Polymorphism of the H-FABP, MC4R and ADD1 genes in the Meishan and four other pig populations in China

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Abstract

Genes such as the Heart Fatty Acid-Binding Protein (H-FABP), the Melanocortin-4 Receptor (MC4R) and the Adipocyte Determination and Differentiation factor-1 (ADD1) play an important role in meat quality in pigs. The Meishan is one of the most prolific pig breeds in the world, but it is in danger, in China, of being replaced by other exotic pig breeds because of its slow growth rate and high body fat content. To know their present genetic status, the polymorphism of the three genes in the Meishan and four other pig populations (Sutai, Yorkshire×Sutai, Landrace×Sutai and Duroc×Landrace×Yorkshire) in China was analysed, using PCR-RFLP and PCR-SSCP, and were further compared with each other. The results showed that gene and genotype frequencies differed significantly between the Meishan and the other four populations. This is suggested to be one of the reasons for the good meat quality of the Meishan compared to the other breeds. In addition, since half of the genetic background of Sutai is from the Meishan, results also indicated that marker assisted selection (MAS) is very important for the development of new pig breeds.

Keywords: Polymorphism, H-FABP, MC4R, ADD1, Meishan
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Introduction

In the past decades the main focus of pig breeders has been on growth rate, feed conversion efficiency and reproductive performance, with less concerned about meat quality such as meat colour, pH, drip loss, intramuscular fat content, tenderness, back fat thickness, etc. This is partially due to the difficulty in improving these type of traits through traditional quantitative genetics. However, the development of molecular genetics this is changing, because many candidate genes have been identified which could be used to improve pork quality through marker assisted selection (MAS) (Dekkers, 2004).

The Heart Fatty Acid-Binding Protein (H-FABP) gene encodes a type of cytosol protein that transports fatty acids from the cell membrane to other sites where 3-acyl-glyceride and phospholipids are synthesized and fatty acids are oxidized. Moreover, H-FABP promotes cells to absorb fatty acids by combining with fatty acids *in vivo* (Frand *et al.*, 1998). Gerbens *et al*. (1997; 1999; 2000) discovered *Msp* I, *Hae* II and *Hinf* I polymorphism of the H-FABP gene that is related to intramuscular fat content. Lin *et al.* (2002) analysed the polymorphism of the H-FABP gene in 10 pig breeds, including six native Chinese breeds and four other breeds. No *Msp* I and *Hae* III polymorphism was found in the six native Chinese pig breeds. Melanocortin-4 Receptor (MC4R) is believed to be a link between feed intake and body weight (Seeley *et al.*, 1997). Polymorphism of the MC4R gene has been reported to be associated with back fat thickness (Kim *et al*., 1998). Adipocyte Determination and Differentiation factor-1 (ADD1) can activate or restrain some genes in fat and glucose metabolism (Foretz *et al*., 1999; Hitoshi, 2001), which include the low-density lipoprotein receptor (LDLR) (Brown *et al.*, 1997), acetyl-CoA cartboxyiase (ACC) (Foretz *et al*., 1999), phosphoenolpyruvate carboxykinase (PEPCK) (Chakravarty *et al*., 2001), fatty acids synthase (FAS) (Soazig *et al*., 2002), lipoprotein lipase (LPL) (Chakravarty *et al*., 1993) and others. Research has suggested that the ADD1 gene can be used as a candidate gene for pork quality (Rebcca *et al*., 2000).

The Meishan breed is renowned for its prolificacy and good meat qualities. It has contributed substantially to pork production and the development of new breeds, and this is sure to continue in future. However, only a small population is presently being conserved. Furthermore, we do not know the status of favourable genes in the breed. The Sutai is a breed that has been developed by The Breeding Centre of Taihu

Pig in China from a cross between the Duroc (50%) and the Meishan (50%). It has taken about 20 years to select for low back-fat thickness, improved growth rate and an improved feed conversion efficiency. The method used in the development of the Sutai breed was the traditional selection method. However, it is important to know whether the traditional selection method can concentrate favourable genes as successfully as the MAS method. Presently the Sutai is used mainly as a maternal line to cross with Landrace or Yorkshire boars for the production of commercial pigs that compete mainly with the Duroc × Landrace × Yorkshire cross in the pork market of China.

In view of the three genes playing an important role in meat quality, this project was conducted to investigate and compare the polymorphism of these genes in the Meishan and four other pig populations, the Sutai, Landrace × Sutai, Yorkshire × Sutai, Duroc × Landrace × Yorkshire, to obtain information which would assist in their conservation and selection.

Materials and Methods

Ninety three individuals were randomly selected from five herds as an experimental sample, and included 18 Meishan (M), 21 Sutai (S), 14 Yorkshire × Sutai (YS), 16 Landrace × Sutai (LS) and 24 Duroc × Landrace × Yorkshire (DLY) pigs.

The DNA was isolated by the standard methods (Cold Spring Harbor), *viz*. *ca.* 10 mg of ear tissue was put into an eppendorff tube and washed with 1 mL PBS. 12 µL proteinase K (0.2 mg/mL) and 600 µL. 10% SDS were added to the tube. The tube was shaken gently, and incubated overnight at 55 °C in a waterbath. 1.2 µL Rnase A (10 mg/mL) was added to the same tube and incubated at 37 °C in a waterbath. 600 µL phenol was added, shaken by hand for 10 min and centrifuged at 10000 rpm for 10 min. The supernatant was pipetted into a new tube, 300 µL phenol and 300 µL chloroform/isoamyl alcohol (24:1) were added, shaken by hand for 10 min, and centrifuged at 10000 rpm for 10 min. The supernatant was pipetted into a new tube, and 600 µL chloroform/isoamyl alcohol (24:1) was added, shaken by hand for 10 min, and centrifuged at 10000 rpm for 10 min. The supernatant was pipetted into a new tube, 1 mL frozen ethanol was added and shaken gently until the DNA precipitated. It was then centrifuged at 10000 rpm for 10 min. The DNA was washed in 70% ethanol and dried at room temperature. The DNA was dissolved in 50 µL sterile water for five hours (or longer if necessary) at 55 °C in a waterbath. The DNA concentration was measured in a spectrophotometer and stored at 4 °C (Sambrook *et al*., 2002).

The characteristics of the H-FABP, MC4R and ADD1 genes were analysed according to the methods of Gerbens *et al*. (1997), Kim *et al*. (1998) and Li *et al*. (Unpublished), respectively. All data were analysed statistically using the χ^2 method (Du, 2003) and the FREQ procedures of SAS (1994).

All primers were synthesized as shown in Table 1. The PCR reaction was run in a thermocycler (MJ, USA). The PCR reaction mixture (total of 30 µL) contained 100 ng of genomic DNA, 3 µL of 10×PCR buffer, 10 pmol of each primer, 170 µM of dNTPs and 1.0 U of *Taq* polymerase (TaKaRa Biotech, China). The cycling profile was: 94 °C (4 min) 1 cycle, 94 °C (30 sec), 55-60 °C (30 s), 72 °C (1 min) 30 cycles, 72 °C (10 min) 1 cycle. The amplified products of H-FABP and MC4R genes were digested with restriction enzymes *Taq* I and *Hae* III (Huamei Biotech, China). The digested PCR fragments were analysed on 2% agarose gels.

Table 1 Primer sequences of H-FABP, MC4R and ADD1 genes

Gene	Analytical technique	Primer sequence	Annealing temperature	References
H-FABP	PCR-RFLP	5'- ATTGCTTCGGTGTGTTTGAG -3' 5'- TCAGGAATGGGAGTTATTGG –3'	60 °C	Gerbens *et al*., 1997
MC4R	PCR-RFLP	5'-TACCCTGACCATCTTGATTG -3' 5'- ATAGCAACAGATGATCTCTTTG-3'	58 °C	Kim *et al*., 2000
ADD1	PCR-SSCP	5'-GGTAGTGGACACTGACAAGCT-3' 5'-GCTTAGCTCAACAGACGGAG-3'	55 °C	Li *et al.*, Unpublished results

H-FABP - Heart Fatty Acid-Binding Protein
MC4R - Melanocortin-4 Receptor
ADD1 - Adipocyte Determination and Differentiation factor-1

The amplified products of ADD1 were mixed with single strand conformation polymorhism (SSCP) buffer, 0.1% bromophenol blue and 0.1% xylene cyanole in formamide. Before being loaded into the gel the samples were denaturized for 5 min at 95 °C and kept on ice for 5 min. Then 10 µL of this mixture was applied to a 9% polyacrylamide gel (49:1 acrylamide:bis), 10% (V/V) glycerol and a 0.5×TBE buffer. Electrophoresis was run overnight at room temperature. The gel was then stained with silver nitrate according to a standard protocol, as follows: The gel was washed in 10% ethanol for 15 min and then transferred to 1% HNO_3 for 10 min. Next, the gel was incubated for 30 min in 0.2% silver nitrate with the addition of formaldehyde (the final concentration of formaldehyde was 0.12%). The developing reaction (*ca.* 20-30 min) was performed with the use of 3% sodium carbonate with the addition of formaldehyde (the final concentration of formaldehyde was 0.05%) and stopped, using 10% acetic acid.

Results and Discussion

H-FABP gene: The PCR-RFLP result of the H-FABP gene is shown in Figure 1 where the AA genotype has 683 bp and 117 bp fragments, the BB genotype has 405 bp, 278 bp and 117 bp fragments and the AB genotype has 683 bp, 405 bp, 278 bp and 117 bp fragments. The reported genotypes AA, BB and AB also contained 16 bp fragments that could not be detected (not scored) when using this technique.

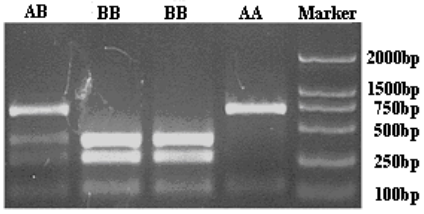

Fig. 1 RFLP analysis on PCR amplification to H-FABP gene

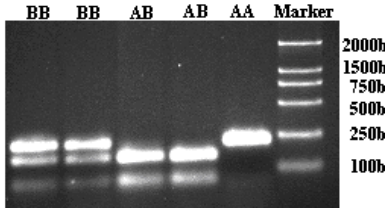

Fig. 2 RFLP analysis on PCR amplification to MC4R gene

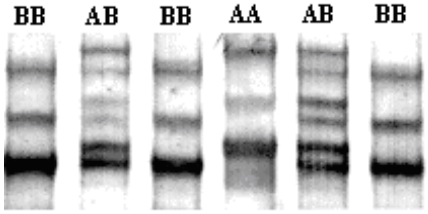

Fig. 3 SSCP analysis on PCR amplification to ADD1 gene

The frequencies of the H-FABP gene and genotype are shown in Table 2 and the results of the χ^2 test between populations in Table 3. The frequencies differed significantly ($P < 0.01$) between the five populations. Furthermore, the frequencies of the H-FABP gene and genotype of the Meishan differed significantly ($P < 0.01$) from the other four populations, while the frequencies of the H-FABP gene and genotype of Sutai were different ($P < 0.01$) from those of the Landrace × Sutai and Duroc × Landrace × Yorkshire populations. The frequencies of the H-FABP gene and genotype did not differ between the latter three populations. The decrease in the frequency of the A allele from 100% in the Meishan to 71.5% in the Sutai reflects its origin as a cross between the Meishan and Duroc.

Table 2 The H-FABP gene and genotype frequencies in the Meishan and four other pig populations in China

Population	n	Genotype Freq. (%)			χ^2	$\chi^2_{0.01\,(8)}$*	Gene Freq. (%)		χ^2	$\chi^2_{0.01\,(4)}$*
		AA	AB	BB			A	B		
M	18	100.0	0	0			100.00	0		
S	21	62.0	19.0	19.0			71.50	28.50		
LS	16	18.8	37.5	43.7	41.56**	20.09	37.55	62.45	51.99**	13.28
YS	14	28.6	35.7	35.7			46.45	53.55		
DLY	24	12.5	33.3	54.2			29.15	70.85		

M - Meishan; S - Sutai; LS - Landrace × Sutai; YS - Yorkshire × Sutai; DLY - Duroc × Landrace × Yorkshire
* $\chi^2_{\alpha\,(df)}$ is the χ^2 value where α is the level of significance and *df* degrees of freedom
** mean frequencies differ significantly ($P < 0.01$)

MC4R gene: The result of PCR-RFLP of the MC4R gene is shown in Figure 2, where the AA genotype has a 226 bp fragment, the BB genotype has 226 bp, 156 bp and 70 bp fragments and the AB genotype has 156 bp and 70 bp fragments.

Table 3 The χ^2 test of the H-FABP gene and genotype frequencies in the Meishan and four other pig populations

Population	Genotype frequency				Gene frequency			
	S	LS	YS	DLY	S	LS	YS	DLY
M	8.60*	23.65**	18.69**	31.50**	10.03**	28.90**	22.28**	39.97**
S		6.93*	3.75	12.24**		7.20**	3.46	14.43**
LS			0.43	0.51			0.19	0.29
YS				1.90				1.62

M - Meishan; S - Sutai; LS - Landrace × Sutai; YS - Yorkshire × Sutai; DLY - Duroc × Landrace × Yorkshire
* mean frequencies differ at $P < 0.05$; ** mean frequencies differ at $P < 0.01$
χ^2 values at 0.05 or 0.01 levels of significance with different degree of freedoms are as follows:
$\chi^2_{0.05\,(2)} = 5.99$; $\chi^2_{0.01\,(2)} = 9.21$; $\chi^2_{0.05\,(1)} = 3.84$; $\chi^2_{0.01\,(2)} = 6.63$

Table 4 The MC4R gene and genotype frequencies in the Meishan and four other pig populations in China

Breeds	n	Genotype Freq. (%)			χ^2	$\chi^2_{0.01\,(8)}$*	Gene Freq. (%)		χ^2	$\chi^2_{0.01\,(4)}$*
		AA	AB	BB			A	B		
M	18	0	0	100.0			0	100.00		
S	21	23.8	66.7	9.5			57.15	42.85		
LS	16	18.8	43.7	37.5	41.98**	20.09	40.65	59.35	34.26**	13.28
YS	14	21.4	42.9	35.7			42.85	57.15		
DLY	24	29.2	54.2	16.6			56.30	43.70		

M - Meishan; S - Sutai; LS - Landrace × Sutai; YS - Yorkshire × Sutai; DLY - Duroc × Landrace × Yorkshire
* $\chi^2_{\alpha\,(df)}$ is the χ^2 value where α is the level of significance and df degrees of freedom
** mean frequencies differ significantly at $P < 0.01$

Table 5 The χ^2 test of MC4R gene and genotype frequencies in the Meishan and four other pig populations

Population	Genotype frequency				Gene frequency			
	S	LS	YS	DLY	S	LS	YS	DLY
M	31.77**	15.94**	16.10**	28.68**	27.10**	15.56**	16.28**	27.36**
S		4.25	3.74	0.84		1.37	0.86	0.02
LS			0.03	2.31			0.01	1.31
YS				1.79				0.80

M - Meishan; S - Sutai; LS - Landrace × Sutai; YS - Yorkshire × Sutai; DLY - Duroc × Landrace × Yorkshire
χ^2 values at 0.05 or 0.01 levels of significance with different degree of freedoms are as follows:
$\chi^2_{0.05\,(2)} = 5.99$; $\chi^2_{0.01\,(2)} = 9.21$; $\chi^2_{0.05\,(1)} = 3.84$; $\chi^2_{0.01\,(2)} = 6.63$
** mean frequencies differ significantly at $P < 0.01$

The frequencies of the MC4R gene and genotype are shown in Table 4 and the results of χ^2 test between populations are shown in Table 5. According to these tables, the frequencies of the MC4R gene and genotype were different ($P < 0.01$) in the five populations. Further, the frequencies differed ($P < 0.01$) between Meishan and other populations, but the differences were not significant ($P > 0.05$) between the Sutai, Landrace × Sutai, Yorkshire × Sutai and Duroc × Landrace × Yorkshire. The B allele is associated with intake and backfat thickness in the Duroc population (Kim *et al.*, 1998), but only B alleles existed in the

Meishan. The frequencies of A and B alleles in the other four populations were almost equal. This suggests that the frequencies of the MC4R gene and genotype changed during the development of the Sutai.

ADD1 gene: The PCR-SSCP results of the ADD1 gene are shown in Figure 3, in which the AA genotype has three bands, the BB three bands and the AB six bands. The frequencies of the ADD1 gene and genotype are shown in Table 6. Neither genotype frequencies nor gene frequencies differed significantly ($P > 0.05$).

Table 6 The ADD1 gene and genotype frequencies in the Meishan and four other pig populations in China

Population	n	Genotype Freq. (%)			χ^2	$\chi^2_{0.05\,(8)}$*	Gene Freq. (%)		χ^2	$\chi^2_{0.05\,(4)}$*
		AA	AB	BB			A	B		
M	18	27.8	50.0	22.2			52.80	47.20		
S	21	23.8	52.4	23.8			50.00	50.00		
LS	16	43.7	37.5	18.8	9.75	15.51	62.45	37.55	8.57	9.49
YS	14	57.1	28.6	14.3			71.40	28.60		
DLY	24	16.7	45.8	37.5			39.60	60.40		

M - Meishan; S - Sutai; LS - Landrace × Sutai; YS - Yorkshire × Sutai; DLY - Duroc × Landrace × Yorkshire
* $\chi^2_{\alpha\,(df)}$ is the χ^2 value where α is the level of significance and *df* degrees of freedom

Conclusion

The gene and genotype frequencies of the H-FABP and the MC4R genes differed significantly among the five pig populations in China. Only the AA genotype of the H-FABP gene and the BB genotype of the MC4R gene were found in the Meishan. This result shows that the Meishan has a special genetic composition, which could be one of the reasons for its good meat quality (Gerbens *et al*., 1997; Seeley *et al*., 1997; Kim *et al*., 1998; Gerbens *et al*., 1999; 2000). Therefore, the Meishan pig should be conserved as a valuable genetic resource.

Although the Sutai was developed from the cross between the Duroc (50%) and the Meishan (50%), the gene and genotype frequencies of the H-FABP and the MC4R genes differed significantly between the Meishan and Sutai populations. The BB genotypes of the H-FABP gene and the AA genotypes of the MC4R gene in the Sutai are suggested to be from the Duroc, in which these two genotypes have been found (Gerbens *et al*., 1999; Kim *et al*., 2000; Lin *et al*., 2002).

The traditional selection method in the development of a new breed or line is based on phenotypic information and not on genotypic information. The result of this research shows that this method cannot identify favoured genes effectively, but that MAS may have that potential.

Acknowledgements

This work was supported by the National Basic Research Program of China (No. 2004CB117502) and the Shanghai Municipal Key Project of Agriculture Science and Technology (No.2003.01-1).

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