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A Novel SNP of *LXRα* Gene Associated with Meat Quality Traits in Cherry Valley Ducks

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Abstract.- Liver X receptor alpha (LXRα) is a nuclear receptor that play a crucial role in regulating the expression of genes involve in lipid metabolism. The aims of this study were to detect the polymorphisms of *LXRα* gene and investigate their associations with Cherry Valley duck meat quality traits (n=450). A novel mutation 277C>G was first identified in exon 2 of *LXRα* gene, which resulted in three genotypes of CC, CG and GG. C allele frequencies and polymorphism information content (PIC) were 0.6200 and 0.3602, respectively. Chi-Square test indicated that genotypes distribution were in accord with Hardy Weinberg equilibrium ($P>0.05$). Least square analysis showed that the birds with genotype CC had significant lower than genotype CG and genotype GG for polyunsaturated fatty acid (PUFA) and essential fatty acid (EFA) ($P<0.05$). The research suggested that the 277C>G mutation of duck *LXRα* gene may be have significant effect on duck fatty acid composition, and G allele may be advantage for improving duck's PUFA and EFA.

Key Words: Cherry Valley duck, *LXRα* gene, gene polymorphism, meat quality traits.

INTRODUCTION

Poultry meat quality traits which including pH-values, shear force, water holding capacity and intramuscular fat are economically important in modern poultry production. The candidate gene approach is widely used to investigate genetic effect of gene polymorphisms on meat quality traits in fowl (Wang *et al.*, 2013). However, selection efficiency depends on allelic frequencies in the population and the power of polymorphisms locus on selected traits (Lenstra *et al.*, 2012). The combination of traditional breeding methods and modern molecular genetics methods may be useful for genetic improvement of the animals with important economic traits in the future (Davis and Hetzel, 2000; Groeneveld *et al.*, 2010).

Liver X receptors (LXRs) are ligand-activated transcription factors that belong to the nuclear receptor superfamily (Willy *et al.*, 1995; Korach-André *et al.*, 2011). The LXR subfamily consists of LXRα and LXRβ, two isoforms that are highly related and share ~78% identity of their amino acid sequences in ligand-binding domains (LBDs), their nuclear retention and localization as well as function display some differences (Grażyna *et al.*, 2007). In mammal, LXRα is specific expression in liver, and at lower levels in the macrophages, intestine, lung, adipose tissue, kidney and adrenal glands, while LXRβ is generally expressed in various tissues. LXRα act as a major regulator of lipid homeostasis through activation of sterol regulatory element binding protein-1c (SREBP-1c) (Howell *et al.*, 2009). It is widely accepted that endogenous LXR agonists are oxidized cholesterol derivatives referred to as oxysterols. Most oxysterols have similar affinity toward both LXR isoforms with the exception of 5,6-24(S), 25-dieopxycholesterol and 6α-hydroxy bile acids which are somewhat selective for LXRα (Song *et al.*, 2000). Polyunsaturated fatty acids (PUFA) were found to be competitive LXR antagonists in various cell lines (Ou *et al.*, 2001). PUFA suppressed transcriptional activity of sterol regulatory element binding protein 1 (SREBP-1) of the major LXR target genes, but this action is independent of LXRα (Pawar *et al.*, 2003). The LXRα autoregulatory loop is generally found to

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human cells since it was not detected in murine preadipocytes and macrophages (Li *et al.*, 2002). *LXRα* transcriptional activity is also regulated post translationally by protein kinase A that phosphorylates receptor protein at two sites thereby impairing its dimerization and DNA-binding (Kase *et al.*, 2007). Recent researches demonstrated that the *LXRα* gene had important effects on mammalian carcass and meat quality (Huang *et al.*, 2010).

Currently, many genes which involve in lipid metabolism related to meat quality traits have been reported in chickens, but the study on duck is still limited. In this study, the polymorphisms of *LXRα* gene were detected and its associations with Cherry Valley duck meat quality traits were also investigated, which may give an insight into the regulatory mechanism of meat quality and be helpful for improving meat quality traits in duck breeding.

MATERIALS AND METHODS

Samples and data collection

Blood samples were collected from 450 healthy Cherry Valley female ducks (aged at 10 weeks). All the ducks were reared under normal management conditions in animal farm of College of Animal Science, Guizhou University, Guiyang, Guizhou, P.R.China. The ducks were raised in a semi-open house and subjected to conventional management conditions, fed commercial corn–soybean diets that met NRC requirements and were slaughtered with appropriate humane methods at 70 days of age.

Meat quality traits, including pH-value, water holding capacity (%), shear force (kg.cm-1), intramuscular fat (IMF)(%), unsaturated fatty acid (UFA)(%), polyunsaturated fatty acid (PUFA) (%), and essential fatty acid (EFA)(%) of breast muscle of each individual duck, were measured within 48 h post mortem as described by Aldai *et al*. (2008) and Huang *et al*. (2007).

DNA isolation

Genomic DNA was isolated from blood samples using standard phenol–chloroform method, and stored at -20°C for use. DNA concentration and quality were measured by the spectrophotometer ND-1000 (Nano-Drop, USA), and the concentrations were adjusted to 100 ng/μl.

Primer sequences

Based on the duck *LXRα* gene mRNA sequences (Accession no. FJ 966078), the following four pairs of primers (LXRα-F1/R1-LXRα-F4/R4, Table I) were designed by using the Oligo 6.0 program to amplify the duck *LXRα* gene.

SNP screening and genotyping

All the DNA samples from Cherry Valley female ducks were used to perform PCR-SSCP. PCR reactions were carried out in a total volume of 20 μl with 100 ng of genomic DNA, 5 pmol each of forward and reverse primer, 2.5 μl 10×buffer, 1.5 mM of $MgCl_2$, 0.16 mM dNTP and 1 U Taq DNA polymerase (Sangon Shanghai, China). PCR program: initial denaturation for 10 min at 95°C, 30 cycles each 45 s at 94°C, 45 s at specific annealing temperatures (Table I), 45 s at 72°C and 10 min final extension at 72°C.

Aliquots of 5 μl PCR products were mixed with 10 μl denaturing solution (98% formamide, 2% glycerine, 25 mM EDTA, 0.025% xylenecyanole and 0.025% bromophenol blue), heated for 10-15 min at 98°C and rapidly cooled on ice. Denatured PCR products were subjected to 12% acrylamide: bisacrylamide (39:1) gels in 1× TBE buffer and constant voltage (130 V) for 12 -14 h. The gel was stained with 0.1 % silver nitrate and visualized with 2% NaOH solution. The PCR fragments from different SSCP patterns in different individuals were sequenced in both directions (Sangon Shanghai, China). Polymorphism sites were analyzed by sequence comparisons using DNAStar software (DNAstar Inc., Madison, WI, USA).

Association analysis

The genetic parameters of allele and genotype frequencies, effective number of alleles (Ne), heterozygosity (h) and polymorphism information content (PIC) were estimated by PopGen32 v1.31 soft. The general linear model (GLM) procedures of SPSS 17.0 was used to determine associations between the different genotypes and duck meat quality traits according to the following model:

$$Y = \mu + G + e$$

Where: Y-dependent variable (analyzed traits), μ-overall mean, G- fixed effects of genotype, and e-random error. Multiple comparisons were performed with the least squares means. Values are considered significant at $P<0.05$ and are presented as least square mean ±standard error.

RESULTS AND DISCUSSION

Polymorphism identification and detection

Genomic DNA of *LXRα* gene for all individuals were successfully amplified using LXRα-F1/R1-LXRα-F4/R4 primers. The PCR products were separated on 1.5% agarose gels. The results showed that amplification fragment sizes were consistent with the target ones and had good specificity, which could directly be analysed by sequencing and SSCP.

No polymorphism were identified in PCR products amplified by primers LXRα-F1/R1, LXRα-F3/R3 and LXRα-F4/R4. The PCR products amplified by primers LXRα-F2/R2 displayed polymorphism, and three genotypes of CC, CG and GG were detected (Fig.1). By aligning the polymorphism sequences, the 277C>G silent mutation (ss831878775) occurred in exon 2 of *LXRα* gene (Fig.2).

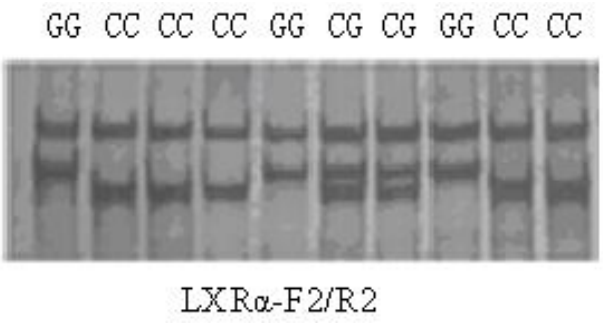

Fig. 1. PCR-SSCP band pattern of primers LXRα-F2/R2.

Allele and genotype distribution

Allele gene and genotype frequencies, heterozygosity (h) and polymorphism information content (PIC) of the 277C>G mutation for Cherry Valley duck *LXRα* gene were presented in Table II. C allele frequencies and PIC of the 277C>G locus were 0.6200 and 0.3602, respectively. Allele C was predominant while CG was most common among the three genotypes. Botstein *et al*. (1980) reported that a PIC value of greater than 0.5 is considered to be highly informative, PIC values from 0.25 to 0.5 is considered to be moderately informative, and the rest (PIC<0.25) is considered to be slightly informative. Under this formulation, the 277C>G locus belonged to medium polymorphism (0.25<PIC<0.5), which implied that own abundant genetic diversity in Xingyi duck populations. The genotypes distribution were in accord with Hardy-Weinberg equilibrium ($P>0.05$), in which genotypes frequencies had not been distorted by mutation, migration, selection or other reasons.

Association of the 277C>G locus of LXRα gene with Cherry Valley duck meat quality traits

In present analyses, the association of the 277C>G mutation of *LXRα* gene with Cherry Valley duck meat quality traits were showed in Table III. The results indicated that the birds with genotype CC had significant lower polyunsaturated fatty acid (PUFA) and essential fatty acid (EFA) than genotype CG and genotype GG ($P<0.05$). No significant associations of other genotypes with other traits were detected ($P>0.05$).

In avian species, several previous studies have reported some genes associated with meat quality traits, such as very low density apolipoprotein-II (*apoVLDL-II*) gene (Zhang *et al*., 2010), krüppel-like factor 7 (*KLF7*) gene (Zhang *et al*., 2013), insulin-like growth factor-1 (*IGF1*) gene (Sato *et al*., 2012). LXRα is important regulator of cholesterol, fatty acid, glucose homeostasis and immune response. Recent studies indicated that the *LXRα* gene had important effects on mammalian carcass, meat quality and growth traits. Yu *et al*. (2006) reported that the *LXRα* intron 8 HpyCH4 III polymorphism was significantly associated with loin eye area and total lipid in individuals from the Berkshire and Yorkshire (BY) resource family, and significant associations were also confirmed between *Bsl* I polymorphism in *LXRα* exon 2 and boneless loin, as well as marbling score in one commercial line. Hoashi *et al*. (2008) reported that the synonymous substitution G>A found in *LXRα*

Table I.- Primers used in PCR amplification.

Primers	Primer sequence (5'→3')	Position	Tm (□)
LXRα-F1/ R1	F1: CTGCTGCTCCTTACTCTGC R1: GTGAAAGCCCTTCCTCCTC	1-156	60
LXRα-F2/ R2	F2: AAGATGCTGGGAAATGAA R2: ACATGTCCATCTCACACTTGC	228-392	58
LXRα-F3/ R3	F3: GACCGCCTGATGTTTCCAC R3: TGGGACATGGTGTTATGTCG	1157-1350	60
LXRα-F4/ R4	F4: GTGTCCCAGCCTTTGCTAAC R4: ATCCCCAGGACATGCTTAGA	1343-1626	60

Table II.- Characteristics of the 277C>G locus of *LXRα* gene in Cherry Valley ducks

Genotype			Allele		H	PIC	χ2
CC	CG	GG	C	G			
0.3933	0.4534	0.1533	0.6200	0.3800	0.4712	0.3602	0.32^{ns}

CV, Cherry Valley ducks; H, heterozygosity; PIC, polymorphism information content; χ2, Chi-square value; ns, non-significant.

Table III.- Association of meat quality traits with the 277C>G locus of *LXRα* gene in Cherry Valley ducks (LSM±SE)

Traits	Cherry Valley ducks (CV)		
	CC(177)	CG(204)	GG(69)
pH-value	5.963±0.070	6.065±0.042	5.920±0.165
Water holding capacity (%)	22.528±0.484	20.512±0.169	21.990±0.875
Shear value(kg.cm^{-1})	1.588±0.147	1.648±0.088	1.741±0.244
Intramuscular fat (IMF) (%)	9.520±0.375	9.922±0.165	9.932±0.608
Unsaturated fatty acid (UFA) (%)	58.807±0.516	60.050±0.464	60.731±0.678
Polyunsaturated fatty acid (PUFA) (%)	$17.844±0.292^{a}$	$19.361±0.226^{b}$	$20.860±0.621^{b}$
Essential fatty acid (EFA) (%)	$17.572±0.485^{a}$	$18.736±0.321^{b}$	$20.418±0.707^{b}$

LSM: least square means; SE: standard error;
^{a,b}Means with no common superscript differ (P<0.05) as found by LSD.

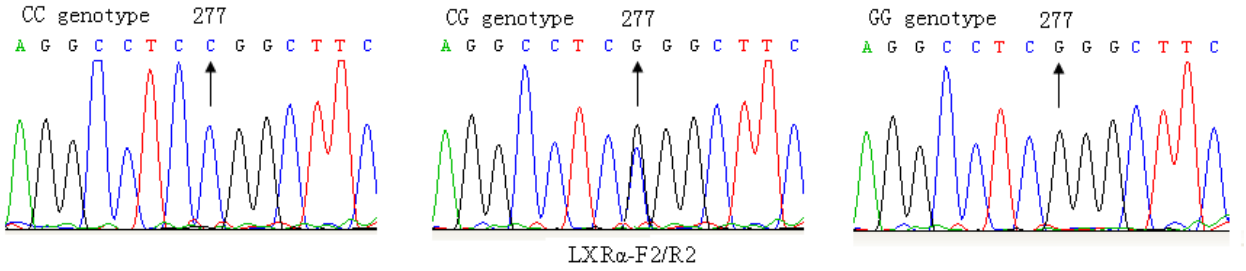

Fig. 2. Sequencing map with different genotypes in primers LXRα-F2/R2

exon 4 causes an amino acid change from valine to isoleucine at the 133 amino acid position, which is significant association between the SNP with linoleic acid (C18:2n-6) of intramuscular fat in the Japanese black cattle. Huang *et al*. (2010) reported that the T1530C mutation in *LXRα* exon 2 had significant effect on backfat thickness, carcass length and marbling score in Qinchuan cattle. Han

et al. (2013) reported that the G>A mutation in *LXRα* exon 4 was associated with the concentration of 9c, 11t C18:2, sum of conjugated linoleic acids (CLA) and 11c C20:1 in Canadian commercial crossbred beef steers. However, there had not reported that polymorphisms of *LXRα* gene associated with economic characters in poultry.

It has been shown that genetic variation is the inner factor for cultivating new varieties or types. Take together, our data suggested that the 277C>G locus may be have significant genetic effect on duck fatty acid compodision, and G allele may be advantage for duck PUFA and EFA. The information on the 277C>G mutation of *LXRα* gene obtained in this study may be applied to effective marker-assisted selection to increase the levels of PUFA and EFA in ducks. Although, the 277C>G mutation has not cause amino acid change, but it also has effect on the expression of *LXRα* gene which may result in the difference of duck meat quality traits. However, because of the lack of functional test and verify data, the conclusion is not solid and further studies are necessary for function validation of polymorphism in question. Meanwhile, studies with larger sample sizes and polymorphism of other regions of *LXRα* gene in duck are recommended in the future.

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Conflict of interest declaration

Our manuscript has no conflict of interest.

REFERENCES

ALDAI, N., DUGAN, M.E., KRAMER, J.K., MIR, P.S. AND MCALLISTER, T.A., 2008. Nonionophore antibiotics do not affect the trans-18:1 and conjugated linoleic acid composition in beef adipose tissue. *J. Anim. Sci.*, **86**: 3522-3532.

BOTSTEIN, D., WHITE, R.L., SKOLNICK, M. AND DAVIS, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.*, **32**: 314-331.

DAVIS, G.P. AND HETZEL, D.J.S., 2000. Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species. *Aquacul. Res.*, **31**: 3-10.

GRAŻYNA, W., ANNA, J.W., KRZYSZTOF, H. AND JERZY, B., 2007. Liver X receptors (LXRs). Part I: Structure, function, regulation of activity, and role in lipid metabolism. *Postepy. Hig. Med. Dosw.*, **61**: 736-759.

GROENEVELD, L.F., LENSTRA, J.A., EDING, H., TORO, M.A., SCHERF, B., PILLING, D., NEGRINI, R., FINLAY, E.K., JIANLIN, H., GROENEVELD, E., WEIGEND, S. AND GLOBADIV, T.C., 2010. Genetic diversity in farm animals – a review. *Anim. Genet.*, **41**: 6-31.

HAN, C., VINSKY, M., ALDAI, N., DUGAN, M.E., MCALLISTER, T.A. AND LI, C., 2013. Association analyses of DNA polymorphisms in bovine *SREBP-1*, *LXRα*, *FADS1* genes with fatty acid composition in Canadian commercial crossbred beef steers. *Meat Sci.*, **93**: 429-436.

HOASHI, S., HINENOYA, T., TANAKA, A., OHSAKI, H., SASAZAKI, S., TANIGUCHI, M., OYAMA, K., MUKAI, F. AND MANNEN, H., 2008. Association between fatty acid compositions and genotypes of *FABP4* and *LXR-alpha* in Japanese black cattle. *BMC Genet.*, **9**: 84.

HOWELL, G., DENG, X., YELLATURU, C., PARK, E.A., WILCOX, H.G., RAGHOW, R. AND ELAM, M.B., 2009. N-3 polyunsaturated fatty acids suppress insulin-induced SREBP-1c transcription via reduced trans-activating capacity of LXRα. *Biochim. Biophys. Acta*, **1791**: 1190-1196.

HUANG, L., ZAN, L.S., WANG, H.B. AND LIU, H.Y., 2010. Study on SNP of *LXRα* gene exon 2 and its association with some meat traits in Qinchuan cattle. *Acta Vet. Zootech. Sin.*, **41**: 531-535.

HUANG ,Y., HALEY, C.S., WU, F., HU, S., HAO, J., WU, C. AND LI, N., 2007. Genetic mapping of quantitative trait loci affecting carcass and meat quality traits in Beijing ducks (*Anas platyrhynchos*). *Anim. Genet.*, **38**: 114-119.

KASE, E.T., THORESEN, G.H., WESTERLUND, S., HOJLUND, K., RUSTAN, A.C. AND GASTER, M., 2007. Liver X receptor antagonist reduces lipid formation and increases glucose metabolism in myotubes from lean, obese and type 2 diabetic individuals. *Diabetologia*, **50**: 2171-2180.

LENSTRA, J.A., GROENEVELD, L.F., EDING, H., KANTANEN, J., WILLIAMS, J.L., TABERLET, P., NICOLAZZI, E.L., SÖLKNER, J., SIMIANER, H., CIANI, E., GARCIA, J.F., BRUFORD, M.W., AJMONE-MARSAN, P. AND WEIGEND, S., 2012.

Molecular tools and analytical approaches for the characterization of farm animal genetic diversity. *Anim. Genet.*, **43**: 483–502.

LI, Y., BOLTEN, C., BHAT, B.G., WOODRING, D.J., LI, S., PRAYAGA, S.K., XIA, C. AND LALA, D.S., 2002. Induction of human liver X receptor alpha gene expression via an autoregulatory loop mechanism. *Mol. Endocrinol.*, **16**: 506-514.

KORACH-ANDRÉ, M., ARCHER, M., BARROS, R.P., PARINI, P. AND GUSTAFSSON, J.-Å., 2011. Both liver-X receptor (LXR) isoforms control energy expenditure by regulating brown adipose tissue activity. *Proc. natl. Acad. Sci. USA*, **108**: 403–408.

OU, J., TU, H., SHAN, B., LUK, A., DEBOSE B.R.A., BASHMAKOV, Y., GOLDSTEIN, J.L. AND BROWN, M.S., 2001. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (*SREBP-1c*) gene by antagonizing ligand-dependent activation of the LXR. *Proc. natl. Acad. Sci. USA*, **98**: 6027-6032.

PAWAR, A., BOTOLIN, D., MANGELSDORF, D.J. AND JUMP, D.B., 2003. The role of liver X receptor-alpha in the fatty acid regulation of hepatic gene expression. *J. biol. Chem.*, **278**: 40736-40743.

SATO, S., OHTAKE, T., UEMOTO, Y., OKUMURA, Y. AND KOBAYASHI, E., 2012. Polymorphism of insulin-like growth factor 1 gene is associated with breast muscle yields in chickens. *Anim. Sci. J.*, **83**: 1.

SONG, C., HIIPAKKA, R.A. AND LIAO, S., 2000. Selective activation of liver X receptor alpha by 6 alpha-hydroxy bile acids and analogs. *Steroid*, **65**: 423-427.

WANG, M., WANG, Q. AND PAN, Y., 2013. From QTL to QTN: candidate gene set approach and a case study in porcine IGF1-FoxO pathway. *PloS One*, **8**: e53452.

WILLY, P.J., UMESONO, K., ONG, E.S., EVANS, R.M., HEYMAN, R.A. AND MANGELSDORF, D.J., 1995. LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Genes Dev.*, **9**: 1033-1045.

YU, M., GEIGER, B., DEEB, N. AND ROTHSCHILD, M.F., 2006. Liver X receptor alpha and beta genes have the potential role on loin lean and fat content in pigs. *J. Anim. Breed. Genet.*, **123**: 81-88.

ZHANG, Y.Y., CHEN, G.H., XU, Q., ZHANG, H.B., DUAN, X.J., ZHAO, W.M. AND SUN, G.B., 2010. Polymorphism in intron-1 of *ApoVLDL-II* gene and its association with production traits and biochemical levels in White Muscovy (Cairina moschata). *J. appl. Anim. Res.*, **38**: 23-28.

ZHANG, Z.W., WANG, Z.P., ZHANG, K., WANG, N. AND LI, H., 2013. Cloning, tissue expression and polymorphisms of chicken Krüppel-like factor 7 gene. *Anim. Sci. J.*, **84**: 535–542.

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