html> (TFSEARCH) and <http://www.cbil.upenn.edu/cgi-bin/tess/tess> (TESS) and then chose the sites which got high score in these software. At last, researchers had a comparison with human and the obtained porcine TCAP promoter sequence to select the highly conservative binding sites.

## RESULTS AND DISCUSSION

**Characterization of porcine TCAP gene promoter:** To investigate the transcriptional regulation of the porcine TCAP gene, the 5'-flanking region was acquired for further analysis. The product was 1662 and 1466 bp was located in 5'-upper stream of start codon (ATG) (Fig. 1). A comparison with the human TCAP 5'-flanking sequence

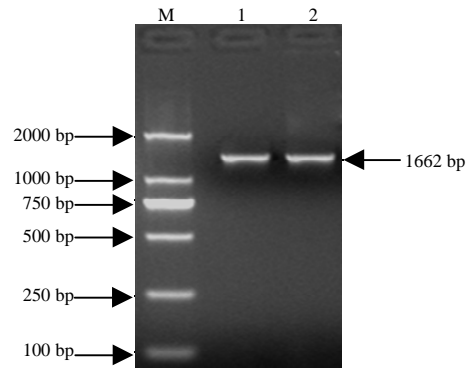


Fig. 1: PCR product of 5' upstream of porcine TCAP gene; M: DL2000 DNA Marker; Lanes 1, 2: PCR product

demonstrated a high degree of homology with approximately 76% nucleotide identity (Fig. 2). Sequence analysis indicated that multiple putative binding sites of several ubiquitous transcription factors such as SP1, AP1 and C/EBP were observed in this region. Some reports indicated that transcriptional factor AP1 had transcriptional activation moreover, AP1 binding site existed in gene promoters of multitude inflammatory cell factors and growth factors which prompted that AP1 might take part in the transcriptional regulation of cell factors and growth factors expression (Mao *et al.*, 2012). C/EBP together with other Rho factors compose complicated but subtle regulational network. They play an important role on cell multiplication and differentiation, tumorigenesis, organism immunity and stress reaction (Zhu *et al.*, 2009; Wang *et al.*, 2009; Xia *et al.*, 2009).

Several putative muscle-specific transcription factor binding sites (MyoD, MyoG and MEF2) were found within the TCAP promoter. Gene regulation in skeletal muscle is controlled by a family of highly related basic Helix Loop Helix (bHLH) transcription factors, the Myogenic Regulatory Factors (MRFs). The MRF family includes Myf5, MyoD, MyoG and Myf6 (also known as Mrf4).

The MRFs dimerize with E-proteins and bind E box sequences (CANNTG) in the regulatory regions of muscle genes (Berkes and Tapscott, 2005). The MRFs work in conjunction with multiple isoforms of the MADS-box factors, Mef2a, Mef2c and Mef2d (Blais *et al.*, 2005). Mef2 factors alone do not have myogenic activity but synergize with the MRFs to enhance gene expression during myogenesis (Molkentin *et al.*, 1995; Wang *et al.*, 2001). MyoD function early in myogenesis to confer a myogenic fate on mesodermal progenitor cells (Rudnicki *et al.*, 1993). MyoG functions later in myogenesis to stimulate



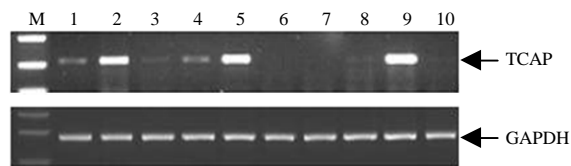


Fig. 3: Tissue expression profile of porcine *TCAP* gene; 1: lung; 2: kidney; 3: liver; 4: adipose tissue; 5: skeletal muscle; 6: stomach; 7: ovary; 8: spleen; 9: heart; 10: uterus. The GAPDH expression level is used for the internal control

expression in uterus, ovary and stomach which were consistent with previous studies (Valle *et al.*, 1997). TCAP is a Z-disc associated protein that is thought to be involved in the regulation and development of normal sarcomeric structure. TCAP co-localizes with titin, the interaction with titin is critical for sarcomeric integrity (Gregorio *et al.*, 1998). In this research, it was also shown that TCAP is localized within the Z-line region of adult striated skeletal and cardiac muscles. In the heart, TCAP is necessary for the cardiomyocyte's stretch sensor and the structural organization of the cardiac sarcomere (Bos *et al.*, 2006; Hayashi *et al.*, 2004).

The studies in cattle indicated that TCAP was highly expressed in striated muscle and was developmentally regulated (Mason *et al.*, 1999; Valle *et al.*, 1997). The over-expression of TCAP in C2C12 cell or primary cardiac myocyte disrupted the process of myofibril, suggesting its requirement for sarcomerogenesis in myocytes (Gregorio *et al.*, 1998). All these evidences suggested that TCAP might have similar biological function on muscle growth and meat quality traits in the pig.

### CONCLUSION

Researchers have isolated and characterized partial porcine *TCAP* gene promoter and found several putative muscle-specific transcription factor binding sites such as MyoD, MyoG and MEF2. The study would lay the foundation for future studies on porcine TCAP function and the regulatory mechanism in porcine skeletal muscle. In addition, the tissues expression profiles of pig TCAP mRNA displayed its abundant expression in skeletal muscle. The results implied that TCAP could be considered as a potential candidate for porcine muscle growth and meat quality.

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