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Department of Animal Sciences

Increased Muscle Mass by CRISPR/Cas9 mediated genome editing in Quail Myostatin

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INTRODUCTION

Poultry is one of the most important meat source and increase meat yield can bring a huge economic benefit to the poultry industry. In addition to selective breeding for bigger muscles, genetic factors that contribute to muscle growth need be investigated for higher muscle gain. Myostatin (*MSTN*) is one of the most well-known and prominent gene that can be targeted to increase muscle growth. *MSTN* is mainly expressed in skeletal muscle and negatively regulates muscle growth. The inhibitory effect on muscle growth has been confirmed by *MSTN* mutation resulting in increased muscle mass in various species including human. However, *MSTN* mutation in avian species has not been reported and needs to be investigated for potential future application in poultry industry.

AIM

The objective of this study was to generate *MSTN* mutation in quail and investigate the effect of *MSTN* mutation in avian muscle growth.

METHODS

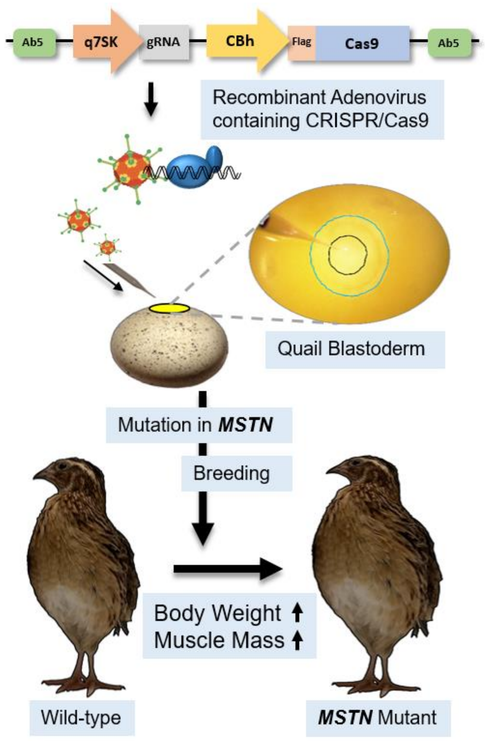

Figure 1. Overview of the study

METHODS

1. Production of Adenovirus containing CRISPR/Cas9 system
2. Injection of the Recombinant Adenovirus into Quail Blastoderm
3. Generation of Germline Chimera
4. Identification of *MSTN* Mutation from Offspring
5. Generation of Homozygous Mutant Quail from Heterozygous Mutant Parents
6. Investigation of Muscle Growth among Groups

RESULTS

Generation of *MSTN* Mutation in Quail Using the Adenovirus-Mediated Method

- *MSTN* gene with non-frameshift three base-pair deletion mutation resulted in deletion of cysteine at the 42th amino acid residue (C42del) in the propeptide region.

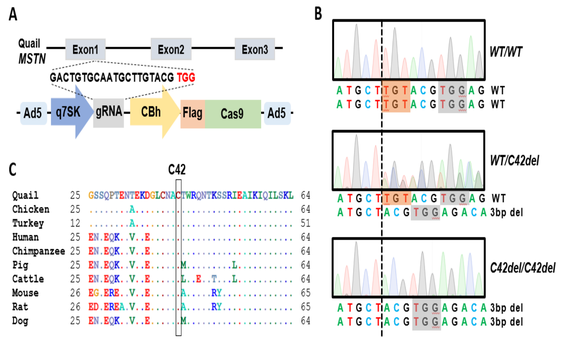

Figure 2. (A) To target quail *MSTN* , guide RNA was designated on exon 1. (B) Sanger sequencing chromatograms of a targeted region in the MSTN gene of wild-type (WT/WT), MSTN heterozygous mutant (WT/C42del), and MSTN homozygous mutant (C42del/C42del) quail were compared. (C) Amino acid sequences of MSTN protein after signal peptide are compared across species.

Positive Effect of MSTN C42del Mutation in Quail Muscle Growth

- *MSTN* homozygous mutant (*C42del/C42del*) quail showed significantly increased body weight compared to *MSTN* heterozygous mutant (*WT/C42del*) and wild-type (*WT/WT*) quail in both male and female.
- Pectoralis major and minor muscles from breast, biceps femoris, semitendinosus, and gastrocnemius muscles from leg, and tricep brachii muscle from wing was significantly heavier in *C42del/C42del* quail compared to *WT/C42del* and *WT/WT* quail in both male and female.
- *C42del/C42del* quail showed decreased fat pad weight and increased heart weight in an sex-dependent manner.

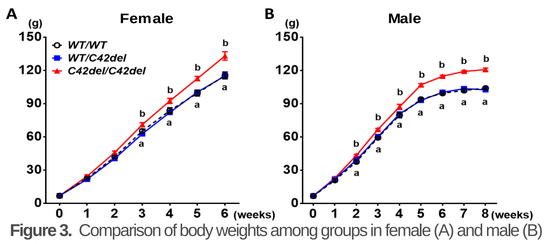

Figure 3. Comparison of body weights among groups in female (A) and male (B) quail.

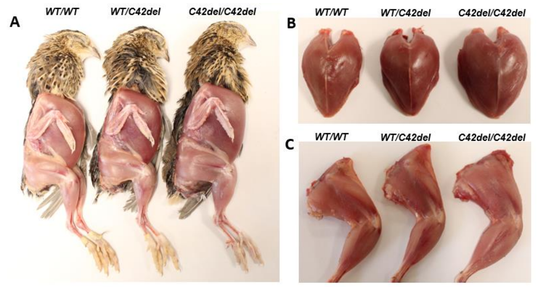

Figure 4. Phenotypic comparisons of whole body (A), breast muscle (B), and leg muscle (C) among groups in 6-week old female quail.

Table 1. Comparison of muscle, adipose tissue, and heart weights in 6-week old female quail.

Tissue	*WT/WT*	*WT/C42del*	*C42del/C42del*
Pectoralis Major	16.19 ± 0.26[a]	16.15 ± 0.5[a]	20.12 ± 0.71[b]
Pectoralis Minor	5.56 ± 0.10[a]	5.61 ± 0.16[a]	6.92 ± 0.20[b]
Biceps Femoris	2.14 ± 0.06[a]	2.20 ± 0.04[a]	2.68 ± 0.08[b]
Semitendinosus	0.95 ± 0.02[a]	0.98 ± 0.03[a]	1.17 ± 0.04[b]
Gastrocnemius	0.80 ± 0.03[a]	0.79± 0.02[a]	0.10 ± 0.04[b]
Tricep Brachii	0.56 ± 0.02[a]	0.54 ± 0.03[a]	0.69 ± 0.02[b]
Leg Fat	0.34 ± 0.03[a]	0.25 ± 0.02[ab]	0.24 ± 0.02[b]
Abdominal Fat	0.23 ± 0.04[a]	0.19 ± 0.02[ab]	0.16 ± 0.02[b]
Heart	0.87 ± 0.03[NS]	0.87 ± 0.03[NS]	0.87 ± 0.02[NS]

Table 2. Comparison of muscle, adipose tissue, and heart weights in 8- and 12-week old male quail.

Tissue	*WT/WT*	*WT/C42del*	*C42del/C42del*
Pectoralis Major	14.00 ± 0.52 [a]	13.95 ± 0.32 [a]	17.96 ± 0.26 [b]
Pectoralis Minor	4.93 ± 0.17 [a]	4.59 ± 0.32 [a]	6.35 ± 0.09 [b]
Biceps Femoris	2.02 ± 0.06 [a]	2.06 ± 0.05 [a]	2.58 ± 0.05 [b]
Semitendinosus	0.89 ± 0.03 [a]	0.92 ± 0.02 [a]	1.14 ± 0.03 [b]
Gastrocnemius	0.67 ± 0.02 [a]	0.70 ± 0.02 [a]	0.91 ± 0.03 [b]
Tricep Brachii	0.48 ± 0.01 [a]	0.50 ± 0.01 [a]	0.61 ± 0.02 [b]
Heart	0.82 ± 0.03 [a]	0.92 ± 0.03 [b]	0.95 ± 0.04 [b]
Leg Fat	0.41 ± 0.06 [NS]	0.30 ± 0.032 [NS]	0.32 ± 0.04 [NS]
Abdominal Fat	0.26 ± 0.04 [NS]	0.17 ± 0.02 [NS]	0.21 ± 0.03 [NS]
Leg Fat (12 wks)	0.70 ± 0.13 [NS]	0.65 ± 0.05 [NS]	0.58 ± 0.09 [NS]
Abdominal Fat (12 wks)	0.44 ± 0.08 [NS]	0.39 ± 0.03 [NS]	0.37 ± 0.07 [NS]

The values are means ± SEM. Statistical analyses were performed by one-way ANOVA followed by Tukey's multiple comparisons test using the Graphpad PRISM 6.02 program. a–b Means sharing the same superscript in a row are not significantly different from each other ($p < 0.05$) and NS means no significant difference.

Muscle Fiber Hyperplasia in C42del/C42del Quail

- C42del mutation caused muscle fiber hyperplasia, rather than fiber hypertrophy in skeletal muscle, resulting in increased body weight and muscle mass of *C42del/C42del* quail.

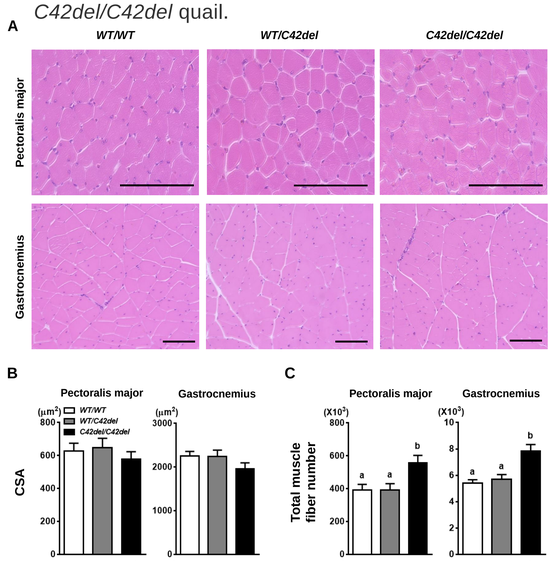

Figure 5. (A) Histological comparison of hematoxylin and eosin stained pectoralis major and gastrocnemius muscles. Scale bar: 100 µm. (B) Comparison of muscle fiber cross-sectional area. (C) Comparison of total muscle fiber number.

CONCLUSIONS

C42del mutation in MSTN protein resulted in increased body weight, muscle mass, and heart weight, along with decreased fat pad weights. The current finding that the C42del mutation positively affected muscle growth in quail indicates an important role of the conserved cysteine 42 residue in MSTN function. Our study provides an important avian model with a novel mutation in MSTN protein for hyperplastic muscle growth and for application to improve genetic traits of poultry with increased meat yield.

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