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Full Length Research Paper

Association of porcine *UCP3* gene polymorphisms with fatness traits in a Pietrain×Jinhua F2 population

Zhe Chen[1], Xiaofeng Zhao[2], Zhu Hao[1], Xiaoling Guo[1], Xiaoling Jiang[1] and Ningying Xu[1]

[1]College of Animal Science, Zhejiang University, Hangzhou 310029, Zhejiang Province, People's Republic of China.
[2]College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, Zhejiang Province, People's Republic of China.

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Uncoupling protein 3 (*UCP3*) is a mitochondrial transmembrane carrier which uncouples oxidative phosphorylation and plays an important role in energy homeostasis and fatty acid transporting. In this study, two missense mutations (T221C and A448G) were detected in porcine *UCP3* gene. Genotype frequencies were analyzed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) in eight pig breeds (n = 384). The results revealed that Chinese native breeds had fixed genotype CC at T221C polymorphic loci. Allele G at the A448G loci has higher frequency than allele A in all pure breeds, except for Pietrain. Association analysis between variants and fatness traits was carried out in a Pietrain×Jinhua F2 population (n = 274). The results showed that two single nucleotide polymorphism (SNPs) were significantly associated with intramuscular fat content (p = 0.023 and p = 0.000, respectively). These results indicated that single nucleotide polymorphism of *UCP3* gene is potentially associated with fatness traits in pig.

Key words: Pig, uncoupling protein 3 (*UCP3*), polymorphism, fatness traits, association analysis.

INTRODUCTION

In pig industry, reducing fat content and improving meat quality are critical issues. Fat accumulation in different breeds is influenced by both environmental and genetic factors. Consecutive evidences demonstrated that most phenotypes are caused by genetic factors rather than the shared environment (Vogler et al., 1995; Taddei et al., 2001; Watson et al., 2006; Prior et al., 2007). Therefore, genes which had potential influence on fat deposition need to be studied extensively.

The uncoupling protein 3 (*UCP3*) is a member of the mitochondrial transporter family. Homologues of *UCP3* (*UCP1* and *UCP2*) have been identified in many tissues. The common effect of all three uncoupling proteins is uncoupling oxidative phosphorylation by mediating proton leak (Gong et al., 1997). Recent studies explored the role of *UCP3* in mitochondrial fatty acid metabolism (Nedergaard and Cannon, 2003), protecting mitochondrial matrix against reactive oxygen species (ROS) (Casteilla et al., 2001; Lombardi et al., 2010) and insulin sensitivity (Mottagui-Tabar et al., 2008).

The porcine *UCP3* gene is located on 9p21-p24 near many quantitative trait locus (QTL) for lipid content and intramuscular fat content (Nowacka-Woszuk et al., 2008). *UCP3* is expressed selectively in skeletal muscle and brown adipose tissue (Boss et al., 1997). Physiological experiments supported the fact that *UCP3* is involved in the regulation of energy expenditure (Boss et al., 2000; Nagy et al., 2004). Genetic studies suggested that *UCP3* plays a role in fatty acid metabolism (Argyropoulos et al., 1998; de Luis Roman et al., 2010). Moreover, genes that regulate energy expenditure have the potential to influence economically important traits in farm animals (Sherman et al., 2008). Thus, *UCP3* gene is considered as a candidate gene for fatness traits in pig.

Polymorphism in *UCP3* gene may change the function

*Corresponding author. E-mail: nyxu@zju.edu.cn. Tel: +86-571-86971308.

Abbreviations: UCP3, Uncoupling protein 3; **SNP,** single nucleotide polymorphism; **BFT,** backfat thickness; **IMF,** intramuscular fat; **PCR,** polymerase chain reaction; **RFLP,** restriction fragment length polymorphism; **PJF2**, Pietrain×Jinhua F2; **LEA,** loin eye area; **WHC,** water holding capacity.

Table 1. Genotype distribution in different breeds.

Breed	N	T221C			A448G		
		CC	CT	TT	AA	AG	GG
Shengxian Hua	66	1.000 (66)	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)	1.000 (66)
Bihu	54	1.000 (54)	0.000 (0)	0.000 (0)	0.018 (1)	0.722 (39)	0.260 (14)
Jiaxing Black	38	1.000 (38)	0.000 (0)	0.000 (0)	0.053 (2)	0.079 (3)	0.868 (33)
Jinhua	96	1.000 (96)	0.000 (0)	0.000 (0)	0.083 (8)	0.313 (30)	0.604 (58)
Pietrain	30	0.367 (11)	0.533 (16)	0.100 (3)	0.367 (11)	0.367 (11)	0.266 (8)
Yorkshire	34	0.912 (31)	0.088 (3)	0.000 (0)	0.235 (8)	0.441 (15)	0.324 (11)
Duroc	30	0.700 (21)	0.200 (6)	0.100 (3)	0.000 (0)	0.500 (15)	0.500 (15)
Landrace	36	0.833 (30)	0.167 (6)	0.000 (0)	0.000 (0)	0.333 (12)	0.667 (24)
PJF2	274	0.759 (208)	0.237 (65)	0.003 (1)	0.263 (72)	0.474 (130)	0.263 (72)

Source of breeds: Jinhua (Zhejiang Jiahua Pig Breeding Co., LTD), Jiaxing Black (Shuangqiao Pig Breeding Farm), Bihu (Lishui Pig Breeding Farm), Shengxian Hua (Shangyu Shenghua Pig Breeding Co., LTD), PJF2 offspring, Yorkshire and Pietrain (The Experimental Pig Farm of Zhejiang University), Landrece (Zhejiang Dengta Pig Breeding Farm) and Duroc (Xiaoshan Duroc Pig Breeding Farm).

or expression level and therefore alter the capacity of storing energy as fat (van Abeelen et al., 2008). Several causative single nucleotide polymorphisms (SNPs) have been found in human *UCP3* gene, but few studies were carried out in pig. Thus, the aim of this study was to search for polymorphisms in porcine *UCP3* gene within different breeds, and to further assess the relationship between polymorphisms and fatness traits in a Pietrain × Jinhua F2 (PJF2) crossbred population.

MATERIALS AND METHODS

Samples and data collection

Preliminary detection for polymorphisms of *UCP3* gene was performed in a multibreed panel (five Jinhua, five Shengxian Hua, five Pietrain and five Yorkshire). Altogether, 384 DNA samples from eight pig breeds were collected, including four European and four Chinese native breeds (Table 1). The sampled pigs were unrelated throughout the last three generations. The further association studies were carried out in a Pietrain×Jinhua F2 (PJF2) crossbred population, which has diverse phenotypes in fatness traits. The PJF2 crossbred population was developed from purebred Pietrain sires and Jinhua dams, including six F1 boars, 20 F1 sows and 527 F2 progeny, in which 274 offspring were used for experiments. All the PJF2 individuals were raised under normal conditions at The Experimental Pig Farm of Zhejiang University, and slaughtered at a commercial abattoir.

PJF2 progenies were fed till the day of slaughter (223 ± 26.88 days, live weight 80.9 ± 8.61 kg) and then dissected. After slaughter, backfat thickness (BFT) were measured by vernier caliper on the left side at four locations (shoulder, 6 to 7th ribs, last rib and gluteus medius). Samples of the longissimus dorsi muscle were taken at the 13th rib for the determination of intramuscular fat (IMF) content using a Meat Analyzer (AntarisII FT-NIR analyzer, Thermo Electric Company, USA). Additionally, recorded traits included loin eye area (LEA), water holding capacity (WHC), muscular pH and muscular temperature.

Genomic DNA was isolated from blood samples using standard phenol protocol (Sambrook and Russell, 2001).

Primers, amplification and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis

The primers used for PCR amplification were designed based on the porcine *UCP3* gene mRNA sequence (GenBank accession No. NM_214049) and human *UCP3* gene DNA sequence (GenBank accession No. NC_000011). Two pairs of primers were designed using Oligo 6 software and synthesized by Invitrogen (Shanghai, P. R. China). The *UCP3*-P1 primers (5' TGCTGGGCACCATTCTCAC 3' and 5' ACTCACGCTCCGATCCCTT 3') amplify 162 base pair (bp) amplicon from exon 2 and partial intron 2 region. The *UCP3*-P2 primers (5' TCCAGACTCCAGCATCACC 3' and 5' CATCGTCCC GCTGTACTT 3') amplify 154 bp fragments of exon3 and partial intron 2 region.

The PCR mix consisted of 50 ng DNA, 400 μM of dNTPs (Sangon, Shanghai, P. R. China), 0.25 μM of each primer and 1 unit (U) of *Taq* polymerase (TaKaRa, Dalian, P. R. China). A total volume of each sample was 25 μl. The PCR procedures were 94℃ for 5 min followed by 36 cycles of denaturation at 94℃ for 30 s, annealing at 60℃ for *UCP3*-P1 or 58℃ for *UCP3*-P2 for 30 s, and extension at 72℃ for 30 s, with a final extension at 72℃ for 10 min. Multiple alignments of the sequences from multibreed panel (five Jinhua, five Shengxian Hua, five Pietrain and five Yorkshire) were carried out using DNAMAN software.

Animal genotyping was performed by restriction fragment length polymorphism (RFLP) with application of restriction enzymes *BsII* and *SmaI* (NEB, Beijing, P. R. China) for *UCP3*-P1 *and UCP3*-P2, respectively. PCR products were digested following the manufacture protocol and then electrophoresize in 2% agarose gel stained with ethidium bromide.

Statistical analysis

The general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA) was performed to analyze the relationship between polymorphisms and fatness traits in PJF2 offsprings. The model included the fixed effect of tested single nucleotide polymorphism (SNP) genotypes, the fixed effect of sex, the fixed effect of sire, linear covariates of age at slaughter and carcass weight, and the random effect of litter. The model for IMF trait included the four-point average BFT as covariate instead of carcass

Table 2. Association analysis between genotype and fatness traits.

Trait	T221C (Mean ± standard error)			P-value	A448G (Mean ± standard error)			P-value
	CC	CT	TT		AA	AG	GG	
BFT at shoulder (cm)	4.324 ± 0.087	4.292 ± 0.096	—	0.736	4.189 ± 0.098	4.338 ± 0.092	4.414 ± 0.103	0.125
BFT at 6-7 ribs (cm)	3.029 ± 0.079	2.999 ± 0.087	—	0.730	2.952 ± 0.089	2.999 ± 0.083	3.110 ± 0.094	0.308
BFT at last rib (cm)	2.237 ± 0.070	2.279 ± 0.078	—	0.589	2.201 ± 0.080	2.240 ± 0.075	2.330 ± 0.084	0.374
BFT at gluteus medius (cm)	2.221 ± 0.077	2.140 ± 0.085	—	0.331	2.127 ± 0.087	2.199 ± 0.081	2.243 ± 0.092	0.505
IMF (%)	2.875 ± 0.114[a]	2.477 ± 0.151[b]	—	0.023	2.440 ± 0.159[b]	2.520 ± 0.128[b]	3.245 ± 0.151[a]	0.000

Means with different superscript a and b within a row for same polymorphic locus are statistically different at $p < 0.05$.

weight.

RESULTS

Detection of single nucleotide polymorphisms

Multiple alignments of the sequences from multi-breed panel revealed two SNPs in the *UCP3* gene. The first one (c.221T > C, relative to the start codon) located in exon 2 caused an amino acid change from leucine to proline (Leu74Pro). The second one (c.448A > G, relative to the start codon) located in exon 3 was also a missense substitution (Arg150Gly). The two SNPs found in our study were earlier discovered by Cieslak et al. (2009) and Li et al. (2005), respectively.

Genotype frequencies in different breeds

Genotype distribution of the two SNPs in different breeds is shown in Table 1. The T221C polymorphism was monomorphic in all Chinese native breeds, in which only CC genotype was found. The allele T exists in European breeds and PJF2 crossbred population. But there was only one TT pig in the PJF2 crossbred population. At the A448G loci, allele G has higher frequency than allele A in all pure breeds, except for Pietrain. Shengxian Hua pig has fixed genotypes in both polymorphism loci.

Relationship between *UCP3* gene variants and fatness traits

The results of association analysis within PJF2 population were presented in Table 2. Since only one TT pig was found in PJF2 population, we excluded it from statistical analysis. Thus, we could analyze associations for two genotypes (CC and CT) only at T221C polymorphism loci. Results showed that the T221C polymorphism was significantly associated with IMF trait ($P = 0.023$), and the higher IMF content was observed for CC genotype. An extremely significant association was found between A448G polymorphism and IMF trait ($P = 0.000$). We observed that GG genotype was associated with the highest IMF (3.245 ± 0.151), when compared with AG (2.520 ± 0.128) and AA genotypes (2.440 ± 0.159). BFT traits and the two polymorphisms were not significantly correlated.

DISCUSSION

Experiments with *UCP3* gene knockout mice showed that lack of *UCP3* represent greater fat storage than wild littermates (Costford et al., 2008), meanwhile, mice overexpressing *UCP3* in skeletal muscle were hyperphagic and lean (Clapham et al., 2000). Human researches have found a number of SNPs in *UCP3* gene, some of them were demonstrated to be associated with human obesity or Type 2 diabetes (Otabe et al., 2000; Liu et al., 2005; van Abeelen et al., 2008). Sherman et al. (2008) observed significant association between bovine *UCP3* gene mutations and feed efficiency traits (average daily gain, feed conversion ratio and growth efficiency). Recently, some statistically significant associations of the *UCP3* gene variants with fatness traits were also found in chicken (Zhao et al., 2006; Liu et al., 2007).

Searching for polymorphisms of the porcine *UCP3* gene have been already carried out in several European and Chinese native breeds (Li et al., 2005). Four deletion polymorphisms, two missense mutations and eight intronic substitutions were discovered. Earlier experiments approved that *UCP3* gene influenced muscle growth and fat convertion (Fang et al., 2002). Cieslak et al. (2009) analyzed the association between T221C polymorphism and fatness trait in a mixed group (Polish Landrace and Polish synthetic line), and a significant correlation with

abdominal fat weight was observed. In this current study, although the T221C and A448G variants were earlier reported by Cieslak et al. (2009) and Li et al. (2005), respectively, unique T221C genotype distribution and novel correlations with IMF trait were observed.

Botstein and Risch (2003) concluded that the majority of identified disease-associated variants were regulatory or missense polymorphisms. They proposed that association analysis should rely on limited potential causative variants, rather than on linkage disequilibrium (LD). Thus, we chose this strategy in our study. The results showed that the T221C polymorphism was significantly associated with IMF trait ($p = 0.023$), and pigs with CC genotype had higher IMF content than CT ones ($p < 0.05$). The A448G polymorphism was extremely significantly associated with IMF trait ($p = 0.000$). Pigs with GG genotype had higher IMF content when compared to the other genotypes ($p < 0.05$). Since the T221C polymorphism is located in the second transmembrane domain, and the A448G polymorphism is located nearly the second mitochondrial carrier signature of UCP3 protein (Laloi et al., 1997; Maia et al., 1998), we speculated that these two mutations affect IMF trait by altering the binding ability and transporting efficiency.

At the T221C polymorphism loci, we observed a significant difference in genotype distribution between European and Chinese native breeds. Chinese native breeds only had fixed genotype of CC, while European breeds were polymorphic. Chinese native breeds, e.g., Jinhua pig, was characterized by lower growth, higher fat content and tender meat. Contrarily, European breeds, e.g., Pietrain, had reputation for its relatively rapid growth, lower fat content and high proportion of lean meat vs. fat content. Moreover, this polymorphism had significant association with IMF trait ($p = 0.023$). Thus, it was indicated that the T221C polymorphism might be an interesting target for studying fatness traits.

Although Cieslak et al. (2009) found a significant association between *UCP3* gene variants and abdominal fat weight in a mixed group (Polish Landrace and Polish synthetic line), no significant association was observed in a separate set of Polish Landrace group and Polish synthetic line. On the other hand, in our study, the *UCP3* gene was associated with intramuscular fat content, but no association was observed with subcutaneous fat deposition. As abdominal subcutaneous, adipose tissue and intramuscular adipose tissue have different metabolic and endocrine degree (Hermsdorff et al., 2004), therefore, we inferred that the *UCP3* gene regulation favors intramuscular fat content over subcutaneous fat deposition.

In conclusion, two missense mutations were detected in porcine *UCP3* gene. The T221C polymorphism has unique distribution between European and Chinese native breeds. Association analysis showed that both genetic variants in porcine *UCP3* gene were associated with IMF trait. Further research, using larger amount of samples and different populations, is needed to better assess the effects of *UCP3* gene on fatness traits.

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