FEBS Letters 582 (2008) 2212–2218

# The ubiquitin ligase gene (*WWP1*) is responsible for the chicken muscular dystrophy

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Received 4 March 2008; revised 7 May 2008; accepted 9 May 2008

Available online 22 May 2008

Edited by Takashi Gojobori

**Abstract** **Chicken muscular dystrophy with abnormal muscle (*AM*) has been studied for more than 50 years, but the gene responsible for it remains unclear. Our previous studies narrowed down the *AM* candidate region to approximately 1 Mbp of chicken chromosome 2q containing seven genes. In this study, we performed sequence comparison and gene expression analysis to elucidate the responsible gene. One missense mutation was detected in *AM* candidate genes, while no remarkable alteration of expression patterns was observed. The mutation was identified in *WWP1*, detected only in dystrophic chickens within several tetrapods. These results suggested *WWP1* is responsible for chicken muscular dystrophy.**


*Keywords:* *WWP1*; Chicken; Muscular dystrophy; Responsible gene

## 1. Introduction

Muscular dystrophy is known as a group of inherited diseases producing progressive weakness and degeneration of skeletal muscles [1]. Many genetically varied diseases are included, and more than 30 causative genes have been identified so far [2]. However, genes responsible for several muscular dystrophies and related diseases have not yet been identified [3].

Asmundson and Julian have first reported dystrophic chickens with abnormal muscle (*AM*) in 1956 [4]. Since then, several strains have been established. The disease is transmitted codominantly by a single gene, but the phenotype is modified by other background genes [5–7]. Although abnormalities in the structural proteins binding sarcolemma to basal lamina were found in many animal models for muscular dystrophy [8], the responsible gene(s) and protein(s) responsible for causing chicken muscular dystrophy have yet to be identified. This study attempted to identify the responsible gene and mutation by sequence comparison and expression analysis.

## 2. Materials and methods

### 2.1. Genetic resource

For sequencing and expression analyses, NH-413 and OPN strains were tested as dystrophic chickens [9], and White Leghorn-F (WL-F) and GSP strains were as normal chickens.

Genomic DNA samples were obtained from total 111 individuals consisting of 16 strains: 20 White Cornishes, 19 White Plymouth Rocks, 20 broilers (White Cornish × White Plymouth Rock), 20 White Leghorns, and 20 Brown Leghorns, maintained at the Tokushima Forest and Forestry Research Institute, Japan, an Onagadori, an Ukokkei, a White Leghorn, a White Plymouth Rock, a Black Minorca, a Fayoumi, a Vietnam native, a Laos native, an Echigo native, a Tosa native, and two Red Jungle fowls from Laos and Indonesia. Muscle cDNA libraries of pigeon, alligator, lizard, turtle and frog, and liver cDNA library of snake, were used to determine partial sequences of *WWP1* gene in this study [10,11].

### 2.2. Sequencing for seven candidate genes

Pectoral muscle cDNAs were used as template DNA to sequence for *WWP1*, *LOC420213*, *LOC420214* and *MMP16*, while genomic DNA for *ATP6V0D2*, *LOC420211* and *LOC428367*. In using cDNA as template, primers were designed to amplify each coding region, and in using genomic DNA, to amplify each exon. The primer sequences and the PCR conditions were summarized in Table 1(a). Sequencing was performed with BigDye® Terminator v3.1 Cycle Sequencing Kit and ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). For polymerase chain reaction (PCR), TaKaRa Ex Taq® Hot Start Version or TaKaRa LA Taq™ with GC buffer (Takara, Tokyo, Japan) was used as polymerase.

### 2.3. Genotyping chickens for WWP1

Genotyping of 111 individuals from 16 strains for *WWP1* was carried out by PCR-restriction fragment length polymorphism (RFLP) method. Primer set was established to amplify the region with a single nucleotide polymorphism (SNP) on *WWP1*. One of them was mismatched primer and designed so that the chicken with wild-type *WWP1* yielded three DNA fragments and the one with mutated *WWP1* yielded two through digestion by a *Mbo*I restricted enzyme (Fermentas International Inc., Ontario, Canada). The primer sequences and the PCR condition applied were shown in Table 1(b).

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*Abbreviations:* *AM*, abnormal muscle; UPP, ubiquitin-proteasome pathway; Ub, ubiquitin

0014-5793/$34.00 
doi:10.1016/j.febslet.2008.05.013

Table 1
Sequences of primers and PCR conditions for sequencing, genotyping and expression analysis

| Name | Primer[a] | Sequence (5′ → 3′) | PCR condition[b] |
|---|---|---|---|
| *(a) Primers used for sequencing to produce template DNA* | | | |
| *ATP6V0D2* | ATP6V0D2s-F1 | tctaaaactatgtgagcctggagtag | Normal PCR (EX-Taq): 58 °C |
| | ATP6V0D2s-R1 | ccaaatcacagtctacacaatcctgc | 1 min, 30 cycles |
| | ATP6V0D2s-F2 | gacacgttataatggtgcaatagtgg | Normal PCR (EX-Taq): 58 °C |
| | ATP6V0D2s-R2 | ccagaccctatacacagtaaagagtc | 1 min, 30 cycles |
| | ATP6V0D2s-F3 | gagatggtgatagtgagtgaaggtac | Normal PCR (EX-Taq): 58 °C |
| | ATP6V0D2s-R3 | tagaagttgttaataaatgttgccag | 1 min, 30 cycles |
| | ATP6V0D2s-F4 | gctgctgcactgattgattccctttg | Normal PCR (EX-Taq): 64 °C |
| | ATP6V0D2s-R4 | tccagacttgccatcagccaggtgac | 1 min, 30 cycles |
| | ATP6V0D2s-F5 | attatgcagtagaaactcaatggagc | Normal PCR (EX-Taq): 58 °C |
| | ATP6V0D2s-R5 | aaaatggtaaggagcaatagtctgag | 1 min, 30 cycles |
| | ATP6V0D2s-F6 | aagtacgtgtgattattgatccttac | Normal PCR (EX-Taq): 58 °C |
| | ATP6V0D2s-R6 | atagcatttaacacagtaagtggaac | 1 min, 30 cycles |
| | ATP6V0D2s-F7 | gaagttcagtgctctctatccaaagg | Normal PCR (EX-Taq): 58 °C |
| | ATP6V0D2s-R7 | ttggtaagagactacagcagcattac | 1 min, 30 cycles |
| | ATP6V0D2s-F8 | agtttcctaagtacagttgtgattgc | Normal PCR (EX-Taq): 58 °C |
| | ATP6V0D2s-R8 | catttaacttcagcaacaggtcacag | 1 min, 30 cycles |
| *LOC420211* | LOC420211s-F | ctgaaggagtccacacgcccaagtca | SH PCR (LA-Taq): 68 °C |
| | LOC420211s-R | cgagcaacagaactagcagacattcc | 3 min, 27 cycles |
| *WWP1* | WWP1s-F1 | aggctccacatgggcagaactttgtc | Normal PCR (EX-Taq): 62 °C |
| | WWP1s-R1 | tcaaataggcagtacatagggttcag | 2 min, 35 cycles |
| | WWP1s-F2 | acttgctcatttccgttacttgtgtc | Normal PCR (EX-Taq): 65 °C |
| | WWP1s-R2 | ttgaagattacctaacatcctcgtgg | 2 min, 30 cycles |
| *LOC420213* | LOC420213s-F | ctcgctcgcaccttctcctcccctgg | SH PCR (EX-Taq): 68 °C |
| | LOC420213s-R | ttcattttcctatgctgcttacatct | 1 min, 40 cycles |
| *LOC420214* | LOC420214s-F | atggcagcacagtgtgtgactaaggt | SH PCR (EX-Taq): 68 °C |
| | LOC420214s-R | atccccgctcaagaaagtaactgatc | 2 min, 32 cycles |
| *LOC428367* | LOC428367s-F1 | gggaagtgaaggcaagagcaccaggc | Normal PCR (EX-Taq): 64 °C |
| | LOC428367s-R1 | gaggagatttagattagatgttagca | 1 min, 30 cycles |
| | LOC428367s-F2 | ctgctacagtattcccagtgagagat | Normal PCR (EX-Taq): 55 °C |
| | LOC428367s-R2 | gatgagatataaatgtggtacaagta | 1 min, 30 cycles |
| | LOC428367s-F3 | atcattctcagcaataaccatctagt | Normal PCR (EX-Taq): 55 °C |
| | LOC428367s-R3 | tttcagataatcttggagcactcata | 1 min, 30 cycles |
| | LOC428367s-F4 | tgaagagataatggaagccaagttct | SH PCR (EX-Taq): 68 °C |
| | LOC428367s-R4 | tgaatactgggtaaatgtggttgcct | 2 min, 32 cycles |
| | LOC428367s-F5 | taatgcttgacttctgcttgtactat | Normal PCR (EX-Taq): 55 °C |
| | LOC428367s-R5 | atgagtaaatgtgatgtggtaagata | 1 min, 30 cycles |
| | LOC428367s-F6 | gaaggttcccaaatactccattaaca | Normal PCR (EX-Taq): 55 °C |
| | LOC428367s-R6 | gaaaacatataatctcacaatctgta | 1 min, 30 cycles |
| | LOC428367s-F7 | atactgtttcttcactggagtaatga | Normal PCR (EX-Taq): 60 °C |
| | LOC428367s-R7 | catatgtacttgagcaggtcagttga | 1 min, 30 cycles |
| | LOC428367s-F8 | tcacagagtaaatacaggtggagagc | Normal PCR (EX-Taq): 60 °C |
| | LOC428367s-R8 | ctgccatccttctaacagttcccat | 1 min, 30 cycles |
| | LOC428367s-F9 | gtcagtgaggatattctgttcaatgt | Normal PCR (EX-Taq): 55 °C |
| | LOC428367s-R9 | agataaatactcagttttctggttaa | 1 min, 30 cycles |
| | LOC428367s-F10 | tacagactgtttcttacacaaccagc | Normal PCR (EX-Taq): 60 °C |
| | LOC428367s-R10 | tcactgattacttaactgcttctgaa | 1 min, 30 cycles |

(*continued on next page*)

Table 1 (*continued*)

| Name | Primer[a] | Sequence (5′ → 3′) | PCR condition[b] |
|---|---|---|---|
| | LOC428367s-F11 | tcagtggagtatcaacttcagagatt | Normal PCR (EX-Taq): 55 °C |
| | LOC428367s-R11 | aagcacctatatgtactagcagacaa | 1 min, 35 cycles |
| *LOC428367* | LOC428367s-F12 | gttgtcaggtgttcatccacacattc | Normal PCR (EX-Taq): 55 °C |
| | LOC428367s-R12 | ctatttctctatccatcatcctctac | 1 min, 35 cycles |
| | LOC428367s-F13 | tgcttcttggttgatctatgctctcc | Normal PCR (EX-Taq): 55 °C |
| | LOC428367s-R13 | ggttttattctgtagccgtctctcct | 1 min, 35 cycles |
| *MMP16* | MMP16s-F | tgaacctgcgttacgggctcctcac | SH PCR (EX-Taq): 68 °C |
| | MMP16s-R | ccttgtttgttggaaaatggctgtc | 2.5 min, 35 cycles |
| *(b) Primers used for genotyping* | | | |
| *WWP1* | WWP1m-F | agagaaaatgagctatgcagtattac | Normal-PCR (EX-Taq): 56 °C |
| | WWP1m-R-mismatch | tatataaaatttaccgaatagagggaa | 1 min, 35 cycles |
| *(c) Primers used for identifying WWP1 partial sequence of other species* | | | |
| *WWP1* | WWP1d-F1 | aacaacvtggcagcgrccwachatgg | DG-PCR (EX-Taq) |
| | WWP1d-R | gtaavccttgrgttckwggrtcttc | |
| | WWP1d-F2 | aaytttgarcagtggcartctcagc | DG-PCR (EX-Taq) |
| | WWP1d-R | gtaavccttgrgttckwggrtcttc | |
| *(d) Primers used for expression analysis* | | | |
| *ATP6V0D2*[c] | ATP6V0D2p-F | atattgtatggattgccgaatgc | Normal-PCR (EX-Taq): 60 °C |
| | ATP6V0D2p-R | cgaaatggtcactgtggggaaca | 0.5 min, 25, 30, 35 cycles |
| *LOC420211*[c] | LOC420211p-F | aaaggactgaataccatctgatt | Normal-PCR (EX-Taq): 55 °C |
| | LOC420211p-R | tacaactgctaaatgctccctca | 0.5 min, 25, 30, 35 cycles |
| *WWP1* | WWP1p-F | tccctcataaatgttgaaagcagaca | Normal-PCR (EX-Taq): 55 °C |
| | WWP1p-R | gtaataacccaaggtaatatgtaaac | 0.5 min, 35 cycles |
| *LOC420213* | LOC420213p-F | agtggcagaagttatagagcaagcag | Normal-PCR (EX-Taq): 60 °C |
| | LOC420213p-R | cgtgtatgtcttctcctgtttgtcca | 0.5 min, 35 cycles |
| *LOC420214* | LOC420214p-F | tggtatgaggttgatcgcacagaaag | Normal-PCR (EX-Taq): 60 °C |
| | LOC420214p-R | ggtgctacagttttgacttccttcgt | 0.5 min, 35 cycles |
| *LOC428367*[d] | LOC428367p-F | tgtcttcgtcttctccagcttaattg | – |
| | LOC428367p-R | ggctaataggctgatctccccaaata | |
| *MMP16*[c] | MMP16p-F | cataatctttcccaagttgtaccaag | Normal-PCR (EX-Taq): 52 °C |
| | MMP16p-R | gcaatatcagagtcatcatttagtt | 0.5 min, 25, 30, 35 cycles |

[a]Each column's upper primers are forward primers and lower ones reverse primers.
[b]Each column shows PCR method (polymerase): annealing temperature, extension time, cyclic number applied by each primer sets. Normal PCR was performed as the following: cyclic number at 94 °C for 30 s, annealing temperature for 30 s, 72 °C for extension time, SH PCR: cyclic number at 98 °C for 10 s, 68 °C for extension time, and DG PCR: 3 cycles at 95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s and 32 cycles at 95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s.
[c]To analyze their expressions, RT-PCR was performed.
[d]Expression of this gene was not confirmed.

### 2.4. WWP1 homology search among species

To obtain *WWP1* partial sequences of pigeon, snake, alligator, lizard, turtle and frog, degenerate primers were designed based on those of chicken (NM_001012554), human (NM_007013), mouse (NM_177327) and rat (NM_001024757) published in NCBI. To amplify each *WWP1* of snake, alligator and pigeon, WWP1d-F1 and WWP1d-R were used as primers, and WWP1d-F2 and WWP1d-R were used for other species. The primer sequences and the PCR condition were shown in Table 1(c). The amplified bands corresponding to *WWP1* cDNAs were extracted from agarose gel and purified with UltraClean™ 15 DNA Purification Kit (MO BIO Laboratories Inc., Carlsbad, CA). The purified cDNA fragments were ligated into pGEM-T vectors (Promega Corporation, Madison, WI) and transformed into *Escherichia coli* JM109 competent cells. To confirm the sequence integrity, eight independent clones were sequenced.

### 2.5. Expression analysis

For Northern blot analysis, pectorals mRNAs of a NH-413 and a WL-F strain female were used. 2 μg of mRNAs were resolved by 1.2% agarose gel electrophoresis in the presence of formaldehyde and blotted on to Hybond-N+ membrane (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The mRNAs were visualized using digoxigenin (DIG) reagents (Roche Diagnostics, Basel, Switzerland).

The DIG-labeled DNA probes were prepared by PCR with DIG-dUTP using pectorals cDNA. The primers used in this procedure were shown in Table 1(d).

For genes whose expression was not detected by Northern blotting, reverse transcription (RT)-PCR method was applied with same condition in Table 1(d).

## 3. Result

### 3.1. Mutations specific to muscular dystrophy

Sequence comparison of seven candidate genes in normal and dystrophic chickens revealed three synonymous mutations and one missense mutation specific to the phenotype of muscular dystrophy. Two of the synonymous mutations were detected in *LOC420214* (C660T and C1009A), and the other in *LOC428367* (C954T). The *WWP1* missense mutation (G1321A) caused amino acid substitution from arginine to glutamine, leading to the molecular alteration from a basic side chain to an uncharged polar side chain. We focused further studies on this missense mutation since it was predicted to influence the function of the WWP1 protein.

The domain structure of human WWP1 was determined previously [12]. It consists of three types of unique domains: one C2 domain, four WW domains and one HECT domain. The amino acid sequences of human and chicken WWP1 share 83% identity, suggesting that the deduced structure of chicken WWP1 was quite similar to that of human's. According to NCBI database, chicken WWP1 possesses one C2 domain, three WW domains and one HECT domain. The detected mutation lay between WW domains 1 and 2 (Fig. 1).

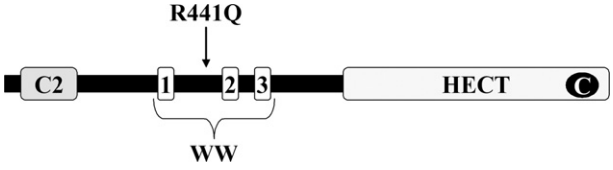


Fig. 1. The domain structure of chicken WWP1 and the site of missense mutation. Chicken WWP1 protein is composed of 922 amino acids. Here are shown WWP1 functional domains: C2 domain, three WW domains and HECT domain. C in HECT domain indicates an active cysteine residue. The arrow indicates the site of missense mutation detected in this study. WW domains bind proline-rich region.

### 3.2. Intra- and inter-species analyses of R441Q WWP1 mutation

In order to exclude the possibility of strain specificity of *WWP1* mutation, this SNP was genotyped in OPN strain to determine if the WWP1 gene has this mutation in any strain with a different genetic background. PCR-RFLP analysis indicated that OPN dystrophic chickens have the identical mutation in the *WWP1* gene. Genotyping was also applied to 111 normal birds from 16 strains with genetically varied backgrounds. None of the normal birds exhibited this type of substitution. The region of *WWP1* including the SNP specific to chicken muscular dystrophy was highly conserved among normal birds. Additional homology research among tetrapods was conducted to estimate amino acid conservation in this region. The sequences of chicken, human, chimpanzee (XP_519843), monkey (XP_001083173), mouse, rat, dog (XP_535119) and cattle (NP_001032540) were available in the NCBI database. To obtain further information from other tetrapod species, we sequenced partial *WWP1* gene of pigeon, snake, alligator, lizard, turtle and frog (DDBJ Accession Nos. AB385863 to AB385868). The amino acid sequence around the region was highly conserved among these tetrapods (Fig. 2), suggesting that the region was important for the function of WWP1.

### 3.3. Expression analysis of candidate genes

Expressions of candidate genes were analyzed by Northern blotting. As depicted in Fig. 3a, the expression of *WWP1*, *LOC420213* and *LOC420214* in the pectoral muscle of both genotypes derived from both normal and dystrophic chicken could be detected by Northern blotting. There was no difference in size of mRNA between affected and normal birds in any of these genes.*LOC420213* was highly expressed in affected individuals. Two bands were detected in *WWP1* and *LOC420214*. *WWP1* was expressed slightly higher in normal than in affected chickens, while a slightly higher level of expression was exhibited for *LOC420214* from affected chickens.

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                                  *
Chicken N[1)]   RNQLQGAMQQFNQRYLYSASMLSAENDPLGPLPPGWERRVDSNDRVYFVNHNTKTTQWED
Chicken A       ............Q...........................................
Human[1)]       ....................A.....Y........K....T.................
Chimpanzee[1)]  ....................A.....Y........K....T.................
Monkey[1)]      ....................A.....Y........K....T.................
Mouse[1)]       ....................A.....Y........K....T.................
Rat[1)]         ....................A.....Y........K....T.................
Dog[1)]         ....................A.....Y........K....T.................
Cattle[1)]      AD..................A.....Y........K....T.................
Pigeon          ....................................................
Snake           ....................................................
Alligator       ....................T...............................
Lizard          ....................T...............................
Turtle          ....................................................
Frog            ....................T...................T.................
```

Fig. 2. Homology study of *WWP1* among tetrapods. Chicken N and A show amino acid sequences of normal and dystrophic chickens, respectively. R441Q WWP1 mutation is specific to chicken muscular dystrophy, and the amino acid sequence on this region is highly conserved among tetrapods. Dots indicate the same amino acids with above sequences. Asterisk indicates the residue that the substitution was detected in dystrophic chicken. (1) Amino acid sequence published in NCBI.

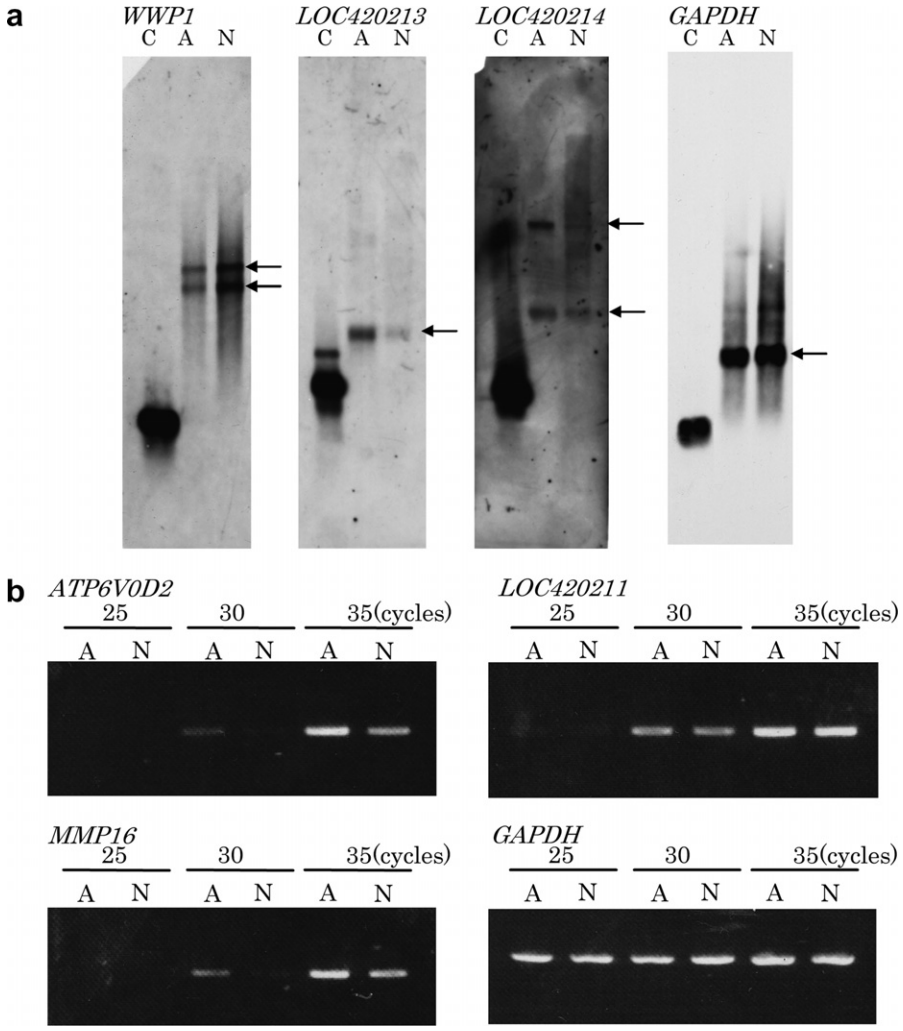


Fig. 3. Expression analysis of candidate genes. Extreme alteration of expression level was not observed in any genes. (a) Northern blotting analysis toward *ATP6V0D2, LOC420211, WWP1, LOC420213, LOC420214, MMP16* using chicken pectoral mRNA (2 μg). In any of these genes, there was no difference in size of mRNA between affected and normal birds. The C indicates loaded PCR product which has the same sequence as probe, the A indicates mRNA of dystrophic chickens and the N indicates mRNA of normal chickens, respectively. The arrows indicate the band detected. b) RT-PCR analysis toward *ATP6V0D2, LOC420211, MMP16* using chicken pectoral cDNA.

The expression of other genes (*ATP6V0D2*, *MMP16*, *LOC420211* and *LOC428367*) was not detected by Northern blotting. RT-PCR analysis in dystrophic chickens revealed higher expression of *ATP6V0D2* and *MMP16* than in normal birds. The pectoral muscles from both genotypes expressed *LOC420211* to identical level. The expression of *LOC428367*-was not confirmed in either genotype.

## 4. Discussion

We previously narrowed down the *AM* candidate region to approximately 1 Mbp on GGA2q. Seven functional genes, *ATP6V0D2*, *LOC420211*, *WWP1*, *LOC420213*, *LOC420214*, *LOC428367* and *MMP16*, in this region were the candidate genes for chicken muscular dystrophy [13,14], but none of them have been determined to be genes for other muscular dystrophy so far. In this study, sequence comparison of normal and dystrophic chickens was conducted to detect a mutation responsible for the disease.

We detected a mutation site specific for the *AM* phenotype in G1321A of the *WWP1* gene that caused amino acid replacement, from arginine with a basic side chain (a basic amino acid) to glutamine with an uncharged polar side chain (a neutral amino acid), which can affect the function of the WWP1 protein. *WWP1* mutated in the coding region of the protein, providing the most likely candidate responsible for causing this disease. This type of mutation was only observed in dystrophic chickens. No mutation was detected in any normal chickens analyzed. Furthermore, the amino acid sequence on this region is highly conserved among tetrapods (Fig. 2). The region is thus probably critical for the function of *WWP1*.

The expression patterns of the candidate genes were analyzed in Fig. 3. *WWP1*, *LOC420213*, *ATP6V0D2* and *MMP16* exhibited some difference in expression level between normal and dystrophic individuals, none of which were drastic alterations. No difference in mRNA size was observed in any gene. Since no extreme alteration of expression level and abnormal splicing were observed, the onset of chicken muscular dystrophy might not be attributed to aberrant expression of these genes.

The WW domain containing E3 ubiquitin protein ligase 1 (*WWP1*), the most likely *AM* candidate gene found by this study, is classified as an ubiquitin ligase (E3) that plays an important role in ubiquitin-proteasome pathway (UPP). Ubiquitination, addition of ubiquitin (Ub) chains to a target protein, is one of the most common forms of posttranslational modification, and it controls important aspects of cell functions [15,16]. In UPP, at least three types of enzymes are required, namely, E1 Ub-activating enzyme, E2 Ub-conjugating enzyme and E3 [17]. E3 recognizes and catalyzes Ub conjugation to specific protein substrates [18]. E3s are structurally divided into several classes: HECT-type E3s, RING-type E3s and others [19].

Accumulating data indicate that some E3s are related to muscular dystrophies [20–22]. E3s that are assumed to be related to muscular dystrophies, such as *Trim32* [25], are all classified as RING-type E3s, while *WWP1* is classified as a HECT-type E3. RING-type E3s facilitate ubiquitination indirectly, while HECT-type E3s transfer Ub directly to substrates bound to a non-catalytic domain of themselves [23]. Though they are structurally and mechanically distinct, their basic role in UPP is common. Therefore, it is possible for some abnormal HECT-type E3s to cause muscular dystrophies. Actually, it has been reported that E6AP, another HECT-type E3, contributes to a severe neurological disorder, Angelman syndrome [24,25] which exhibits muscular hypotonia [26].

*WWP1* is expressed ubiquitously but more strongly in liver, bone marrow, testis, and muscle [12]. Flasza et al. also mentioned at least six splice variants of *WWP1* [12]. Two of the six products are commonly observed among multiple tissues, and their expression levels are higher than the others. The bands detected by Northern blotting (Fig. 3a) may correspond to these two major products, but little is known about the function of these transcript variants.

E6AP is known as the gene responsible for Angelman syndrome [24]. Cooper et al. reported that two mutations lying in the non-catalytic amino-terminal portion of E6AP cause Angelman syndrome [25]: one of them affects E6AP enzymatic activity, and the other may disturb substrate binding, subcellular localization or protein stability. The mutation of WWP1 detected in this study is located between WW domains 1 and 2 (Fig. 1). Though the mutation is outside all domains, it is predicted that the region near the mutation is functionally important because of its high homology (Fig. 2). The R441Q mutation probably influences the WWP1 function like Angelman syndrome causative mutations, so that it triggers the onset. Since the mutation lies between two domains both of which recognize target proteins [27], it could alter the conformation of WWP1 [28], the physical relationship among domains and the preference for substrates.

Aberrant regulation of membrane protein may lead to the onset of chicken muscular dystrophy. The HECT-type E3s with WW domains generally regulate membrane proteins [29]. Though much remains unclear about WWP1's substrates, it has been demonstrated that WWP1 could interact with β-dystroglycan, an important muscle protein consisting of membrane [30]. Abnormal glycosylation of α-dystroglycan in chicken muscular dystrophy was also reported [31]. Some E3s are known to recognize sugar chain [32–34], leading to the hypothesis that WWP1 might be able to recognize the sugar chain of α-dystroglycan and regulate the glycosylated molecules, and that insufficiently glycosylated α-dystroglycan, which originally requires degradation, accumulates and causes the disease because altered WWP1 can not recognize and degrade it.

This study identified R441Q WWP1 mutation as being specific to chicken muscular dystrophy. This is the most likely candidate mutation responsible for chicken muscular dystrophy because the region nearby is highly conserved among species and no drastic alteration of expression patterns of the candidate genes was observed. In order to clarify the mechanism by which mutated WWP1 triggers the onset of chicken muscular dystrophy, further biochemical research is required. The information must be useful for determining the corresponding human dystrophy and for providing new insights for understanding muscular dystrophies.

*Acknowledgements:* This work was supported in part by Grant-in-Aid for Scientific Research (C), No. 19580338, and the Global COE Program "Global Center for Education and Research in Integrative Membrane Biology" (A-8) from the Ministry of Education, Science, Sports and Research on Nervous and Mental Disorders (16B-2, 19A-7) from the Japanese Ministry of Health, Labor and Welfare.

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