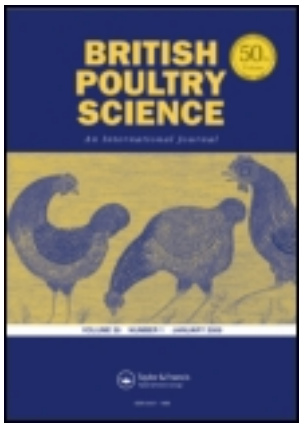

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H.Y. Huang [a], S.F Li [a], Z.H. Zhao [a], Z. Liang [a], J. Zhang [a] & Y.R. Ding [a]
[a] Institute of Poultry Science, Chinese Academy of Agriculture Science, Yangzhou 225003, P.R. China
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Association of polymorphisms for nuclear receptor coactivator 1 gene with egg production traits in the maternal line of Shaobo hens

H.Y. HUANG†, S.F LI†, Z.H. ZHAO, Z. LIANG, J. ZHANG AND Y.R. DING

Institute of Poultry Science, Chinese Academy of Agriculture Science, Yangzhou 225003, P.R. China

Abstract 1. The objectives of the study were to find polymorphic sites and elucidate the association between SNPs in the nuclear receptor coactivator 1 (NCOA1) gene and reproductive traits.
2. SNPs were detected by PCR-SSCP and DNA sequencing. Four SNPs were detected, including T10155007A, T10125838C, G10118492A and G10109315T. Three polymorphisms were associated with total egg production at the age of 300 d and the G10109315T polymorphism was associated with age at first egg.
3. In conclusion, the NCOA1 gene can be used as a molecular marker for reproductive traits in hens.

INTRODUCTION

The nuclear receptor coactivator 1 (NCOA1), also known as the steroid receptor coactivator 1 (SRC-1), was the first coactivator of steroid receptors to be discovered and belongs to a larger p160 family of nuclear receptor coactivators (Onate *et al*., 1995). The role of NCOA1 has been confirmed *in vivo* and *in vitro* in mammals. *In vitro*, the NCOA1 can enhance the transcriptional activity of the oestrogen receptor (ER) and progesterone receptors (PR) in a ligand-dependent manner (Onate *et al.*, 1995; Wong *et al*., 2001). The research revealed that the ER exist in two forms, α and β (Kuiper *et al.*, 1996), which were both essential for normal reproductive behaviour (Rissman *et al.*, 1997; Ogawa *et al*., 1999). The A gene knockout experiment also showed that female mice lacking both ERα and ERβ were infertile (Couse *et al.*, 1999; Dupont *et al*., 2000). Moreover, disruption of either oestrogen or progesterone receptors in female mice completely diminished their sexual receptivity (Mani *et al*., 1997; Rissman *et al*., 1997). NCOA1 has been found to profoundly affect hormone-dependent sexual differentiation of the brain and adult sexual behaviours (Auger *et al*., 2000; Auger *et al*., 2002). Therefore, we speculated that the NCOA1 played a central role in regulating reproductive behaviour in animals. *In vivo*, NCOA1 was expressed in a variety of hormone-responsive tissues such as brain, uterus, prostate and breast (McKenna *et al*., 1998; Misiti *et al*., 1998, 1999; Xu *et al*., 1998; Shim *et al.*, 1999; Auger *et al*., 2000; Meijer *et al*., 2000; Ogawa *et al*., 2001). Mediated steroid hormone responses and loss of steroid hormone coactivator function led to a partial resistance to hormone stimulation in mice (Xu *et al.*, 1998). Recently, it was reported that NCOA1 genotypes were associated with prolificacy and productivityin the Meishan pig, affecting traits such as individual weight at birth and individual weight at 30 d of hybrid pigs (Landrace ♀ × Wild boar ♂) (Melville *et al.*, 2002; Zhang *et al.*, 2010). However, the relationship between NCOA1 genotypes and egg productivity of hens has yet to be not been determined.

The maternal line of Shaobo hen is a dual purpose chicken with low egg productivity. However, genetic progress to enhance egg productivity has been slow using conventional breeding methods. Therefore, the objectives of the present study were to find polymorphic sites in

Correspondence to: Researcher Shoufeng Li, Institute of Poultry Science, Chinese Academy of Agriculture Science, Yangzhou 225003, P.R. China. E-mail: yzlsf3333@126.com
†Both authors contributed equally to the work.
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NCOA1 and to elucidate the association between NCOA1 genotypes and egg production, which could then be used for marker assisted selection for improved egg production in Shaobo hens.

MATERIALS AND METHODS

Birds and traits

The maternal line of Shaobo hens ($n = 348$) were raised at the Poultry Institute, Academy of Chinese Agricultural Science. All hens were housed and reared in individual cages and fed the same diets under the same management protocols. Egg-laying performance, including age at first egg (AFE), weight at first egg (WFE) and total egg production to 300 d of age was recorded on a daily basis.

DNA extraction

Blood samples were collected from a wing vein for each bird at 300 d, and genomic DNA was isolated by a phenol-chloroform method. The DNA purity was detected by 1·5% agarose gel and UV spectrophotometer, ratio of OD 260/280 was 1·762.

Primer design

Twenty-three pairs of primers were synthesised based on the published sequence of the NCOA1 gene (Genebank accession No. NC_006090·2).The primers were designed and synthesised by TaKaRa Biotechnology (Dalian) Co, Ltd., China (Table 1).

Polymerase chain reactions and sequencing

PCR reactions were carried out in a 25 μL volume comprising 20–50 ng of genomic DNA, 2 pmol of each primer, 0·2 μL of 5 U/μL Taq polymerase, 0·8 μL of 10 mmol deoxynucleotide triphosphate, 2 μL of 10 × buffer and 2 μL of 25 mmol MgCl. All PCR reactions were performed using Mastercycle Gradient PCR machines (Eppendorf, Westbury, NY). Annealing temperature is shown in Table 1. Individual birds with different genotype were sequenced by TaKaRa Biotechnology (Dalian) Co, Ltd., China.

SSCP methodology

Four μL of PCR products were mixed with 8 μL loadinq buffer (98% formamide, 0·025% bromophenol blue, 0·025% xylene cyanol, 10 mM EDTA, 10% glycerol) and the mixture denatured at 98°C for 10 min, placed on ice for 10 min, and electrophoresed for 11 to 12 h at 10 V on a 12% PAG. The silver stain method was used to display the bands And individual PCR-SSCP banding patterns were determined under visible light.

Statistical analysis

The model of $y_{ij} = \mu + G_i + e_{ij}$ was used to analyse the association between NCOA1 genotypes and reproductive traits, where y_{ij} is the dependent variable of the jth bird with the ith genotype, μ is the overall population mean, G_i is the fixed effect of the ith NCOA1 marker genotype and e_{ij} as the random error effect. Statistical analysis was carried out with SAS 9.0

Table 1. *PCR Primers of NCOA1 gene in a maternal line of Shaobo hens*

Location	Sense primers (5′-3′)	Anti-sense primers (5′-3′)	Length bp	Tm (°C)
Exon 1	TTGGCAGGATTCAGGAGT	TTTGCCAGAGGAAAGGAAG	231	55·6
Exon 2	TCCAGGTGCAAAGTAGGTCA	ACACGTACCTCTGGGCTGTATC	114	57·5
Exon 3	AACATGGCATTTCATTGGTG	ACCTTGCTCCAACCTCTTCA	204	56
Exon 4	GGAGAATTACAGTTCTTTGGCTGT	CTTACTGCACCCACCTCCAG	157	56·8
Exon 5	CATTGGATGGCTTCTTCTTCGTCGT	CTTTCTTACCTAGAGACTTGGG	186	59
Exon 6	CCTCAGGAAGCGACCCGACGCAAC	CTGCCCTACCTTCTCCTTCCT	172	58
Exon 7	GCTTTGGCAGATTTCCAGTC	TGCATGGACATTACCTGTGG	123	57
Exon 8	GCTCTCCTCTCTCCACCCAG	CCTTCTTGAAACAGCTGCTTG	162	56
Exon 9	TGGTTGTCCCCTTCAGTGAT	ACCTGTCGATGATGTGGATG	166	58·5
Exon 10	TGTTTCTGACAGGGATCACG	AGAACAGCCGAAGCTTGTTT	228	58·3
Exon 11	GCTCTCCTTTCCTTCCTCAGTTTGC	ACAGCCACGCCGCTCTCACTCC	137	58·5
Exon 12	GAGCCCCCTTTGAGTTCTG	TACCTGTGCAGGTGGGAAG	156	59
Exon 13	CCGCAGGGATGTCAGAGCT	CTTACTCTTCAGGTTTACTCAG	138	59·5
Exon 14	GGACGAGCTCCTGTGTCCT	AGCCTCTCTACCTGCACCAG	159	55
Exon 15	CCCTGACGGAGCGGTTCCAGCCCC	GGCCCTGCAGCCGCTGCT	291	57
Exon 16	CTGTCCTCCCTCCCCTGCAGCTGAT	CCCTTACCTGCGGGGTCATC	128	57
Exon 17	CCCACAGCCTCCCCTCAAC	CGGAATACTGAAAGATGTTCT	136	58
Exon 18	CTGATGCTGTCTGTCCTTCCTTC	TGACCTTACTTGCTGTTCCCC	212	59·5
Exon 19	CCTTTTCTCCCTCTC CATC	CAGGCATCTGCAGTGAGTTC	180	55·6
Exon 20	TATTGCTTTTGACAGGTAAACGAC	TACCTGAGATGTGCTTAGCAGT	155	57·8
Exon 21	ACAGCAGGTTCAGGTGTTTGCCGA	TTCACATTCCTCGAAAAGCGGT	184	56
Intron 8	GAA GGT AACATGCTCTCTTCGC	GTCATCACTGAAGGGGACAACC	358	57
Intron 15	CTC CCA ACCAGCTGCGACTTCA	TGGTTGAGGACTGCCTGCCG	262	59·5

for Windows (SPSS Inc., Chicago, IL). The differences were tested with ANOVA using a general linear model (GLM). A *P*-value less than 0·05 was considered significant.

RESULTS

PCR-SSCP analysis

SSCP analysis showed that a single nucleotide polymorphism (SNP) was detected in exon3, exon10, exon12 and intron15. Sequence analysis revealed a T/A substitution at position 10155007, genotypes A_1A_1, A_1A_2 and A_2A_2 were found in exon3 (Figure A), a T/C point mutation at position 10125838, B_1B_1, B_1B_2, and B_2B_2 in exon10 (Figure B), a G/A point mutation at position 10118492, C_1C_1, C_1C_2 and C_2C_2 were detected in exon12 (Figure C) and a G/T substitution at position 10109315, D_1D_1 and D_1D_2 in intron15; but a D_2D_2 genotype was not found (Figure D). However, the three mutations in the exon regions did not cause an alteration of the corresponding amino acid.

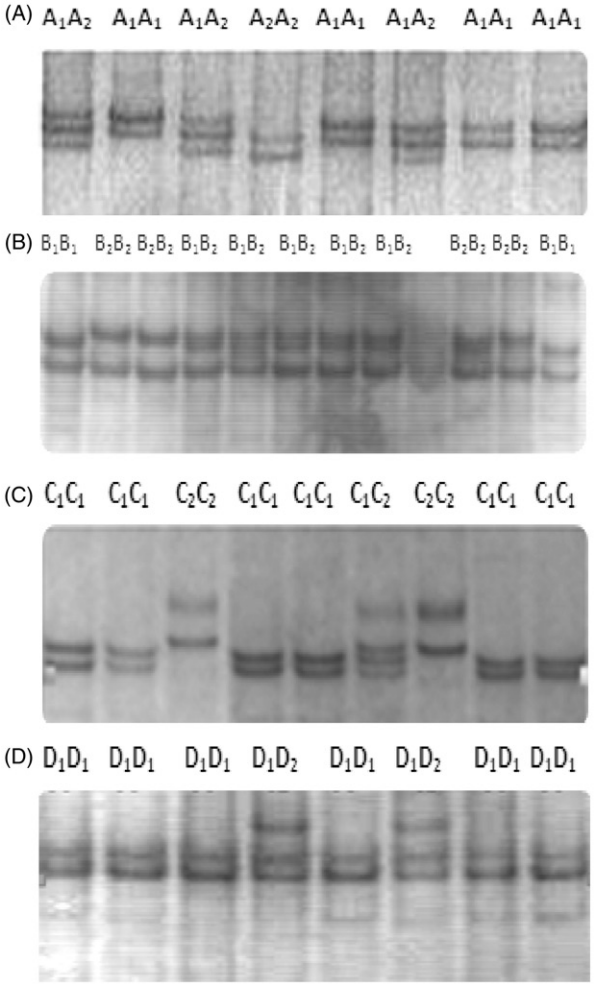

Figure. *(A) SSCP analysis on PCR amplification in exon 3, (B) SSCP analysis on PCR amplification in exon 10, (C) SSCP analysis on PCR amplification in exon 12 and (D) SSCP analysis on PCR amplification in intron 15 in a maternal line of Shaobo hens.*

Genotypic and allelic gene frequency

The frequency of allele A_1 and A_2 were 0·49 and 0·51, B_1 and B_2 were 0·66 and 0·34, C_1 and C_2 were 0·53 and 0·47 respectively. The observed distribution of genotypes was not different from the distribution expected under the assumption of Hardy-Weinberg equilibrium (Table 2).

Correlation between genotypes and reproductive traits

A_1A_1 genotype birds laid significantly more eggs to 300 d than A_1A_2 and A_2A_2 birds, B_1B_1 had significantly more than B_1B_2 and B_2B_2 birds, and C_1C_1 had significantly better egg production than C_1C_2 and C_2C_2 birds. A_1A_1 birds had the highest egg numbers, 89·0 eggs compared with 87·5 eggs for B_1B_1 and 89·0 eggs for C_1C_1. AFE in D_1D_1 birds was significantly earlier than D_1D_2 birds; egg numbers in D_1D_1 birds were numerically higher than that D_1D_2 birds, but the difference was not significant. The WFE was not significantly between genotypes (Table 3).

DISCUSSION

A nuclear receptor coactivator can enhance transcriptional activation and interact with nuclear receptors bound to DNA. Nuclear receptors play important roles in a wide variety of biological processes, including development, homeostasis and reproduction (Leo and Chen, 2000). The role of NCOA1, which was a member of the superfamily of nuclear receptor

Table 2. *Gene frequency and genotype frequency in a maternal line of Shaobo hens*

Location	Genotype frequence			Allelic frequence		x^2 value[1]
Exon 3	0·27 (A_1A_1)	0·44 (A_1A_2)	0·29 (A_2A_2)	0·49 (A_1)	0·51 (A_2)	5·38
Exon 10	0·49 (B_1B_1)	0·35 (B_1B_2)	0·16 (B_2B_2)	0·66 (B_1)	0·34 (B_2)	15·08
Exon 12	0·32 (C_1C_1)	0·41 (C_1C_2)	0·26 (C_2C_2)	0·53 (C_1)	0·47 (C_2)	11·21
Intron 15	0·54 (D_1D_1)	0·46 (D_1D_2)		0·77 (D_1)	0·23 (D_2)	8·18

[1] χ^2 (df = 2,0·05) = 5·99, χ^2 (df = 2,0·01) = 9·21.

Table 3. *Correlation analysis between NCOA1 genotypes and mean(±sed) egg-laying traits in a maternal line of Shaobo hens*

Loci	Genotype	Trait		
		AFE (d)	WFE (g)	Eggs laid to 300 d
Exon 3	A_1A_1	153·3 ± 12·25	39·5 ± 3·27	89·0 ± 16·78[a]
	A_1A_2	154·4 ± 11·31	40·0 ± 2·56	82·9 ± 17·21[bc]
	A_2A_2	155·6 ± 13·27	40·1 ± 4·13	82·6 ± 18·36[b]
Exon 10	B_1B_1	155·3 ± 10·63	39·6 ± 4·10	87·4 ± 11·41[a]
	B_1B_2	157·1 ± 10·69	41·0 ± 3·68	83·0 ± 13·97[b]
	B_2B_2	160·4 ± 12·81	40·2 ± 3·57	80·3 ± 10·36[bc]
Exon 12	C_1C_1	152·8 ± 12·62	39·8 ± 3·15	89·0 ± 19·20[a]
	C_1C_2	154·2 ± 13·55	40·0 ± 3·76	84·9 ± 17·35[bc]
	C_2C_2	154·9 ± 11·37	40·9 ± 3·87	81·6 ± 17·53[b]
Intron 15	D_1D_1	155·2 ± 10·57[a]	39·5 ± 3·74	86·2 ± 10·71
	D_1D_2	158·7 ± 11·49[b]	40·6 ± 3·93	82·8 ± 14·47

Within a column and within a locus, means without superscripts or the same superscript are not significantly different at $P > 0·05$.
All values are expressed as means ± SE.

coactivators, has been studied *in vivo* and *in vitro* in mammals. However, behavioural tests also revealed that NCOA1-null mice exhibited normal hippocampal function, but moderated motor dysfunction. The disruption of NCOA1 specifically delayed the development and maturation of Purkinje cells (PCs) in the early stages of growth and resulted in moderate motor dysfunction in adult mice (Nishihara *et al*., 2003). Although NCOA1-null mutant mice did not become infertile, they did have decreased growth of steroid-responsive tissues, such as the uterus, prostate and testes, compared with wild-type mice (Xu *et al*., 1998). The relationship of NCOA1 with prolificacy in pigs has been reported; a SNP was found that was correlated with prolificacy in pigs (Melville *et al*., 2002) and provided the scientific basis for the present research.

The analysis of the relationship between genotypes and egg traits indicated that three SNP sites located in the exon regions of the NCOA1 gene have significantly increased egg production in hens, whilst the only SNP site located at intron 15 significantly advanced AFE. No significant differences in WFE were detected among the different genotypes, however, a close inspection of the data in Table 3 suggests a trend for earlier-AFE birds (lighter weight) to produce more eggs and have lighter WFE, and so we putatively conclude that A_1A_1, B_1B_1 and C_1C_1 genotypes are the dominant genotypes in this maternal line of Shaobo hens. Whilst it is possible that this will not be the case in all breeds and under all management systems, higher egg productivity may be obtained if the breeding is based on the effects of genotype found in this maternal line of Shaobo hens. At the same time, these findings provide important information for selection for egg productivity in other breeds of hen.

The current results confirmed the association between NCOA1 and egg productivity in this line of Shaobo hens, and so NCOA1 genotypes may act as genetic markers for egg productivity in this breed. How the NCOA1 gene influences reproductive performance in hens needs further study.

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