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Association Analysis of Polymorphism in Intron-10 of Porcine *HK2* Gene with Meat Quality and Carcass Traits

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Absract: Hexokinase II (HK2) has been demonstrated to play as a key enzyme member in the glycolysis reaction. It catalyzes the conversion glucose to glucose-6-phosphate thus committing glucose to the glycolytic pathway. The objective of this study was to investigate the polymorphism in candidate gene *HK2* affectting on meat quality and carcass traits in pigs. The polymorphism (G981A) in intron 10 of porcine *HK2* gene which resulting in the changes of restriction site for enzyme Msp I was genotyped in the population of 309 F2 pigs of two Large White x Meishan reference family, result of statistical analysis of variance showed that significant difference between AA and BB was found in meat marbling (m. Biceps Femoris, BF) traits. This locus is significantly additive in action; there are significant associations of the detected locus between AA and BB were found with loin eye height, average skin thickness and significantly additive in action.

Key words: Pig, HK2, PCR-RFLP, association analysis, breeding, genotyped

INTRODUCTION

Recently intensive selection in the pig has made great improvement in lean meat percentage and feed efficiency but meat quality is reduced. It is necessary for us to understand the factors influencing meat quality. The traditional breeding methods are based on phenotype and physiological and biochemical characteristics. With the development of molecular biology technologies to the field of pig breeding, the molecular breeding techniques including the Marker-Assisted Selection (MAS) and Marker-Assisted Introgression (MAI) have emerged. The basis of MAS or MAI was to seek the major genes which are closely connected with the target traits. The use of molecular markers combining with traditional breeding methods accelerated the progress of genetic improvement in pigs.

Studies have been showed the precursor and macromolecule of glycogen do not directly participate in the glycolysis pathway, they firstly convert them to glycogen without protein and glycose. Content of glycogen in muscle could affect the level and rate of glycolysis pathway then result in change of pH and generation of lactic acid thus, affect some meat quality traits, such as meat color value, drip loss rate, meat pH and so on (Bee *et al*., 2007; Hamilton *et al*., 2003; Rosenvold *et al*., 2003). In mammalian tissues, the phosphorylation of intracellular glucose to Glucose-6-Phosphate (Glu-6-P) is facilitated by four distinct Hexokinase (HK) isoenzymes, designated as HKI-IV. Because of Hexokinases II mediates phosphorylate glucose into glucose-6-phosphate, thus committing glucose to the glycolytic pathway. Hexokinase II is reported to play as a key enzyme member in the glycolysis reaction, studies in rats suggest that hexokinase II is involved in the increased rate of glycolysis seen in rapidly growing cancer cells (Mathupala *et al*., 1997). In addition, Hexokinase II was regarded as a leading glycolytic enzyme in insulin-sensitive tissues such as skeletal muscle, heart and adipose tissue (Heikkinen *et al*., 2000) defects in HKII function could contribute to the important direct roles for traits related to skeletal muscle and fat (Wilson, 2003). HK2 which encodes Hexokinase II is the predominant form found in skeletal muscle (Jun *et al*., 2006). Therefore, researchers chose HK2 as a candidate gene to study the effects of gene to the target traits.

In the previous study, results showed that the expression of HK2 mRNA was found only in pig skeletal muscle (Jun *et al*., 2006). Further. the polymorphism in intron 10 of *HK2* gene was found and genotyped in six different pig breeds populations and its association analysis with some meat quality and carcass traits was

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studied (Jun *et al*., 2006). In order to further validate the polymorphism in candidate gene *HK2* affect on meat quality and carcass traits, the current study was designed to evaluate associations between the polymorphism and meat quality and carcass traits in a enlarge group.

MATERIALS AND METHODS

Sample collection and treatments: A total of 309 pigs of F2 cross-breeding population between Large White and Meishan pigs which was constructed during 2000 and 2003. All the animals had unlimited access to food and water and were born and raised in Huazhong Agriculture University Jingpin pig statioin. The 180 days old pigs were slaughtered and measured according to the methods of Xiong and Deng (1999). The measured carcass traits: Dressing Percentage (DP), Carcass Length (CL), Loin Eye Height (LEH), Loin Eye Area (LEA), Backfat Thickness at shoulder (BFT1), Backfat Thickness at thorax-waist (BFT2), Backfat Thickness at buttock (BFT3), average Backfat Thickness (BFT4) (average backfat thickness is from three point: backfat thickness at shoulder, backfat thickness at thorax-waist, backfat thickness at buttock), Ratio of Lean meat versus Fat meat (RLF), Lean Meat Percentage (LMP), Fat Meat Percentage (FMP), Average Skin Thickness (AST). The measured meat quality traits: Meat pH (m. Longissimus Dorsi, LD) [pH (LD)], Meat pH (m. Semispinalis Capitis, SC) [pH (SC)], Meat pH (m. Biceps Femoris, BF) [pH (BF)], Drip Loss Rate [DLR], Water Holding Capacity [WHC], Meat Color Score (LD) [MCS (LD)], Meat Color Score (BF) [MCS (BF)], Meat Marbling (LD) [MM (LD)], Meat Marbling (BF) [MM (BF)] Intramuscular Fat (LD) [IMF (LD)] and Water Moisture (LD) [WM (LD)].

Blood samples were collected from 309 F2 individuals. Genomic DNA was isolated by phenol/chloroform purification based protocols (Sambrook *et al*., 1989) and stored at -20°C.

Primer design and PCR-RFLP: A pair of primers (F :5'-CTGCTCCCAATTCAGAAAA-3' and R: 5'-GGATG-AGTGATGATTTGTTTG-3') were designed to ampilfy the tenth intron in *HK2* gene. PCR amplificationwas carried out in 25 μL volume containing standard 1×PCR buffer and 1 U Taq-polymerase (Jingmei Biotech, China), 200 μM of each dNTP, 10 pmol of each primer and 2.0 μL of first strand cDNAmix. The template was denaturated for 4 min at 94°C, followed by 35 cycles of amplification at 94°C for 50 sec, 57°C for 50 sec, 72°C for 50 sec and terminated with an additional extension step for 10 min at 72°C.

A fragment of 840 bp was amplified by H4 primers, A base mutation was detected by aligning Landrace, Large White and Meishan, the position within the restriction site for enzyme Mspl have been found; sequence CCGG (in Meishan) is cut (allele B) while sequence CCAG (in Landrace and Large White) is not cut (allele A) (Jun *et al*., 2006).

Statistical analysis: The association between genotype and carcass and meat quality traits was performed with the Least-Square Method (GLM procedure, SAS 8.0). The additive and dominance effects were estimated using REG procedure of SAS 8.0. The additive effect was defined as -1, 0 and 1 for AA, AG and GG, respectively and the dominance effect represented as 1, -1 and 1 for AA, AG and GG, respectively. The statistical model was assumed to be (Liu, 1998):

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

Where:

Y_{ijk} = The observed values of traits
μ = The least-square mean
S_i = Effect of sex (i = 1 for male or 2 for female)
F_j = The effect of family
G_k = The effect of genotype (K = AA, AB and BB)
b_{ijk} = The regression coefficient of the slaughter age
e_{ijk} = The random residual

A level of $p<0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

PCR and the analysis of polymorphisms by RFLP: The result of the fragment of 840 bp amplified was shown in Fig. 1. One SNP (G981A) in the 10th intron was validated as studied former (Jun *et al*., 2006). The polymorphisms of 309 F2 pigs were analyzed by PCR-RFLP (Fig. 2).

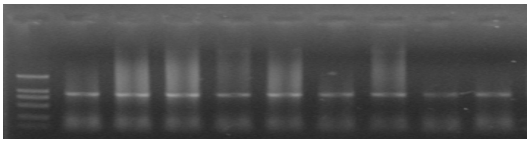

Fig. 1: The amplified fragement of F2 generation from the Large White x Meishan resource family; M: DL2000 maker

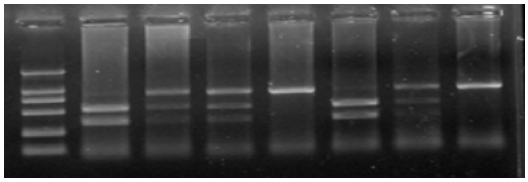

Fig. 2: The MspI-RFLP results of *HK2* gene. Lane M: DNA molecular marker DL2000; Lane 4, 7: genotype AA, 840 bp; Lane 2, 3, 6: genotype AB, 840 bp, 533 bp, 307 bp; Lane 1, 5: genotype BB, 533 bp, 307 bp

Table 1: The phenotype value of carcass traits with different genotype of *HK2* gene

Traits	Genotype AA (n = 82)	Genotype AB (n = 147)	Genotype BB (n = 80)	Additive effect	Dominance effect
DP (%)	72.288±0.473	72.023±0.351	72.119±0.475	0.084±0.336	0.090±0.243
BP (%)	12.656±0.244	13.134±0.181	13.319±0.245	0.331±0.173	-0.074±0.126
CL (cm)	90.814±0.495	91.001±0.367	91.043±0.497	0.114±0.351	-0.036±0.254
LEH (cm)	6.883±0.130^{a}	6.650±0.097^{ab}	6.467±0.131^{b}	-0.207±0.093*	0.010±0.067
LEA (cm^2)	29.745±0.523	28.864±0.388	28.406±0.525	-0.669±0.372	0.103±0.270
BFT1 (cm)	3.057±0.086	3.550±0.064	3.494±0.086	-0.007±0.061	-0.025±0.044
BFT2 (cm)	2.792±0.069	2.762±0.052	2.736±0.070	-0.027±0.050	0.001±0.036
BFT3 (cm)	2.082±0.064	2.031±0.047	2.027±0.064	-0.027±0.045	0.011±0.033
BFT4 (cm)	1.915±0.071	1.844±0.053	1.764±0.072	0.076±0.051	0.002±0.037
ABFTR (cm)	2.503±0.650	2.461±0.048	2.427±0.065	-0.038±0.046	0.002±0.033
RLF	2.723±0.103	2.673±0.077	2.710±0.104	-0.007±0.073	0.622±0.053
LMP	54.919±0.449	54.446±0.333	54.269±0.451	-0.324±0.319	0.073±0.231
FMP	22.434±0.533	22.071±0.395	21.962±0.535	-0.236±0.379	0.063±0.273
AST	0.379±0.011^{a}	0.380±0.008^{ab}	0.406±0.011^{b}	0.014±0.0088*	0.006±0.005

Table 2: The phenotype value of meat quality traits with different genotype of *HK2* gene

Traits	Genotype AA (n = 82)	Genotype AB (n = 147)	Genotype BB (n = 80)	Additive effect	Dominance effect
pH (LD)	6.343±0.0200	6.328±0.015	6.353±0.020	0.005±0.014	0.010±0.010
pH (SC)	6.446±0.0140	6.444±0.010	6.441±0.014	-0.003±0.010	-0.001±0.007
pH (BF)	6.420±0.0150	6.414±0.011	6.417±0.015	-0.002±0.017	0.002±0.007
DLR (%)	6.602±0.4130	6.819±0.306	6.782±0.414	0.090±0.293	-0.063±0.211
WHC (%)	91.060±0.5690	90.656±0.422	90.812±0.571	-0.123±0.404	0.140±0.292
MCS (LD)	20.389±0.3360	20.490±0.249	19.951±0.338	-0.219±0.239	-0.161±0.173
MCS (BF)	19.240±0.1490	19.158±0.110	18.926±0.149	-0.157±0.106	-0.037±0.077
MM (LD)	3.450±0.0220	3.415±0.016	3.426±0.022	0.003±0.010	0.011±0.011
MM (BF)	4.135±0.0194^{a}	4.097±0.014^{ab}	4.077±0.020^{b}	-0.029±0.014*	-0.004±0.010
IMF (LD)(%)	3.173±0.0710	3.112±0.053	3.234±0.071	0.031±0.050	-0.046±0.036
WM (%)	73.752±0.0850	73.795±0.063	73.816±0.085	0.032±0.060	0.006±0.043

All the data in the table are least square means±standard error. Letters denoting significant difference between groups: a, b, *p<0.05

Association of the polymorphisms with carcasss traits: About 309 F2 pigs of a Large White x Meishan were used to identify polymorphisms by PCR-RFLP. From Table 1 and 2 researchers found that the genotype frequencies are AB>AA>BB.

The results of tests for HK2 genotypes and carcass traits were shown in Table 1. Table 1 shows statistically significant associations of the detected locus with loin eye height and average skin thickness were found, the associations of loin eye area and bone percentage in AA genotype versus BB were close to be significant (p<0.06), no significant effect can be made on other carcass traits. This locus seemed to be significantly additive in action on some carcass traits such as loin eye height and average skin thickness.

The results of tests for HK2 genotypes and meat quality traits were shown in Table 2. Significant difference between AA and BB was found in meat marbling (m. Biceps Femoris, BF) traits. This locus is significantly additive in action.

Studies had showed that seeking SNP of the important function region of the candidate gene and taking the association analysis with the economic traits is very useful tool to study gene function (Jun *et al*., 2006). In pig breeding, meat quality and carcass traits are considered to be important economic traits and some candidate genes such as *HSL* , *LPL*, *H-FABP* and *CAST* for these traits have been identified (Harbitz *et al*., 1999; Gu *et al*., 1992; Gerbens *et al*., 1997; Ernst *et al*., 1998). These genes all play as a key role in some physiological and biochemical metabolic pathways. Therefore, *HK2* gene as a key enzyme member in the glycolysis reaction was selected as a candidate gene for the pig production traits.

A mutation site from G to A was identified by RFLP at 981 in the tenth intron of the *HK2* gene, a significant difference of pig average backfat at rump was found between AB and BB genotypes (p<0.05) in 135 F2 cross-breeding population (Jun *et al*., 2006). In this study, researchers enlarge population of F2 group. Study showed that pigs with AA genotype of G981A in HK2 had more loin eye height (+0.416 cm) than pigs with BB genotype and pigs with AA genotype had less average skin thickness (-0.027 cm) than pigs with BB genotype, no significant effect can be made on other carcass traits, the associations of loin eye area and bone percentage in AA genotype versus BB were close to be significant (p<0.06). In meat quality traits, pigs with AA genotype had better meat marbling in m. biceps femoris (-0.058) than pigs with BB genotype. Marbling in the muscle are the response to the distribution of the fat layer, they have closely relation with the meat juicy, flavor and tenderness. As the comparative values showed above, pigs that with AA genotype would be avail to the aim of breeding. Thus, increasing the frequency of the favorable genotype could be beneficial to accelerate the genetic improvement of these traits.

The probable reason of different results emerged in two studies are that the 309 F2 cross-breeding population between Large White and Meishan pigs which was constructed during 2000 and 2003, they had different construction on sex or original male parent and female parent had different polymorphism in the site, major reason from Meishan pigs because of Large White pig only have AA genotype however, Meishan pigs have three genotypes (Jun *et al*., 2006). On the other hand, perhaps have more or less difference on season, climate and feeding between 2000 F2 and 2003 F2 group. Above this problem, analyzing more animals is necessary to confirm the association between the HK2 genotype and some traits in crossbreds and purebreds.

On the other hand, from the analysis of gene function, we inferred the gene will result in change of pH and generation of lactic acid in muscle, thus affect some meat quality traits such as meat color value, drip loss rate, meat pH and so on (Bee *et al*., 2007; Hamilton *et al*., 2003; Rosenvold *et al*., 2003). Therefore, seeking other SNPs in *HK2* gene to research interrelationship of pig carcass and meat quality traits and polymorphisms by above pathway researchers can get correct, all round conclusion for the *HK2* gene.

CONCLUSION

The results suggest that *HK2* gene could be related to some porcine carcass traits and meat quality but further study should be needed to decide whether this gene could be regarded as a molecular marker for pig breeding.

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