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Myostatin (*GDF8*) gene and its intriguing role in regulating growth in poultry

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ABSTRACT

Myostatin, also called as growth differentiating factor 8 (*GDF8*), a negative regulator of growth in chicken has been characterized at nucleotide and protein level. The total length of the coding frame is 1128 bp encoding 375 amino acids pro-myostatin consisting of first 23 amino acids forming the signal peptide, next 243 amino acids as pre-protein and remaining 109 amino acids forming mature peptide. The protein in dimeric form is biologically active in chicken. This gene harbours many SNPs at promoter, 5'-UTR, exons, introns and 3'-UTR across the breeds of chicken in which coding region had higher level of polymorphism than non-coding regions. The SNPs had significant association with many economic traits such as growth traits, carcass traits, and immune response traits. The body weights of poultry can be rapidly enhanced by knocking down the expression of myostatin gene by RNAi and knocking out the gene by gene-editing, and in one generation more than 26% improvement in body weight was achieved. It is concluded that myostatin has been a promising molecule associated with controlling growth in chicken which may be considered to determine genetic markers for growth and other economically important traits for further use in selection programme, and to improve growth traits rapidly by inhibiting its expression through RNAi and gene editing in poultry.

Keywords: Association, Gene editing, Growth marker, Myostatin, RNAi, SNPs

Skeletal muscle is the most commercially important tissue in meat-producing animals, serving as a quality protein source for human. Hence, it is important to improve muscle growth in animals where growth has been known to be controlled by multiple genes including major genes such as growth hormone (GH), growth hormone receptor (GHR), insulin like growth factors (IGFs), insulin like growth factor binding proteins (IGFBPs), follistatin (FSTN) (Dushyanth *et al.* 2016a, b), bone morphogenetic proteins (BMPs) (Divya *et al.* 2018), myostatin (*MSTN*) (Bhattacharya *et al.* 2013), activin receptor type IIA (ACVR2A) (Satheesh *et al.* 2016), and activin receptor type IIB (ACVR2B) (Guru *et al.* 2016, Vishnu *et al.* 2016). The myostatin is also called as growth differentiating factor 8 (*GDF*8). However, these genes can be classified into two groups such as positive regulatory and negative regulatory genes. The major positive regulatory genes of growth are GH, GHR, IGFs and their receptors viz. insulin like growth factor receptors (IGFRs), IGFBPs, FSTN, BMPs and their receptors (Bhattacharya *et al.* 2015a, Dushyanth *et al.* 2016a, Divya *et al.* 2018). The major negative regulatory genes are myostatin and its receptors viz. ACVR2A, and ACVR2B (Bhattacharya *et al.* 2015b, 2019). The positive regulators mostly act through their receptors for increasing growth of animals by enhancing muscle mass during both embryonic and post-embryonic periods. Similarly, negative regulators act through their receptors for exerting their biological functions through inhibiting muscular growth (Bhattacharya *et al.* 2016). Myostatin being one of the most powerful negative regulators of growth acts through its receptors viz. ACVR2A and ACVR2B (Bhattacharya *et al.* 2019, Satheesh *et al.* 2016, Guru *et al.* 2016).

Conventional selection has been applied rigourously for more than 50 years in poultry field for improving the desired economic traits like growth, egg production, skeletal integrity, and immune response traits. We have achieved remarkable improvement in growth and production traits in pure line chicken through traditional selection, but, rate of genetic gain in this process was slow over the last 50 years. In pure chicken lines, the enhancement of growth and egg production may lead to the appearance of certain undesired traits such as muscle and egg fat deposition, low fitness, abnormal anatomical traits, lameness, low bone mineralization, and calcium deficiency. These undesired characters become harmful and have been appeared to be worrisome in poultry industry. With the advancement of science, researchers have developed many advanced biotechnological tools/techniques such as marker assisted selection, gene knock down technology

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by RNAi, and CRISPR/Cas mediated gene editing to improve the performance of birds at a very rapid rate in poultry (Bhattacharya *et al.* 2016, 2019; Kim *et al.* 2020, Lee 2020). In our laboratory (ICAR-Directorate of Poultry Research, Hyderabad, India), we have employed some of the advanced biotechnological tools such as RNAi and marker assisted selection (MAS) to improve growth and production traits in chicken (Bhattacharya 2017, 2018; Bhattacharya and Chatterjee 2014). The present review discusses the recent *MSTN* applications through detection of SNPs, gene-trait association, gene knock-down by RNAi, gene editing and some fundamental aspects such characterization, gene function etc. It would help researchers to find out genetic markers related to growth, and advanced techniques to improve growth at a faster rate by manipulating the activity of *MSTN* through RNAi and gene editing in poultry. Additionally, this review focusses on the role of myostatin, its association with growth traits, and its manipulation for lowering its expression along with its effect on growth and other traits in poultry.

Structure

The *MSTN* gene is located on chromosome 7 in chicken (NCBI Accession No. NC 006094.5). On downstream of the gene, there is a 362 bp minimal promoter where there are sites for binding transcription factors such as fork-head activin signal transducer (FAST-1) involved in regulating gene expression (Paswan *et al.* 2014). Besides, there is a motif in the promoter, which showed mutation forming two promoter alleles viz. A and B (Nucleotide, A to T at 214th position of the promoter) (Paswan *et al.* 2014). Another transcription factor, Smad also binds to the promoter of the *MSTN* gene and plays a critical role in up-regulation of the gene expression (Allen and Unterman 2007, Du *et al.* 2007a).

The coding region of the open reading frame in chicken is of 1128 bp in length comprising of 3 exons viz. exon 1, exon 2 and exon 3, where their corresponding lengths are 372, 374 and 381 bp (Bhattacharya and Chatterjee 2013). However, the total length of the gene is of 6742 bp in which the non-coding parts viz. 5'-UTR, 3'-UTR, intron 1, and intron 2 are of 117, 1131, 2091 and 2275 bp, respectively (Fig. 1). The 1128 bp *MSTN* coding region encodes 375 amino acids (Pro-myostatin) consisting of 46 strongly basic, 46 strongly acidic, 117 hydrophobic and 107 polar amino acids (Bhattacharya *et al.* 2015b). The comparison of the coding frame of the gene in broiler and layer chicken revealed variation of the gene where there are many substitutions of nucleotides between these varieties. The nucleotide substitutions were observed at C65T (alanine22valine), C306T and C1094T (proline365leucine) of the gene (Bhattacharya *et al.* 2015b). The first 23 amino acids of

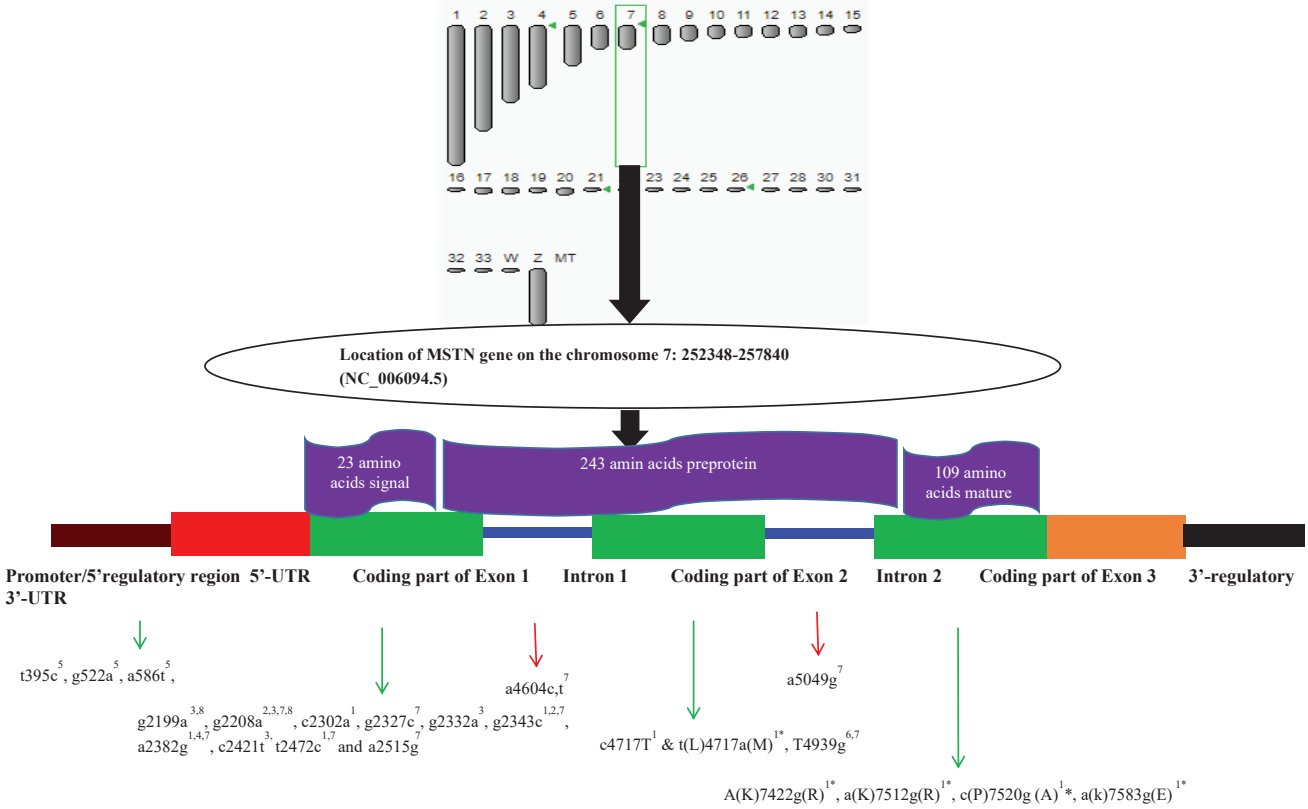

Fig. 1. Structure and location of mutation (SNPs) in myostatin gene of chicken. Capital letters within parenthesis corresponding to the SNP mutation are amino acids in abbreviated form indicating functional/missense/non-synonymous types of substitutions in the gene. *indicates functional mutation. Numbers in superscripts indicate references where SNPs have been reported. [1]Bhattacharya and Chatterjee 2013, [2]Dementeva *et al.* 2016, [3]Zhang *et al.* 2019, [4]Zhang *et al.* 2011, [5]Paswan *et al.* 2014, [6]Khaerunnisa *et al.* 2016, [7]Ye *et al.* 2007, [8]Zhu *et al.* 2007.

the protein form the signal peptide, next (NH_2-terminal) 243 amino acids (24-266) form preprotein and remaining COOH-terminal 109 amino acids (267-375) form mature peptide (McPherron *et al.* 1997, Tries *et al.* 2001, Thomas *et al.* 2000, Zimmers *et al.* 2002, Wolfman *et al.* 2003, Rios *et al.* 2004, Bhattacharya *et al.* 2015b). The molecular weight of mature peptide is 13 kDa while the function unit forms a dimer of mature peptide having molecular weight of 26 kDa (Bhattacharya *et al.* 2015b). The *MSTN* dimer is formed at cysteine knot comprising cysteine residues in the C-terminal region of the protein (Baron *et al.* 2002, Starck *et al.* 2011). In fact, the C-terminal dimer binds with the activin type II receptors such as ACVR2B and ACVR2A for exerting its biological activity. However, the functional pattern of *MSTN* protein is similar in nature across the species such as cattle, buffalo, African catfish, and croceine croaker (Jeanplong *et al.* 2001, Mota *et al.* 2006, Xue *et al.* 2006, Lee 2010, Kanjanaworakul *et al.* 2016). Little mutation in the structure of the cysteine knot may lead to dissociation of dimers into 2 dysfunctional *MSTN* units causing double-muscling phenotype (Mota *et al.* 2006).

Function

The *MSTN* functions during both embryonic and adult stages in poultry (Kocamis *et al.* 1999, Dushyanth *et al.* 2016c). It is expressed throughout the life and has become the most powerful inhibitor for development of skeletal muscle (Abdel-Gawad *et al.* 2017). During embryonic stage, *MSTN* inhibits activation, proliferation, and differentiation of myogenic satellite cells responsible for skeletal muscle formation (Kocamis *et al.* 1999, McFarland *et al.* 2007). In fact, *MSTN* acts through a pathway where it downregulates expression of Pax-3 gene, which is the causal agent for proliferation of myogenic cells, and also prevents expression of Myo-D involved in activation of the myogenic programme (Amthor *et al.* 2002). This mechanism of *MSTN* activity in poultry and other species is more or less similar making this protein as one of the most conserved proteins expressed through out the life across the species (McPherron *et al.* 1997, Gonzalez-Cadavid *et al.* 1998, Ivey *et al.* 2000, Reardon *et al.* 2001, McCroskery *et al.* 2003, Schuelke *et al.* 2004, Scheuermann *et al.* 2004).

Expression

Myostatin is expressed in wide range of tissues such as brain, eye, gizzard, muscle, heart, small gut, large gut, testis, and ovary in chicken making the protein as ubiquitous (Kubota *et al.* 2007, Bhattacharya and Chatterjee 2013, Dou *et al.* 2018). In our lab, findings showed *MSTN* gene expression profile in muscle was different between PB-1 or CB broilers and IWI White Leghorn layer strains chicken (IWI) at different time points (Bhattacharya *et al.* 2015b). Its expression is not uniform in slow and fast-growing chicken where 8.5, 5.8 and 6.3 folds higher expression was observed in slow growing layer chicken compared to the fast-growing broiler ones at 4th, 6th and 7th week of age, respectively (Bhattacharya *et al.* 2015b). As age progressed, the expression is gradually decreased from day old to 6th week of age in fast growing broiler chicken (Bhattacharya *et al.* 2015b). Such variability of expression is regulated by genetic means where expression is controlled by cis-regulatory elements viz. enhancer box (E-box) motifs. A total of 13 E-box motifs (E1 to ED13) were identified in the promoter of *MSTN* gene of chicken, which regulates *MSTN* expression (Hu *et al.* 2013).

Polymorphism and its effect on economic traits

The polymorphisms in terms of SNPs are located in the promoter, 5'UTR, 3'UTR, exons and introns of the *MSTN* gene in chicken (Ye *et al.* 2007, Zhu *et al.* 2007, Zhang *et al.* 2011, Bhattacharya and Chatterjee 2013, Paswan *et al.* 2014, Khaerunnisa *et al.* 2016, Dementeva *et al.* 2017, Zhang *et al.* 2019). Of all exonic SNPs, some of the SNPs viz. T4717A in exon 2 and A7422G, A7512G, C7520G, A7583G and T7634G in exon3 were of mis-sense types substituting amino acids viz. leucine to methionine, lysine to arginine, lysine to arginine, proline to alanine, lysine to glutamic acid and cysteine to glycine in the corresponding positions of the exons (Bhattacharya and Chatterjee 2013). The SNPs located at the coding as well as non-coding, and regulatory regions were significantly associated with many traits of economic importance such as growth (Zhang *et al.* 2011, Bhattacharya and Chatterjee 2013, Zandi *et al.* 2013, Paswan *et al.* 2014, Dementeva *et al.* 2017, Zhang *et al.* 2019), carcass (Gu *et al.* 2004, Zhu *et al.* 2007, Khaerunnisa *et al.* 2016), and immune response traits (Ye *et al.* 2007) in chicken (Table 1). These information on SNPs-traits association provide baseline data for exploring SNPs markers for further use in breeding programme to improve economically important traits in chicken.

Myostatin knock-down by RNAi and genome editing

Protocols such as embryo manipulation (EM), embryo electroporation (EP), and sperm mediated gene transfer (SMGT) methods were developed by the scientists to produce knock-down chicken. Two methods, viz. EM and SMGT have been successfully used to produce knock-down chicken for silencing expression of the *MSTN* gene (Rao *et al.* 2004, Andermatt and Stoeckli 2014, Bhattacharya *et al.* 2016, 2019). It was revealed that the EM method was better than the SMGT method as the first one has higher hatching rate (58% vs 42%) to produce chicks (Bhattacharya *et al.* 2016). However, in our lab, myostatin gene knockdown increased body weight by silencing expression of the *MSTN* gene in control broilers which makes the knock-down birds heavier (26%) than the non-knockdown birds (1209.8 *vs* 948.3 g) (Bhattacharya *et al.* 2016). Silencing the *MSTN* gene along with its 2 receptor genes viz. ACVR2A and ACVR2B in PD-1 broiler chicken also influences body weight where at the age of 35 days, in myostatin knockdown, ripple knockdown and control birds, the body weights were 668.3±12.2, 657.2±32.4 and 581.5±8.8 g, respectively (Bhattacharya *et al.* 2019). Our

Table 1. Mutations (SNPs) present in myostatin gene and its association with economic traits

Fragment	Alleles/haplotype	Mutation (SNPs)	Genotype/Haplogroup-trait association	References
1128 bp open reading frame	Haplotypes: h1, h2, h3, h4, h5, h6, h7, h8, h9, h10, h11, h12 and h13	C154A, C195G, G234A, C324T, T477C, A478T, G908A, G998A, G1006C, G1069A and G1120T	Body weights at 4, 6 and 7 weeks of age in PB-1 broiler; Body weights at 2 and 7 weeks of age in CB broiler	Bhattacharya and Chatterjee (2013)
Exon 1 (AF346599)	G1 and A	G2109A	-	Dementeva *et al*. 2017
	C and G2	C2244G	Body weight at 7 days in Smena-8 chicken cross	Dementeva *et al*. 2017
Exon 1	SNP1	Chromosome 7: G218133A	Body weights at hatch, 1 and 8 weeks in Daheng broiler	Zhang *et al*. 2019
	SNP2	Chromosome 7: A218142G	SNP2 Body weights at hatch and 1 week in Daheng broiler	Zhang et al. 2019
	SNP3	Chromosome 7: C218277G	SNP3 body weight at hatch in Daheng broiler	Zhang *et al*. 2019
	SNP4	Chromosome 7: G218316A	-	Zhang *et al.* 2019
	SNP5	Chromosome 7: C218406T	SNP5 Body weights at hatch, 1 week and 8 week in Daheng broiler	Zhang *et al*. 2019
Exon 1	G and A alleles	G234A	Body weights at 6, 8, 10, 12, 14, 16 and 18 weeks in Bian chicken	Zhang *et al.* 2011
5'-regulatory region	A and B alleles	G304A, A322G and C334T	Abdominal fat% and breast muscle% at 12 weeks of age; birth weight in F2 population of Broiler × Silky chicken	Gu *et al.* 2004
	E and F alleles	G167A	Breast muscle% at 12 weeks of age in F2 population of Broiler × Silky chicken	Gu *et al*. 2004
3'-regulatory region	C and D allele	A7263T	Breast muscle% at 12 weeks of age in F2 population of Broiler × Silky chicken	Gu *et al*. 2004
362-bp partial promoter (371 – 732 of HQ171974)	A and B alleles	T23C, G150A, A214T and A241T	Body weights at 2, 4, 5 and 6 weeks in control broiler chicken	Paswan *et al.* 2014
599 bp of promoter and exon 1	A, B and C alleles	-	Body weight at 12 weeks in Western Azerbaijan Native Chickens	Zandi *et al*. 2013
Exon 1	M and N alleles	G2100A	Abdominal fat weight and carcass % in Wenling grass-chicken	Zhu *et al*. 2007
	A and B alleles	G2109A	Breast muscle% in Wenling grass-chicken	
Exon 2 (AF346599.2)	T and G alleles	T4842G	Carcass weight, breast weight, thighs weight, drums sticks weight, wings weight and free water in carcass in Indonesian chickens	Khaerunnisa *et al.* 2016
Promoter (AF346599)	-	G2100A	-	Ye *et al.* 2007
Exon 1 (AF346599)	-	G2109A, G2244C, A2283G, C2346T, C2373T and A2416G	Antibody titer to infectious bursal disease virus vaccine, mortality from hatching to 14 days and 14 to 40 days of age; body weights at 7 and 40 days of age; breast meat%, feed conversion efficiency in commercial broiler chicken	
Exon 2 (AF346599)	-	T4842G		Ye *et al.* 2007

findings by silencing of ACVR2B gene through shRNAs in PD-1 chicken line showed significantly lowest serum cholesterol and HDL content.

The *MSTN* gene was edited by CRISPR/Cas9 mediated method to develop knock-out (KO) chicken (Kim *et al.* 2020). The editing of this gene enhances muscle mass in birds due to hyperplasia and hypertrophy of muscle fibres. The editing also reduces the abdominal fat deposition in the birds (Kim *et al.* 2020). The *MSTN* edited quail was also developed through CRISPR/Cas9 method where deletion of a cluster of 3 bases in the *MSTN* gene was observed (Lee *et al.* 2020). Such nucleotides deletion caused deletion of an amino acid, cysteine in the protein. The editing of this gene increased body weight and heart weight, and decreased fat pad weight in the KO birds (Lee *et al.* 2020). Thus, by silencing the *MSTN* gene expression in birds has potential impact on enhancing body weight, which is considered as one of the major economic traits in meat animals. These advanced technologies could be suggested as potential means to augment the productivity of birds.

Conclusion

The structure and function of the myostatin gene has been thoroughly studied in poultry and other species. It is expressed during embryonic as well as adult stages. The expression of the myostatin gene is negatively associated with growth where higher myostatin expression is associated with slow growth. The structural variants of the gene revealed polymorphisms which were found to be associated with growth and other economic traits in poultry. The expression of the myostatin gene has been altered through gene editing methodologies. These tools were used effectively to develop knock-down and knock-out birds with higher body weights. Hence, it is suggested that an insight in analyzing myostatin through genetic markers, RNA interference, and gene editing, may explore effective means in near future to improve growth traits in poultry and in other species.

REFERENCES

Abdel-Gawad M M, Azab M E, Ismail R S, Nafeaa A A and A El-Magd M E. 2017. The action of myo-pro gene on the morphology of skeletal muscle of Fayoumi chicken. *Benha Veterinary Medical Journal* **33**(2): 129–36.

Abdel-Gawad M M, Azaba M E, Ismaila R S, Nafeaa A A and El-Magd M E A. 2019. Effect of myostatin inhibitor (myostatin-propeptide) on expression of myogenic genes in chick embryo. *Arabian Journal of Medical Sciences* **2**(2): 30–34.

Allen D L and Unterman T G. 2007. Regulation of myostatin expression and myoblast differentiation by FoxO and SMAD transcription factors. *American Journal of Physiology-Cell Physiology* **292**(1): 188–99.

Amthor H, Huang R, McKinnell I, Christ B, Kambadur R, Sharma M and Patel K. 2002. The regulation and action of myostatin as a negative regulator of muscle development during avian embryogenesis. *Developmental Biology* **251**(2): 241–57.

Andermatt I and Stoeckli E T. 2014. RNAi-based gene silencing in chicken brain development. *Methods in Molecular Biology* **1082**: 253–66.

Baron E E, Wenceslau A A, Alvares L E, Nones K, Ruy D C, Schmidt G S and Ledur M C. 2002. High level of polymorphism in the myostatin chicken gene. *Proc. 7th World Congress on Genetics Applied to Livestock Production*, Montpellier, France.

Bhattacharya T K. 2017. Development of an improved coloured broiler chicken line through MAS. *ICAR-DPR News* **17**(2): 2.

Bhattacharya T K and Chatterjee R N. 2013. Polymorphism of the myostatin gene and its association with growth traits in chicken. *Poultry Science* **92**(4): 910–15.

Bhattacharya T K and Chatterjee R N. 2014. 'Improvement in meat production with RNAi in poultry.' *ICAR News* **20**(4): 7.

Bhattacharya T K, Chatterjee R N, Dushyanth K, Paswan C, Shukla R and Shanmugam M. 2015a. Polymorphism and expression of insulin-like growth factor 1 (IGF1) gene and its association with growth traits in chicken. *British Poultry Science* **56**(4): 398–407.

Bhattacharya T K, Chatterjee R N, Dushyanth K and Shukla R. 2015. Cloning, characterization and expression of myostatin (growth differentiating factor-8) gene in broiler and layer chicken (*Gallus gallus*). *Molecular Biology Reports* **42**(2): 319–27.

Bhattacharya T K, Chatterjee R N, Sharma R P, Niranjan M and Rajkumar U. 2011. Associations between novel polymorphisms at the 5′-UTR region of the prolactin gene and egg production and quality in chickens. *Theriogenology* **75**(4): 655–61.

Bhattacharya T K, Chatterjee R N, Sharma R P, Raj Kumar U and Niranjan M. 2011. Genetic polymorphism of exon 1 of myostatin gene in a broiler and a layer chicken line. *Indian Veterinary Journal* **88**(5): 34–36.

Bhattacharya T K, Chatterjee R N, Sharma R P, Rajkumar U, Niranjan M and Reddy B L N. 2011. Exon-2 of Myostatin gene in chicken. *Indian Veterinary Journal* **88**: 39–40.

Bhattacharya T K, Paswan C, Chatterjee R N, Bhanja S K and Dange M. 2018. Development of an improved coloured broiler chicken line through MAS. *ICAR-DPR News* **18**(1): 3.

Bhattacharya T K, Shukla R, Chatterjee R N and Bhanja S K. 2019. Comparative analysis of silencing expression of myostatin (*MSTN*) and its two receptors (ACVR2A and ACVR2B) genes affecting growth traits in knock down chicken. *Scientific Reports* **9**(1):1-13.

Bhattacharya T K, Shukla R, Chatterjee R N and Dushyanth K. 2017. Knock down of the myostatin gene by RNA interference increased body weight in chicken. *Journal of Biotechnology* **241**: 61–68.

Boschiero C, Gheyas A, Godoy T, Moreira G, Grade C V C, Gasparin G and Coutinho L. 2013. SNPS and INDELS detection in the chicken myostatin gene and its intergenic regions. *5th Symposium on Animal Functional Genomics-ISAFG 2013*.

Chen P R, Suh Y, Shin S, Woodfint R M, Hwang S and Lee K. 2019. Exogenous expression of an alternative splicing variant of myostatin prompts leg muscle fiber hyperplasia in Japanese quail. *International Journal of Molecular Sciences* **20**(18): 4617.

Crisa A, Marchitelli C, Savarese M C and Valentini A. 2003. Sequence analysis of myostatin promoter in cattle. *Cytogenetic and Genome Research* **102**(1-4): 48–52.

Dementeva N V, Mitrofanova O V, Tyshchenko V I, Terletskiy V P and Yakovlev A F. 2017. The rate of weight gain and productivity of a chicken broiler cross with various polymorphic types of the myostatin gene. *Russian Journal of*

Genetics: Applied Research **7**(1): 1–5.

Divya D, Bhattacharya T K, Gnana Prakash M, Chatterjee R N, Shukla R, Vishnu G and Dushyanth K. 2018. Molecular characterization and expression profiling of BMP 3 gene in broiler and layer chicken. *Molecular Biology Reports* **45**(4): 477–95.

Dou T, Li Z, Wang K, Liu L, Rong H, Xu Z and Jia J. 2018. Regulation of myostatin expression is associated with growth and muscle development in commercial broiler and DMC muscle. *Molecular Biology Reports* **45**(4): 511–22.

Dushyanth K, Bhattacharya T K, Paswan C, Sitaramamma T, Shukla R and Chatterjee R N. 2016a. Structural variation in Fstn Exon 3 gene and its association with growth traits in chicken. *Journal of Animal Research* **6**: 471–74.

Dushyanth K, Bhattacharya T K, Paswan C, Shukla R, Vishnu P G and Chatterjee R N. 2016b. Characterization of exon4 of FSTN gene and its association with growth traits in PD-1 broiler chicken. *Journal of Animal Research* **6**(5): 967–69.

Dushyanth K, Bhattacharya T K, Shukla R, Chatterjee R N, Sitaramamma T, Paswan C and Guru Vishnu P. 2016c. Gene expression and polymorphism of Myostatin gene and its association with growth traits in chicken. *Animal Biotechnology* **27**(4): 269–77.

Dybus A, Proskura W S, Sadkowski S and Pawlina E. 2013. A single nucleotide polymorphism in exon 3 of the myostatin gene in different breeds of domestic pigeon (*Columba livia* var. domestica). *Veterinarni Medicina* **58**(1): 32–38.

Gonzalez-Cadavid N F, Taylor W E, Yarasheski K, Sinha-Hikim I, Ma K, Ezzat S and Bhasin S. 1998. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proceedings of the National Academy of Sciences* **95**(25): 14938–43.

Gu Z L, Zhang H F, Zhu D H and Li H. 2002. Single nucleotide polymorphism analysis of the chicken Myostatin gene in different chicken lines. *Acta Genetica Sinica* **29**(7): 599–606.

Gu Z, Zhu D, Li N, Li H, Deng X and Wu C. 2004. The single nucleotide polymorphisms of the chicken myostatin gene are associated with skeletal muscle and adipose growth. *Science in China Series C: Life Sciences* **47**(1): 25–30.

Guru Vishnu P B, Bhattacharya T K, Kumar P, Chaterjee R N, Ravi Kumar G V P P S, Paswan C and Rajendra Prasad A. 2017. Expression profiling of activin type IIB receptor during ontogeny in broiler and indigenous chicken. *Animal Biotechnology* **28**(1): 26–36.

Heintzman N D and Ren B. 2007. The gateway to transcription: identifying, characterizing and understanding promoters in the eukaryotic genome. *Cellular and Molecular Life Sciences* **64**(4): 386–400.

Hill E W, McGivney B A, Gu J, Whiston R and MacHugh D E. 2010. A genome-wide SNP-association study confirms a sequence variant (g. 66493737C> T) in the equine myostatin (*MSTN*) gene as the most powerful predictor of optimum racing distance for Thoroughbred racehorses. *BMC Genomics* **11**(1): 1–10.

Hu W, Chen S, Zhang R and Lin Y. 2013. Single nucleotide polymorphisms in the upstream regulatory region alter the expression of myostatin. *In Vitro Cellular and Developmental Biology-Animal* **49**(6): 417–23.

Ivey F M, Roth S M, Ferrell R E, Tracy B L, Lemmer J T, Hurlbut D E and Hurley B F. 2000. Effects of age, gender, and myostatin genotype on the hypertrophic response to heavy resistance strength training. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **55**(11): 641–48.

Jain H, Singh S, Kadam M and Sarkhel B C. 2010. Knockdown of the myostatin gene by RNA interference in caprine fibroblast cells. *Journal of Biotechnology* **145**(2): 99–102.

Jeanplong F, Sharma M, Somers W G, Bass J J and Kambadur R. 2001. Genomic organization and neonatal expression of the bovine myostatin gene. *Molecular and Cellular Biochemistry* **220**(1): 31–37.

Kanjanaworakul P, Sawatdichaikul O and Poompuang S. 2016. cDNA sequence and protein bioinformatics analyses of *MSTN* in African catfish (*Clarias gariepinus*). *Molecular Biology Reports* **43**(4): 283–93.

Khaerunnisa I, Pramujo M and Arief I. 2016. Polymorphism of the T4842G myostatin gene is associated with carcass characteristics in Indonesian chickens. *International Journal of Poultry Science* **15**(8): 316–24. carcass characteristics in Indonesian chickens.

Kim G D, Lee J H, Song S, Kim S W, Han J S, Shin S P and Park S. 2020. Generation of myostatin-knockout chickens mediated by D10A-Cas9 nickase. *FASEB Journal* **34**(4): 5688–96.

Kim S, Park K, Shin C, Cho N H, Ko J J, Koh I and Kwack K. 2013. Diplotyper: diplotype-based association analysis. *BMC Medical Genomics* **6**(2): 1–8.

Kocamis H, Kirkpatrick-Keller D C, Richter J and Killefer J. 1999. The ontogeny of myostatin, follistatin and activin-B mRNA expression during chicken embryonic development. *Growth, Development, and Aging: GDA* **63**(4): 143–50.

Kubota K, Sato F, Aramaki S, Soh T, Yamauchi N and Hattori M A. 2007. Ubiquitous expression of myostatin in chicken embryonic tissues: its high expression in testis and ovary. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* **148**(3): 550–55.

Kumar R, Singh S P, Kumari P and Mitra A. 2014. Small interfering RNA (siRNA)-mediated knockdown of myostatin influences the expression of myogenic regulatory factors in caprine foetal myoblasts. *Applied Biochemistry and Biotechnology* **172**(3): 1714–24.

Kumar S B, Dilbaghi N, Ahlawat S P S, Mishra B, Tantia M S and Vijh R K. 2007. Genetic relationship among chicken populations of India based on SNP markers of myostatin gene (GDF 8). *International Journal of Poultry Science* **6**(9): 684–88.

Lee J, Kim D H and Lee K. 2020. Muscle hyperplasia in Japanese quail by single amino acid deletion in *MSTN* propeptide. *International Journal of Molecular Sciences* **21**(4): 1504.

Lee J H, Kim S W and Park T S. 2017. Myostatin gene knockout mediated by Cas9-D10A nickase in chicken DF1 cells without off-target effect. *Asian Australasian Journal of Animal Sciences* **30**(5): 743.

Lee S J. 2010. Extracellular regulation of myostatin: a molecular rheostat for muscle mass. *Immunology, Endocrine and Metabolic Agents in Medicinal Chemistry* (*Formerly Current Medicinal Chemistry-Immunology, Endocrine and Metabolic Agents*) **10**(4): 183–94.

McCroskery S, Thomas M, Maxwell L, Sharma M and Kambadur R. 2003. Myostatin negatively regulates satellite cell activation and self-renewal. *Journal of Cell Biology* **162**: 1135–47.

McFarland D C, Velleman S G, Pesall J E and Liu C. 2007. The role of myostatin in chicken (*Gallus domesticus*) myogenic satellite cell proliferation and differentiation. *General and Comparative Endocrinology* **151**(3): 351–57.

McPherron A C, Lawler A M and Lee S J. 1997. Regulation of skeletal muscle mass in mice by a new TGF-p superfamily member. *Nature* **387**(6628): 83–90.

Mota L S, Curi R A, Palmieri D A, Borges A S, Lopes C R, Barbosa J D and Gimenes M A. 2006. Sequence characterization of coding regions of the myostatin gene (*GDF8*) from Brazilian Murrah buffaloes (*Bubalus bubalis*) and comparison with the *Bos taurus* sequence. *Genetics and Molecular Biology* **29**: 79–82.

Paswan C, Bhattacharya T K, Nagaraj C S, Chaterjee R N and Jayashankar M R. 2014. SNPs in minimal promoter of myostatin (GDF-8) gene and its association with body weight in broiler chicken. *Journal of Applied Animal Research* **42**(3): 304–09.

Petersen J L, Mickelson J R, Rendahl A K, Valberg S J, Andersson L S, Axelsson J and McCue M E. 2013. Genome-wide analysis reveals selection for important traits in domestic horse breeds. *PLoS Genetics* **9**(1): e1003211.

Rao M, Baraban J H, Rajaii F and Sockanathan S. 2004. *In vivo* comparative study of RNAi methodologies by *in ovo* electroporation in the chick embryo. Developmental dynamics: an official publication of the *American Association of Anatomists* **231**(3): 592–600.

Reardon K A, Davis J, Kapsa R M, Choong P and Byrne E. 2001. Myostatin, insulin-like growth factor-1 and leukemia inhibitory factor mRNAs are upregulated in chronic human disuse muscle atrophy. *Muscle and Nerve*: *Official Journal of the American Association of Electrodiagnostic Medicine* **24**(7): 893–99.

Ríos R, Carneiro I, Arce V M and Devesa J. 2002. Myostatin is an inhibitor of myogenic differentiation. *American Journal of Physiology-Cell Physiology* **282**(5): C993-99.

Salerno M S, Thomas M, Forbes D, Watson T, Kambadur R and Sharma M. 2004. Molecular analysis of fiber type-specific expression of murine myostatin promoter. *American Journal of Physiology-Cell Physiology* **287**(4): C1031-40.

Satheesh P, Bhattacharya T K, Kumar P, Chatterjee R N, Dhara S K, Paswan C and Dushyanth K. 2016. Gene expression and silencing of activin receptor type 2A (ACVR2A) in myoblast cells of chicken. *British Poultry Science* **57**(6): 763–70.

Sato F, Kurokawa M, Yamauchi N and Hattori M A. 2006. Gene silencing of myostatin in differentiation of chicken embryonic myoblasts by small interfering RNA. *American Journal of Physiology-Cell Physiology* **291**(3): 538–45.

Scheuermann G N, Bilgili S F, Tuzun S and Mulvaney D R. 2004. Comparison of chicken genotypes: myofiber number in pectoralis muscle and myostatin ontogeny. *Poultry Science* **83**(8): 1404–12.

Schuelke M, Wagner K R, Stolz L E, Hübner C, Riebel T, Kömen W and Lee S J. 2004. Myostatin mutation associated with gross muscle hypertrophy in a child. *New England Journal of Medicine* **350**(26): 2682–88.

Sharma M, Langley B, Bass J and Kambadur R. 2001. Myostatin in muscle growth and repair. *Exercise and Sport Sciences Reviews* **29**(4): 155-58.

Song X C, Xu C, Yue Z G, Wang L, Wang G W and Yang F H. 2016. Bioinformatic analysis based on the complete coding region of the *MSTN* gene within and among different species. *Genetics and Molecular Research* **15**(10.4238).

Spiller M P, Kambadur R, Jeanplong F, Thomas M, Martyn J K, Bass J J and Sharma M. 2002. The myostatin gene is a downstream target gene of basic helix-loop-helix transcription factor MyoD. *Molecular and Cellular Biology* **22**(20): 7066–82.

Starck C S and Sutherland-Smith A J. 2011. The C313Y Piedmontese mutation decreases myostatin covalent dimerisation and stability. *BMC Research Notes* **4**(1): 1–12.

Tao Z, Zhu C, Song C, Song W, Ji G, Shan Y and Li H. 2015. Lentivirus-mediated RNA interference of myostatin gene affects MyoD and Myf5 gene expression in duck embryonic myoblasts. *British Poultry Science* **56**(5): 551–58.

Tries R S, Chen T, Da Vies M V, Tomkinson K N, Pearson A A, Shakey Q A and Wolfman N M. 2001. GDF-8 propeptide binds to GDF-8 and antagonizes biological activity by inhibiting GDF-8 receptor binding. *Growth Factors* **18**(4): 251–59.

Thomas M, Langley B, Berry C, Sharma M, Kirk S, Bass J and Kambadur R. 2000. Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. *Journal of Biological Chemistry* **275**(51): 40235–43.

Tripathi A K, Aparnathi M K, Vyavahare S S, Ramani U V, Rank D N and Joshi C G. 2012. Myostatin gene silencing by RNA interference in chicken embryo fibroblast cells. *Journal of Biotechnology* **160**(3-4): 140–45.

Vishnu P G, Bhattacharya T K, Kumar P, Chatterjee R N, Paswan C and Dushyanth K. 2016. Single nucleotide polymorphism in the 5′-upstream region of activin A receptor, type IIB in Aseel Chicken. *Journal of Animal Research* **6**(2): 221–25.

Wang Y, Xu H Y, Gilbert E R, Peng X, Zhao X L, Liu Y P and Zhu Q. 2014. Detection of SNPs in the TBC1D1 gene and their association with carcass traits in chicken. *Gene* **547**(2): 288–94.

Wilt F H and Hake S. 2004. *Principles of Developmental Biology*. Inc. ISBN 0-393-97430-8: pp450.

Wolfma N M, McPherron A C, Pappano W N, Davies M V, Song K, Tomkinson K N and Lee S J. 2003. Activation of latent myostatin by the BMP-1/tolloid family of metalloproteinases. *Proceedings of the National Academy of Sciences* **100**(26): 15842–46.

Xue L, Qian K, Qian H, Li L, Yang Q and Li M. 2006. Molecular cloning and characterization of the myostatin gene in croceine croaker, *Pseudosciaena crocea*. *Molecular Biology Reports* **33**(2): 129–35.

Ye X, Brown S R, Nones K, Coutinho L L, Dekkers J C and Lamont S J. 2007. Associations of myostatin gene polymorphisms with performance and mortality traits in broiler chickens. *Genetics Selection Evolution* **39**(1): 73–89.

Zammit P S. 2017. Function of the myogenic regulatory factors Myf5, MyoD, Myogenin and MRF4 in skeletal muscle, satellite cells and regenerative myogenesis. *In Seminars in Cell and Developmental Biology* **72**: 19–32.

Zandi S, Zamani P and Mardani K. 2013. Myostatin gene polymorphism and its association with production traits in western azerbaijan native chickens. *Iranian Journal of Applied Animal Science* **3**: 611–15.

Zhang G X, Zhao X H, Wang J Y, Ding F X and Zhang L. 2012. Effect of an exon 1 mutation in the myostatin gene on the growth traits of the Bian chicken. *Animal Genetics* **43**(4): 458–59.

Zhang X X, Ran J S, Lian T, Li Z Q, Yang C W, Jiang X S and Liu Y P. 2019. The single nucleotide polymorphisms of myostatin gene and their associations with growth and carcass traits in Daheng broiler. *Brazilian Journal of Poultry Science* **21**(3).

Zhiliang G U, Zhu D, Li N, Li H, Deng X and Wu C. 2004. The single nucleotide polymorphisms of the chicken myostatin gene are associated with skeletal muscle and adipose growth. *Science in China Series C: Life Sciences* **47**(1): 25–30.

Zhu J, Li Y, Shen W, Qiao C, Ambrosio F, Lavasani M and

Huard J. 2007. Relationships between transforming growth factor-β1, myostatin, and decorin: implications for skeletal muscle fibrosis. *Journal of Biological Chemistry* **282**(35): 25852–63.

Zimmers T A, Davies M V, Koniaris L G, Haynes P, Esquela A F, Tomkinson K N and Lee S J. 2002. Induction of cachexia in mice by systemically administered myostatin. *Science* **296**(5572): 1486–88.