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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2023.380.385

Research Article
Novel Polymorphisms in Caprine *Myostatin* Gene and its Relationship with Growth Traits in Saburai Does (*Capra hircus*)

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Abstract

Background and Objective: Saburai goat (*Capra hircus*) is a crossbred goat from Boer buck (75%) and Ettawa doe (25%) for meat production purposes. This study was carried out to detect the mutation points or Single Nucleotide Polymorphisms (SNPs) in the myostatin (*MSTN*) gene of Saburai with the forward sequencing method. **Materials and Methods:** A total of twenty-one blood samples of Saburai does (75% Boer, 25% Ettawa) were collected from the Villager Breeder Center (VBC) at Tanggamus Regency of Lampung Province, Indonesia. The DNA analysis consists of DNA isolation, PCR analysis and sequencing analysis. The record data were used for association study with the mathematical model: $Y_{ij} = \mu + G_i + \epsilon_{ij}$. **Results:** Research showed that one common SNP of g.217_218.indel.TTTTA (5'UTR) and three novel SNPs of c.386G>C (exon 1), g.641_642.indel.T (intron 2) and c.4957G>C (exon 3) were detected in the present study. In this study, a novel SNP on exon 1 and intron 2 of Saburai *MSTN* gene has a moderate PIC value (>0.30). In addition, a novel SNP on exon 1 and exon 3 of the Saburai *MSTN* gene was detected as a missense mutation of A55P and A43P, respectively. Goats with the heterozygous genotype have higher growth traits compared to goats with the homozygous genotype. **Conclusion:** The goats with heterozygous genotypes can be further developed to increase the productivity of Saburai goats.

Key words: Novel polymorphisms, caprine myostatin gene, Saburai does, growth traits, heterozygous genotypes

Citation: Dakhlan, A., W.P.B. Putra, P.E. Santosa and D. Kurniawati, 2023. Novel polymorphisms in caprine myostatin gene and its relationship with growth traits in Saburai does (*Capra hircus*). Pak. J. Biol. Sci., 26: 380-385.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Multiple genes control physiological rules for muscle growth in animals. Polymorphism shows relationships with important traits economically and becomes a useful marker for the selection process. The most common form of genome variation that can be used to study the relationship between polymorphism and the nature of the individual is a single nucleotide polymorphism[1]. For the study of genetic associations, this is an increasingly general approach.

Members of the changed β growth factor that usually acts to limit muscle mass by controlling the amount and fiber of muscle growth are myostatin which is encoded by the *MSTN* gene[2]. This superfamily includes a large number of differentiation factors and growth that play an important role in maintaining homeostasis of tissue and regulating the development of embryonic in animals[3]. According to Zhang *et al.*[4] myostatin (MSTN), a member of the transforming growth factor superfamily, is crucial in controlling meat quality and muscle hypertrophy[4]. The *MSTN* gene encodes a glycoprotein that is broadly expressed in skeletal muscle and has 3 exons and 2 introns[5].

Myostatin-deficient mice had reduced the formation of adipocytes (fat cells) from stem cells[6] as a result of reduced secretion and production of leptin and tendons that were brittle, small and hypocellular[7]. Other, natural mutations that have been identified in human subjects are mutations that reduce the number of myostatin and/or inhibit their functions[8] and also in the sheep[9] breeds and in several cattle[10]. An increase in the frequency of mutations in the *MSTN* gene has been thoroughly researched due to the role of MSTN identification essential in the development of growth and skeletal muscle. The sheep *MSTN* gene is found on chromosome 2 and studies have demonstrated that an SNP called DQ530260: g.6223G>A in the gene's intron 2 affects muscularity[11,12].

Many species exhibit remarkable muscularity and a "double-muscling" phenomenon as a result of mutations in the *MSTN* gene, which can inhibit its production or result in a non-functional protein[13]. Numerous studies on pigs and cattle have been conducted recently[14,15]. Because of this, the *MSTN* gene, which may one day serve as a candidate gene for animal muscle growth, is primarily responsible for the development of muscles. Similar research into the *MSTN* gene's characteristics in various breeds hasn't been done as much, either. Therefore, in this study, DNA sequencing and PCR-based caprine myostatin gene polymorphism were used to find SNPs of the *MSTN* gene in reference Saburai does populations in Tanggamus, which are crucial for understanding. Based on the above explanations, the objectives of the research were to investigate the polymorphism of the Saburai goat *MSTN* gene and the associations of polymorphism with growth traits.

MATERIALS AND METHODS

Study site: This study was carried out for 6 months from August, 2022 to February, 2023 at Tanggamus Regency of Lampung Province, Indonesia for the data collected and the Laboratory of Research Center for Applied Zoology, National Research and Innovation Agency, Bogor, West Java, Indonesia to analyze the blood samples.

Data collection and blood sampling: A total of twenty-one blood samples of Saburai does (75% Boer and 25% Ettawa) were collected from the Villager Breeder Center (VBC) at Tanggamus Regency of Lampung Province, Indonesia. Body measurements such as weight, length, shoulder height, chest girth and blood samples were also taken. A 15 mL propylene tube containing the anticoagulant EDTA was filled with 5 mL of the blood that was drawn from each cow. At the National Research and Innovation Agency's Laboratory of Research Center for Applied Zoology in Bogor, West Java, Indonesia, blood samples were analyzed.

Molecular DNA analysis: The DNA extraction of each sample was conducted using Genomic Extraction Kit (GeneAid, Taiwan) following the manufacturer's protocol. The PCR analysis was performed in a total volume of 30 µL consisting of 3 µL of DNA template, 0.60 µL of each primer, 10.8 µL water free-nuclease and 15 µL of PCR mastermix (Bioline, USA). A primer pair of *MSTN*1-F: 5'- AGG CAT TAA CGT TTG GCT TG-3' and *MSTN*1-R: 5'- ACA CTA GAA CAG CAG TCA GCA GA -3' was used to amplify *caprine MSTN* gene (GenBank: DQ167575.1) in the exon 1 region along 516 bp. While primer pairs of *MSTN*3-F: 5'- TCT TTA ATA ATG ACT CCC TGC G -3' and *MSTN*3-R: 5'- GAA CAC CCA CAG CGA TCT ACT -3' was used to amplify *MSTN* gene in exon 3 region along 450 bp[16]. With pre-denaturation at 95°C for 5 min, denaturation at 95°C for 15 sec, annealing at 60°C for 15 sec, extension at 72°C for 20 sec and final extension at 72°C for 3 min, the PCR program for both regions was carried out by the Mastercycler gradient (Eppendorf, Germany) in 35 cycles. In this investigation, forward sequencing was carried out by a for-profit laboratory (First Base Laboratory, Malaysia) to find the *MSTN* gene's SNP.

Statistical analysis: The Nei and Kumar[17] method was used to determine the genetic diversity parameters of genotypic frequency, phenotypic frequency, observed heterozygosity (Ho), anticipated heterozygosity (He), number of effective alleles (ne), polymorphic informative content (PIC) and Chi-square (2). In order to conduct association research with the mathematical model, records of growth traits, such as birth weight, weaning weight (4 months of age), yearling weight (12 months of age) and ADG1 (pre-weaned daily gain), were used:

$$Y_{ij} = \mu+G_i+\epsilon_{ij}$$

where, Y_{ij} is the observed trait, μ is the common mean, G_i is the effect of i^{th} genotype and ϵ_{ij} is the experimental error. Confidence level of 95% or with a significance level of 5% ($\alpha = 0.05$).

RESULTS AND DISCUSSION

According to the sequencing analysis, four SNP's were detected in the *MSTN* gene of Saburai goats. One common SNP of g.217_218.indel.TTTTA (Fig. 1) and three novel SNP's of c.386G>C (Fig. 2), g.641_642.indel.T (Fig. 3) and c.4957G>C (Fig. 4) were detected in the *MSTN* gene of Saburai goats.

In addition, two misssense mutations were determined in SNP c.386G>C (A55P) and c.4957G>C (A43P). In this study, an SNP g.217_218.indel.TTTTA has two genotypes of II (g.217_218.ins.TTTTA) and DD (g.217_218.del.TTTTA) with the allelic frequency of 0.05 for I and 0.95 for D (Table 1).

Hence, the genetic diversity in SNP g.217_218.indel.TTTTA was low in Saburai goats. In contrast, the genetic diversity in SNP g.217_218.indel.TTTTA was high in the Nubi goat breed with an allelic frequency of 0.49 for I and 0.51 for D[4]. Commonly, the D allele is the common allele in many goat breeds i.e., Boer (0.70), Matou (0.90), Haimen (0.70), Black Bengal (0.99), Sirohi (0.94), Osmanabadi (0.98), Jakhrana (1.00), Jamunapari (0.96), Barbari (0.95), Marwari (0.90), Mohabadi (0.85), Markhoz (0.94), Lori (0.91) and 0.88 for Bital[4,18-20].

A novel SNP g.641_642.indel.T (intron 2) in caprine *MSTN* gene has not been reported before. In Saburai goats, this SNP has high genetic diversity with a PIC value of 0.37. The PIC value consisted of three categories i.e. low (0.00-0.30), moderate (0.31-0.50) and high (0.51-1.00)[17]. An SNP with a high PIC value indicates high genetic diversity and can be used as the molecular selection. Therefore, two missense mutations in Saburai goats i.e., A55P (exon 1) and A43P (exon 3) have not been reported before. Previous studies have reported many novel SNP's in the caprine *MSTN* gene (GenBank: DQ167575.1) at exon 1 (g.197G>A, g.269T>A, g.297G>A, g.345T>A, g.368A>C) and exon 3 (g.4911C>T, g5046T>A, g.5055C>T) regions[4,20,21]. Mostly the novel SNP's of caprine *MSTN* gene in the previous studies was not detected in Saburai goats. The genetic mutation in the livestock can be affected by selection, crossbreeding and inbreeding.

In this study, SNP of g.641_642.indel.T (intron 2) and c.386G>C (exon 1) have a moderate PIC value (Table 2). Hence, both SNP's can be used as the genetic marker for the growth traits of Saburai goats in the future. However, the association study with a limited sample revealed that no significant effect on SNP for growth traits of Saburai does. Previous studies reported that SNP in the exon 1 of g.269T>A, g.345T>A and 368A>C has a significant association with growth traits of sheep[4,16,22]. In addition, SNP g.217_218.indel.TTTTA has a significant association with the body weight of Boer goats[4]. In this study, goats with the heterozygous genotype have higher growth traits than homozygous genotype (SNP c.386G>C) (Table 2). In the future, a depth study to confirm this SNP with a large sample is important to obtain the genetic marker of growth traits in Saburai goats.

The implication of this research is to enhance the genetic quality of livestock, particularly Saburai goats, with a focus on their application in Tanggamus, Lampung, Indonesia. The recommendation stemming from this study is that Saburai goats with heterozygous genotypes should be further developed to enhance productivity specifically in Tanggamus, Lampung, Indonesia. Moreover, the findings of this research can serve as a foundation for

Table 1: Genetic diversity in the four SNP's of Saburai MSTN goat

		Genotypic frequency			Allelic frequency						
SNP	N						H_o	H_e	n_e	PIC	χ^2
g.217_218.indel.TTTTA	21	II (0.05)	DD (0.95)	ID (0.00)	I (0.05)	D (0.95)	0.00	0.09	1.10	0.09	21.00
c.386G>C	12	GG (0.50)	CC (0.00)	GC (0.50)	G (0.75)	C (0.25)	0.50	0.38	1.60	0.31	1.33
g.641_642.indel.T	15	II (0.53)	DD (0.47)	ID (0.00)	I (0.53)	D (0.47)	0.00	0.50	1.99	0.37	15.00
c.4957G>C	21	GG (0.90)	CC (0.00)	GC (0.10)	G (0.95)	C (0.05)	0.09	0.09	1.10	0.09	0.05

SNP: Single Nucleotide Polymorphism, N: Number of observation, H_o: Observed heterozygosity, H_e: Expected heterozygosity, n_e: Number of effective allele, PIC: Polymorphic informative content and χ^2: Chi-square

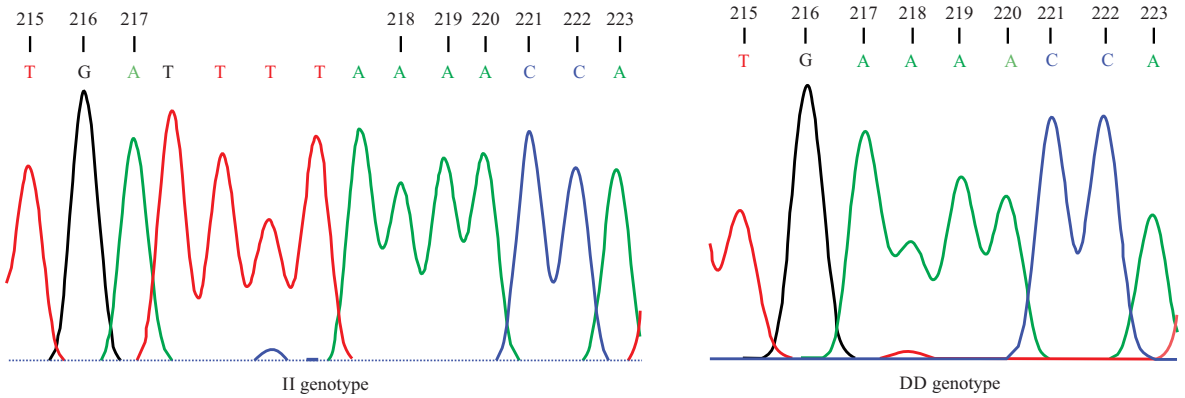

Fig. 1: Detection of common SNP g.217_218.indel.TTTTA in the 5'UTR of Saburai *MSTN* gene

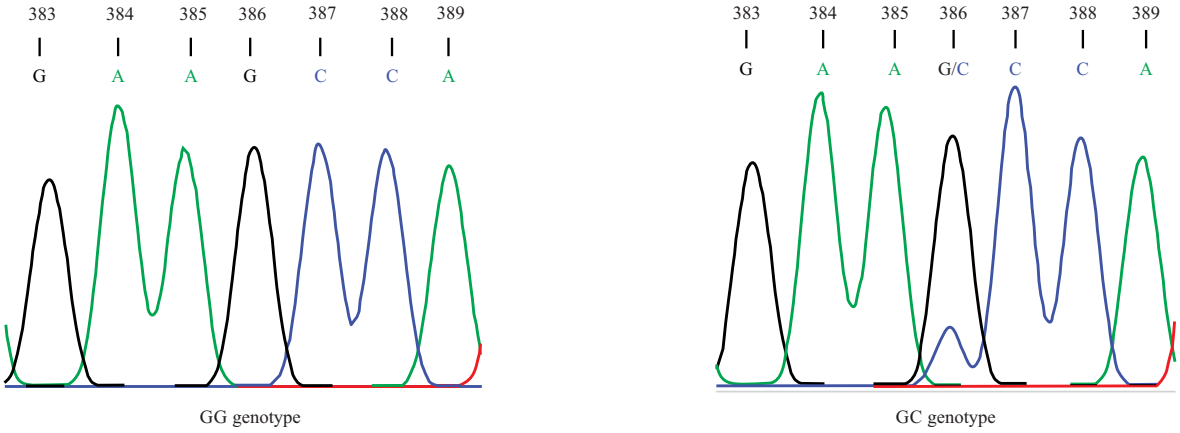

Fig. 2: Detection of novel SNP c.386G>C (A55P) in the exon 1 of Saburai *MSTN* gene

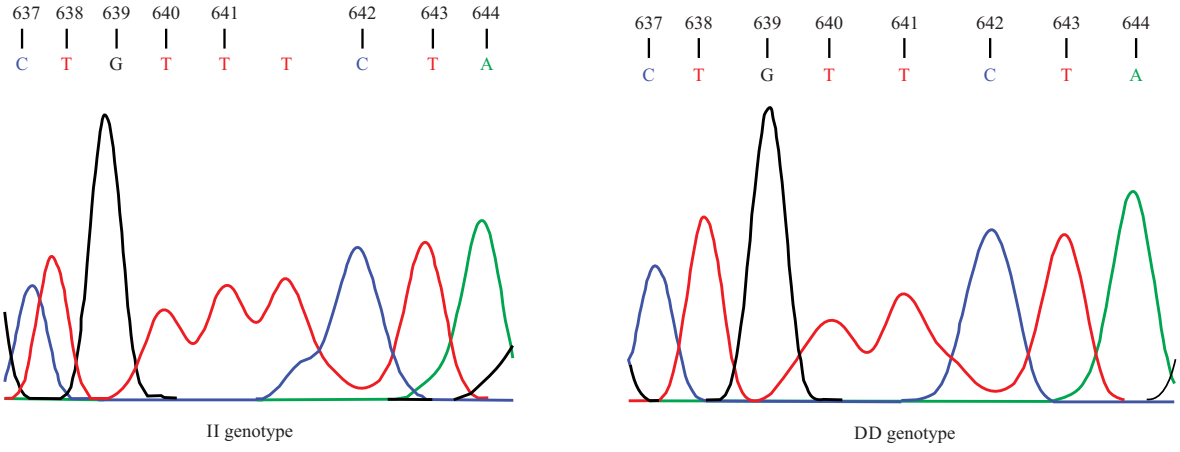

Fig. 3: Detection of novel SNP g.641_642.indel.T in the intron 2 of Saburai *MSTN* gene

selecting other goat breeds in Indonesia. One limitation of this research is the limited number of goat samples available in Tanggamus Regency. In the future, it is crucial to conduct more extensive studies with larger sample sizes to validate these SNPs as genetic markers for growth traits in Saburai goats.

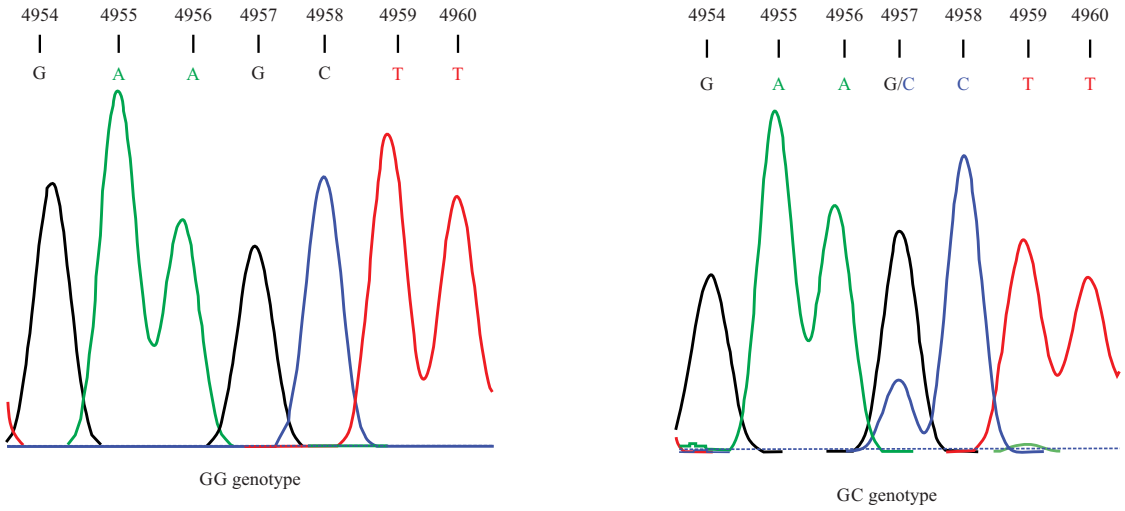

Fig. 4: Detection of novel SNP c.4957G>C (A43P) in the exon 3 of Saburai *MSTN* gene

Table 2: Effect of novel polymorphisms in *MSTN* gene with growth traits of Saburai does

		Growth traits*				
SNP	N	BW	WW	YW	ADG1	ADG2
c.386G>C						
GG genotype	6	2.89±0.60	15.54±2.42	33.63±7.25	0.10±0.01	0.07±0.02
GC genotype	6	3.05±0.49	16.19±1.95	35.58±5.85	0.11±0.01	0.08±0.01
g.641_642.indel.T						
DD genotype	7	2.98±0.49	15.92±1.95	34.78±5.85	0.11±0.01	0.08±0.01
II genotype	8	2.98±0.70	15.91±2.81	34.74±8.44	0.11±0.02	0.08±0.02
c.4957G>C						
GG genotype	19	3.30±0.69	17.20±2.77	38.60±8.30	0.11±0.02	0.09±0.02
GC genotype	2	2.38±0.25	13.51±1.01	27.55±3.04	0.09±0.01	0.05±0.01

SNP: Single Nucleotide Polymorphism, N: Number of observations, BW: Birth weight (kg), WW: Weaning weight at 4 months of age (kg), YW: Yearling weight at 12 months of age (kg), ADG1: Pre-weaned daily growth (kg day^{-1}), ADG2: Post-weaned daily growth (kg day^{-1}) and *Non-significant (p>0.05)

CONCLUSION

The SNP at g.641_642.indel.T (intron 2) and the one at c.386G>C (exon 1) both have a moderate PIC value. Therefore, both SNPs can be used as genetic markers for growth traits in Saburai goats in the future. In this study, goats with the heterozygous genotype have shown higher growth traits compared to those with the homozygous genotype (SNP c.386G>C). This suggested that goats with heterozygous genotypes can be further developed to increase the productivity of Saburai goats.

SIGNIFICANCE STATEMENT

This research is necessary to identify novel polymorphisms in the caprine myostatin gene and investigate their relationship with growth characteristics in Saburai goats. The significance of this study lies in the discovery of genetic markers that exert the most influence on growth traits, enabling more effective selection of Saburai goats in the future. A unique contribution of this research is the identification of SNPs g.641_642.indel.T and c.386G>C as potential genetic markers for growth traits in Saburai goats. Notably, in this study, goats with the heterozygous genotype exhibited higher growth characteristics compared to those with the homozygous genotype, suggesting that further development of goats with the heterozygous type can significantly boost the productivity of Saburai goats.

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