New Evidences of Effect of Melanocortin-4 Receptor and Insulin-like Growth Factor 2 Genes on Fat Deposition and Carcass Traits in Different Pig Populations

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ABSTRACT : The Melanocortin-4 Receptor (MC4R) and Insulin-like Growth Factor 2 (IGF2) are two important candidate genes related to fat deposition and carcass traits. MC4R was found on study on human obesity and then was studied as candidate gene affecting food intake and fat deposition traits in mice and pigs. Insulin-like Growth Factor 2 (IGF2) gene plays an important role on tumor cell proliferation and muscle growth. It also affects fat traits and live weight in pigs. In this paper, MC4R and IGF2 were studied as two candidate genes associated with important economic traits such as fat deposition and carcass traits in five different pig populations. *Taq* I-PCR-RFLP and *Bcn* I-PCR-RFLP were respectively used to detect the polymorphism of genotypes of MC4R and IGF2 genes. Different MC4R genotype frequencies were observed in four populations. IGF2 genotype frequencies were also different in two populations. The results of association analysis show both MC4R and IGF2 genes were significantly associated with fat deposition and carcass traits in about 300 pigs. This work will add new evidence of MC4R and IGF2 affecting fat deposition and carcass traits in pigs and show that two genes can be used as important candidate genes for marker assistant selection (MAS) of growth and lean meat percentage in pigs. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1542-1547)***

Key Words : MC4R Gene, IGF2 Gene, Fat Deposition and Carcass Traits, Different Pig Populations

INTRODUCTION

MC4R is a G protein coupled (Yeo et al, 1998; Kim et al., 2000a, b) secreted by ventromadial hypothalamus (VWH) (Fan et al., 1997). It was found initially being a locus leading obesity on study on human (Ho et al., 1999). As a part of the central melanocortin system (CMS) which regulates food intake and metabolize, MC4R regulates the feeding behavior and body weight cooperated with Leptein Neuropeptide (NPY) and Alpha-melanocyte-stimulating hormone (Alpha-MSH) (Jiang et al., 2002). Many evidences showed MC4R and the melanocortin pathway are important determinants of controlling appetite, weight and obesity in mice and human. (Yeo et al., 1998; MacNeil et al., 2002; MacIntyre et al., 2003). It suggested that MC4R could regulate feeding behavior and body weight in pigs as well as in human and mice, and sequences analysis result showed there is a missense variant G→C which makes an amino acid substitution at codon 298 and then makes the functional change of MC4R (Kim et al., 2000b). This study will use *Taq* I-PCR-RFLP to analyze the association of this variant with fat deposition and carcass traits in a total of 275 pigs belonging to different populations.

The insulin-like growth factors (IGFs) and their receptors as well as their binding proteins play key roles in regulating cell proliferation and apoptosis (Jones and Clemmons, 1995; Yu and Rohan, 2000; Kim et al., 2005). IGF2 is one of IGFs, a growth-promoting peptides, which are structurally homologous with insulin and also their biological effects are similar to those of insulin (Kolarikova et al., 2003). IGF2 gene was mapped to the distal end of Chromosome 2 in pigs (SSC2) (Jeon et al., 1999; Nezer et al., 1999). It plays an important role on tumor cell proliferation, muscle growth and genomic imprinting (Florini et al., 1991; Latham et al., 1994; Magri et al., 1994). Studies showed IGF2 is associated with fat traits and live weight in pigs (Lamberson et al., 1996; Kolarikova et al., 2003). A G→A substitution in intron 2 has been detected (Knoll et al., 2000), and this substitution caused an increase in lean meat yield in the F2 population of hybrids between Large White and Pietrain pigs (Nezer et al., 1999). In intron 8, two variants G→C which may influence splicing of intron and the structure of protein have also been found to affect on fat and carcass traits significantly (Liu et al., 2003). Based on these two variances G→C, we designed two pairs of primers and used *Bcn* I-PCR-RFLP to analyze the association of the variances with fat and carcass traits in a total of 311 pigs belonging to different populations.

Fat deposition and growth are important economic traits in pig industry. As we know, fat deposition and carcass traits correspond to obesity and weight. Those genes including MC4R and IGF2 affecting animal obesity could be candidate genes associated with fat thickness and carcass character of pigs. The result of this study could add new

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Received December 21, 2004; Accepted May 15, 2005

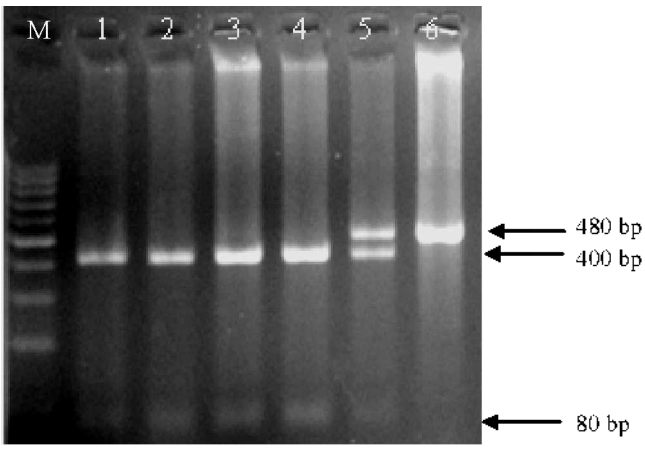

Figure 1. *Taq* I-PCR-RFLP test of MC4R gene. Lane1-4: genotype BB; Lane5: genotype AB; Lane6: genotype AA; Lane M: DNA Ladder (1,000 bp, 900 bp, 800 bp, 700 bp, 600 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp).

important evidences that MC4R, IGF2 are two important candidate genes to be used for selecting of fat deposition and lean meat percentage in pig industry.

MATERIALS AND METHODS

Animals

All pigs were chosen from pureblood and crossbreeding populations founded in Agricultural Ministry Key Laboratory of Pig Breeding and Genetics, Huazhong Agricultural University. Population A, B and D represents Large White (LW), Landrace (L) and Chinese breed Meishan (MS), respectively.

For MC4R gene, population C includes LW, L and crossbreeding population between LW and L (LW×L) pig breeds. Population E includes crossbreeding populations between LW and MS (LW×MS and MS×LW) pig breeds.

For IGF2 gene, population C includes LW, L, LW×L and L×LW. Population E includes MS, LW×MS and MS×LW.

The pigs were raised under the same condition, and they were slaughtered at a live weight around 80-kg by standard procedures. We have measured and calculated some important fat deposition and carcass traits as follows: average backfat thickness (ABT, mm), buttock fat thickness (BFT, mm), shoulder fat thickness (SFT, mm), thorax-waist fat thickness (TFT, mm), 6-7th rib fat thickness (RFT, mm), fat meat percentage (FMP, %), internal fat rate (IFR, mm), lean meat percentage (LMP, %), and dress percentage (DP, %).

PCR amplification

For MC4R gene, a known pair of primers (Liu et al., 2002) was used to amplify a 480 bp PCR product including polymorphic locus for G→A which makes an amino acid substitution at codon 298. For IGF2 gene, primer 1 (F: 5' CCT TCG GGA TGG ATG GTG 3', R: 5' GCC TTT ATT CTT ATT GGT TTT CAA C 3') and primer 2 (F: 5' AAG CGC CTT TCA CTT CTG 3', R: 5' TCT GAA GTC TCA GAC CCT CG 3') were designed from pig IGF2 DNA sequence (GenBank access number AY044828). They individually gives 451 bp and 557 bp PCR products which include two different polymorphic loci which were reported by Liu et al. (2003) for G→C.

All PCR amplifications were performed in a 25 μl reaction solution which contain 2.5 μl 10×PCR buffer (with NH_4^+), 1.5 μl 25 mM $MgCl_2$, 1.5 μl 2 mM each dNTP, 0.5 μl each primer (5 μM), 0.5 U *Taq* DNA Polymerase and 50 ng DNA. The PCR conditions were preheated at 94°C for 4 min, then 35 cycles with 50 sec denaturation at 94°C, 50 sec annealing and 50 sec extension at 72°C, and then followed by a final extension of 10 min at 72°C in a GeneAmp PCR System 9600. The annealing temperature of MC4R and primer 1 of IGF2 are 58°C, that of primer 2 of IGF2 is 60°C.

PCR-RFLP polymorphism examination

PCR products were digested (10 μl reaction solution contain 5 U restriction enzyme) with *Taq* I (for MC4R gene) and *Bcn* I (for both two polymorphic loci IGF2 gene) and then separated by 2% agrose gels.

Statistical analyses

The associations between the MC4R, IGF2 genes polymorphisms and fat deposition and carcass traits were tested using mixed-model procedures (SAS procedure MIXED; SAS Institute), which includes the fixed effects of genotype, sex of animals and family. The procedure was described as followed: $Y_{ijk} = \mu+G_i+S_j+F_k+e_{ijk}$ Consider of the pig populations are not large enough, some populations of which genetic background is close were pooled for an across-population (population C and population E) analysis. Breed was added as a fixed effect for these populations. Similar analysis ways was used respectively by Kim et al. (2000b) and Ciobanu et al. (2001). LS means (Least-squares means) for the genotype classes were obtained in five populations for MC4R and IGF2 gene (primer 1) respectively.

RESULTS

Identification of mutation in porcine MC4R and IGF2 gene

MC4R genotypes marked with AA (480 bp), AB (480 bp+400 bp+80 bp) and BB (400 bp+80 bp) (See Figure 1). IGF2 (primer 1) genotypes marked with CC (451 bp), CD (451 bp+84 bp+367 bp) and DD (84 bp+367 bp) (See Figure 2). Genotypic frequencies of MC4R, IGF2 (primer

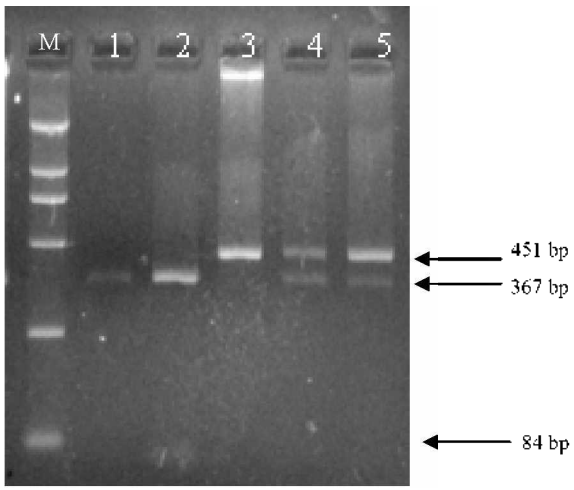

Figure 2. *Bcn* I-PCR-RFLP test of IGF2 gene (primer 1). Lane1-2: genotype DD; Lane3: genotype CC; Lane4-5: genotype CD; Lane M:DNA Ladder (2,000 bp, 1,000 bp, 750 bp; 500 bp; 250 bp; 100 bp).

1) were obtained in all populations (See Tables 1 and 2). Genotype frequency of IGF2 (primer 2) was not showed due to there is no polymorphism on this locus which was reported by Liu et al. (2003) in all populations. For MC4R, in each population, frequency of allele A is much lower than that of allele B, and allele A frequency is zero in MS (population D), a Chinese pig breed especially. For IGF2, population A, B and C are all DD genotype, however, frequency of allele D is lower than that of allele C in MS (population D) and the crossbreeding populations between LW and MS (LW×MS and MS×LW) (population E).

Association between the genotypes of MC4R, IGF2 genes and fat deposition, carcass traits

The fat deposition and carcass traits were measured and calculated. To investigate the effects of these mutations, some populations of which genetic background is close were pooled for an across-population (population C and population E) analysis.

Association analysis indicated that MC4R had significant ($p<0.05$) and highly significant ($p<0.01$) effects on most of traits we have measured in different populations, and IGF2 gene (primer 1) had significant ($p<0.05$) effects on two traits (TFT and DP) in two populations (See Tables 3 and 4). For IGF2 (primer 2), association analysis result was not obtained because of only one genotype in five populations.

For MC4R, in population B and population C, pigs with genotype BB had a significant ($p<0.05$) lower LS means than those with AA on almost all fat thickness traits except TFT. In population A, pigs with genotype AA had lower LS means on ABT, SFT, TFT, BFT and RFT, and the difference LS means on RFT is significant ($p<0.05$). Significant ($p<0.05$) differences of FMP, IFP and highly significant ($p<0.01$) difference LMP were only observed in population E, in which lower LS means of FMP, IFP and higher LMP on genotype BB were observed. Higher LS means of DP on genotype BB was also observed in population E, though it is not significant (See Table 3). Besides these, higher LS means of DP and LMP on allele B are also observed in other populations, though it is also not significant (not included in Table 3). We suggest MC4R gene genotype BB is favorable in term of fat deposition and carcass traits in

Table 1. Genotypic frequencies for the G→A substitution in the MC4R gene in five populations[1]

Population	Number of pigs	Genotype and frequency			Allele frequency	
		AA	AB	BB	A	B
A	32	2 (0.06)	16 (0.50)	14 (0.44)	0.31	0.69
B	43	1 (0.02)	14 (0.33)	28 (0.65)	0.19	0.81
C	103	4 (0.04)	40 (0.39)	62 (0.57)	0.23	0.77
D	50	0 (0.00)	0 (0.00)	50 (1.00)	0.00	1.00
E	122	3 (0.02)	38 (0.31)	81 (0.65)	0.18	0.82

[1] All pigs are chosen from populations founded on Key Laboratory of Pig Breeding and Genetics, Agricultural Ministry, Huazhong Agricultural University. Population A, B and D represents Large White (LW), Landrace (L) and Chinese breed Meishan (MS) respectively. Population C includes LW, L and crossbreeding population between LW and L (LW×L) pig breeds. Population E includes crossbreeding populations between LW and MS (LW×MS and MS×LW) pig breeds.

Table 2. Genotypic frequencies for the G→C substitution in the IGF2 gene (primer 1) in five populations[1]

Population	Number of pigs	Genotype and frequency			Allele frequency	
		CC	CD	DD	C	D
A	32	0 (0.00)	0 (0.00)	32 (1.00)	0.00	1.00
B	43	0 (0.00)	0 (0.00)	43 (1.00)	0.00	1.00
C	138	0 (0.00)	0 (0.00)	138 (1.00)	0.00	1.00
D	50	24 (0.48)	18 (0.36)	8 (0.16)	0.66	0.34
E	173	24 (0.14)	126 (0.73)	23 (0.13)	0.51	0.49

[1] All pigs are chosen from populations founded on Key Laboratory of Pig Breeding and Genetics, Agricultural Ministry, Huazhong Agricultural University. Population A, B and D represents Large White (LW), Landrace (L) and Chinese breed Meishan (MS) respectively. Population C includes LW, L, LW×L and L×LW. Population E includes MS, LW×MS and MS×LW.

Table 3. Association results between the genotypes of MC4R gene and fat deposition and carcass traits in five populations[1]

Genotype	SFT			TFT		
	AA	AB	BB	AA	AB	BB
Population A	2.81±0.23	2.88±0.08	2.84±0.09	1.36±0.19	1.54±0.07	1.45±0.07
Population B	2.68±0.43	2.92±0.11^{a*}	2.55±0.08^{b*}	1.61±0.35	1.50±0.09	1.39±0.06
Population C	2.76±0.22	2.85±0.06^{a}	2.67±0.05^{b}	1.48±0.20	1.50±0.05	1.45±0.04
Population D			4.15±0.07			2.16±0.06
Population E	4.36±0.31	4.01±0.09	4.04±0.06	2.03±0.28	2.14±0.08	2.07±0.05
Genotype	BFT			ABT		
	AA	AB	BB	AA	AB	BB
Population A	0.66±0.17	0.78±0.06	0.71±0.06	1.61±0.12	1.74±0.04	1.67±0.05
Population B	1.34±0.30	1.04±0.08^{a}	0.84±0.06^{b}	1.87±0.25	1.82±0.07^{a}*	1.60±0.05^{b}*
Population C	0.94±0.17	0.91±0.05	0.82±0.04	1.71±0.13	1.75±0.04^{a}	1.64±0.03^{b}
Population D			2.20±0.08			2.84±0.06
Population E	2.06±0.30	1.80±0.08	1.85±0.06	2.81±0.18	2.65±0.05	2.65±0.03
Genotype	RFT			Population E			
	AA	AB	BB	Genotype	AA	AB	BB
Population A	1.19±0.22^{a}	1.64±0.08^{b}	1.64±0.09^{b}	FMP	24.78±2.15^{a}	21.11±0.59	20.92±0.40^{b}
Population B	2.15±0.44	2.12±0.12^{a}	1.81±0.08^{b}	LMP	51.00±1.69^{a}*	55.85±0.46 ^{b}*	55.97±0.32^{b}*
Population C	1.62±0.23	1.90±0.06	1.80±0.05	IFR	5.37±0.52^{a}	4.56±0.14	4.29±0.10^{b}
Population D			3.00±0.07	DP	70.14±1.73	70.36±0.47	70.61±0.32
Population E	3.01±0.31	2.66±0.09	2.78±0.06				

[1] All estimates are least-squares means±standard error. Estimates with the different letters are significantly different.
Letter with * represent $p<0.01$, without * represent $p<0.05$.

Table 4. Association results between the genotypes of IGF2 gene (primer 1) and fat deposition and carcass traits in five populations[1]

Genotype	SFT			TFT		
	CC	CD	DD	CC	CD	DD
Population A			2.86±0.06			1.49±0.05
Population B			2.67±0.07			1.43±0.05
Population C			2.79±0.46			1.49±0.38
Population D	4.03±0.11	4.25±0.13	4.28±0.20	2.19±0.06^{a}	2.24±0.08^{a}	1.89±0.11^{b}
Population E	3.85±0.14	4.12±0.05	4.03±0.11	2.15±0.12	2.10±0.05	2.14±0.10
Genotype	RFT			ABT		
	CC	CD	DD	CC	CD	DD
Population A			1.61±0.06			1.70±0.03
Population B			1.92±0.07			1.67±0.04
Population C			1.86±0.44			1.71±0.30
Population D	3.03±0.11	2.92±0.13	3.09±0.19	2.76±0.09	2.92±0.10	2.79±0.15
Population E	2.80±0.14	2.83±0.05	2.82±0.11	2.61±0.11	2.74±0.04	2.66±0.09
Genotype	DP					
	CC	CD	DD			
Population A			72.46±0.53			
Population B			73.29±0.38			
Population C			72.95±2.63			
Population D	66.71±0.56^{a}*	69.33±0.68^{b}*	67.77±1.01			
Population E	67.70±0.75^{a}*	70.07±0.28^{b}*	70.04±0.62 ^{b}*			

[1] There are significant ($p<0.05$) difference on TFT (fat deposition trait) and highly significant ($p<0.01$) differences on DP (carcass trait). All estimates are least-squares means±standard error. Estimates with the different letters are significantly different. Letter with * represent $p<0.01$, without * represent $p<0.05$.

population B, C and E, but a different trend in population A.

For IGF2, significant ($p<0.05$) difference was found on TFT, and highly significant ($p<0.01$) differences were found on DP. It shows lower LS means of TFT on genotype DD in population D and lower LS means of DP on genotype CC in both population D and E. The other deposition traits such as RFT, SFT and ABT show lower LS means on genotype CC in population D and E, but no significant difference was observed (See Table 4).

Besides differences among different genotypes, we

showed higher ABT. TFT. BFT. SFT. RFT. FMP, IMF and lower DP. LMP in Chinese breed and its intercross pigs than European breeds and their intercross pigs in the tables (See Tables 3 and 4).

DISCUSSION

The MC4R gene has been mapped to human chromosome 18q21.3 (Gantz et al., 1993) and SSC1q22–q27, a region with expected correspondence to HSA9 and HSA14 (Yerle et al., 1996; Kim et al., 2000a). It secreted by ventromadial hypothalamus (VWH) (Fan et al., 1997). As a part of hypothalamus which regulates feeding and drinking, VWH destroy will make fat thickness increase and lead to obesity (Huszar et al., 1997). Pro-opiomelanocortin (POMC)(a part of CMS) cDNA produces a mutant b melanocyte-stimulating hormone (b-MSH)-endorphin fusion protein which can bound to the human melanocortin-4 receptor (hMC4R) and had a markedly reduced ability to activate the receptor. The functional loss of both alleles of the human POMC gene will leads to a very rare syndrome of hypoadrenalism, early-onset obesity (Challis et al., 2002). A lot of studies showed MC4R affects obesity, feeding intake and fat deposition in human, mice and pigs (Fan et al., 1997; Ho et al., 1999; Kim et al., 2000b; Challis et al., 2002; Jiang et al., 2002; MacIntyre et al., 2003).

IGF2 gene is located in the region on the distal end of Chromosome 2 in pig (SSC2), where the QTL can explain 15-30% of the variation in muscle mass and 10-20% of the variation in back fat thickness (Jeon et al., 1999; Nezer et al., 1999).

The results reported in this work provide new important evidence in favor of the mutations we have detected in MC4R and IGF2 genes affecting fat deposition and carcass traits in pigs. This conclusion is based on two points: (1) the mutations detected in five different populations in total of about 300 pigs were all observed polymorphisms and associated with five fat deposits and four carcasses traits;(2) Aspartic acid found at position 298 of the seventh transmembrane domain is very highly conserved in the MCR proteins, and the mutation in highly conserved regions may cause structural changes and alter the function of the receptor (Kim et al., 2000b).

For MC4R, most of the traits were observed significant ($p<0.05$) differences in five populations, and average backfat thickness and shoulder fat thickness were observed highly significant ($p<0.01$) differences in Landrace breed pigs. Results showed that allele B frequency is much higher than that of allele A in all populations, and genotype BB is the favorable one which can decrease the fat thickness in population B, C, D. Besides these, genotype BB showed a tendence to decrease the fat meat percentage, internal fat rate and increase lean meat percentage in population E. But population A. Large white breed pigs showed the different results. We speculate the reason is that the breeding background among Large White, Landrace and Chinese breed Meishan pig are different. Similar result was observed by Kim et al. (2000b), who explained growth and fatness are complex polygenic traits. No QTL conducted for fatness and growth traits has been reported near the MC4R locus, which maps to SSC1 at approximately 80 cM on the linkage map (Kim et al., 2000a, b). Besides these reason, the number of genotype AA of Large White we have detected is not enough.

For IGF2, although there is no polymorphism in population A, B and C, significant difference and highly significant difference on TFT and DP was respectively observed in Chinese breed (Population D). The highly significant difference on DP was also observed in population E. LS means of DP of genotype CD is the highest. In population D and E, the dominant effect of DP reach significance ($p<0.034$ and $p<0.050$), respectively (p value not showed in the table), so we suggest dominant effect is the main effect on DP for IGF2.

In this paper, two candidate genes MC4R and IGF2 can explain significant differences for backfat, internal fat, fat meat percentage, lean meat percentage and dress percentage in five populations of pigs. These results illustrate the potential value of Marked Assistance Selection (MAS) with candidate genes in livestock industry.

ACKNOWLEDGEMENTS

This work was supported by the grants from National Natural Science Foundation of China (30371028), National Key Foundation Research and Development Program of China (TG2000016105) and the National High Technology Research and Development Program of China (2004AA213111).

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