

Missense mutations in exon 2 of the porcine leptin receptor gene and their associations with litter size and body weight

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ABSTRACT: Leptin receptor (*LEPR*) gene is regarded as a “candidate-gene” of production traits. The aims of this study were to detect polymorphisms of exon 2 within *LEPR* gene and to investigate their associations with production traits, litter size and live weight in Luchuan and Large White pig breeds. For this purpose, the single nucleotide polymorphisms (SNPs) of exon 2 within *LEPR* were detected using the PCR-SSCP procedure and their association with litter size and live weight was also analysed in Luchuan ($n = 446$) and Large White pigs ($n = 405$). The results showed that the C155T mutation was found in exon 2 of porcine *LEPR* gene in analyzed populations, which caused a missense mutation (Met to Thr). The different genotypes of this locus had the effects on total number of piglets born, born alive of the first (1st) and the first to the fourth (1st–4th) litters. The individuals with allele A had a higher number of total piglets born and a higher number of born alive piglets in the first litter ($P < 0.05$) while the differences in birth weight, weaning weight and average daily gain were not significant among different genotypes of this locus in analyzed populations. It implies that the exon 2 (Thr/Met) mutation of *LEPR* gene is a potential gene marker of pig reproduction. Therefore, it can be used in the marker-assisted selection (MAS) of pig breeding work.

Keywords: *LEPR*; SNPs; pigs; litter size; live weight

Leptin plays an important role in regulating feed intake, live weight, energy balance and fertility (Liefers et al., 2002). *LEPR* is a high affinity acceptor and important mesmerisms of leptin, it belongs to the class I cytokine receptor superfamily. *LEPR* has two modes of action (Avcin et al., 2006): short-range regulation, it mediates bellyful signal's input via the brain stem vagus nerve to the centre (Sawhney et al., 2001), and long-range regulation, including the mediation from adipocyte signal and neuropeptide (NP), it is the main mode of leptin action. So, *LEPR* gene is a candidate gene for detecting production traits, such as litter size and body weight.

Several years ago, *LEPR* gene was found for the first time in the mouse choroid plexus and set up cDNA Lib (Tartaglia et al., 1995). Then, different animal *LEPR* genes were investigated in locations, mutations and functions. E.g. mouse *LEPR* mapped to Chr 4 (Chua et al., 1996) as well as human *LEPR* in Chr 1 (Tartaglia et al., 1995), porcine *LEPR* gene in Chr 6 q3.3–3.5 (Ernst et al., 1997). Based on a comparison between human *LEPR* (Chen et al., 2000) and mouse *LEPR* (Jin et al., 2000), the nucleotide homology of porcine *LEPR* gene was 89.3% and 80.3%, respectively, as well as the amino acid homology of porcine *LEPR* gene was 80.3% and 76.6%, respectively.

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As the leptin gene is a candidate gene for production traits, its polymorphisms and its associations with commercial traits were reported (Nephawe and Spurlock, 2007). The *LEPR* also was related to growth and body composition (Olivo et al., 2005). Polymorphisms of porcine *LEPR* were respectively associated with backfat thickness, intramuscular fat content in Large White pigs and live weight at 21st weaning weight in L990 sows, and litter size traits in Duroc and Yorkshire (Kopečný et al., 1997; Vincent et al., 1997). In addition, single nucleotide polymorphisms (SNPs) of *LEPR* gene were associated with human familial combined hyperlipidaemia (van der Vleuten et al., 2006), human type 2 diabetes (Moritani et al., 2007), decreased leptin response and weight loss (de Luis Roman et al., 2006), obesity and T2DM (Park et al., 2006), and atherogenesis (Zhang et al., 2007). Although there are many reports on the porcine *LEPR* gene, few of them reported these results about the association analysis in litter sizes and body weight in China local pigs.

Luchuan pig is a superordinary local variety in Guangxi Province of China. Its superordinary characteristics are larger litter sizes, better maternity, higher survival rate, better antireversion force, and so on; so it was recorded in the livestock and poultry variety conservation directory by the Ministry of Agriculture in 2000 (Xie and Qiu, 2003). Large White pig is the main lean variety with fast growth and high lean meat percentage. Presently, these two breeds greatly contribute to the economic development of pig productions. To date, the study of the polymorphism of *LEPR* gene and its association with production traits has been limited, which constrains more benefits of pig production.

So, the objective of this study is to detect the polymorphism of porcine *LEPR* gene using the PCR-SSCP method in Luchuan ($n = 446$) and Large White pigs ($n = 405$) and to analyze the associations with litter size and body weight, which will find a novel genetic marker, provide scientific evidence for subsidiary selection to advance the productivity and growth rate of pigs.

MATERIAL AND METHODS

Pig and DNA sources

Purebred Luchuan pigs, 164 males and 282 females, were supplied by the Luchuan pig farm of Guangxi Livestock Research Institute. Purebred Large white pigs, 185 males and 220 females, as the check breed, were supplied by the Guangxi Livestock Research Institute. The samples collected from pig ear tissues were put in a centrifuge tube which contained 70% alcohol, and they were preserved at -70°C .

PCR amplification

The primers for *LEPR* gene were designed based on the known sequence of exon 2 (GenBank accession No. AF092422). The primer information was as follows:

forward primer:

5'-GTGATAACTGCATTTGACTTGGC-3';

reverse primer:

5'-CTGCAATGTTGTCTGCATGTACAG-3.0'

PCR system (25 μl): 10 \times PCR buffer 2.0 μl , dNTPs (2.5 mmol/l) 2.5 μl , upstream and downstream primers (10 $\mu\text{mol/l}$) each 1 μl , genome DNA 100 ng, Taq Plus 1U, added di-distilled water to 25 μl . The amplification parameters consisted of initial denaturation at 95°C for 8 min, denaturation at 95°C for 40 s, annealing at 59.4°C for 40 s, and extension at 72°C for 50 s, for 30 cycles, extreme extension at 72°C for 8 min.

Single stranded conformation polymorphism (SSCP) and DNA sequencing

Aliquots of 10 μl PCR products were mixed with 10 μl denaturing solution (95% formamide, 25mM EDTA, 0.025% xylene-cyanole and 0.025%

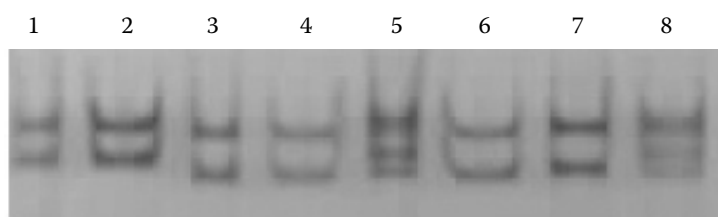


Figure 1. SSCP patterns of PCR amplifications of *LEPR* exon 2 locus in Luchuan and Large White pigs

Note: 3, 4, 6, 7: AA genotype; 1, 2: BB genotype; 5, 8: AB genotype

bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to PAGE in 1 × TBE buffer and at constant voltage (180–300 V) for 10–16 h. The gel was stained with 0.1% silver nitrate. The DNA samples showing different patterns on SSCP gels were selected for sequencing. The PCR products were purified by PCR purification kits (Unigen, Hangzhou, P.R. China). The most purified PCR amplification fragments were sequenced in the forward and reverse directions using ABI 3730 (Applied Biosystem, USA) and a few of them were subcloned to T-vector and sequenced in both directions.

Statistical analysis

Total number born (TNB) and number born alive (NBA) were used to describe the born characters birth weight, weaning weight and average daily gain were used to express weight. According to the fixed effect model, the relationship between total number born and number born alive of different breeds and genotypes was analyzed. Because

of the difference between the breeds, the statistical analysis in Luchuan pig and Large White pig was done separately. The model of statistical analysis is as follows:

$$Y_{ijkl} = \mu + HYS_i + P_j + G_k + e_{ijkl}$$

where:

Y_{ijkl} = litter size

μ = group mean

HYS_i = the fixed effect of one year one quarter on the animal farm i

P_j = litter size effect

G_k = genotype effect of *LEPR* gene mutable site

e_{ijkl} = random residual error effect

All data were analyzed by GLM (SPSS 13.0 software).

RESULTS

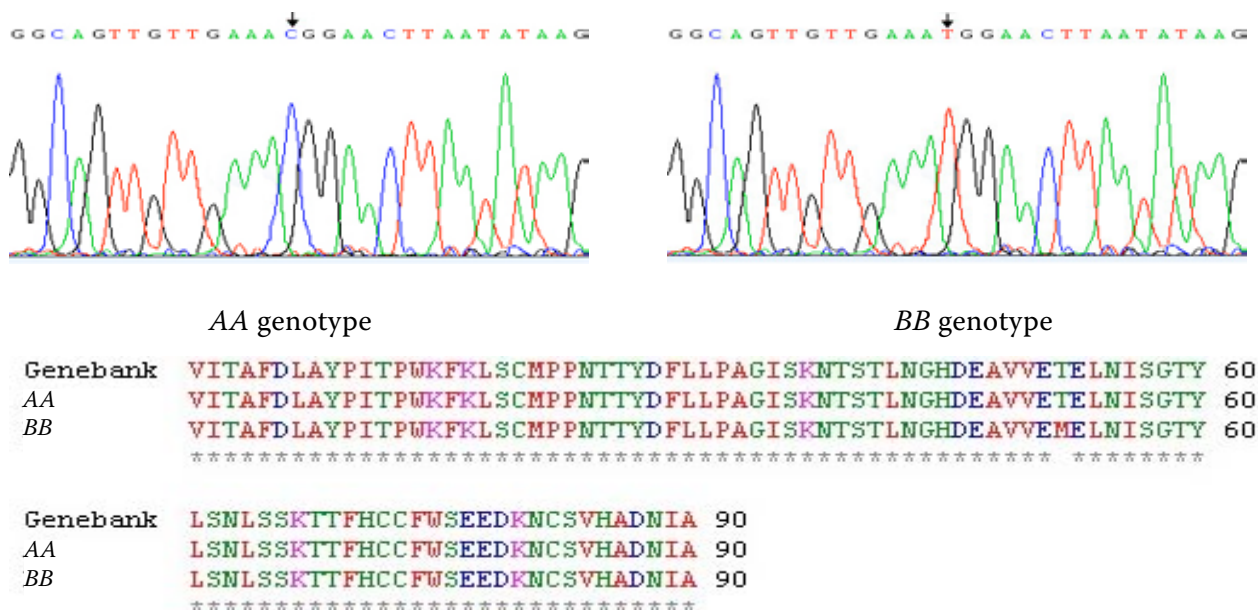
In this study, exon 2 of *LEPR* gene demonstrated polymorphism (namely, genotype *AA*, *BB* and *AB*) by the PCR-SSCP method (Figure 1).

Table 1. Allele and genotype frequencies of *LEPR* gene in Luchuan and Large White pigs

		Luchuan	Large White
Genotype frequencies	<i>AA</i>	0.7534 (336)	0.0494 (20)
	<i>AB</i>	0.1659 (74)	0.0617 (25)
	<i>BB</i>	0.0807 (36)	0.8889 (360)
Allele frequencies	<i>A</i>	0.8363	0.0802
	<i>B</i>	0.1637	0.9198
χ^2 value		1.826	2.980

Table 2. Associations of *LEPR* genotypes with litter size in Luchuan and Large White pigs (LSM ± SE)

Breeds	Genotypes	Numbers	1 st litters		1 st –4 th litters	
			TNB(n)	NBA(n)	TNB(n)	NBA(n)
Luchuan	<i>AA</i>	214	10.71 ± 0.13 ^a	9.30 ± 0.10 ^a	12.44 ± 0.08 ^a	10.80 ± 0.10 ^a
	<i>AB</i>	46	9.96 ± 0.10 ^b	8.70 ± 0.08 ^b	11.75 ± 0.07 ^b	10.24 ± 0.11 ^b
	<i>BB</i>	22	9.91 ± 0.16 ^b	8.82 ± 0.12	11.70 ± 0.07 ^b	9.91 ± 0.16 ^b
Large White	<i>AA</i>	10	11.50 ± 0.50 ^a	10.5 ± 0.50 ^a	12.00 ± 0.25	10.50 ± 0.25
	<i>AB</i>	15	11.0 ± 0.58 ^a	9.33 ± 0.67	11.91 ± 0.22	10.25 ± 0.29
	<i>BB</i>	195	8.46 ± 0.90 ^b	8.60 ± 0.13 ^b	11.48 ± 0.13	9.54 ± 0.11

Figure 2. The result of homology alignment for *LEPR* gene sequence

Different polymorphic PCR products were sequenced. Then, the sequencing results (EU366316 and EU366317) were compared with porcine *LEPR* gene DNA sequence (GenBank Accession No. AF092422) (Figure 2). The result showed that the AA genotype sequence was the same as the porcine *LEPR* gene DNA sequence in GenBank, while in BB genotype a point mutation occurred in exon 2 (C155T), which caused a missense mutation (Thr > Met).

From Table 1, in the Luchuan group, AA genotype gained absolute ascendancy because the disposition frequency of AA genotype was 67.27% and 58.75% higher than that of BB and AB genotype, respectively. Moreover, it indicated that the distribution frequency of A allele was much higher than that

of B allele. But in the Large White pig group, the distribution frequency of BB genotype was much higher than AA (higher 83.95%) genotype and AB (higher 82.72%) genotype, B allele gained absolute ascendancy. Chi-square test indicated that this site is in Hardy-Weinberg equilibrium ($P > 0.05$).

The least-squares analysis between all genotypes of *LEPR* exon 2 and the litter sizes in Luchuan pigs and Large White pigs were described (Table 2). In Luchuan pigs, the total number born of first-born, average total number born and number born alive of first four born with AA genotype were higher than those in AB and BB genotype ($P < 0.05$) ($AA > AB > BB$). Compared with AB genotype, the number born alive of first born showed a significant difference in AA genotype ($P < 0.05$), but the

Table 3. Association of different *LEPR* genotypes with WB, WW and ADG in Luchuan and Large White pigs (LSM \pm SE)

Breeds	Genotypes	Numbers	WB/kg	WW/kg	ADG/g
Luchuan	AA	122	0.59 \pm 0.10	5.74 \pm 0.10	171.63 \pm 3.25
	AB	28	0.62 \pm 0.19	6.13 \pm 0.24	183.81 \pm 7.90
	BB	14	0.61 \pm 0.27	6.08 \pm 0.34	182.48 \pm 11.11
Large White	AA	10	1.26 \pm 0.20	6.64 \pm 0.26	179.33 \pm 9.33
	AB	10	1.28 \pm 0.75	6.91 \pm 0.63	187.83 \pm 23.50
	BB	165	1.39 \pm 0.26	7.71 \pm 0.15	210.70 \pm 4.12

difference between *BB* and *AB* was not statistically significant ($P > 0.05$). In Large White pigs, the total number born and number born alive of first born showed significant differences ($P < 0.05$) in different genotypes, but the average total number born and number born alive of first four born were not significant in each genotype ($P > 0.05$).

Finally, the least-squares analysis between the Thr mutation of *LEPR* exon 2 and the body weight in Luchuan and Large White pigs was also done (Table 3). The result indicated no matter whether in Luchuan or Large White pig group, the Thr mutation of *LEPR* exon 2 in birth weight, weaning weight and average daily gain was not significant ($P > 0.05$).

DISCUSSION

This study reported a point mutation in exon 2 (C155T) within *LEPR* gene in Luchuan and Large White pigs, which caused a missense mutation (Met to Thr). This mutation had a significantly higher effect on litter sizes in Luchuan pigs.

About ten years ago, *LEPR* polymorphisms detected by PCR-DGGE and PCR-RFLP were reported in pigs (Kopečný et al., 1997; Vincent et al., 1997). Polymorphic short tandem repeat (STR) sequences of intron 3 within *LEPR* gene in L990 were revealed, and the association studies of productive traits indicated that the polymorphisms had significant effects on the backfat thickness trait in pigs (Chmurzynska et al., 2004). T232A in exon 4 of the porcine *LEPR* gene in Polish Landrace and synthetic line 990, and these SNPs associated with backfat thickness were reported (Mackowski et al., 2005). In 2007, weaning weight was also found to be associated with the *LEPR* gene transcription expression level (Kojima et al., 2007). Besides, it was reported that SNPs of *LEPR* had a significant effect on milk and milk fat content in cows (Komisarek and Dorynek, 2006).

The association analysis in the analyzed locus of *LEPR* gene exon 2 showed that: (1) in Luchuan and Large White pigs, the majority of analyzed traits with *AA* genotype were higher than those of *AB* and *BB* genotypes ($P < 0.05$). It indicated that *AA* genotype surpassed *AB* and *BB* genotypes in litter size traits. In the Luchuan group, *AA* genotype occupied the main position in the group, which implied that *AA* genotype displayed more excellent litter size traits than *BB* genotype in Luchuan pigs.

It may owe to the long-term artificial selection for the breeding trait in Luchuan pigs. In Large White pigs, *BB* genotype frequency was 88.89%, *AA* genotype frequency was 4.94%, but the litter size traits of *AA* genotype were better than in *BB* genotype. This may be due to the long-term artificial selection for growth rate and lean meat rate, causing a breeding performance loss.

Chen et al. (2004) found that the effects of exon 6 and 18 within *LEPR* gene on backfat thickness were significant in Landrace and Yorkshire, respectively. Effects of polymorphisms of intron 2, exon 2, and exon 18 on the reproduction traits such as litter size of sows were evident in Duroc and Yorkshire. In this study, the Thr mutation of *LEPR* gene exon 2 had a significant effect on total number of piglets born, born alive of the first (1st) and the first to the fourth (1st–4th) litters in Luchuan pigs ($P < 0.05$) and Large White pigs ($P < 0.05$), which was coincident with the research of Chen et al. (2004).

CONCLUSIONS

This study indicated that there occurred a point mutation in exon 2 (C155T) of *LEPR* gene, which caused a missense mutation (Met to Thr). This mutation changed the expression axiom of *LEPR*, it may even change the spatial structure of protein. From this, we can conclude that the *LEPR* gene is a potential genetic marker of the litter size traits in pigs, and it can be used for the marker-assisted selection (MAS) in pig breeding work. But whether the point mutation causes a change in the protein structure, expresses a quantity change and affects the litter size traits in Luchuan pigs still needs further studies.

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