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Significant Association of One Intronic SNP Within *PPARδ* Gene with Carcass Length in Pigs

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Abstract: Significant QTL for carcass length and rib number were located on pig chromosome 7 in the earlier studies. The Peroxisome Proliferator-Activated Receptor δ (PPARδ) plays an important role in the regulation of lipogenesis and fatty acid oxidation and is considered the most important positional and functional candidate gene for QTL within QTL region. The association between one SNP (EU169095: g.40395T>G) and carcass length and rib number was investigated by single marker association analysis and QTL Mapping Method. Significant associations of the intronic polymorphism with carcass length were found. The present study provided the useful marker information for the potential maker assisted selection for carcass length in pigs.

Key words: Pig, carcass length, Peroxisome Proliferator-Activated Receptor δ (PPARδ), Single Nucleotide Polymorphism (SNP), QTL region

INTRODUCTION

The carcass length and rib number are important economically traits in pig production as meat productivity and body size are associated with carcass length. Compared with Chinese native pig breeds, Western commercial pig breeds such as Large White and Landrace have longer carcass length due to their long-term selection for large body size. Mikawa *et al*. (2007) repored that NR6A1 on *Sus Scrofa* Chromosome (SSC) SSC7 was a strong candidate for one of the QTL that influence number of vertebrae in pigs. But most of the major genes cotrolling the carcass length remained unclear. The earlier studies also showed that significant QTL for carcass length and rib number were located between microsatellite marker Sw252 and Sw581 on SSC7 (Zhang *et al*., 2007). Both detected a positive effect on increasing the rib number by the QTL allele from Large White. The Peroxisome Proliferator-Activated Receptor δ (PPARδ) plays an important role in the regulation of lipogenesis and fatty acid oxidation and is considered the most important positional and functional candidate gene for QTL within this QTL region. One SNP (EU169095: g.40395T>G) located in the intronic region of *PPARδ* gene was detected and significantly associated with fat deposition in the earlier studies (Huang *et al*., 2011, 2012).

MATERIALS AND METHODS

Animal and traits: The study was approved by the Animal Care Committee at the Huazhong Agricultural University. The Large White x Meishan resource population used in the linkage and QTL analysis was derived from the intercross of Large White and Meishan pigs in 37 full-sib families (Huang *et al*., 2011). The animals were born and raised in Huazhong Agricultural University Jingpin pig farm. All pigs were fed twice daily with diets formulated according to age under the standardized feeding and management regimen and free access to water. The F_2 pigs were slaughtered at average 200 days with the slaughter weight of 87.0±7.07 kg. After the pigs were slaughtered, carcass length from the first cervical vertebra to anterior border of pubic symphysis (CL1, cm), carcass length from the first thoracic vertebra to anterior border of pubic symphysis (CL2, cm) and Rib Number (RN) traits were measured according to the literature (Xiong and Deng, 1999).

SNP identification and genotyping: The markers including six SNPs and one insertion or deletion within seven genes (*BTNL1*, *COL21A1*, *PPARδ*, *GLP1R*, *MDFI*, *GNMT* and *PLA2G7*) in SSC7 q1.1-q1.4 region were developed by comparative sequencing then detected using PCR or

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PCR-Restriction Fragment Length Polymorphisms (RFLP) (Huang *et al*., 2011, 2012). Animal genotyping was performed by PCR or PCR-RFLP with the application of the restriction endonucleases and separated in agarose gel or polyacrylamide gel.

Statistical analysis: The effects of single genotypes on the traits studied were analyzed by the Least-Squares Method as applied in the General Linear Model (GLM) procedure of SAS (SAS Institute, Cary, NC.) according to the following statistical model:

$$T_{ijkl} = \mu + S_i + Y_j + G_k + F_l + b_{ijkl}X_{ijkl} + e_{ijkl}$$

Where:

T_{ijkl} = The observed values of a given trait
μ = The overall mean
S_i = Effect of sex (i = 1 for male or 2 for female)
Y_j = The effect of year (j = 1 for year 2000 or 2 for year 2003)
G_k = The effect of genotype (k = GG, GT and TT), F_l is the effect of family (l = 37)
b_{ijkl} = The regression coefficient of the slaughter age for carcass length
X_{ijkl} = The slaughter age
e_{ijkl} = The random residual

Both additive and dominance effects were estimated using the REG procedure of SAS Version 8.0. In order to determine whether the significant associations of *PPARδ* gene were due to the marker or due to other co-inherited blocks, the genetic mapping were performed using the SNP map available on SSC7 (Huang *et al*., 2011, 2012). Least-square regression interval mapping was used for QTL detection (Haley *et al*., 1994). QTL analysis was carried out on the Internet (http://qtl.cap.ed.ac.uk). About 6 years and full-sib family were included as fixed effects with the slaughter weight as a covariate for carcass length.

RESULTS AND DISCUSSION

The results of association analysis between SNP (EU169095: g.40395T>G) within *PPARδ* gene and traits in pigs are shown in Table 1. Differences among genotypes were highly significant for carcass length (p = 0.01) and significant for rib number (p≤0.05). The pigs with genotype TT had significantly more carcass length than GG or GT pigs but GG pigs had significantly more rib number than GT or TT pigs. Linkage analysis showed that the PPARδ marker was located at the 10.3 cM (Huang *et al*., 2011). In the QTL mapping, the significant QTL affecting CL1, CL2 and RN were detected for which the respective F-statistics of 9.6, 8.3 and 6.38 was obtained (Table 2). The highest probability for QTL position was at 11 or 12 cM flanked by PPARδ and GLP1R. Compared with those homozygous for Meishan allele, individuals homozygous for the Large White allele had more carcass length and the alleles increasing carcass length were inherited from Large White breed which were in agreement with its breed phenotype and the earlier reported QTL effects (Zhang *et al*., 2007). However, the opposite occurred for rib number. The results from QTL mapping were consistent with that of the single marker analysis. There are two reasons which could explain this phenomenon. First, there are different mutations underlying the QTL of carcass length and rib number. Second, one SNP might have pleiotropic effects on carcass length and rib number.

Significant QTL for traits such as growth, carcass length, skeletal morphology and fat deposition have been found in many pig resource populations including Meishan-derived population according to PigQTLdb (http://www.animalgenome.org/QTLdb/pig.html). Because *PPARδ* gene plays an important role in diverse biological functions including fat metabolism, cartilage development, chondrocyte proliferation and differentiation, it is speculated that mutations in *PPARδ* gene might have pleiotropic effects on carcass length, rib number and fatness traits in pigs. This pleiotropic phenomenon could be consistently evidenced in human and pig genetics. For example, polymorphisms of *PPARδ* gene were found to be associated with bipolar disorder (Zandi *et al*., 2008). Burch *et al*. (2009) found one mutation site within *PPARδ* gene and height was significantly associated with height in 11000 individuals. One missense mutation in *PPARδ* gene causes a major QTL effect on ear size in pigs as it mediates down-regulation of β-catenin and its target gene expression that is crucial for fat deposition in skin (Ren *et al*., 2011). So, it is necessary to further investigate and validate the above hypothesis.

Table 1: Association between SNP within *PPARδ* genotype and traits

	PPARδ genotype (μ±SE)			p-value		
Traits	GG (n = 133)	GT (n = 135)	TT (n = 30)	GG-GT	GG-TT	GT-TT
CL1	89.80±0.34	91.70±0.33	93.30±0.70	<0.0001	<0.0001	0.041
CL2	76.11±0.28	77.60±0.28	78.80±0.58	0.0001	<0.0001	0.063
RN	14.83±0.06	14.62±0.05	14.47±0.12	0.0140	0.0110	0.273

Table 2: Results of QTL mapping for carcass length and rib number

Traits	Location (cM)	Marker interval	F-value	Additive effect	Dominant effect
CL1	12	PPARδ-GLP1R	9.60**	1.337±0.366	0.838±0.482
CL2	11	PPARδ-GLP1R	8.37**	1.099±0.314	0.749±0.445
RN	11	PPARδ-GLP1R	6.38*	-0.245±0.069	0.067±0.097

*Significance at chromosome-wide level with p≤0.05; **Significance at chromosome-wide level with p≤0.01. Negative values of the additive effects denote a decrease of the trait due to the Meishan alleles

CONCLUSION

The association between this SNP and carcass length remained unknown. In this study, the association between SNP and carcass length and rib number was investigated by single marker association analysis and QTL Mapping Method.

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