Journal of Animal and Veterinary Advances 10 (5): 663-667, 2011
ISSN: 1680-5593

Association of *Leptin* Gene Polymorphisms with Litter Performance Traits in Four Chinese Indigenous Pig Breeds

Xiaoling Ding, Yan Yan, Chenghua Zhang, Yueyun Ding and Zongjun Yin
College of Animal Science and Technology,
Anhui Agricultural University, 230036 Hefei, P.R. China

Abstract: In this study, *leptin* gene was chosen as a candidate gene to evaluate its effect on litter performance traits in four indigenous pig population of Anhui province in China. Two SNPs (U_66254 g.3469 T>C; g. 3714 G>T) in Exon 3 of leptin gene were demonstrated by PCR-SSCP and sequencing of PCR product. Litter performance traits including Numbers of Born Alive (NBA) of the first parity and latter parities were recorded in four pig breeds population. The further association analysis of the 2 SNPs with litter performance traits were conducted in four pig breeds population including 213 sows. Researchers found the 2 SNPs are significantly associated with litter performance traits. The effect of CC genotype of SNP (U_66254 g.3469 T>C) and TT genotype of SNP (U_66254 g.3714 G>T) were significantly higher than other genotypes in both litter traits ($p<0.05$). The study suggested that lepetin gene could be used for marker assisted selection program as a molecular genetic marker for litter sizes in Chinese indigenous pig breeding.

Key words: Pig, PCR-SSCP, association, *leptin* gene, SNP, litter performance traits, China

INTRODUCTION

Leptin (LEP), the product of *leptin* gene is a 16 kDa protein synthesized by adipose tissue and is involved in regulation of feed intake, energy balance, fertility and immune functions (Campfield *et al*., 1995; Chen *et al*., 2004).

LEP also regulates animal's reproductive function and leptin receptors are also found in hypothalamus, pituitary, gonads of female mouse (Jin *et al*., 2000). Exogenous LEP can promote hyperplasia development of oviduct epithelium and endometrial of the female animal so that increased weight of the fallopian tubes, ovaries and uterus at the same time, Luteinizing Hormone (LH) levels of serum was increased these showed that LEP can promote genital organs maturation of the female animals and establish a good foundation for implantation of fertilized egg, embryonic development (Zamorano *et al*., 1997). In addition, leptin also can control the expression of hypothalamic Neuropeptide Y (NPY) and therefor regulation the secretion of reproduction hormones. In this pathway, LEP play a important role on controlling the reproduction (Terman, 2005).

Genetic differences in the *leptin* gene were first reported in obese ob/ob mice that lack functional LEP due to a mutation in *leptin* gene (Hamann and Matthaei, 1996). In human, polymorphisms of *leptin* gene are related to low circulating LEP level and morbid obesity (Hager *et al*., 1998; Ohshiro *et al*., 2000). In pigs, polymorphisms of *leptin* gene were associated with fatness in Duroc, Hampshire, Landrace and Large White pig (Jiang and John, 1999). Polymorphisms of *leptin* gene were also reported that it is significantly associated with litter sizes after the first parity in a synthetic line 990 which was resulted from crossing productive breeds such as Hampshire, Duroc, Polish Large White and various lines of Landrace (Korwin-Kossakowsk *et al*., 2002).

Considering the biological function of *leptin* gene in this study, researchers chose *leptin* gene to investigate its polymorphisms and evaluate its effect on litter performance traits in four indigenous pig breeds of Anhui province of China.

MATERIALS AND METHODS

Animals: Ear tissue samples of 213 sows were collected from four pig breeds population which included Anqing Liu White pig (34) from Anqing Anqing Liu White pig conservation farms, Wannan Black pig (51) from pig farm of forestry development company in Ningguo city, Wannan Hua pig (55) from Anqing Liu White pig conservation farm in Yixian county and Dingyuan Black pig (73) from breeding stock farm in Dingyuan county. Those samples are stored at -20°C. The phenotype of litter performance traits (NBA of the first parity and latter parities) for those sows in 4 different population were

Corresponding Author: Yin Zongjun, Department of Animal Science, College of Animal Science and Technology, Anhui Agricultural University, 230036 Hefei, China

Table 1: Primers used for SNP identification of the porcine *leptin* gene

Fragments	Primers sequence (5'~3')	Product size (bp)	Annealing Tm (X°C)
Leptin-exon 2	GTGGTTCCCTCTGTTTCCGGT TCG ACCTTGTCTCCC	223	59.5
Leptin-exon 3	TCGGGCCTAGATAGGATTGGAA GGCAGACTGGTGAGG	279	60.5
Leptin-exon 3	GAAATGTGATCCAAATATCGCTT CAA GGCTTCAGCAGC	240	58.0

obtained from the records of the four pig farms which is composed of five parities records corresponding to different pig population.

PCR amplification of *leptin* gene: Genomic DNA was extracted from ear tissue samples of sows using the phenol-chloroform method. Using Primer 5.0 software, three pairs of primers corresponding to Exon 2 and Exon 3 regions were designed according to the genomic sequence of *leptin* gene in NCBI (GenBank accession no. U_66254) (Table 1).

The PCR was carried out in a total volume of 25 μL including 60-80 ng of template DNA, 10 pmol of each primer, 250 μM dNTPs, 2.5 μL 10×PCR buffer (with $MgCl_2$) and 1.0 U Taq polymerase (TaKaRa Biotechnology, China). PCR was performed in a MJ Research PTC-200 Thermal Cycler (BIO-RAD, USA) under the following reaction procedure: initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 40 sec, annealing at X°C for 40 sec (X was shown in Table 1) and extension at 72°C for 45 sec with a final extension step at 72°C for 5 min.

Single-stranded conformation polymorphism and sequencing: PCR-SSCP method was used to find the mutation site in the amplified regions. Aliquots of 5 μL PCR products were mixed with 6 μL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated at 98°C for 10 min and then chilled in ice immediately. Denatured DNA was subjected to 10% Polyacrylamide Gel Electrophoresis (PAGE) in 1×TBE buffer and constant voltage (140 V) for 16 h at a constant temperature of 4°C.

After the process above, the gels were stained with 0.1% silver nitrate and visualized with 2% NaOH solution (containing 0.1% formaldehyde). The PCR products which represented different PCR-SSCP genotype including both homozygous and heterozygous genotypes were purified with the Gel Midi PCR DNA Purification Kit (Tiangen Biotechnology, China) and sequenced using the ABI377 sequencer. Those tested sequences were aligned by using the DNAMAN software (version 5.2.2) to identify the mutation site.

Association analysis: Association analysis between genotypes of the SNPs and litter performance traits were tested by using in SAS9.1.3 software based on the general linear models. The general linear model is as follows:

$$Y_{ijkl} = \mu + B_i + M_j + G_k + p_l + e_{ijkl}$$

Where:

Y_{ijkl} = The vector of the phenotypic value for litter traits of sow k
B_i = The vector of the breed effect
M_j = The vector of the parity effect
G_k = The vector of the fixed effect corresponding to the genotypes of SNPs
p_l = The random environmental effect
e_{ijkl} = The random residuals

RESULTS AND DISCUSSION

Identification of polymorphisms: Through the three pairs of primers, successfully amplified out all the expected fragments for Exon 2 and Exon 3 region of *leptin* gene. After PCR fragments were detection by PCR-SSCP method and sequencing, two Single-Nucleotide Polymorphisms (SNPs) was identified in Exon 3 of *leptin* gene (Fig. 1). By comparing the sequences of two types of homozygote on SNP found that two SNPs showed nucleotide transition from T-C at 3469 bp and G-T at 3714 bp in Exon 3 of *leptin* gene called SNP (U_66254:g.3469 T>C) and SNP (U_66254 g.3714 G>T).

After peptide-binding region predicted by DNAMAN software (version 5.2.2), the two SNPs in the peptide-binding region of *leptin* gene are all synonymous substitution. A total number of 213 DNA samples from four indigenous pig breeds population were genotyped and allele frequencies of the two SNPs are shown in Table 2. The frequency of T allele of SNP (U_66254 g.3469 T>C) was higher than that for C and the frequency of G allele of SNP (U_66254 g.3714 G>T) was higher than that for T allele, the two alleles are obviously dominant alleles in four Anhui indigenous pig population.

Association analyses: Based on statistical analysis, supporting association between different genotypes of SNPs and litter performance traits were tested. Comparisons between the least squares means of the litter size phenotypes evaluated and their respective standard errors for the genotypes of the two SNPs are shown in

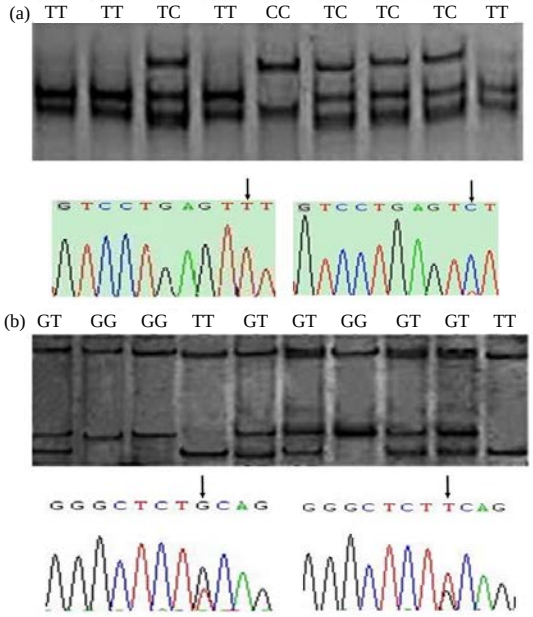

Fig. 1: The electrophoresis patterns of PCR-SSCP and sequencing maps for porcine *leptin* gene, two SNPs are indicated by black arrows; a) The electrophoresis patterns of PCR-SSCP for SNP (U_66254:g.3469 T>C); b) The electrophoresis patterns of PCR-SSCP for SNP (U_66254:g.3714 G>T)

Table 3 and 4. There were significant associations with two litter performance traits ($p<0.05$). Both Numbers of Born Alive (NBA) of the first parity and latter parities of sows with CC genotype and TT genotype are higher than those genotype TT and genotype GG in four indigenous pig breeds populations.

The *leptin* gene is composed of three exons and two introns with the coding area being formed by the Exon 2 and Exon 3 (Bidwell *et al*., 1997) and it has been mapped on pig chromosome 18q13-q21 (Neuenschwander *et al*., 1996). The comparative studies showed high homology between the pig and human, mouse and rat sequence and is highly conservative gene (Peixoto *et al*., 2006).

In the study, the polymorphisms of Exon 1 of *leptin* gene were not found in four Anhui indigenous pig populations there is different form other western pig population i.e., Landrace, Duoroc (Kennes *et al*., 2001; Stachowiak *et al*., 2007). In the first parity and latter parities of four Anhui indigenous pig population, the results showed that the effect on Numbers of Born Alive (NBA) of litter among SNP genotypes of Exon 3 in *leptin* gene were significant ($p<0.05$). These data are consistent with those by Chen *et al*. (2004) reported and are some different from those of Korwin-Kossakowsk *et al*. (2002) who found that there was no relationship ($p>0.05$) between polymorphisms and litter sizes in the first parity.

Table 2: Genotype and allele frequencies of the two SNPs detected in the porcine *leptin* gene

	U_66254:g.3469 T>C					U_66254: g.3714 G>T				
	Genotype frequencies			Allele frequencies		Genotype frequencies			Allele frequencies	
Breeds (N = 213)	TT	TC	CC	T	C	GG	GT	TT	G	T
Anqing Liu White (34)	0.324	0.588	0.088	0.618	0.382	0.471	0.471	0.059	0.706	0.294
Wannan Black (51)	0.353	0.569	0.078	0.637	0.363	0.333	0.490	0.177	0.578	0.422
Wannan Hua (55)	0.218	0.618	0.164	0.527	0.473	0.418	0.491	0.091	0.664	0.336
Dingyuan Black (73)	0.370	0.480	0.151	0.610	0.390	0.452	0.370	0.178	0.637	0.363

Table 3: Least square means of Numbers of Born Alive (NBA) of the first parity of the two SNPs in four Anhui indigenous pig populations

	Numbers of Born Alive (NBA) of the first parity					
	U_66254:g.3469 T>C			U_66254: g.3714 G>T		
Breeds (N = 213)	TT	TC	CC	GG	GT	TT
Anqing Liu White (34)	6.81±0.58^{A}	8.38±0.33^{B}	8.57±0.45^{B}	7.56±0.15	8.00±0.55	8.20±0.12
Wannan Black (51)	7.11±0.35^{A}	7.62±0.39^{A}	8.20±0.42^{B}	7.11±0.45^{a}	7.90±0.68^{b}	7.92±0.38^{b}
Wannan Hua (55)	7.78±0.30^{A}	8.26±0.54^{B}	8.70±0.45^{B}	8.20±0.37	8.24±0.62	8.30±0.73
Dingyuan Black (73)	8.30±0.14	8.50±0.65	8.71±0.21	7.96±0.56^{A}	8.61±0.36^{B}	8.94±0.27^{B}

$^{a, b}$Statistically different of least square means ($p<0.05$); ^{A,B} Statistically different of least square means ($p<0.01$)

Table 4: Least square means of Numbers of Born Alive (NBA) of the latter parities of the two SNPs in four Anhui indigenous pig populations

	Numbers of Born Alive (NBA) of the latter parities					
	U_66254:g.3469 T>C			U_66254: g.3714 G>T		
Breeds (N = 213)	TT	TC	CC	GG	GT	TT
Anqing Liu White (34)	9.72±0.47^{A}	12.05±0.64^{B}	12.46±0.62^{B}	10.95±0.77^{a}	11.38±0.89^{a}	11.90±0.68^{b}
Wannan Black (51)	10.97±0.59^{a}	11.45±0.78^{a}	12.05±0.82^{b}	10.92±0.17^{a}	11.75±0.72^{b}	11.80±0.58^{b}
Wannan Hua (55)	12.45±0.23	12.59±0.56	12.72±0.22	11.56±0.20^{A}	12.65±0.58^{B}	13.55±0.50^{B}
Dingyuan Black (73)	11.00±0.66	11.36±0.86	11.78±0.72	10.50±0.41^{A}	11.40±0.61^{B}	12.24±0.64^{B}

$^{a, b}$Statistically different of least square means ($p<0.05$); ^{A,B}Statistically different of least square means ($p<0.01$)

These differences between Chinese indigenous pig and western commercial pig should be explained for their different genetic background and sample sizes of population in this study, four populations in this study are from pig conservation farm of Anhui province in China and need to further investigation for genetic variation among other pig populations including western commercial pig in China.

Furthermore, the two SNPs (U_66254: g.3469 T>C; g.3714 G>T) are located in coding sequence of the porcine *leptin* gene but both are all synonymous substitutions. A silent polymorphism changes substrate specificity has revealed (Kimchi-Sarfaty *et al*., 2007). The synonymous SNPs can affect protein expression by alteration or increase in the stability of the mRNA was also reported (Capon *et al*., 2004: Liu *et al*., 2009). Therefore, the association results indicated that the two SNPs of *leptin* gene also may simply be used as a genetic molecular marker with effects on litter traits for marker assisted selection in Anhui indigenous pig breeding. Further studies on the relationship between the *leptin* gene and production performance traits in other indigenous pig and western commercial pig breed populations are currently in progress.

CONCLUSION

In summary, the association analysis revealed two SNPs in Exon 3 of *leptin* gene which showed significant associations with two litter performance traits in four Anhui indigenous pig breeds population. Based on this study, researchers suggested that *leptin* gene could be a potential genetic marker for marker assist selection of litter traits in Chinese indigenous pig breeding.

ACKNOWLEDGEMENT

This research was supported by the Key Scientific Technology Project of Chinese Ministry of Education (No. 208059), the Project of Anhui Province Scientific Technology Plan (Grant No. 07010302136) and Natural Science Foundation of Education Department of Anhui Province (KJ2009A114).

REFERENCES

Bidwell, C.A., S. Ji, G.R. Frank, S.G. Cornelius, G.M. Willis and M.E. Spurlock, 1997. Cloning and expression of the porcine obese gene. Anim. Biotechnol., 8: 191-206.

Campfield, L.A., F.J. Smith, Y. Guisez, R. Devos and P. Burn, 1995. Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science, 269: 546-549.

Capon, F., M.H. Allen, M. Ameen, A.D. Burden, D. Tillman, J.N. Barkerand and R.C. Trembath, 2004. A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups. Human Mol. Genet., 13: 2361-2368.

Chen, C.C., T. Chang and H.Y. Su, 2004. Genetic polymorphisms in porcine leptin gene and their association with reproduction and production traits. Aust. J. Agric. Res., 55: 699-704.

Hager, J., K. Clement, S. Francke, C. Dina and J. Raison *et al*., 1998. A polymorphism in the 5 untranslated region of the human ob gene is associated with low leptin levels. Int. J. Obes. Relat. Metab. Disorders, 22: 200-205.

Hamann, A. and S. Matthaei, 1996. Regulation of energy balance by leptin. Exp. Clin. Endocrinol. Diabetes, 104: 293-300.

Jiang, Z.H. and P.G. John, 1999. Genetic polymorphisms in the leptin gene and their association with fatness in four pig breeds. Mammalian Genome, 10: 191-193.

Jin, L., S.Y. Zhang, B.G. Burguera, M.E. Couce, R.Y. Osamura, E. Kulig and R.V. Lloyd, 2000. Leptin and leptin receptor expression in rat and mouse pituitary cells. Endocrinology, 141: 333-339.

Kennes, Y.M., B.D. Murphy, F. Pothier and M.F. Palin, 2001. Charaterization of swin leptin (LEP) polymorphisms and their association with production traits. Anim. Genet., 32: 215-218.

Kimchi-Sarfaty, C., J.M. Oh, I.W. Kim, Z.E. Sauna, A.M. Calcagno, S.V. Ambudkar and M.M. Gottesman, 2007. A silent polymorphism in the MDR1 gene changes substrate specificity. Sciences, 315: 525-528.

Korwin-Kossakowsk, A., M. Kamyczek, D. Cieslak, M. Perzchaia and J. Kuryl, 2002. The effect of the polymorphism of leptin (LEP), leptin receptor (LEPR) and osteopontin (OPN) genes on selected reproduction traits of synthetic Line 990 sows. Anim. Sci. Papers Rep., 20: 159-168.

Liu, Y., X. Lu, Y.R. Luo, J.P. Zhou, X.Y. Liu, Q. Zhang and Z.J. Yin, 2009. Effect of single nucleotide polymorphism of IRF1 gene on cytokine traits in three pig breeds. J. Anim. Vet. Adv., 18: 2346-2350.

Neuenschwander, S., G. Rettenberg, E. Meijerink, H. Jorg and G. Stranzinger, 1996. Partial characterization of porcine obesity gene (OBS) and its localization to chromosome 18 by somatic cell hybrids. Anim. Genet., 27: 275-278.

Ohshiro, Y., K. Ueda, M. Nishi, M. Ishigame and H. Wakasaki *et al*., 2000. A polymorphic marker in the leptin gene associated with Janpanese morbid obesity. J. Mol. Med., 78: 516-520.

Peixoto, J.D.O., S.E.F. Guimaraes, P.S. Lopes, M.A.M. Soares and A.V. Pires *et al*., 2006. Association of leptin gene polymorphism with production traits in pigs. J. Anim. Breed. Genet., 123: 378-383.

Stachowiak, M., M. Mackowski, Z. Madeja, M. Szydlowski and A. Buszka *et al*., 2007. Polymorphism of the porcine leptin gene promoter and analysis of its association with gene expression and fatness traits. Biochem. Genet., 45: 245-253.

Terman, A., 2005. Effect of the polymorphism of prolactin receptor(*PRLR*) and Leptin (*LEP*) genes on litter size in Polish pigs. J. Anim. Breed. Genet., 122: 400-404.

Zamorano, P.L., V.B. Mahesh, L.M. de Svilla, L.P. Chorich, G.K. Bhat and D.W. Brann, 1997. Expression and localization of the leptin receptor in endocrine and neuroendocrine tissues of the rat. Neuroendocrinolgy, 65: 223-228.