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Characterization of Myostatin (*MSTN*) Gene in Rabbit

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Abstract: Three exonic regions of *MSTN* gene was resequenced in hundred rabbits (*Oryctolagus cuniculus*) of five different breeds viz.,White Giant, Grey Giant, Soviet Chinchilla, New Zealand White and Angora) to make comparative study. The comparison of three MSTN exonic region of these animals revealed in total four polymorphisms of which three were located in the exon I (g.108T>C, g.111C>G, g.247C>A) and one was located in exon II (g.711T>A), respectively. Among those identified polymorphisms only g.247C>A observed to be missense mutation which changes p.K83Q (Lysine to Glutamate) at the amino acid level. The mutation at the position g.247C>A was further analysed with PANTHER, cSNP and SNAP tool and considered for correlation with the production traits. In the analysis 92% of the White Giant rabbits were heterozygous for the identified mutation (g.247C>A), rest 8% of White Giant and all other breeds under study showed g.247A>A genotype. The phenotypic analysis inferred that White Giant had better performance (body weight) than the other breeds, i.e., Grey Giant, Soviet Chinchilla, New Zealand White and Angora.

Key words: *MSTN* gene, oryctolagus cuniculus, polymorphism, angora, production

INTRODUCTION

Rabbits are small mammals found in almost all parts of the world and used for mainly meat and fur production. Much candidate gene approach has already been successfully applied to identify several DNA markers associated with production traits in livestock (Rothschild and Soller, 1997). The principle is based on the fact that the variability within genes coding for protein products involved in key physiological mechanisms and metabolic pathways directly or indirectly involved in determining an economic trait might probably explain a fraction of genetic variability for the production itself (Fontanesi *et al*., 2008). Myostatin (MSTN), a member of the Transforming Growth Factor-β (TGF-β) superfamily has been shown to be a negative regulator of myogenesis and disruption in the gene leads to double muscled phenotype (Grobet *et al*., 1997). Numerous mutations in the *MSTN* gene have been identified in livestock which includes cattle (Grobet *et al*., 1997; McPherron and Lee, 1997), sheep (Boman *et al*., 2009; Clop *et al*., 2006), goat (Zhang *et al*., 2012) and rabbit (Fontanesi *et al*., 2011; Sternstein *et al*., 2014). Until now, only few attempts have been done to study *MSTN* gene polymorphism in rabbit (Fontanesi *et al*., 2011; Sternstein *et al*., 2014). Moreover, association of these polymorphisms with the production traits could not be clearly detected. Hence, the aim of the study was to identify polymorphisms in the *MSTN* gene of White Giant, Grey Giant, Soviet Chinchilla, New Zealand White and Angora and its association with production trait.

MATERIALS AND METHODS

Collection of blood samples from rabbit: Blood samples of 5 different breeds of rabbit (White Giant, Grey Giant, Soviet Chinchilla, New Zealand White and Angora) were collected by 2 mL sterile disposable syringe in a sterile vial containing 0.5 mM EDTA. In total 100 blood samples (20 in each breed) were collected and stored at 4°C for DNA isolation.

Isolation of genomic DNA from blood: Genomic DNA was isolated from the blood by using the Standard Salt Precipitation Method and further purified with Chloroform:Phenol (1:1) Method. The quality and quantity of isolated DNA samples were measured using a Genesys 10 UV spectrophotometer (Thermo, USA) by calculating the ratio of optical density at A260/A280. The integrity of DNA samples were checked using 0.8% agarose gel electrophoresis. The good quality DNA samples were stored at -20°C for further analysis.

Primers selection and amplification of *MSTN* gene: Three pairs of primer MSTN1F and MSTN1R, MSTN2F andMSTN2R, MSTN3F and MSTN3R (Table 1) spanning

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Table 1: Primers used in the amplification of exon I, II and III of *MSTN* gene from rabbits

Primers	Sequence (5'-3')	Location	Expected size (bp)	Annealing temperature (°C)
MSTN1F	AATTTTGCTTGCCATTACTGA	Exon I	499	58
MSTN1R	TCAGCAGAACTGTTGACATACAC			
MSTN2F	TGCATGCATTATCCCAATAGA	Exon II	570	
MSTN2R	TCGGTAGTTGTTTCCCACTTT			
MSTN3F	AAAGGTATTCCAAGCAAAATGA	Exon III	523	
MSTN3R	GGGGAAGACCTTCCATGTTT			

the MSTN exon I, II and III were selected from the published literature (Fontanesi *et al*., 2008) and procured from SIGMA, Bangalore. Polymerase Chain Reaction (PCR) amplifications using the three primer pairs were done separately. And carried out in a final reaction volume of 25 μL containing 1x PCR Buffer (Sigma), 1.5 mM MgCl2, 0.2 mM dNTPs, 0.4 μm Forward and Reverse Primers, 1.25U Taq Polymerase and 50-80 ng template DNA with the following cycling condition: initial denaturation at 94°C for 3 min followed by 30 cycles of 94°C for 40 sec, 58°C for 30 sec and 72°C for 35 sec with a final extension at 72°C for 5 min. The size of PCR products were of 499, 570 and 523 bp of MSTN exon I, II and III, respectively. In every experiment, negative controls were performed containing all reagents except DNA, aiming to avoid contamination. The reactions were performed in a thermal cycler (eppendorf Master cycler gradient) and the amplicons were analyzed on 1.5% agarose gel containing 0.5 μg mL^{-1} ethidium bromide in horizontal gel electrophoresis and visualized under UV light by gel documentation system (Alpha imager). The PCR products were purified with the column (Neucleospin) as per the manufacturer's instruction and sequenced with SciGenom Labs Pvt. Ltd., Kerala.

Sequence analysis: Nucleic acid and protein database searches were performed using BLAST at the NCBI server. The DNA sequence data were analyzed using DNASTAR 5.0 Software (Dayhoff *et al*., 1978). The alignment of the nucleotide and amino acid sequences of MSTN were performed using ClustalW (Thompson *et al*., 1994).

***In silico* functional analysis with PANTHER cSNP and SNAP tool:** *In silico* functional analysis of missense mutations were obtained using PANTHER (Thomas *et al*., 2003). PANTHER estimates the likelihood of a particular amino acid substitution to cause a functional impact on the protein. It calculates the substitution Position-Specific Evolutionary Conservation (subPSEC) score based on an alignment of evolutionarily related proteins (Thomas *et al*., 2003, 2006; Thomas and Kejariwal, 2004). The probability that a given variant will cause a deleterious effect on protein function is estimated by $P_{deleterious}$ such that a subPSEC score of -3 corresponds to a $P_{deleterious}$ of 0.5 (Brunham *et al*., 2005). The subPSEC score is the negative logarithm of the probability ratio of the wild-type and mutant amino acids at a particular position. PANTHER subPSEC scores are continuous values from 0 (neutral) to Ca -10 (most likely to be deleterious).

SNAP (Bromberg and Rost, 2008) is a neural-network based tool used to evaluate the functional effects of single amino acid substitutions in proteins. It utilizes various biophysical characteristics of the substitution as well as evolutionary information, predicted or observed structural features and annotations, to predict whether or not a mutation is likely to alter protein function (in either direction: gain or loss). Higher Reliability Indices (RI) are strongly correlated with higher prediction accuracy.

RESULTS AND DISCUSSION

Resequencing of the three coding exons was carried out using a panel of genomic DNA from 100 rabbits belonging to 5 different breeds (White Giant, Grey Giant, Soviet Chinchilla, New Zealand White and Angora). The multiple sequence alignment of sequenced *MSTN* gene revealed 4 single nucleotide polymorphisms of which three were located in the exon I (g.108T>C, g.111C>G, g.247C>A) and one was located in exon II (g.713T>A), respectively. Among those two SNPs (g.108T>C, g.713T>A) were already been described by Fontanesi *et al*. (2011) and Sternstein *et al*. (2014) and two SNPs (g.111C>G, g.247C>A) were found to be novel. In the newly identified polymorphisms only g.247C>A observed to be missense (amino acid changing) mutations which changes p.K83Q (Lysine to Glutamate) at the amino acid level (Fig. 1). The mutation identified in the coding region may have functional impact. Further, *in silico* functional analysis by PANTHER, cSNP of p.K83Q supports the putative functional role of these mutations (subPSEC = -4.96794; $P_{deleterious}$ = 0.87739). This was further evidenced by the SNAP tool (non-neutral; RI-1; EA-63%). These predictions also suggest that the mutation at the position g.247G>A may have functional impact on the phenotype. Moreover, mutations in the *MSTN* gene leading to hyper muscularity in mammals (Grobet *et al*., 1997; McPherron and Lee, 1997). Hence, the mutation at the position g.247C>A was further considered for the correlation analysis with the production traits. In the

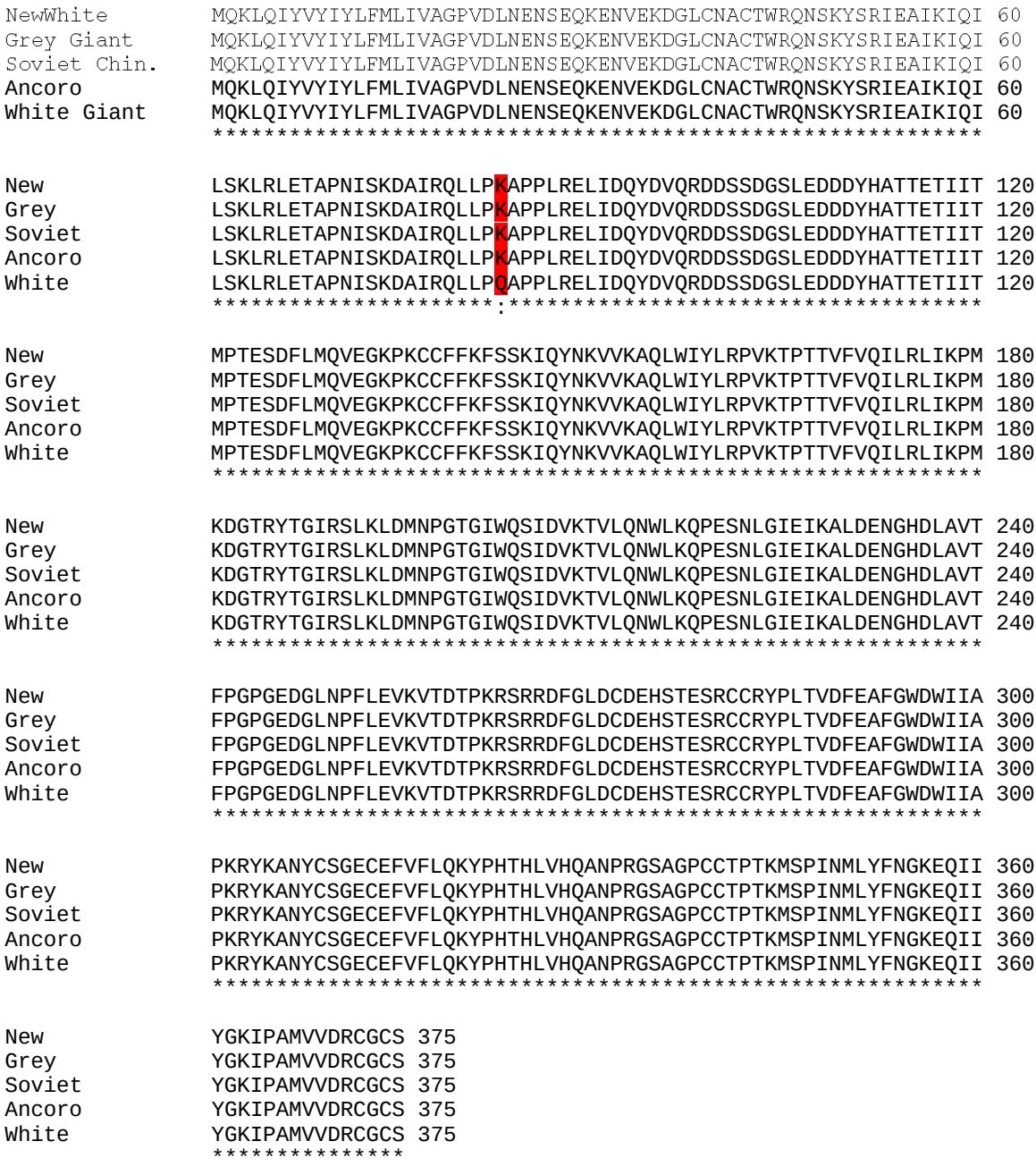

Fig. 1: MSTN protein alignment showing the amino acid changes in different breeds of rabbit under study

analysis 92% of the White Giant rabbits were heterozygote for the identified mutation (g.247C>A), rest 8% of White Giant and all other breeds under study showed g.247A>A genotype. The phenotypic analysis inferred that white giant had more performance (body weight) than the other breeds, i.e., Grey Giant, Soviet Chinchilla, New Zealand White and Angora.

CONCLUSION

As some breeds of rabbit are significant meat producers, the double-muscling phenotype can be potentially exploited for economic gain. In summary, we have characterized the MSTN coding regions of rabbits and the identified the mutation (amino acid changing)

g.247C>A with White Giant had high correlation with body weight. Thus, the present study conclude that the mutation g.247C>A could be consider as marker in the breeding programme of rabbit focussed on meet production. Further, characterization is going on to conclude the above mentioned fact in rabbits.

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