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Polymorphic Diversity of UBX Domain D from cDNA Isolated from Pectoral Muscle of Korean Native Chicken

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ABSTRACT The objectives of this study are to identify specific functional genes which are related with growth and protein structure of the pectoral muscle of Korean native chicken. Pectoral muscle was isolated from three Korean native chickens (KNC, red brown, 12 months old, 2.41 ± 0.24 kg) and three Cornish chickens (16 month old, 2.76 ± 3.0 kg). The subtraction cDNA library was prepared in PCR4 Blunt-TOPO vector. The DNA sequence homology was compared with other breeds and species in GenBank. A clone NDS-81 was found to be unique for the DNA sequence homology with UBX family. Their partial sequence has high homology (98%) with chicken UBX domain D. Chicken UBX domain has chicken (93%), cattle (68%), dog (67%), mouse (64%) and, human (63%) nucleotide sequence homology. Several regions were mutated from T in chicken to C or G in the NDS-81 clone. The first site is LAD in chicken, but it was expressed as (L)RM in clone NDS-81. In this site, amino acids were changed from Ala to Arg, and from Asp to Met. The second site was changed from ER (Arg) in chicken to ED (Asp) in clone NDS-81. They are both containing functional side chains and play an important role in binding other proteins. Therefore, the clone NDS-81 could be a different candidate gene for the UBX family gene and could related with pectoral muscle structure of Korean native chicken.

(Key words : UBX domain D, Korean native chicken, cDNA, muscle growth)

INTRODUCTION

Genetic resources of native animals would be important in world markets for future bio-industry. Meat quality is especially closely related to the DNA expression of muscle proteins (Caravatti et al., 1982, Sun et al., 2006). Large-scale genomic and cDNA sequencing projects have revealed thousands of new genes whose open reading frames (ORFs) are highly conserved during vertebrate evolution, but whose precise cellular functions remain unclear. Only full-length cDNAs unambiguously define the boundaries of the transcription units within whole-genome assemblies. Cloned full-length cDNAs are also of immense practical value to complement mutant phenotypes and artificially express the encoded protein (Caldwell et al., 2005).

Korean native chicken (KNC) is a conserved breed for pedigree, family line, and improving economic value. Commercial broilers have low preference and palatability although they have good economic value with high growth rate. Therefore, the KNC needs to be conserved for unique taste and meat quality (Sang et al., 2003). Also, KNC may have strong resistance against some pathogenic microorganisms (personal communication).

Ubiquitin regulator-X (UBX, erasin) is a discrete protein domain that binds p97/valosin-containing protein (VCP), a molecular chaperone involved in diverse cell processes, including endoplasmic-reticulum-associated protein degradation (ERAD). UBXD2 is highly conserved in mammals compared to poultry and other species. Erasin is an integral membrane protein of the endoplasmic reticulum and nuclear envelope with both its N- and C-termini facing the cytoplasm or nucleoplasm (Liang et al., 2006). A widely expressed protein containing UBX (ubiquitin-like) domains was identified as a substrate of SAPKs (stress-activated protein kinases) (McNeill et al., 2004). These proteins may be involved in skeletal muscle growth and structure.

The objectives of this study were to identify specific functional genes which are related with animal growth and structure of pectoral muscle in Korean native chicken.

MATERIALS AND METHODS

1. Animals

Experimental animals were described in a previous paper (Sun et al., 2006). Three Korean native chickens (KNC, red

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brown, 12 months old, 2.41 kg ± 0.24) and three Cornish chickens (16 month old, 2.76 kg ± 3.04) were obtained from the Daejeon branch of the National Livestock Research Institute, Korea. Pectoral muscles were dissected and frozen immediately in liquid nitrogen. Total RNA was isolated by RNA isolation kit (Clonetech, Korea). mRNA was analyzed by the methods of Poly (A)+ purification using PolyATract mRNA isolation System (Promega, USA).

2. mRNA Subtraction and Cloning

Two breed mRNAs were subtracted by the methods of subtractive suppression hybridization. mRNA was subtracted by suppression hybridization method using PCR-select cDNA Subtraction Kit. Cloned cDNA were inserted into PCR4 Blunt-TOPO vector. The cloned vector was transformed in TOP 10 electroporation cells. The cDNA was then used as a probe to screen 0.5×10^6 lysate plates. Specific clones were constructed by the subtraction from cDNA of Korean native chicken to cDNA of Cornish chicken.

3. cDNA Library Preparation

Isolated RNA was used Sub-Lib DNA (Eugentech, Korea). To select *E. coli* contained incorporated plasmid, *E. coli* (0.1 mL) was spread on LB/AMP/ KAN/ X-Gal plates and were incubated overnight at 37℃. The cultured colony was adjusted to 1×10^4 cfu per 10 ng of vector DNA. From cultured colony, the single white colony was inoculated in LB/AMP/KAN, and was incubated for 16~18 hrs in shaking (170 rpm) incubator until 0.6~0.8 O.D. value at 37℃. The cultured liquid (817 uL) was mixed with 80% glycerol (183 uL), and then this culture stock was stored in −70℃. This culture stocks (1.0 mL) were centrifuged in 1.5 mL tube at 3,000 rpm for 15 min, and the *E. coli* cell pellet was collected.

Vector ligation was used with 80 ng of cDNA and 10 ng of PCR4 Blunt-TOPO vector. cDNA inserted vector was transformed into TOP 10 electroporation cells (Invitrogen). The cell pellet was dissolved completely in 250 uL resuspension buffer (P1) in 1.5 mL tube, and then the plasmid was isolated using QIAprep Miniprep kit (Invitrogen). The mixture was gently mixed with 250 uL lyses buffer (P2), and incubated for 4 min at RT. 250 uL Neutralization buffer (N3) was added, incubated for 5 min, and then centrifuged for 10 min at 10,000 rpm. Supernatant was transferred into a binding column tube and was centrifuged 60 sec, and filtered liquid was discarded. PE buffer (750 uL) was added into the binding column tube and it was centrifuged for 1 min at 12,000 rpm. The binding column tube was completely dried without ethanol. The binding column tube was connected into 1.5 mL tube, 50 uL Elution buffer was added in the middle, rested for 1 min, and centrifuged for 1 min at 13,000 rpm. The isolated plasmid was digested with restriction enzymes, and the DNA size was identified with electrophoresis. The insert was sequenced (Macrogen, Korea), and the homology was compared with other breeds and species in GenBank.

RESULTS AND DISCUSSION

Six-hundred fifty nucleotides were sequenced from two-thousand clones. Six unique clones were registered in GenBank. The six clones were NDS-7 (accession no, AY466164, 847 nt), NDS-10 (accession no, EF117324.1, 495 nt), NDS-13 (accession no, EF 117325.1, 452 nt), NDS-22 (accession no, EF117326.1, 1730 nt), NDS-24 (accession no, EF117327.1, 1003 nt), NDS-81 (accession no, EF117328.1, 612 nt). A clone NDS-7 was actin-like protein and was published (Sun et al., 2006).

A clone NDS-81 was also derived from cDNA subtraction of pectoral muscle of Korean native chicken. This clone is very specific for the DNA sequence homology with UBX domain. NDS-81 has 612 nucleotides, and the translated region is 17~593 nt. The untranslated region (UTR) was expressed underlined in italics (Fig. 1). Every ten nucleotides were marked by dot. This partial sequence has high homology (98%) with chicken UBX domain D (Liang et al., 2006). They reported that the erasin, the new name of UBX family, may be involved in human disease but it is related with the structure of chicken pectoral muscle. Therefore, the expression of DNS-81 could change three-dimensional structure of skeletal muscle and could modify texture of chicken breast muscle.

DNA sequence was compared with other organisms. The clone NDS-81 is matched (327 nt) with chicken (*Gallus gallus*) cDNA clone ChEST293i14 (Boardman et al., 2004). In humans (*Homo sapiens*), it may be related with GAS7 (growth arrest-specific protein) and/or MIH (molt-inhibiting hormone) (Devaraj and Natarajan, 2006) and PAC clone RP5-968I16 from 7 (Arnett et al., 1998). Also, the sequence will be a fragment of

1 *GCGTGGTCGC.GGCCGA*GGTA.CAGTTACCTC.TGCTGCATTA.TTCAGCCAGG.CTCTGATCAC.
61 CAAAATTAGT.TGAAAATATT.TTTGTTACAG.CAGATTACAG.CACAGCACAC.ATTTGCTCTC.
121 TAAGATCACA.CTAGGTAAGA.ATGGTAGTGC.ATCCAGATAT.TGCTGATTAC.ATTTTGTCTA.
181 GTTCTGCCTG.TGTGTAGCTT.TCAGTCAGTA.ATAAACTAAT.TTGAACTTGT.GCTTCTGATA.
241 GCATTTGTTT.GCTCATTCTT.GAGCCGTCCA.TTAGGTGTAG.CAGCAGTTTT.ACAGTCGTCA.
301 TCAGGAGCTT.TCATTGCTAA.CAGTCTAAAA.GGTTCGGTTG.TTTTCCGAAG.TCTCTGAACA.
361 ACAACAGCAT.TTTGCAACTT.TGCTTCCTGT.AGTGTGAGCG.TCTCATCTTG.CAGTTCTCGA.
421 AAGGGCAGCG.AAGTAGTCAG.CATGAAGGGA.ACACCTCCCT.CTGATCCTTG.ATACTTTGTT.
481 ATGAAGTCTC.TGACATGGCT.TATTCTGTGG.GAAACGTTGA.ACTTCTGAAT.ATCCTCTCCC.
541 ATCCGCTACC.AGATCTGGAT.GTTAGTGATA.GGCTCCAAAT.CATTTAAAG.GT*ACCTGCCC*.
601 *GGGGCGGNCG.CTC*

Fig. 1. Partial cDNA sequence (612 bases) of clone NDS-81 derived from Korean native chicken. The translated region is 17～593 nt and untranslated region was expressed boxed *italic* character.

undulin-2 in humans (Just et al., 1991). In chimpanzees (*Pan troglodytes*), a similar sequence was reported from chromosome 7 clone RP43-9F6. Another possibility was suggested that our clone is similar with hypothetical protein in mouse (*Mus musculus*) (Kawai et al., 2002). Haptoglobin was partially related with clone NDS-81 in rabbits (*Oryctolagus cuniculus*). However, all sequence ho-mology was not representing the clone NDS-81 information.

Nucleotide sequence of NDS-81 was compared with chicken UBX domain D4 (Fig. 2). Several regions were mutated from T in chicken to C or G in NDS-81. It may have severe structural modification due to not making a H-bond between T and A. They are going to make G-C linkage for their three-dimensional structure. The late regions were point deleted and then can not be translated or expressed to different protein. These specific sites are expressed in the gray box in Fig. 2. These compounds will have an effect on GLUT system for glucose transport in mouse model (Schertzer et al., 2009; Kim et al., 2011).

Chicken UBX domain containing D4 (UBXD4) and mRNA for hypothetical protein from clone 10c14 ND were compared. They are 98% (571/579 nt) homology of amino acid sequence (Fig. 3). Chicken UBX domain has chicken (93%), cattle (68%), dog (67%), mouse (64%) and human (63%) amino acid sequence homology. Clone NDS-81 match with *Gallus gallus* UBX domain containing 4 (UBXD4) mRNA (Caldwell et al., 2005). A similar sequence was found to in the UBX domain containing 4 in cattle, dog, monkey, and rat (Carim-Todd et al., 2001).

In this comparison, we found several major mutated sites. Those sites are very conservative among other species including chicken. Therefore, two sites would play an important role in muscle gene expression. The first site is LAD in chicken, but it was expressed as (L)RM in clone NDS-81. In this site, amino acids were changed from Ala to Arg, and from Asp to Met. The second site was changed from ER (Arg) in chicken to ED (Asp) in clone NDS-81. They both contain functional side chains and play an important role in binding other proteins and protein structure. A large number of cDNA inserts were sequenced from a high-quality library of chicken breast muscle cDNAs. Comparisons to public gene databases indicate that the cDNA collection represents new gene, full-length transcripts. This resource defines the structure and the coding potential of a large fraction of chicken growth gene. The changed structure will also modify functional characteristics (Albrecht et al., 2004). Therefore, we conclude that the clone NDS-81 could be a different candidate gene for UBX family gene and could related with pectoral muscle structure of Korean native chicken.

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NDS-10      GTACAGTTACCTCTGCTGCATTATTCAGCCAGGCTCTGATCACCAAAATTAGTTGAAAAT      77
Chicken     GTACAGTTACCTCTGCTGCATTATTCAGCCAGGCTCTGATCACCAAAATTAGTTGAAAAT      1096

NDS-10      ATTTTTGTTACAGCAGATTACAGCACAGCACACATTTGCTCTCTAAGATCACACTAGGTA      137
Chicken     ATTTTTGTTACAGCAGATTACAGCACAGCACACATTTGCTCTCTAAGATCACACTAGGTA      1036

NDS-10      AGAATGGTAGTGCATCCAGATATTGCTGATTACATTTTGTCTAGTTCTGCCTGTGTGTAG      197
Chicken     AGAATGGTAGTGCATCCAGATATTGCTGATTACATTTTGTCTAGTTCTGCCTGTGTGTAG      976

NDS-10      CTTTCAGTCAGTAATAAACTAATTTGAACTTGTGCTTCTGATAGCATTTGTTTGCTCATT      257
Chicken     CTTTCAGTCAGTAATAAACTAATTTGAACTTGTGCTTTTCATAGCATTTTTTTGCTCATT      916

NDS-10      CTTGAGCCGTCCATTAGGTGTAGCAGCAGTTTTACAGTCGTCATCAGGAGCTTTCATTGC      317
Chicken     CTTGAGCCGTCCATTAGGTGTAGCAGCAGTTTTACAGTCGTCATCAGGAGCTTTCATTGC      856

NDS-10      TAACAGTCTAAAAGGTTCGGTTGTTTTCCGAAGTCTCTGAACAACAACAGCATTTTGCAA      377
Chicken     TAACAGTCTAAAAGGTTCGGTTGTTTTCCGAAGTCTCTGAACAACAACAGCATTTTGCAA      796

NDS-10      CTTTGCTTCCTGTAGTGTGAGCGTCTCATCTTGCAGTTCTCGAAAGGGCAGCGAAGTAGT      437
Chicken     CTTTGCTTCCTGTAGTGTGAGCGTCTCATCTTGCAGTTCTCGAAAGGGCAGCGAAGTAGT      736

NDS-10      CAGCATGAAGGGAACACCTCCCTCTGATCCTTGATACTTTGTTATGAAGTCTCTGACATG      497
Chicken     CAGCATGAAGGGAACACCTCCCTCTGATCCTTGATACTTTGTTATGAAGTCTCTGACATG      676

NDS-10      GCTTATTCTGTGGGAAACGTTGAACTTCTGAA-TATCCTCT-CCCATCCGCT-ACCAGAT      554
Chicken     GCTTATTCTGTGGGAAACGTTGAATTTCTGAATTATCCTCTCCCCATCCGCTAACCAGAT      616

NDS-10      CTGGATGTTAGTGATAGGCTCCAAATCATTTAAAGGTAC                           593
Chicken     CTGGATGTTAGTGATAGGCTCCAAATCATTTAAGGGTAC                           577
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Fig. 2. Nucleotide sequence comparison of NDS-81 (576 nt) and mRNA of chicken UBX domain containing 4 (UBXD4).

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NDS-81      VPLNDLEPITNIQIW?RMGEDI?QKFNVSHRISHVRDFITKYQGSEGGVPFMLTTSLPFR
Chicken     VPLNDLEPITNIQIWLADGERIIQKFNVSHRISHVRDFITKYQGSEGGVPFMLTTSLPFR
Cattle      VPLNNLEPITNIQIWLANGKRIVQKFNISHRISHIKDFIEKYQGSQRSPPFSLATALPFL
Dog         VQLNNLEPITNVQIWLANGKRIVQKFNISHRISHIKDFIEKYQGSQRSPPFSLATALPFL
Mouse       VSLNNLEPITRIQIWLANGERTVQRFNVSHRVSHIKDFIEKYQGSQRSPPFALATALPFL
Human       VPLNNLEPITNIQIWLANGKRIVQKFNITHRVSHIKDFIEKYQGSQRSPPFSLATALPVL
Frog        VDLNNLEPITNIKVWLADGKRIVQKFNTSHRISDVRDFLERIPCKPGNAPFTLATSFPLH
Catfish     VELDEAIPITCIQIWLADGRRIVQRFNISHRISDVQGFVEKAQSSSSPFIITTSLPFREL

NDS-81      ELQDETLTLQEAKLQNAVVVQRLRKTTEPFRLLAMKAPDDDCKTAATPNGRLKNEQTNAI
Chicken     ELQDETLTLQEAKLQNAVVVQRLRKTTEPFRLLAMKAPDDDCKTAATPNGRLKNEQKNAM
Cattle      KLLDETLTLEEADLQNAVIIQRLKKTAEPFKELS
Dog         KLLDETLTLEEADLQNAVIIQRLQKTAEPFRELS
Mouse       RFLDETLTLEEADLKNAVIIQRLQKTAEPFRKL
Human       RLLDETLTLEEADLQNAVIIQRLQKTAS-FRELS
Frog        DLLDESLTLQEADLQNSVIVQKLQKTTEPFRNS
Catfish     TQGNLSLEEADITNAVIVQRPVSTQAPFGQS
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Fig. 3. Comparison of a partial sequence of 192 amino acids from clone NDS-81 with other species. The major sites (478~476, 473~472, 431~434, 417~416, 398~397 nt) of mutated were expressed in box.

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