Association of Mahogany/Attractin Gene (*ATRN*) with Porcine Growth and Fat

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ABSTRACT : Associations of restriction fragment length polymorphism in porcine attractin (ATRN) gene with average daily gain (ADG), live weight (LWT) and back fat thickness (BFT) were studied with one exotic composition population (Xianghuang) and three local breeds (Ningxing, Shaziling and Daweizi) in China. Two types of alleles were detected; one with the band size of 700 bp (A) and the other with the band size of 450 and 250 bp (B), and their genotypes were classified as AA, AB and BB. Polymorphisms were observed in the exotic breed, but not in the three local breeds. Individuals with the genotype BB showed larger ADG and LWT than those with AA in Xianghuang population ($p<0.05$). The breed specific effects of the porcine attractin gene in this study implied that identification of the polymorphisms and determination of genetic effects on phenotypes should be performed in the target populations. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 : 1383-1386)***

Key Words : Genetic Effect, Genotype Frequency, Mahogany Gene, RFLP

INTRODUCTION

Mahogany gene was characterized as a recessive coat color mutation in mouse (Lane and Green, 1960), and its high homology with human attractin gene led to another name called ATRN (Gunn et al., 1999; Nagle et al., 1999). The ATRN gene was mapped in human, mouse, bovine, and porcine chromosomes (Gunn et al., 1999; Edeal et al., 2000). The ATRN gene is widely expressed and encodes two types of proteins, transmembrane and secretory types. While the human and rat ATRN genes encode membrane- and secreted-type proteins, the hamster and mouse genes encode only membrane-type protein (Kuramoto et al., 2002). ATRN mRNAs were found in central nervous system (CNS) such as limbic structures, brainstem, cerebellum, spinal cord, and hypothalamus. Such a wide distribution in CNS suggested a broad spectrum of physiological functions for the ATRN gene product including immune system regulation, body weight control, pigmentation, myelination, and tumor susceptibility (Lu et al., 1999). Mutations at the ATRN locus that encodes a transmembrane protein were responsible for a juvenile-onset neurodegeneration manifest as hypomyelination and cerebral vacuole development in several rodent species (Bronson et al., 2001; Kuramoto et al., 2001; Kuramoto et al., 2002; Kuwamura et al., 2002; Tang and Duke-Kohan, 2002).

It has been reported that the mutations in ATRN locus suppressed the pleiotropic phenotypes (including obesity) of the agouti-lethal-yellow mutant (Miller et al., 1997; Dinulescu et al., 1998). Furthermore, mahogany mice, mutant type in ATRN locus, were abnormal in feeding behavior, motor activity, and metabolic rate, even in the absence of agouti overexpression (Dinulescu et al., 1998). Nagle et al. (1999) found that ATRN gene of mouse was expressed in the ventromedial hypothalamic nucleus, a region intimately involved in the regulation of body weight and feeding, and suggested that the mahogany locus could suppress diet induced obesity. Gunn et al. (2001) found that mice carrying a homozygous mutant genotype led to reduced body weight, reduced adiposity, and increased locomotor activity under normal food intake. These results implied the associations of ATRN locus products with growth and carcass composition of animals, especially focusing on the mouse, rat, hamster and human. Our study was conducted on some pig populations to investigate the restriction fragment length polymorphism (RFLP) in attractin (ATRN) gene and the relationships of the RFLP markers with economically important traits.

MATERIALS AND METHODS

Phenotypic records and samples for genomic DNA extraction were obtained from one exotic (Xianghuang) and three local (Daweizi, Shaziling and Ningxiang) pig populations in China. Boars from generation 9 of Xianghuang population that was established by crossing of Pietrain with Duroc were selected for the experiment. Animals were raised under feeding standards of the experimental station of Hunan provincial government, China. Genotyping was conducted on 129 pigs, and back fat

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Table 1. Allele and genotype frequencies of ATRN gene in the pig populations used in this study

Origin	Population	No. of samples	Genotype frequency			Allele frequency	
			AA	AB	BB	A	B
Exotic breed	Xianghuang	129	0.18 (23)[1]	0.60 (78)	0.22 (28)	0.48	0.52
Local breed	Ningxiang	76	0.00 (0)	0.00 (0)	1.00 (76)	0.00	1.00
Local breed	Shaziling	84	0.00 (0)	0.00 (0)	1.00 (84)	0.00	1.00
Local breed	Daweizi	97	0.00 (0)	0.00 (0)	1.00 (97)	0.00	1.00

[1] The figures in parentheses are the number of observations.

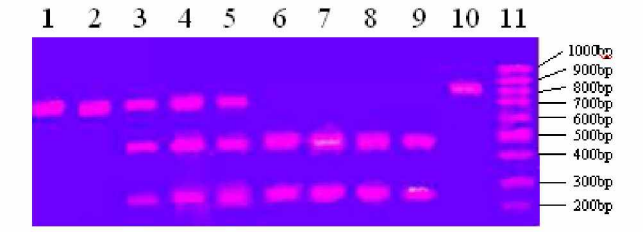

Figure 1. Testing results of ATRN gene in experimental swine. Lane 1-2: AA genotype (700 bp); lane 3-5: AB genotype (700 bp, 450 bp and 250 bp); lane 6-9: BB genotype (450 and 250 bp); lane 10: uncut; lane 11: 100 bp DNA ladder.

thickness (BFT), average daily gain (ADG), and live weight (LWT) were measured on 124 pigs out of the genotyped. The BFT was averaged over three metal probe measurements at the first rib, at the last rib, and at the last lumbar vertebra. The ADG was measured as the weight difference from birth to end of test divided by the period. The LWT was measured after 12 h fasting on May 16-18, 2002. Daweizi, Shaziling and Ningxiang were raised in local areas of Hunan Province, China. The numbers of genotyped pigs were 97, 76 and 76 for Daweizi, Shaziling and Ningxiang pig, respectively.

Genomic DNAs were extracted from ear tissue collected from pigs. Most of hair was removed from the ear tissue, and the tissue was minced and placed in a tube with 0.6 ml digestion buffer (10 mM EDTA, 2% SDS, 300 mM NaCl, 10 mM Tris-HCL, pH 7.4). Proteinase K (10 μl of 10 mg/ml) was added to the tube and the solution was incubated for 3 h in a shaking water bath at 45°C until the tissue was digested. Same volume of digestion buffer (0.6 ml) added to digested tissue and mixed gently, followed by centrifugation (11,000 rpm) and removal of supernatant. Two volumes of 95% EtOH were added to the supernatant and the DNA pellet was removed and washed in 70% EtOH.

The forward and reverse primers (Forward primer: 5`-GTGTACAAGGAGAAGTCAGGAG-3`; Reverse primer: 5`-GATCTATTTAAAGTCTAGGCAC-3`) were designed based on homologous regions of human and mouse ATRN cDNA sequences (GeneBank accession No. AB011120 and AF120318, respectively). These primers amplified a region of the porcine and bovine ATRN gene spanning from exon 5 to 3' untranslated region (Edeal et al., 2000).

The PCR was performed using PCR buffer solution containing 4.75 of ddH_2O, 1.00 μl of 10×Buffer, 1.00 μl of $MgCl_2$ (25 mM), 0.5 μl of dNTP (4 mM), 2.00 μl Template DNA (25-50 ng/μl), 0.05 μl of Taq Gold (Perkin Elmer, Forster City, CA), and 0.35 μl of each primer in a final volume of 10 μl. The PCR reactions were carried through follow thermal profile: pre-denaturation at 95°C for 3 min, 10 cycles of amplification with annealing temperatures beginning at 64°C and decreasing 1°C per cycle (denaturation at 95°C for 15 seconds, annealing at 64°C for 30 seconds and decreasing 1°C per cycle, and extension at 72°C for 1 min), 25 cycles with an annealing temperature with 55°C (denaturation at 89°C for 15 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 1 min), and a final 5-minute extension at 72°C. Digestion of the PCR product was conducted by incubating 10 μl PCR product with 10 U/μl of Taq I for 3 h at 65°C, and the digested fragments were separated by electrophoresis on a 4% agarose gel.

Linkage analyses were performed using the CRIMAP program (Green et al., 1990). Chi-square tests were conducted for homogeneity of genotypic frequencies across populations using SAS package (version 6. SAS Inst. Inc., Cary, NY.). Genetic analyses were made for the Xianghuang pig data using also the SAS to see ATRN genotype effects on ADG, LWT and BFT.

RESULTS

The size of ATRN-PCR product was 890 bp. Taq I digestion of the PCR product fragment revealed a polymorphism with two alleles; one with the band size of 700 bp and the other with the band size of 450 and 250 bp. Additional smaller bands were not visualized on the 4% gel. Genotypes were classified by the number and the size of band: AA genotype (700 bp), AB genotype (700 bp, 450 bp and 250 bp) and BB genotype (450 and 250 bp) (Figure 1).

Allele and genotype frequencies are presented in Table 1. In Xianghuang population, both alleles A and B were observed. On the other hand, allele A was not observed in the three local populations. The frequency of allele A was similar to that of allele B in Xianghuang population. Chi-square statistics for homogeneity of genotypic frequencies between Xianghuang population and the others were all highly significant ($p<0.001$, Data are not shown).

Table 2. LOD scores for linkage between ATRN gene and other markers on chromosome 17

Marker	Recombination rate	LOD score
SWR1004	0.50	0.00
SW24	0.50	0.00
SW2142	0.35	0.31
SW1920	0.30	1.32
SW1031	0.21	3.19
SW2427	0.31	0.90

LOD scores were calculated between ATRN marker and other genetic markers on chromosome 17 (SSC17) by two-point linkage analyses (Table 2). The ATRN gene was significantly linked to marker SW1031 ($p<0.05$), and recombination rate was 0.21. This concurred with Edeal et al. (2000) study where they were linked with recombination rate of 0.20 and LOD score of 4.17. Larger recombination rates (0.30-0.50) and smaller LOD scores (0.00-1.32) were observed between ATRN locus and the other markers.

The effects of the ATRN genotypes on the ADG, LWT and BFT in Xianghuang population are presented in Table 3. Individuals with genotype BB had larger ADG and LWT than those with genotype AA ($p<0.05$). While BFT did not differ between the homozygous genotypes AA and BB ($p< 0.05$). BFT of heterozygous genotype AB was thicker than the homozygous genotypes ($p<0.05$).

DISCUSSION

After first characterization of ATRN as a recessive coat color mutation in the mouse in 1960, its location, structure, and biological functions were extensively studied for mouse, rat, hamster and human with the rapid progress of the knowledge and experimental skills in molecular genetics. Its gene products had multiple functions such as immune system regulation (Duke-Cohan et al., 2000; Kuramoto et al, 2001), body weight and obesity control (Dinulescu et al., 1998; Nagle et al., 1999; Gunn et al., 2001), pigmentation (Miller et al., 1997; Dinulescu et al., 1998; Jackson, 1999), and myelinazation and tumor susceptibility (Bronson et al., 2001; Kuramoto et al., 2001; Kuramoto et al., 2002; Kuwamura et al., 2002; Tang and Duke-Cohan, 2002). Edeal et al. (2000) found the polymorphism by using the primers amplifying a region of porcine and bovine ATRN gene spanning from exon 5 to 3' UTR, and suggested that ATRN locus might be an attractive candidate gene responsible for genetic variation in body composition. Following Edeal et al. (2000), we confirmed the polymorphism in the ATRN gene in pig populations. Regarding genetic composition of the populations, Xianghuang population was originated from Duroc and Pietrain, and the other populations (Ningxiang, Shaziling, and Daweizi) were native breeds raised in the central china. The polymorphism was discovered in Xianghuang population, but not in the local breeds.

Table 3. Effects of ATRN genotypes on average daily gain (ADG), live weight (LWT), and backfat thickness (BFT) in Xianghuang population (least square mean±standard error)

Genotypes	No of samples	ADG (g)	LWT (kg)	BFT (cm)
AA	20	462±17b	101.60±2.01b	1.30±0.02b
AB	76	468±6ab	105.58±0.98a	1.41±0.03a
BB	28	509±6a	106.14±1.14a	1.27±0.03b

Since there has been a great concern on producing the swine with low back fat and high lean pork, we further investigated the associations of the polymorphism with ADG, LWT, and BFT in the Xianghuang population. Individuals with different homozygous genotypes effects had significantly different LWT and ADG, but not BFT. Selection for salutary allele (i.e. B in this study) might increase body weight without any change of BFT in Xianghuang population. Yet, cautions should be given for the use of ATRN polymorphism in breeding schemes of complex traits such as body weight and fatness. Three scenarios could be considered for explanation of the ATRN effects detected in this study. The first scenario was that the ATRN locus might have the causal polymorphisms of the traits. The second scenario was that the ATRN locus itself might not be responsible for the traits, but linked closely to the genes responsible for the traits. The third scenario was that the ATRN locus has direct effects and the effects of the linked genes. Under any of the scenarios, the ATRN effects on the traits could be inflated or deflated by sampling error or interaction with other loci affecting the same traits. Functional studies would be required in order to find what underlying biological mechanism of the association might be.

IMPLICATIONS

Genetic markers having association with phenotypes have been particularly interesting for their potential application to marker assisted selection (MAS) schemes (Lee and Wu, 2003), but such genetic markers have been quite limitedly known for porcine complex traits such as body weight and fatness (Zhu et al., 2004; Zeng et al., 2005). We found that the ATRN gene was associated with the traits in swine, which might be applicable to the practical use of MAS. The population specific effects in this study suggested that identification of the polymorphisms and determination of the genotype effects on the traits should be studied in the target population beforehand.

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