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Single Nucleotide Polymorphism of *Nuclear Receptor Coactivator 1* Gene and Association with Semen Quality in Chicken

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Abstract: Single Nucleotide Polymorphisms (SNPs) of *Nuclear receptor Co-activator 1* gene (NCOA1), gene of shaobo breed were detected by using PCR-SSCP and sequencing reaction and its associations with semen quality was done. Three SNPs were detected, T/A substitution at position 10155007 in exon 3 and resulted in 3 genotypes AA, AB and BB, C/G substitution at position 108273257 in exon 10 and resulted in 3 genotypes CC, CD and DD, C/T substitution at position 108273423 in exon 12 and 3 genotypes of SS, ST and TT were found. AA genotype had more semen volume than BB ($p<0.05$) and it had significantly higher than AB at sperm density ($p<0.05$). No difference was found between CC, CD and DD ($p>0.05$) at semen volume but CC significantly higher than DD at sperm density ($p<0.05$). Sperm volume and sperm density among genotypes of SS, ST and TT were no significant significance ($p>0.05$). There were no significant differences at the sperm motility and the rate of abnormal sperm between all different genotype and haplotype ($p>0.05$). The haplotype effect of AACC was higher than the single genotype effect and significant higher than BBDD at sperm density and semen volume ($p<0.05$). We tentatively conclude that *NCOA1* gene is the major gene affecting semen quality or linked to the major gene and the mutation could be used as the molecular genetic marker to select shaobo rooster for high semen quality.

Key words: Rooster, *NCOA1* gene, SNPs, semen quality, genotypes, sperm, China

INTRODUCTION

The shaobo chicken is a special meat breed in China and the qualities of the chicken meat suit Chinese consumers' tastes. They have some good qualities including good endurance, strong antidisease, flavor and getting on well with others easily. Therefore, it is necessary to study the shaobo chicken by the molecule marker method in an effort to efficiently improve its reproductive traits which will help to meet the market demand for increased production.

The nuclear receptor coactivator can enhance transcriptional activation by interacting with nuclear receptors bound to DNA. The nuclear receptors play important roles in a wide variety of biological processes included development, homeostasis and reproduction (Leo and Chen, 2000). The Nuclear receptor Coactivator 1 (NCOA1) as a member of superfamily of nuclear receptor coactivators, an also enhance transcriptional activity of Estrogen Receptor (ER) and Androgen Receptor (AR) (Onate *et al*., 1995; Wong *et al*., 2001) and affect hormone-dependent sexual differentiation of the brain and adult sexual behaviors (Auger *et al*., 2000, 2002).

Behavioral tests revealed that NCOA1 null mice exhibited normal hippocampal function but moderated motor dysfunction and that disruption of NCOA1 specifically delayed the Purkinje Cells (PCs) development and maturation in early stages and resulted in moderate motor dysfunction in adulthood mice (Nishihara *et al*., 2003). Moreover, although NCOA1 null mutant mice not resulted in infertile, exhibit decreased growth of steroid-responsive tissues such as the uterus, prostate and testes, compared with wild-type mice (Xu *et al*., 1998). The association of NCOA1 with prolificacy trait in pig has been reported and a SNP was found and confirmed *NCOA1* gene can be known as a candidate gene correlated with prolificacy trait in pig (Melville *et al*., 2002) therefore, *NCOA1* gene play central role in mediating reproduction performance in animals.

The effects of NCOA1 on semen quality performance in chicken have not been reported. These findings provide scientific basis for the research, it is necessary to study the effect of NCOA1on semen quality performance in chicken. The index of chicken production efficiency is not only the laying rate but more important lies in its egg rate of fertilization and the hatching rate. Despite of the

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performance of egg production has already got more notable genetic improvement, the rate of fertilization and hatching rate of Chinese nature breeds which associated with semen quality of rooster were low than commercial breeds of egg-type chickens. Traditional selection depending on phenotypic value of broiler has made a little improvement in the rate of fertilization and hatching rate because of the heritability is very low.

Understanding the genetic control of reproduction in chickens will provide an opportunity for genetic enhancement of production performance and physiology. Molecular marker assisted selection may be required to increase selection efficiency and further improve production performance.

The objectives of the present study were to identify single nucleotide polymorphisms of *NCOA1* genes by developing PCR-SSCP method to detect those DNA polymorphisms and to elucidate the association between NCOA1 genotypes and semen quality and to estimate the possibility of selection for improvement of semen quality by NCOA1 genotype in chicken.

MATERIALS AND METHODS

Experimental chickens and traits: A total of sixty roosters of shaobo chicken which were bred in Poultry Institute, Chinese Academy of Agricultural Sciences, China were used for test. Semen collection for individual rooster was proceeded for every 3-4 days after 30 weeks old. Immediately after collection, the ejaculates were stored at 37°C in a water bath prior to evaluation for fresh semen quality. The fresh semen was then diluted with glycerol-citrate. Data on Semen Volume (SV), Sperm Density (SD), Sperm Motility (SM), the Rate of Abnormal Sperm (RAS) were inspected and measured to identify individual birds.

All birds were housed and reared in individual cages under the same management system. Birds were fed with commercial corn-soybean-based diets that met all NRC requirements.

DNA extraction and primer design: About 1 mL blood was sampled from plumage veins and sampled into test tubes containing an anticoagulant solution. Genomic DNA was isolated from whole blood by the phenol/chloroform method and elution into 350 μL of TE. The DNA purity was detected by 1.5% agarosegel and UV spectrophotometer, OD value is 1.762. About 21 pairs of PCR primers were designed according to the sequence of NCOA1 (Gene Bank Accession No. NC_006090.2), primers were shown in Table 1.

Polymerase chain reactions: PCR reactions were carried out in a 25 μL volume comprising 2050 ng of genomic DNA, 2 pmol of each primer, 0.2 μL of 5 U μL^{-1} Taq polymerase, 0.8 μL of 10 mmoL deoxynucleotide triphosphate, 2 μL of 10× buffer and 2 μL of 25 mmoL MgCl. PCR amplifications were performed as follows: an initial denaturation step at 95°C for 5 min followed by 30 cycles of 1 min at 94°C and a final extension step at 72°C for 10 min. Annealing temperature were shown in Table 1.

Statistical analyses: Statistical analysis of associations between genotypes of *NCOA1* gene and production performance was determined by ANOVA using a General

Table 1: Primer information of *NC0A1* gene

Location	Forward primer	Reverse primer	Length (bp)	Temperature (°C)
Exon				
1	TTGGCAGGATTCAGGAGT	TTTGCCAGAGGAAAGGAAG	231	55.6
2	TCCAGGTGCAAAGTAGGTCA	ACACGTACCTCTGGGCTGTATC	114	57.5
3	AACATGGCATTTCATTGGTG	ACCTTGCTCCAACCTCTTCA	204	56.0
4	GGAGAATTACAGTTCTTTGGCTGT	CTTACTGCACCCACCTCCAG	157	56.8
5	CATTGGATGGCTTCTTCTTCGTCGT	CTTTCTTACCTAGAGACTTGGG	186	59.0
6	CCTCAGGAAGCGACCCGACGCAAC	CTGCCCTACCTTCTCCTTCCT	172	58.0
7	GCTTTGGCAGATTTCCAGTC	TGCATGGACATTACCTGTGG	123	57.0
8	GCTCTCCTCTCTCCACCCAG	CCTTCTTGAAACAGCTGCTTG	162	56.0
9	TGGTTGTCCCCTTCAGTGAT	ACCTGTCGATGATGTGGATG	166	58.5
10	TGTTTCTGACAGGGATCACG	AGAACAGCCGAAGCTTGTTT	228	58.3
11	GCTCTCCTTTCCTTCCTCAGTTTGC	ACAGCCACGCCGCTCTCACTCC	137	58.5
12	GAGCCCCCTTTGAGTTCTG	TACCTGTGCAGGTGGGAAG	156	59.0
13	CCGCAGGGATGTCAGAGCT	CTTACTCTTCAGGTTTACTCAG	138	59.5
14	GGACGAGCTCCTGTGTCCT	AGCCTCTCTACCTGCACCAG	159	55.0
15	CCCTGACGGAGCGGTTCCAGCCCC	GGCCCTGCAGCCGCTGCT	291	57.0
16	CTGTCCTCCCTCCCCTGCAGCTGAT	CCCTTACCTGCGGGGTCATC	128	57.0
17	CCCACAGCCTCCCCTCAAC	CGGAATACTGAAAGATGTTCT	136	58.0
18	CTGATGCTGTCTGTCCTTCCTTC	TGACCTTACTTGCTGTTCCCC	212	59.5
19	CCTTTTCTCCCTCTC CATC	CAGGCATCTGCAGTGAGTTC	180	55.6
20	TATTGCTTTTGACAGGTAAACGAC	TACCTGAGATGTGCTTAGCAGT	155	57.8
21	ACAGCAGGTTCAGGTGTTTGCCGA	TTCACATTCCTCGAAAAGCGGT	184	56.0

Linear Model (GLM) performed, using the SAS 9.0 software. The model is Y = μ+genotype effect+breed effect+random error effect.

RESULTS AND DISCUSSION

Single nucleotide polymorphisms in the chicken *NCOA1* gene: SSCP analysis showed that PCR products amplified detected Single Nucleotide Polymorphism (SNP) in exon 3, 10 and 12. Sequence analysis revealed a T/A substitution at position 10155007 in exon 3, AA, AB and BB genotype were found. T/C point mutation at position 108273393 in exon 10, TT, TS and SS genotype were found. C/T point mutation at position 108273423 in exon 12 CC, CD and DD genotype were found (Fig. 1) but these three mutations did not cause alteration of the corresponding amino acid.

Association of the *NCOA1* gene SNPs with semen quality traits: There were no significant differences between genotypes in exon 10 ($p>0.05$). AA genotype birds had significant higher SV than BB ($p<0.05$) and no difference was founded with AB genotype birds ($p>0.05$). There are no significant difference among the genotype birds of CC, CD, DD ($p>0.05$) at SV. AA had significant higher SD than AB ($p<0.05$) and CC significant higher than DD genotype birds ($p<0.05$). CC had significant higher SD than CD and DD genotype birds.

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Genome      GCTCTGCCAGCTCCTCCAAATATTTGTTCTCTTGCTCCCGC
BB Genoype  GCTCTGCCAGCTCCTCCAAATATTTGTTCTCTTGCTCCCGC
AA Genoype  GCTCTGCCAGCTCCTCCAAATATTTGTTCTCTAGCTCCCGC
              *10155007
Genome      GCGCCGGCCGAGCAGGGCAGGGGCACTGTGCCAGGACCA
TT Genoype  GCGCCGGCCGAGCAGGGCAGGGGCACTGTGCCAGGACCA
SS Genoype  GCGCCGGCCGAGCAGGGCAGGGGCACCGTGCCAGGACCA
              *108273393
Genome      TGCCTGCTCGGCCGGCGCTCGGGGCCGCGGGCTGCTGCC
DD Genoype  TGCCTGCTCGGCCGGCGCTCGGGGCCGCGGGCTGCTGCC
CC Genoype  TGCCTGCTCGGCCGGCGCTCGGGGCCGTGGGCTGCTGCC
              *108273423
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Fig. 1: Alignment of sequences representing each of the three homozygotes in exon 3, 10 and 12 identified by SSCP analysis with original genome sequence

AA genotype birds also had a litter higher SM and RAS than AB and BB genotype birds but the difference was not significant. So, we could conclude that there is a relationship between this SNP site and semen quality content in chickens. Haplotypes between exon 3 and 12 were also analyzed. Birds with AACC haplotypes were higher SV, SD and SM than in those with BBDD haplotypes indicating that an additive effect exists in haplotypes homozygotes which may be transmitted to the next generation. Larger additive effect could be obtained when combining excellent genotypes (Table 2).

NCOA1which was the 1st coactivator of steroid receptors to be discovered belong to a larger p160 family of nuclear receptor co-activators (Onate *et al*., 1995). The role of NCOA1 has been confirmed *in vivo* and *in vitro* mammals. NCOA1 have been found to profoundly affect hormone-dependent sexual differentiation of the brain and adult sexual behaviors (Auger *et al*., 2000). Therefore, we speculated that the NCOA1 play central role in regulating reproductive behavior in animals. *In vivo*, NCOA1 was expressed in a variety of hormone-responsive tissues such as brain, uterus, prostate and breast (Misiti *et al*., 1998, 1999; Shim *et al*., 1999; McKenna *et al*., 1998; Auger *et al*., 2000; Meijer *et al*., 2000; Ogawa *et al*., 2001) and mediated steroid hormone responses and that loss of its co-activator function lead to partial resistance to hormone in mice (Xu *et al*., 1998). The effects of NCOA1 on prolificacy trait of pig have been reported (Melville *et al*., 2002). Therefore, there is a biological rationale for the observed finding of an association between polymorphism in NCOA1 and reproductive traits in chicken.

Chicken semen quality traits, like other economically important traits are controlled by a lot of minor modification genes and s everal major genes (Stock and

Table 2: The semen quality of individual rooster with different genotype and haplotype

Locus	Genotypes	Numbers	SV (mL)	SD (100 million mL^{-1})	SM	RAS (%)
Exon 3	AA	40	0.58 ± 0.25^{a}	35.44 ± 1.31^{a}	8.31 ± 0.18^{a}	4.21±0.35
	BB	12	0.55 ± 0.21^{b}	35.12 ± 1.23^{a}	8.12 ± 0.15^{a}	4.12±0.28
	AB	8	0.57 ± 0.24^{a}	34.62 ± 1.52^{b}	8.30 ± 0.16^{a}	4.20±0.31
Exon 12	CC	13	0.59 ± 0.22^{a}	35.50 ± 1.02^{a}	8.33 ± 0.13^{a}	4.31±0.33
	CD	2	0.58 ± 0.23^{ab}	35.02 ± 1.21^{ab}	8.24 ± 0.23^{a}	4.30±0.36
	DD	26	0.58 ± 0.25^{ab}	34.48 ± 1.22^{b}	8.16 ± 0.12^{a}	4.10±0.18
Haplotype	AACC	8	0.59 ± 0.42^{a}	35.87 ± 1.23^{a}	8.50 ± 0.15^{a}	4.20±0.36
	AACD	4	0.57 ± 0.25^{ab}	35.54 ± 1.28^{a}	8.37 ± 0.23^{a}	4.20±0.52
	ABCC	14	0.60 ± 0.28^{a}	35.54 ± 1.29^{a}	8.11 ± 0.31^{ab}	4.18±0.39
	ABCD	4	0.58 ± 0.16^{ab}	34.98 ± 1.47^{ab}	7.99 ± 0.29^{b}	4.01±0.62
	BBCC	18	0.58 ± 0.28^{ab}	34.81 ± 1.42^{ab}	7.86 ± 0.19^{b}	4.21±0.39
	BBCD	4	0.60 ± 0.37^{a}	34.59 ± 1.18^{b}	8.11 ± 0.26^{ab}	4.18±0.47
	BBDD	4	0.56 ± 0.36^{b}	34.04 ± 1.37^{b}	8.11 ± 0.07^{ab}	4.44±0.68

Means with different superscripts within the same column and locus differ significantly at small letters ($p<0.05$), capital letters ($p<0.01$). The haplotype which is <4 were note statistics

Distl, 2009). The minor genes are small but their effects are large in most cases. Some discoveries have suggested that the large effects of removal or mutation have on an animal's function (Dunn *et al*., 2004). Most traits with economic interest in animal production show continuous variation. However, their underlying genetic nature is very complex. It is possible to identify the chromosome regions containing genes affecting these traits because of todays availability of neutral polymorphisms scattered throughout the genome (Andersson *et al*., 1994).

NCOA1 as the candidate gene correlated with semen quality, was studied at first time. Three mutation points were found in *NCOA1* gene which located exon 3, 10, 12. Regarding to the effect of genetic substitution individuals with AA genotype had 0.03 mL of SV and 0.3200 million mL^{-1} of SD higher than those with BB genotype in exon 3; birds with CC genotype had 1.0200 million mL^{-1} higher SD than those with DD genotype in exon 12 in individuals with AACC haplotypes was 0.03 mL of SV and 1.8300 million mL^{-1} of SD higher than in those with BBDD haplotypes indicating that an additive effect was existed in haplotypes homozygotes which may be transmitted to the next generation.

This may be of use in marker assisted selection for meat quality traits in chicken. Molecular marker assisted selection is efficient and leads to improvements in production performance. A candidate gene approach is a powerful method for understanding the direct genetic basis involved in the expression of quantitative differences between individuals (Nagaraja *et al*., 2000). We applied the candidate gene approach to semen quality performance of shaobo chicken.

The candidate gene approach is a powerful method for finding the QTL which is responsible for genetic variation in the traits of interest in agricultural animal species (Rothschild and Soller, 1997).

Genetic polymorphism of candidate genes and their association with performance traits of animals is intensively studied in many laboratories all over the world. A number of successes have been claimed for the physiological candidate gene approach to explain trait variance (Rothschild *et al*., 1996; Meng *et al*., 2002).

CONCLUSION

It is observed that the research was discussed the association between phenotype and trait. Statistical analyses revealed that these SNPs in chicken NCOA1 are associated with semen quality traits. *NCOA1* gene can be of use in marker assisted selection for semen quality traits in shaobo chicken, therefore *NCOA1* gene how to influence on semen quality performance in chicken need further to study.

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