



## Structural Variation in Fstn Exon 3 Gene and Its Association With Growth Traits In Chicken

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### ABSTRACT

Follistatin gene is a member of transforming growth factor – super family involved in muscle growth. The present study was carried out to characterize the nucleotide variability in the exonic (coding) region of FSTN gene in boiler (PD-1) line and control layer chicken lines. A PCR product of 219 bp of exon-3 was amplified and nucleotide variability was studied using PCR-SSCP technique. The PCR products were sequenced to confirm the variability in coding sequence. The present study revealed that the exon3 sequence of FSTN gene was monomorphic having similar sequence pattern in all individuals. Growth data was also analyzed, the growth performance of male and female differed significantly at six week of age. It is concluded that exon3 of FSTN gene was monomorphic without showing any variation in the nucleotide composition both in broiler and layer chicken lines. The codon did not show any effect on variation in growth traits in chicken.

**Keywords:** Chicken, growth performance, FSTN coding region, nucleotide variability

Follistatin (FSTN) is one of the important candidate genes for the skeletal muscle mass growth and muscle fibre formation (Lee *et al.* 2007; Medeiros *et al.* 2009). In chickens, follistatin was first detected in the cranial segmental plate and mesoderm and subsequently in all somites (Amphor *et al.* 1996). Structurally, follistatin is monomeric glycosylated protein, encoded by two splice variants containing six codons, (Patel *et al.* 1998; Shimasaki *et al.* 1991).

This protein is broadly distributed in all tissues in embryonic as well as adult tissues. It is also presents in circulatory system and binds to activin via their subunits (Patel *et al.* 1998). Follistatin is one of the receptors of myostatin which is a very crucial negative regulator of growth of skeletal muscles in livestock (Helene Gilson *et al.* 2009) Most of the works related to FSTN genes have concentrated on protein characterization and gene expression patterns, while reports on variation of the gene was very scanty. Therefore, the study was carried out to study the polymorphism in the exon3 of FASTN gene and its association with body weights in chicken.

### MATERIALS AND METHODS

#### Experimental birds

The present study was conducted on a broiler line (PD-1) and a layer line (Control Layer) maintained at the Directorate of Poultry Research, Hyderabad, India. The PD-1 line is a color broiler chicken line and its body weight at 6 and 20 weeks of age was 668 and 1986g, respectively (PDP Annual Report, 2012). The broiler birds were kept in the brooder house till the age of 6 weeks and then, shifted to the grower house. The birds were reared on deep litter system under intensive management up to 6 weeks of age. The PD-1 chicks up to 3 weeks were fed with 2800 Kcal ME and 21% crude protein. The chicks of PD-1 from 3 to 6 weeks were fed with 2600 Kcal ME and 16% crude protein. During the brooding stage, proper heating was provided with bulb and the temperature was 32°C for 1<sup>st</sup> week with a weekly gradual decrease from 1<sup>st</sup> to 5<sup>th</sup> week. The vaccination schedule followed in the farm was Marek's disease vaccine at day 1, Newcastle disease vaccine on day 7, Infectious bursal disease vaccine on day



14 and day 24, and New Castle disease vaccine on day 28. From day 1 to 6<sup>th</sup> week, 0.03 to 0.09 m<sup>2</sup> of space for female chicks and 0.09 m<sup>2</sup> of space for male chicks were provided in the deep-litter system.

Cooling arrangements during the summer season through water sprinkling on the roof was provided in the shed so that birds have a congenial environment for expressing their optimum potential. The control layer (CL) was a random-bred control population developed from white leghorn breed and was used as control population to estimate genetic progress in the selected layer lines. The birds were reared on deep litter system under intensive management in the brooder house till the age of 16 weeks and then, shifted to the cage house.

### Isolation of Genomic DNA and amplification of FSTN coding region

About 0.1 ml of blood from 274 birds of PD-1 line and 227 birds of control layer line were collected from the wing vein into vacutainer tube containing 2.7% 0.5M EDTA (60-70µl per ml of blood) as anticoagulant. Genomic DNA was isolated from blood cells following standard protocol (Bhattacharya *et al.* 2011). The quality of DNA was verified by 0.8% agarose gel electrophoresis while quantity was checked by spectrophotometer (Genova, UK). All DNA stocks were diluted using nuclease free water to create a standard DNA concentration of 100 µg/µl.

### Polymerase Chain Reaction

A pair of primers was designed from the exon 3 of chicken FSTN sequence available at the National Centre for Biotechnology Information (Accession number: NC\_006127) using DNASTAR software (LasergeneInc) where the forward primer was 5'-AACATGTGAGAATGTGGACTG-3' and the reverse primer was 5'TTGCACTTGCCCTGATATTG-3'. The PCR reactions included 50µg of DNA template, 10 pM of each primer, 1.5 mM of MgCl<sub>2</sub>, and 100µM of DNTP Mix. The PCR amplification conditions for two exons were initial denaturation at 94°C at 5 min, 30 cycles of denaturation at 94°C for 45s, annealing at 56°C for 30 s and extension at 72°C for 45s with final extension at 72°C for 10 min.

### Single-Stranded Conformation Polymorphism (SSCP)

The SSCP protocol was used to detect variation. The protocol involved PCR amplification of the target fragment, denaturation of the double-stranded PCR product with heat (95°C for 5min) and formamide dye (95% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue, 0.5M EDTA). It was followed by snap cooling on ice for 15 min, and the product was loaded in 12% polyacryl amide gel (PAGE) the gel and electrophoresis was performed at 4°C for 12h at 200V. After electrophoresis was over, the gel was stained with 0.1% silver nitrate to visualize banding patterns of the fragments (Bhattacharya *et al.* 2011; Paswan *et al.* 2013).

### Sequencing

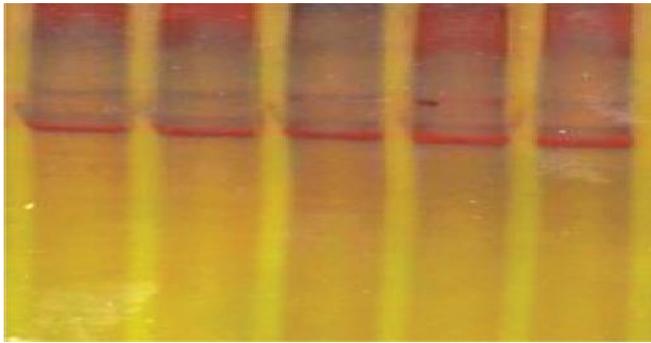
Three PCR products amplified from each genotyping group were sequenced using fragments-specific primers from both ends by the automated dye terminator cycle sequencing method in ABI PRIZM 377 DNA sequence (Perkin-Elmer). Sequenced fragment FSTN exon3 fragment was aligned with the reported sequence.

## RESULTS AND DISCUSSION

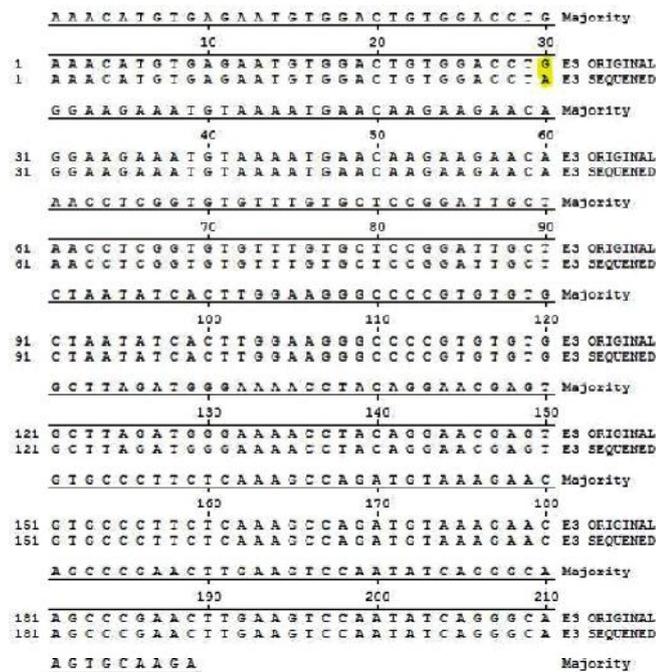
### Structural variation of FSTN exon3

The 219 bp of exon-3 coding region was amplified in PD-1 and control layer line (Fig. 2). PCR-SSCP followed by sequencing of representative samples of genotypes revealed monomorphic pattern of FSTN exon-3 in both the lines (Fig. 1), which is in contrast to previous reports in human (Jones *et al.* 2007; Sean *et al.* 2007), also reported different haplotypes of follistatin gene which were associated with skeletal muscle mass in humans. The PCR products in this study were sequenced using fragment specific primers from both the ends by the automated dye-terminator cycle sequence method.

The nucleotides sequences were compared with the original sequences (Accession number: NC\_006127) from which primer was designed. A mutation was observed at c.30G>A, but this was common in all individuals of two populations leading to monomorphic pattern of FSTN exon3 fragment (Fig.1).



**Fig. 1:** SSCP patterns of FSTN Exon3 in PD-1 and control layer lines of chicken



**Fig. 2:** Alignment of FSTN Exon3 fragment under study with original sequence (Accession number: NC\_006127), point of mutation is shown by yellow colour

**Growth performance**

In PD-1 line, the body weight of male at day1, wk2, wk4, and wk6 were 40.48±0.38, 138.67±2.16, 438.63±42.50 and 730.41±11.07g, respectively. The weight of females at the corresponding ages was 40.27±0.35, 135.88±2.22, 380.03±6.05, and 698.21±10.11g (Table.1). The body

weight of male and female did not differ significantly (P<0.05) at day1, wk2 and wk4. However, at 6<sup>th</sup> week the body weight differed significantly between male and female. . It may be because of similar feed given to male and female as reported Haunshi *et al.* (2015). In control layer, the body weights at day1, wk2, wk4 and wk6 were 35.21±0.34, 66.56±0.84, 127.93±2.48 and 231.85±2.93g, respectively while the weights of females at corresponding ages was 35.56±0.29, 65.43±0.11, 124.80±1.85 and 226.80±3.68g (Table.2). The body weight of male and female did not differ significantly at day1, 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> weeks of age.

**Table 1: Growth Performance of PD-1 line**

Parameters	Male	Female	p-value
Bwt (Day1) ±SE	40.482±0.375 <sup>a</sup>	40.27±0.348 <sup>a</sup>	0.689
Bwt (2 <sup>nd</sup> wk) ±SE	138.673±2.163 <sup>a</sup>	135.88±2.215 <sup>a</sup>	0.376
Bwt (4 <sup>th</sup> wk) ±SE	438.633±42.50 <sup>a</sup>	380.03±6.049 <sup>a</sup>	0.132
Bwt (6 <sup>th</sup> wk) ±SE	730.411±11.07 <sup>a</sup>	698.21±10.11 <sup>b</sup>	0.034

Bwt (Body Weight), Day1 (Hatch Day), 2<sup>nd</sup> wk (Second Week), 4<sup>th</sup> wk (Fourth Week), 6<sup>th</sup> wk (Sixth Week), SE (Standard error). Row-wise superscripts with same alphabet indicates non-significant difference and different superscript indicates significant difference along row.

**Table 2: Growth Performance of Control layer line**

Parameters	Male	Female	p-value
Bwt (Day1) ±SE	35.21±0.34	35.56±0.29	0.57
Bwt (2 <sup>nd</sup> wk) ±SE	66.56±0.84	65.43±.114	0.28
Bwt (4 <sup>th</sup> wk) ±SE	127.93±2.48	124.8±1.85	0.68
Bwt (6 <sup>th</sup> wk) ±SE	231.85±2.93	226.80±3.68	0.45

Bwt (Body Weight), Day1 (Hatch Day), 2<sup>nd</sup> wk (Second Week), 4<sup>th</sup> wk (Fourth Week), 6<sup>th</sup> wk (Sixth Week), SE (Standard error).

**CONCLUSION**

Exon3 of Follistatin gene was found to be monomorphic in PD-1 as well as control layer chicken population. One transition at nucleotide 30bp (postion no-2847 as per ncbi reference sequence) was observed which was common to all individual. The body weight of PD-1 was higher than Layer control.



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## REFERENCES

- Annual report, 2011 and 2015. Department of Animal husbandry, dairying and fisheries, Ministry of agriculture, Govt of India.
- Bhattacharya, T.K. and Chatterjee, R.N. 2013. Polymorphism of the myostatin gene and its association with growth traits in chicken. *Poult. Sci.*, **92**: 910-915.
- Haunshi, S., Reddy, B.L.N., Niranjana, M., Padhi, M.K., Rajaravindra, K.S. and Chatterjee, R.N. 2015. *Status of genetic resources of chicken evolved at ICAR-DPR*. ICAR-Directorate of Poultry Research, Rajendra nagar, Hyderabad, page 4-11.
- Helene Gilson, Oliver Schakman, Stephanie Kalista, Pascale Lause, Kunihiro, Tsuchida and Jean-Paul Thissen 2009. *American. J. Physiol. Endocrinol. Metbol.*, **297**: E157-E164.
- Hosseini, A.A. and Mohsen, K. 2011. Detection of polymorphism at the Insulin like growth factor-1 gene in Mazandaran Native chicken using polymerase chain-restriction fragment length polymorphism method. *American. J. Anim. Vet. Sci.*, **6**: 80-83.
- Jones, M.R., Wilson, S.G., Mullin, B.H., Mead, R., Watts, G.F. and Stuckey, B.G., 2007. Polymorphism of the follistatin gene in polycystic ovary syndrome. *Mol. Hum. Reprod.*, **13**(4): 237-241.
- Lee, S.J. and McPherron A.C. 2001. Regulation of myostatin activity and muscle growth. *Proc.Natl.Acad.Sci.USA.*, **(98)**: 9306-9311.
- Matzuk, M.M., Lu, N., Vogel, H., Sellheyer, K., Roop D.R. and Bradley, A. 1995. Multiple defects and prenatal death in mice deficient in follistatin. *Nature*, **374**: 360-363.
- Otsuka, F., Yao, Z., Lee, T., Yamamoto, S., Erickson, G.F. and Shimasaki *et al.* 2001a. Bone morphogenetic protein- Identification of target cells and biological functions. *J. Biol Chem.*, **275** (39): 523-39.
- Paswan, C., Bhattacharya, T.K., Nagaraj, C.S., Chatterjee, R.N. and Jayashankar, M.R. 2013. SNPs in minimal promoter of myostatin (GDF-8) gene and its association with body weight in broiler chicken. *J. Appl. Anim. Res.*, **(42)**: 304-309.
- Patel, K. 1998. Follistatin. *The Int. J. Biochem Cell Biol.*, **(30)**: 1087-1093.
- Sean Walsh, Jeffrey, M.E, Ferrucci, L. and Stephen M.R. 2007. Activin-type II receptor B (ACVR2B) and follistatin haplotypes association with muscle mass and strength in humans. *J. Appl. Physiol.*, **102**(6): 2142-2148.