

***Tropomodulin 1 (TMOD1)* is associated with lean meat growth and meat quality in the pig** (Brief Report)

***Tropomodulin 1 (TMOD1)* beeinflusst Fleischansatz und -qualität beim Schwein**
(Brief report)

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Background

Tropomodulin 1 (TMOD1) is a member of the tropomodulin family, which are highly conserved capping proteins of the pointed ends of the erythrocyte membrane and sarcomeric actin filaments. Tropomodulins are involved in the architecture of the sarcomere in muscle cells and the membrane skeleton in nonmuscle cells (COLUCCIO *et al.* 1994, GREGORIO *et al.* 1995). *TMOD1* is predominantly expressed in vertebrate cardiac muscle and slow twitch muscle fibers, and it binds to one end of tropomyosin (TM) that plays important roles in regulating the function of actin filament (FISCHER *et al.* 2003, GUNNING *et al.* 2008). Here we identified a single nucleotide polymorphism (SNP) in the porcine *TMOD1* gene and further analyzed the effects of this gene on the lean meat growth, meat quality and other related traits in pigs.

Procedures

The human *TMOD1* mRNA (acc. no. NM_003275) was used to search for porcine ESTs in the EST-others database through BLAST (<http://www.ncbi.nlm.nih.gov/blast>). Porcine ESTs sharing more than 80% identity were aligned using SeqMan program of DNASTar (DNASTAR, Inc., Madison, WI, USA). The aligned sequence was corresponding to human exon7 and exon8 (acc. no. FJ428533). Two pairs of primers (F1: AGTTCAGCATCGTGGGGACA/R1: AATGCACCTGAAACACCACACAG and F2: AGATGCTCAAAGTGAACAAGGTG/R2: CCAGAGAAGTGTGTATGGAAGA) were designed for PCR amplification from pig genomic DNA with Primer Premier 5.0 (PREMIER Biosoft Inc, Palo Alto, CA, USA). SNP discovery was implemented by sequencing the pooled PCR products amplified from six DNA samples and each two were from Yorkshire, Landrace and Tongcheng pigs. One SNP c.816A>G (dbSNP acc. no. 107795099) in exon 7 causing a synonymous mutation Leu239Leu was identified with primers F2/R2, and it can be distinguished by *Bst*NI with allele A revealing a 111bp and allele G revealing a 78bp and a 33bp fragments. A PCR-RFLP for SNP genotyping was developed as the followings, PCR mixture (10 µL) included 1×PCR buffer, 0.2 µM each primer, 150 µM each dNTP, 1.5 mM MgCl₂, 2U Taq DNA polymerase (Takara Company, Dalian, China) and 12.5ng genomic DNA. PCR reaction comprised of the initial denaturation at 95°C for 5 min, 30 cycles with 94°C for 30s, 62°C for 30s, 72°C for 30s,

followed by a final extension at 72°C for 5 min; RFLP reaction mixture (10 µL) consisted of 1 µL 10×buffer, 2U restricted enzyme BstNI and 5 µL PCR products. Samples were kept in 37°C incubator overnight.

SNP genotyping was performed in two different pig populations. Population A ($n=205$) included Yorkshire (Y, $n=26$), Landrace (L, $n=26$), Tongcheng (T, $n=49$), $L \times (Y \times T)$, $n=54$) and $Y \times (L \times T)$, $n=50$). The association analysis was implemented using mixed procedure (SAS 9.0; SAS Institute, Cary, NC, USA) and this model treated population/population combination, sex, slaughter date and marker genotyping as fixed effects, dam as random effect and body weight as covariate (TANG *et al.* 2008). The genotyping was also performed in the ISU Berkshire \times Yorkshire ($B \times Y$) pig resource family comprised of 515 F_2 animals (MALEK *et al.* 2001). The association analyses was implemented using mixed model procedure, including sex, slaughter date and marker genotypes as fixed effects, dam (litter) as random effect and body weight as covariate.

Results

In population A, SNP c.816A>G was significantly associated ($p < 0.05$) with loin pH. In $B \times Y$ family, c.816A>G was significantly associated with ($p < 0.05$) loin eye muscle area, average back fat, back fat at the lumbar, at 10th rib and at last rib, ham pH and lab loin pH, and was suggestively associated ($p < 0.10$) with hormel lion pH and average drip loss (Table 1). TM is one of the important regulatory proteins during the process of muscle contraction, and it is dominantly expressed in the thin filament with actin double helix, influencing interaction in actin and myosin (GREENFIELD *et al.* 2005, GUNNING *et al.* 2008). It is possible that *TMOD1* functions in lean meat growth and affects water holding capacity of muscle cells by changing TMOD-TM binding ability (KOSTYUKOVA *et al.* 2004). *TMOD1* was mapped between *S0331* and *Sw974* on SSC1, where numbers of QTL related to lean meat weight, LEA and pH have been located (<http://www.animalgenome.org/cgi-bin/QTldb>; MALEK *et al.* 2001, BEECKMANN *et al.* 2003, GELDERMANN *et al.* 2003). Combining the association analyses and linkage mapping promote the porcine *TMOD1* gene as a candidate gene for muscle production. Further work on additional causative mutation discovery and function analysis of *TMOD1* gene in pigs are warranted.

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Table 1

Association of Leu239Leu of *Tmod-1* gene with the analyzed traits in two pig populations respectively
Assoziation des SNP c.816A>G im TMOD1 Gen mit Schlachtkörper- und Fleischbeschaffenheitsmerkmalen in zwei Populationen

Population	Trait	LSM (SE)			P-value
		AA	AG	GG	
A	loin pH	6.36 (0.04) ^a	6.48 (0.03) ^b	6.49 (0.03) ^b	0.0210
B	LEA	33.22 (0.87) ^a	35.29 (0.62) ^b	36.87 (0.61) ^c	<0.0001
	AVBF	3.54 (0.09) ^a	3.33 (0.05) ^b	3.18 (0.05) ^c	<0.0001
	last rib BF	3.40 (0.09) ^a	3.23 (0.05) ^a	3.05 (0.05) ^b	<0.0001
	lumbar BF	3.83 (0.11) ^a	3.63 (0.06) ^a	3.44 (0.06) ^b	0.0002
	10th rib BF	3.39 (0.11) ^a	3.13 (0.06) ^b	3.44 (0.06) ^b	0.0059
	ham pH	5.95 (0.03) ^a	5.86 (0.01) ^b	5.91 (0.01) ^a	0.0248
	hormel pH	5.77 (0.02) ^{ab}	5.74 (0.01) ^a	5.78 (0.01) ^b	0.0989
	lab loin PH	5.81 (0.02) ^{ab}	5.79 (0.01) ^a	5.84 (0.01) ^b	0.0209
	AVDRIPPR	5.92 (0.30) ^{ab}	5.97 (0.15) ^a	5.58 (0.15) ^b	0.0560

loin pH loin pH at 45 min *post mortem*, LEA loin eye muscle area (cm²), AVBF average back fat (cm), last rib BF last rib back fat (cm), Lumbar BF lumbar back fat (cm), 10th rib BF 10th rib back fat (cm), ham pH ham pH at 24 h *post mortem*, hormel loin pH loin pH at 24 h *post mortem* measured at the Hormel slaughter plant lab loin pH loin pH at 48 h *post mortem* measured at the Iowa State University Meat Laboratory, AVDRIPPR average drip loss (%) LSM (SE) represents least squares means and their standard errors, Superscripts a, b, and/or c differ significantly ($P < 0.05$) from each other

References

- BEECKMANN P, SCHRÖFFEL JR J, MOSER G, BARTENSCHLAGER H, REINER G, GELDERMANN H (2003) Linkage and QTL mapping for *Sus scrofa* chromosome 1. *J Anim Breed Gen* 120, 1-10
- COLUCCIO LM (1994): An end in sight: tropomodulin. *J Cell Biol* 127, 1497-9
- FISCHER RS, FOWLER VM (2003) Tropomodulins: life at the slow end *Trends. Cell Biol* 13, 593-601
- GELDERMANN H, MULLER E, MOSER G, REINER G, BARTENSCHLAGER H, CEPICA S, STRATIL A, KURYL J, MORAN C, DAVOLI R, BRUNSCH C (2003) Genome-wide linkage and QTL mapping in porcine F2 families generated from Pietrain Meishan and Wild Boar crosses. *J Anim Breed Gen* 120, 363-93
- GREENFIELD NJ, KOSTYUKOVA AS, HITCHCOCK-DEGREGORI SE (2005) Structure and tropomyosin binding properties of the N-terminal capping domain of tropomodulin 1. *Biophys J* 88, 372-83
- GREGORIO CC, WEBER A, BONDAD M, PENNISE CR, FOWLER VM (1995) Requirement of pointed-end capping by tropomodulin to maintain actin filament length in embryonic chick cardiac myocytes. *Nature* 377, 83-6
- GUNNING P, O'NEILL G, HARDEMAN E (2008) Tropomyosin-based regulation of the actin cytoskeleton in time and space. *Physiol Rev* 88, 1-35
- KOSTYUKOVA AS, HITCHCOCK-DEGREGORI SE (2004) Effect of the Structure of the N Terminus of Tropomyosin on Tropomodulin Function. *J Biol Chem* 279, 5066-71
- MALEK M, DEKKERS JCM, LEE HK, BAAS TJ, ROTHSCHILD MF (2001) A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig II Meat and muscle composition. *Mamm Genome* 12, 637-45
- TANG ZL, PENG ZZ, LIU B, FAN B, ZHAO SH, LI XP, XU SP, LI K (2008) Effect of breed, sex and birth parity on growth carcass and meat quality in pigs. *Front Agric China* 2, 331-7

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