Association Analyses with Carcass Traits in the Porcine *KIAA1717* and *HUMMLC2B* Genes*

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ABSTRACT : By screening a subtracted cDNA library constructed with mRNA obtained from the *longissimus dorsi* muscles of F1 hybrids Landrace×Yorkshire and their Yorkshire female parents, we isolated two partial sequences coding for the H3-K4-specific methyltransferase (*KIAA1717*) and skeletal muscle myosin regulatory light chain (*HUMMLC2B*) genes. In the present work we investigated two SNPs, one (C1354T) at the 3' untranslated region (UTR) of *KIAA1717* and one (A345G) at the SINE (PRE-1) element of *HUMMLC2B*, in a resource population derived from crossing Chinese Meishan and Large White pig. The selected pigs were genotyped by means of a PCR-RFLP protocol. Significant associations were observed for the *KIAA1717* C1354T polymorphic site with thorax-waist backfat thickness ($p<0.05$), buttock backfat thickness ($p<0.05$), average backfat thickness ($p<0.05$), loin eye height ($p<0.05$), loin eye area ($p<0.05$), carcass length to 1st spondyle ($p<0.01$) and carcass length to 1st rib ($p<0.01$). *HUMMLC2B* A345G were significantly associated with loin eye width ($p<0.05$), loin eye area ($p<0.05$). Further studies are needed to confirm these preliminary results. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1519-1523)***

Key Words : H3-K4-Specific Methyltransferase, Myosin Regulatory Light Chain, Pigs, Polymorphism, Carcass Traits

INTRODUCTION

Continued genetic improvement of swine requires molecular markers to assist selection. One method of developing such markers is by studying candidate genes. A quantitative trait (QT) is controlled by several or many genes (quantitative traits loci-QTLs) which may contribute to the phenotype to a different extent, and be also affected by environmental factors. Genes involved in the biology of a trait of interest are candidate genes for association studies (Brunsch et al., 2002). In the last decade, animal genomics has contributed to the mapping and identification of genes responsible for several traits. In some cases both the genes and the underlying causal mutations were identified by the candidate gene approach (Harlizius and van der Lende, 2001; Li et al., 2002; Yan et al., 2004; Zhang et al., 2005).

Muscle comprises as much as 45% of an animal's body mass (Young, 1970). Hence there is much interest in understanding the development, physiology and metabolism of this tissue. The regulation of muscle development is complex, involving many integrated biochemical pathways that interact with the environment to ultimately control the rate of protein accretion. One strategy in dissecting out these networks is to identify genes that are differentially expressed between properly defined breeds, selection lines, or developmental stages. The isolation of these differentially expressed genes will give insight into their biological function, provide clues as to what molecular pathway they participate in, and suggest potential candidate genes for breeding programs or genetic methods of manipulating growth in livestock (Janzen et al., 2000). In addition, the phenomenon of heterosis is in fact the external exhibition of gene expression and regulation in the heterozygote (Liu et al., 2004). The development of cDNA libraries from tissues directly related to important production traits is also important for the identification of candidate genes (Gellin et al., 2000). For these reasons we developed a porcine longissimus dorsi subtracted cDNA library between F1 hybrids Landrace×Yorkshire and their Yorkshire female parents. Recently, from this subtracted cDNA library we isolated partial cDNAs representing *KIAA1717* and *HUMMLC2B*, which were highly expressed in the female Yorkshire parents.

In this study we investigated the polymorphisms of *KIAA1717* and *HUMMLC2B* and evaluated the effects of *KIAA1717* and *HUMMLC2B* Msp I PCR-RFLP on carcass traits in the population derived from crossing Chinese Meishan and Large White pigs.

MATERIALS AND METHODS

Animals and data collection

The animals were produced by crossing Large White×Meishan and raised in Huazhong Agriculture University. They were fed twice daily diets formulated according to age under a standardized feeding regimen and had free access to water. The finishing animals were

* The nucleotide sequence data reported in this paper have been submitted to the GenBank under accession number: AY900164 (*KIAA1717*), AY900165 (*HUMMLC2B*).

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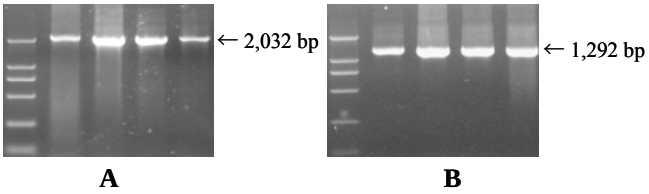

Figure 1. PCR products of *KIAA1717* and *HUMMLC2B*. A corresponds to the 3′-untranslated region (3′-UTR) of *KIAA1717*; B corresponds to part of exon 4 and 3′ UTR, complete exons 5 to 6 and introns 4 to 6 of *HUMMLC2B*.

slaughtered and the carcass traits were measured according to the method of Xiong and Deng (1999). Genomic DNA was prepared from blood samples using a standard phenol: chloroform extraction method.

Primer design and PCR amplification

According to the cDNA sequences, obtained from the porcine longissimus dorsi subtracted cDNA library between F1 hybrids Landrace×Yorkshire and their Yorkshire female parents, and the results of database searches performed with BLASTN (Altschul et al., 1997), PCR primers (forward: 5′-AAACAACCGTAAAGGACTG-3′; reverse: 5′-GCAAGA AATGCCTAAAGC-3′) were designed to amplify *KIAA1717*; and PCR primers (forward: 5′-AGCGGTCCC ATCAACTTC-3′; reverse: 5′-GTCCCCTACTCCTGGTC CT-3′) amplified *HUMMLC2B*. The reaction mixture comprised 25 ng porcine genomic DNA as template, 0.25 μM of each primer, 0.25 μM of each dNTP, 1×PCR buffer and 1 U Taq DNA polymerase (Biostar Internation, Toronto, Canada). The PCR amplifications were performed in 20 μl on a GeneAmp PCR system 9600 (Perkin Elmer, Foster City, CA, USA) with the following cycling parameters: 95°C initial denaturation for 4 min, 35 cycles of 95°C denaturation for 40 s, 53°C (*KIAA1717*) or 64°C (*HUMMLC2B*) annealing for 40 s, and 72°C extension for 2 min. A final extension was performed at 72°C for 10 min.

PCR-RFLP analysis

The alleles of *KIAA1717* and *HUMMLC2B* were analysed by using the restriction fragment length polymorphism (RFLP) protocol. 8.5 μl of PCR products were digested with 5 U of Msp I (MBI Fermentas, Vilnius, Lithuania) at 37°C for 4 h in a volume of 10 μl and the digested products were electrophoresed on 1.5% argarose gels and stained with ethidium bromide.

Statistics analysis

The genotype distribution was tested for Hardy-Weinberg equilibrium as described by Falconer and Mackay (1996). Associations between genotypes and carcass traits were evaluated by means of the least square method (GLM procedure, SAS version 8.0). According to the method of Liu (1998), both additive and dominant effects were also

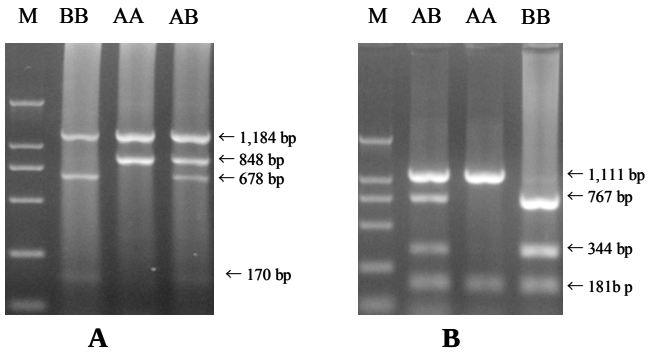

Figure 2. Agarose gel electrophoresis (1.5%) showing polymorphisms in PCR fragments of *KIAA1717* (A) and *HUMMLC2B* (B) after digestion with *Msp* I. The genotypes (AA, AB and BB) are shown at the top of the lanes. M, marker DL 2000 DNA Ladder (TaKaRa).

estimated by using the REG (regression) procedure of SAS version 8.0, where the additive effect was denoted as -1, 0 and 1 for AA, AB and BB, respectively, and the dominance effects represented as 1, -1 and 1 for AA, AB and BB, respectively. The model used to analyze the data was assumed to be:

$$Y_{ijk} = \mu + S_i + B_j + G_k + b_{ijk}X_{ijk} + e_{ijk}$$

Where Y_{ijk} is the observation of the trait; μ is the least square mean (LSM), S_i is the effect of i-th sex (i = 1 for male or 0 for female), B_j is the effect of j-th year (j = 0 for 2,000 or 4 for 2,004), G_k is the effect of k-th genotype (k = AA, AB, BB), b_{ijk} is the regression coefficient of the carcass weight and e_{ijk} is the random residue.

RESULTS AND DISCUSSION

In total, 2,032 bp, corresponding to the 3′-untranslated region (3′-UTR) of *KIAA1717* (GenBank accession number AY900164) and 1,292 bp, encompassing part of exon 4 and 3′ UTR, complete exons 5 to 6 and introns 4 to 6 of *HUMMLC2B* (GenBank accession number AY900165) sequences were obtained (Figure 1). The fragments of *KIAA1717* and *HUMMLC2B* both had two digested sites for Msp I. The polymorphic Msp I sites were at position C1354T, in the 3′-UTR of *KIAA1717*, and A345G, in the SINE (PRE-1) element of the fifth intron of *HUMMLC2B*, respectively. The Msp I digestion of *KIAA1717* generated two (genotype AA, 1,184+848 bp), three (genotype BB, 1,184+678+170 bp) or four (genotype AB, 1,184+848+ 678+170 bp) fragments (Figure 2A). The Msp I digestion of *HUMMLC2B* generated two (genotype AA, 1,111+181 bp), three (genotype BB, 767+344+181 bp) or four (genotype AB, 1,111+767+344+181 bp) fragments (Figure 2B).

In order to study the possible associations between carriers of the different genotypes and the trait values, the

Table 1. Association between *KIAA1717* genotypes and carcass traits

Trait	*KIAA1717* genotype (μ±SE)			Effect (μ±SE)	
	AA (n = 205)	AB (n = 71)	BB (n = 9)	Additive	Dominance
Dressing percentage (%)	71.634±0.263	72.561±0.459	72.374±1.307	0.288±0.630	-0.291±0.385
Lean meat percentage (%)	57.171±0.263	56.685±0.453	56.014±1.242	-1.070±0.639	-0.236±0.390
Shoulder backfat thickness (cm)	3.205±0.0476	3.313±0.082	3.555±0.225	0.118±0.116	0.006±0.071
6-7th thorax backfat thickness (cm)	2.543±0.041	2.677±0.072	2.928±0.196	0.165±0.101	0.014±0.062
Thorax-waist backfat thickness (cm)	1.802±0.038^{a}	1.980±0.065^{b}	2.183±0.179^{b}	0.216±0.092*	0.018±0.056
Buttock backfat thickness (cm)	1.693±0.045^{a}	1.873±0.078^{b}	2.052±0.214^{ab}	0.142±0.110	-0.021±0.067
Average backfat thickness (cm)	2.310±0.037^{a}	2.470±0.064^{b}	2.678±0.176^{b}	0.159±0.090	0.005±0.055
Loin eye width (cm)	4.727±0.047	4.625±0.082	5.022±0.224	0.109±0.116	0.102±0.071
Loin eye height (cm)	9.411±0.054^{a}	9.148±0.093^{b}	9.091±0.255	-0.053±0.127	0.075±0.077
Loin eye area (cm^2)	31.171±0.352^{a}	29.703±0.608^{b}	31.242±1.666^{ab}	0.110±0.859	0.665±0.524
Carcass length to 1st spondyle (cm)	92.931±0.296^{Aa}	91.307±0.512^{Bb}	88.321±1.403^{Bc}	-2.164±0.721**	-0.280±0.440
Carcass length to 1st rib (cm)	78.508±0.267^{A}	76.984±0.462^{B}	74.818±1.266^{B}	-1.824±0.649**	-0.150±0.396
Rib number	14.798±0.048	14.654±0.083	14.437±0.227	-0.147±0.115	0.000±0.070

Least square means (LSM) estimated for each polymorphism is indicated with its standard error (SE).
Significant differences (within a trait) between the genotype classes indicated with different lower case superscripts are significant at $p<0.05$, those with capital superscripts differ at $p<0.01$. * $p<0.05$ and ** $p<0.01$. n = Number of individuals.

Table 2. Association between *HUMMLC2B* genotypes and carcass traits

Trait	*HUMML2B* genotype (μ±SE)			Effect (μ±SE)	
	AA (n = 8)	AB (n = 87)	BB (n = 207)	Additive	Dominance
Dressing percentage (%)	72.651±1.293	71.885±0.395	71.722±0.257	-0.469±0.659	0.124±0.386
Lean meat percentage (%)	56.691±1.336	57.144±0.404	57.016±0.262	0.154±0.684	-0.138±0.400
Shoulder backfat thickness (cm)	3.497±0.238	3.181±0.072	3.224±0.047	-0.141±0.122	0.102±0.072
6-7th thorax backfat thickness (cm)	2.839±0.209	2.629±0.063	2.524±0.041	-0.163±0.107	0.033±0.063
Thorax-waist backfat thickness (cm)	2.134±0.193	1.833±0.059	1.838±0.038	-0.149±0.099	0.077±0.058
Buttock backfat thickness (cm)	2.068±0.225	1.756±0.068	1.692±0.044	-0.187±0.116	0.072±0.068
Average backfat thickness (cm)	2.631±0.188	2.348±0.057	2.323±0.037	-0.159±0.096	0.072±0.056
Loin eye height (cm)	9.192±0.273	9.361±0.083	9.317±0.054	0.081±0.135	-0.075±0.079
Loin eye width (cm)	4.947±0.233^{ab}	4.828±0.071^{a}	4.643±0.046^{b}	-0.159±0.119	-0.020±0.070
Loin eye area (cm^2)	31.674±1.739^{ab}	31.718±0.526^{a}	30.282±0.341^{b}	-0.683±0.892	-0.464±0.522
Carcass length to 1st spondyle (cm)	90.433±1.510	92.424±0.457	92.508±0.296	1.026±0.774	-0.525±0.453
Carcass length to 1st rib (cm)	77.486±1.355	78.034±0.410	78.066±0.266	0.274±0.695	-0.130±0.407
Rib number	14.865±0.239	14.842±0.072	14.685±0.047	-0.077±0.121	-0.047±0.071

Significant differences (within a trait) between the genotype classes indicated with different lower case superscripts are significant at $p<0.05$.
Least square means (LSM) estimated for each polymorphism is indicated with its standard error (SE).
n = Number of individuals.

Msp I PCR-RFLP of *KIAA1717* and *HUMMLC2B* amplicons were typed in 285 and 302 F2 animals of a Large White×Meishan population, respectively. The results of tests for *KIAA1717*, *HUMMLC2B* genotypes and meat quality traits are given in Tables 1 and 2. Within F2 animals of the Large White×Meishan population the genotype distributions were at Hardy-Weinberg equilibrium. Statistically significant associations with thorax-waist backfat thickness ($p<0.05$), buttock backfat thickness ($p<0.05$), average backfat thickness ($p<0.05$), loin eye height ($p<0.05$), loin eye area ($p<0.05$), carcass length to 1st spondyle ($p<0.01$) and carcass length to 1st rib ($p<0.01$) were found for the *KIAA1717* Msp I PCR-RFLP site. This site seemed to have a significantly additive effect on carcass length to 1st spondyle ($p<0.01$), carcass length to 1st rib ($p<0.01$) and thorax-waist backfat thickness ($p<0.01$). Allele A was favourable for these traits. Statistically significant associations with loin eye width ($p<0.05$), loin eye area ($p<0.05$) were found for the *HUMMLC2B* Msp I PCR-RFLP site. No significantly additive or dominance effect was detected for this site.

KIAA1717 encodes a H3-K4-specific methyltransferase (H3-K4-HMTase) (also called SET7/9) with 3 MORN repeats and a SET domain (Nagase et al., 2000; Wang et al., 2001). The evolutionarily conserved SET (Suvar3-9, Enhancer-of-zeste, Trithorax) domain occurs in most proteins known to possess histone lysine methyltransferase activity, and methylates diverse proteins, such as, histones, Rubisco and cytochrome C. In particular, they play an important role in the dynamics of the eukaryotic chromatin and are present in several chromatin-associated proteins (Wilson et al., 2002; Aravind and Iyer, 2003). It had also

been reported that histone methylation had significant effects on heterochromatin formation and transcriptional regulation (Nishioka et al., 2002). In addition, SET domains have been identified as protein-protein interaction domains and they interact with members of a family of proteins that display similarity to dual specificity phosphatases and could link the epigenetic regulatory machinery with signalling pathways involved in growth and differentiation (Cui et al., 1998; Firestein et al., 2000). Chuikov et al. (2004) reported a novel mechanism of p53 regulation through lysine methylation by Set9 methyltransferase. Set9 specifically methylates p53 at lys372 within the C-terminal regulatory region. Methylated p53 is restricted to the nucleus and the modification positively affects its stability. Set9 regulates the expression of p53 target genes in a manner dependent on the p53 methylation site. *HUMMLC2B* encodes a Ca^{2+} binding protein, skeletal muscle myosin regulatory light chain (RLC) with EF-hand calcium binding motif. Ca^{2+} binding protein is involved in Ca2+/CaM-mediated signaling pathways related to morphogenesis, cell division, cell elongation, ion transport, gene regulation, cytoskeletal organization, cytoplasmic streaming, pollen function, and stress tolerance (Reddy et al., 2002). RLC is also a primary regulatory component of the thick filament-linked systems. It had been suggested that phosphorylation of RLC served as an efficient mechanism during cross-bridge cycle, calcium sensitivity and other parameters strengthening muscle performance (Sweeney et al., 1993). In molluscan muscle, direct binding of Ca^{2+} by the regulatory RLCs promotes actin activation of the myosin ATPase (Claudia and Philip, 1988). Therefore, *KIAA1717* and *HUMMLC2B* seem to be involved in the regulatory mechanisms of genes with responsibility for some carcass traits.

The International Radiation Hybrid Mapping Consortium has mapped the human *KIAA1717*gene to HSA4q28. No mapping information is available for porcine *KIAA1717*. But the Sus scrofa chromosome 8 (SSC8) is homologous to most of the Homo sapiens chromosome 4 (HSA4) as reported previously (Goureau et al., 1996). Another gene, FGG, on HSA4q28 has also been assigned to SSC8 (Jiang et al., 2002). So it is probable the porcine *KIAA1717*gene is located on SSC8. In addition, *HUMMLC2B* has already been localized to SSC3 (Davoli et al., 2003). On SSC8, QTLs affecting backfat thickness, loin eye area and carcass length have been detected. On SSC3, QTLs affecting loin eye area have also been detected. Therefore, *KIAA1717* and *HUMMLC2B* are probably linked with the causal mutations and the genes with responsibility for some carcass traits, too.

In addition, in this study, the polymorphic Msp I sites were at position in the 3′-UTR of *KIAA1717*, and in the SINE (PRE-1) element of *HUMMLC2B*, respectively. The important role that UTRs of eukaryotic mRNAs may play in gene regulation and expression is now widely recognized. Indeed, experimental studies have demonstrated that sequence motifs located in the UTRs are involved in crucial biological functions. SINEs (short interspersed nucleotide elements) have also been taken as important elements to generate variations in genome structure and expression. Therefore, the variations in these sequences may have important regulation roles and be directly related to functional variations, too.

The specific gene function, and the results obtained from the PCR-RFLP analyses of *KIAA1717* and *HUMMLC2B* are worth being further investigated using larger samples to better evaluate their effects on carcass traits

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