Archiv Tierzucht 53 (2010) 2, 242-245, ISSN 0003-9438

Investigation of effects of the *MKK3* and *MKK6* genes on meat production traits in the pig (Brief Report)

Untersuchung der Auswirkungen der Gene *MKK3* und *MKK6* auf Merkmale der Fleischleistung beim Schwein (Brief Report)

HUI WU[1], SHUHONG ZHAO[1] and BIN FAN[1,2]

[1]Key Laboratory of Agricultural Animal Genetics, Breeding & Reproduction of Ministry of Education, Key Laboratory of Swine Genetics & Breeding of Ministry of Agriculture, Huazhong Agricultural University, Wuhan, People's Republic of China, [2]Department of Animal Science and Centre for Integrated Animal Genomics, Iowa State University, Ames, USA

Background

Skeletal muscle development is a complexity process involved in spatial and temporal expressions of numerous muscle differentiation-specific genes, which are controlled by a series of basic helix-loop-helix myogenic regulatory factors (MRFs) (HOUBA and TE PAS 2004). The recent studies have demonstrated that the p38 mitogen-activated protein kinase (MAPK) pathway is probably one of the major intracellular signaling pathways affecting myogenesis (KEREN *et al.* 2006, LIUÍS *et al.* 2006). The p38 MAPKs regulate the transcriptional activities of MRFs and function in the remodeling of chromatin at specific muscle-regulatory regions (LI *et al.* 2000, ZARUBIN *et al.* 2005). The genes involved in the p38 MAPK pathway have been poorly studied in pigs. The primary work on SNP discovery and association analyses of two important MAPK kinases encoding genes *MKK3* (*MAP2K3*) and *MKK6* (*MAP2K6*) was presented in the study, so as to identify the potential genetic markers useful for marker assisted selection (MAS) improving lean meat production.

Procedures

The human *MKK3* and *MKK6* mRNA sequences (acc. no. NM_145109; NM_002758) were used to search for porcine ESTs through BLAST (http://www.ncbi.nlm.nih.gov/blast). Porcine ESTs sharing more than 85% identity were aligned using SeqMan program of DNAStar (DNASTAR, Inc., Madison, WI, USA). The aligned sequences were corresponding to human exons 7 and 8 of the *MKK3* gene, exons 4 and 5, and exons 9 and 10 of the *MKK6* gene, respectively. One pair of *MKK3* primers (F1: 5′-GAG CAC CTG CAC AGC AAG CT-3′/ R1: 5′-ACG GAG TCC ACC AAG TAG CC-3′) and two pairs of primers (F2: 5′-TTG AGG TGA AGG CGG ATG A-3′/R2: 5′-CCG TAG AAG GTG ACC GTA AAA G-3′; F3:5′-ACA TTT GGA GTC TGG GCA TCA-3′/R3: 5′-GTC GTA GGG AAA CCG AAG GA-3′) were designed for PCR amplification. The PCR products were commercially sequenced and then deposited in GenBank (acc. no. GQ169691, GQ169690). 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. The primers were redesigned to facilitate allele calling after PCR products were digested by restriction enzymes (Table 1).

SNP genotyping were implemented in two different pig populations. Population A (n=205) included Yorkshire (Y, n=26), Landrace (L, n=26), Tongcheng (T, n=49), L×(Y×T) (n=54) and Y×(L×T) (n=50). The association analysis was implemented using mixed procedure (SAS 9.0; SAS Institute, Cary, NC, USA) and this model treated breed, sex, slaughter date and marker genotype 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 × Yorkshire (B×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

Totally four SNPs were detected in porcine *MKK3* and *MKK6* genes. The associations between SNPs and the traits at significant ($P<0.05$) and suggestively significant ($P<0.1$) levels are shown in Table 2. *MKK3_Ava*I genotypes were significantly associated with carcass body length and loin muscle area. The SNPs within the *MKK6* gene were significantly associated with carcass body length, loin muscle area and fat traits; surprisingly, these SNPs were not associated with common traits, which implied they are in different haplotype blocks or *MKK6* has multiple effects through these different mutations. On the basis of SNPs genotyping results from population B, the porcine *MKK3* gene was mapped the end of *SSC12* and was 34.8cM to *SW2180*. The *MKK6* gene was assigned between *S0229* (9.5cM) and *SW874* (29.3cM) on SSC12. A few of QTL related to loin muscle area and backfat traits have been mapped around these regions (http://www.animalgenome.org/cgi-bin/QTLdb).

In vertebrate, there are four p38 MAP kinases being mainly phosphorylated and activated by the MAPK kinases *MKK6* and *MKK3*. Once being activated, these p38 MAPKs phosphorylate serine/threonine residues of their substrates, which include a number of transcription factors and protein kinases such as MEF2 and MRF4 (YANG *et al.* 1999, DE ANGELIS *et al.* 2005). The p38 MAPKs are also involved in the regulation of chromatin remodeling activities and RNA polymerase II recruitment to muscle-specific promoters (LIUÍS *et al.* 2006). These characteristics make the p38 MAPKs exhibit roles to activate different subsets of muscle genes in spatial and temporal patterns. The studied SNPs within *MKK3* and *MKK6* genes showed associations with loin muscle area and fat traits in pigs, implying the p38 MAPKs may play the same essential roles on muscle differentiation and growth. However, these intronic SNPs are possibly linked to causative mutations that actually exist in exons or other regulatory regions. The discovery of the causative mutations and function analyses are needed to confirm the association of the *MKK3* and *MKK6* genes with meat production in pigs.

Table 1
The identified SNPs within porcine *MKK3* and *MKK6* genes
Die identifizierten SNPs innerhalb der MKK3- und MKK6-Gene des Schweins

Gene	*MKK3*	*MKK6*	*MKK6*	*MKK6*
SNP type	C/T	C/T	A/G	C/T
SNP location	Intron 7	Intron 4	Intron 9	Intron 9
Primer (5′→3′)	GAC ACG CCC TCT TGG CTA CA/ CAA GGC TTC TGA GAC TCA CCC	GTG AGG CTG GAA TGG AGT GG/ CCT GGA GCA GAA GTC AGA ACC T	ATG GCG GTG AAG GTA GAG TT/ CTT GGG CAG AGG GTC ATA GT	ATG GCG GTG AAG GTA GAG TT/ CTT GGG CAG AGG GTC ATA GT

Table 2
Genotyping and association analysis of SNPs from porcine *MKK3* and *MKK6* genes in two pig populations
Genotypisierung und Assoziationsananlyse von SNPs der MKK3- und MKK6-Genen des Schweins in zwei Populationen

SNP (dbSNP acc. no.)	PCR-RFLP pattern (11/22, bp)	Genotyped population (sample size)	Trait, unit	Least Square Means (SE) 11	12	22	*P*
*MKK3_Ava*I (ss136268337)	504+149/276+228+149	A (n=205)	Carcass body length, cm	88.75 (0.39)^{a}	89.97 (0.47)^{b}	90.55 (0.79)^{bc}	0.04
		B (n=515)	Loin muscle area, cm^2	–	34.64 (0.80)^{a}	36.15 (0.56)^{b}	0.03
*MKK6_Alu*I (ss136268344)	259/188+71	A (n=205)	Marbling score	2.37 (0.09)^{a}	2.40 (0.06)^{a}	2.01 (0.14)^{b}	0.03
		B (n=515)	Last rib backfat, cm	3.20 (0.07)^{a}	3.17 (0.05)^{a}	3.01 (0.07)^{b}	0.04
			Carcass body length, cm	83.99 (0.22)^{a}	84.08 (0.16)^{a}	84.69 (0.24)^{b}	0.03
			Birth body weight, kg	1.51 (0.04)^{a}	1.53 (0.04)^{a}	1.61 (0.05)^{b}	0.04
*MKK6_Nsi*I (ss136268348)	591/320+271	B (n=515)	Water holding capacity, g	0.19 (0.01)^{a}	0.22 (0.01)^{b}	0.18 (0.03)^{ab}	0.03
			AVGP, µmol/g	103.70 (1.13)^{a}	106.07 (1.39)^{a}	113.72 (3.60)^{b}	0.01
			AVLAC, µmol/g	86.49 (0.88)^{a}	87.96 (1.07)^{a}	93.93 (2.8)^{b}	0.02
*MKK6_Taq*I (ss136268351)	591/482+109	B (n=515)	Loin muscle area, cm^2	35.75 (0.76)	35.54 (0.60)	36.44 (0.60)	0.08
			Carcass body length, cm	84.55 (0.27)	84.24 (0.17)	84.00 (0.17)	0.08

11 and 22 represent homozygote uncut by the restriction enzyme and homozygote cut by the restriction enzyme, respectively. AVGP average content of glycolytic potential, AVLAC average content of lactate of the loin at 48 h *post mortem*. ^{a,b,c}Different subscripts in the same row indicate significant differences between means ($P<0.05$). 11, 12 and 22 represent homozygote uncut by the restriction enzyme, heterozygote and homozygote cut by the restriction enzyme, respectively.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (30771169), Wuhan Youth Chenguang Project (200750731282) and Key Project of National Basic Research and Developmental Plan (2006CB102105) of China. The authors thank the members of Dr. Max F. Rothschild's research group at Iowa State University for their assistance on this research.

References

De Angelis L, Zhao J, Andreucci JJ, Olson EN, Cossu G, Mcdermott JC (2005) Regulation of vertebrate myotome development b the p38 MAP kinase-MEF2 signaling pathway. Dev Biol 283, 171-9

Houba PHJ, te Pas MFW (2004) The muscle regulatory factors gene family in relation to meat production. In: te Pas MFW, Everts ME, Haagsman HP (eds) Muscle development of livestock animals: physiology, genetics and meat quality. CABI Publishing, Wallingford, Oxfordshire, UK, 201-4

Keren A, Tamir Y, Bengal E (2006) The p38 MAPK signaling pathway: A major regulator of skeletal muscle development. Mol Cell Endocrinol 252, 224-30

Li YQ, Jiang BH, Ensign WY, Vogt PK, Han JH (2000) Myogenic differentiation requires signaling through both phosphatidylinositol 3-kinase and p38 MAP kinase. Cellular Signalling 12, 751-7

Liuís F, Perdiguero E, Nebreda AR, Muñoz-Cánoves P (2006) Regulation of skeletal muscle gene expression by p38 MAP kinases. Trends Cell Biol 16, 36-44

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

Williamson D, Gallagher P, Harber M, Hollon C, Trappe S (2003) Mitgoen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle. J Physiol 547, 977-87

Yang SH, Galanis A, Sharrocks AD (1999) Targeting of p38 mitogen-activated protein kinases to MEF2 transcription factors. Mol Cell Biol 19, 4028-38

Zarubin T, Han J (2005) Activation and signaling of the p38 MAP kinase pathway. Cell Res 15, 11-8

Received 16 October 2009, accepted 10 March 2010.

Corresponding author:

BIN FAN
email: bfan@iastate.edu

Department of Animal Science, Iowa State University, 2255 Kildee Hall, Ames, IA 50011-3150, USA