



A Systematic Review of Myostatin Gene Variations and their Association with Growth Traits in Sheep

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Abstract | Myostatin (MSTN), a member of the transforming growth factor superfamily, plays a role in the regulation of skeletal muscle growth and development in animals. The single nucleotide polymorphisms (SNPs) of the MSTN gene have been implicated with growth parameters in a multitude of livestock species. The aim of the study was to conduct a comprehensive literature evaluation of the association between ovine growth traits and the SNPs of the MSTN gene. Four databases (PubMed, Web of Science, ScienceDirect and Google Scholar) were systematically evaluated using the keywords sheep, MSTN, polymorphism, genetic variation, growth traits, gene polymorphism, skeletal muscle growth, GDF-8, without any year of publication restrictions and 8 studies were found to be eligible. The results indicated that eight (n = 8) publications which were examined observed of 13 unique MSTN SNPs with multiple occurring throughout different breeds and 3 articles identified 4 SNPs which had an association to at least 1 growth trait. The SNP 2379C>T found in the promoter region exhibited significance with 80% of associated results and can potentially be utilised as a candidate genetic in marker assisted selection for growth traits in sheep. In conclusion, this systematic review concludes that there exists a relationship between the SNPs of the MSTN gene and growth traits in sheep and that more research is necessary to explore this relationship in a greater number of growth traits to further understand the relationship that exists.

Keywords | Genetic variation, Genetic marker, Marker-assisted selection, Sheep breeding, Body weight, Growth rate

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INTRODUCTION

The myostatin (MSTN) gene, otherwise known as the growth and differentiation factor 8 (GDF8), genetically controls the growth of muscle cells through the myostatin protein, a member of transforming growth factor β superfamily, which acts to negatively regulate muscle development through the inhibition of skeletal muscle development (Han *et al.*, 2010; Sahu *et al.*, 2017). The MSTN gene is associated with the “double-muscling” phenotype in multiple mammalian species including sheep

(Han *et al.*, 2010; Haren *et al.*, 2020; Osman *et al.*, 2021). As quantitative trait growth performance is polygenic, and one of the genes which influences this is MSTN, the genetic basis of any such influence on the production traits of an animal should be understood (Kolenda *et al.*, 2019; Osman *et al.*, 2021). They are SNPs of the MSTN gene that have been found to have significant associations to the performance of multiple quantitative characteristics in various sheep breeds, and the variability that has been observed in the MSTN gene can viably be employed in the development of marker-assisted selection (MAS) breeding

programs to improve animal production (Wang *et al.*, 2016; Sahu *et al.*, 2017). To the best of our knowledge, no systematic review has examined the relationship between growth traits and the ovine MSTN gene's single nucleotide polymorphisms. As such, the objective of the study was to thoroughly examine the literature on the observed impact of the ovine MSTN gene single nucleotide polymorphisms on growth traits. The study will aid in highlighting and detailing the recent developments and discoveries about the MSTN gene's SNPs and their potential use as a genetic marker in sheep during marker-assisted selection.

MATERIALS AND METHODS

ELIGIBILITY CRITERIA

Population, Exposure, and Outcomes (PEO) components of the research issue, as described by Bettany-Saltikov (2010), were identified. The population was described as Sheep with Polymorphisms exposure and Growth traits outcomes. A preliminary search of the PEO components on the PubMed database was conducted before deciding to conduct the systematic review.

LITERATURE SEARCH

The search for the research publications was conducted using the Google Scholar, PubMed, Science Direct and Web of Science databases up to the 20th of February 2023 by the two authors (Tebogo Letsukulo Percy Thepa and Thobela Louis Tyasi) where the keywords used were: myostatin (MSTN)/ GDF-8, polymorphisms/genetic variations, growth traits, and sheep.

INCLUSION CRITERIA

The following criteria had to be met for any of the acquired papers to be eligible: (1) the MSTN gene was being investigated; (2) include polymorphism characterization of the MSTN gene; (3) include the species of interest, sheep.

EXCLUSION CRITERIA

The exclusion criteria involved: (1) duplicate studies; (2) no association between MSTN polymorphisms and growth traits; (3) studies that inferred association with growth traits without association analyses.

DATA EXTRACTION

The two authors independently extracted the content. The first author's name, publication year, nation, species, breed, population size, and observed genotypes are among the information extracted from the papers.

RESULTS AND DISCUSSION

SEARCH RESULTS

A total of eighty-three (n= 83) articles were retrieved for

the systematic review from the databases; Google Scholar (n = 30), PubMed (n = 19), Science Direct (n = 8), and Web of Science (n = 26). Twenty-three (n=23) articles which occurred as duplicates among databases were removed, and the remaining articles were analysed for exclusion and inclusion criteria. The articles that remained (n=60) were screened for their title, abstract, full paper and twenty-seven (n=27), ten (n=10), and fifteen (n=15) were excluded respectively emanating in a total of eight (n=8) articles which are to be included in the systematic review.

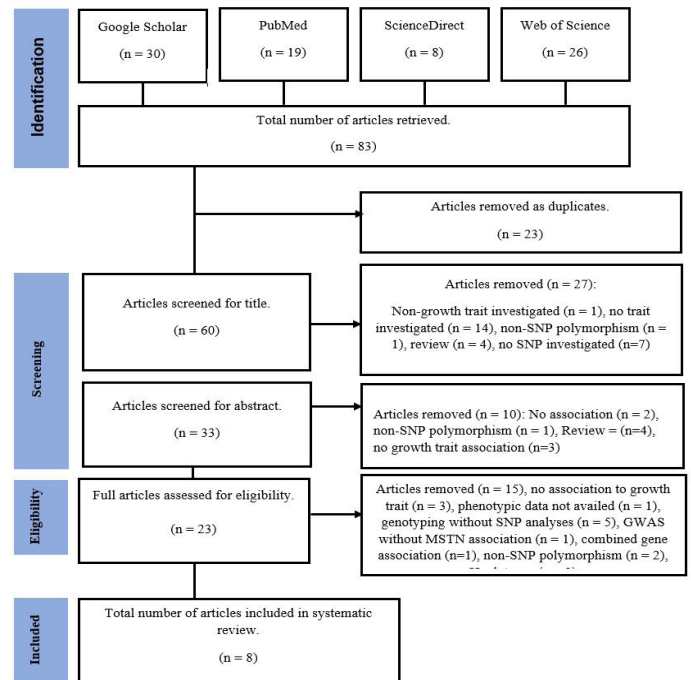


Figure 1: Study selection flow diagram.

CHARACTERIZATION OF INCLUDED STUDIES

Eighty-three (n=83) articles were analysed and eight (n=8) were retained to be included in the systematic review (Table 1). The articles included in the review ranged from 2010 to 2021. 100% of the included studies evaluated body weight, 28.57% evaluated average daily gain and another 28.57% evaluated weaning weight along with the body weight.

PUBLICATION BY COUNTRY

Figure 2 below displays publication by country of included studies in the systematic review. Of the papers analysed Asia produced 50% (China = 1, India =2, Indonesia = 1), Oceania produced 25% (New Zealand = 1, Australia =1) and the remaining 25% was split between Africa (n=1) and Europe (n=1). Of the included papers investigating the MSTN genes SNP's association with growth traits, 100% of the papers investigated body weight or its variants.

IDENTIFIED SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) AND REGIONS

The MSTN SNPs described in the eight included articles are detailed in Table 2. The reviewed articles detailed

Table 1: General characteristics of studies included in the review.

Author	Year	Country	Breed	N	Trait	Genotyping method
Han <i>et al.</i>	2010	New Zealand	New Zealand Romney	79	BW	PCR-SSCP
Haren <i>et al.</i>	2020	Indonesia	Batur	30	BW	PCR Assay
Hope <i>et al.</i>	2013	Australia	(Poll Dorset × White Suffolk ram) × (White Suffolk × (Border Leicester × Merino) dam)	24	BW	PCR Assay
Kolenda <i>et al.</i>	2019	Poland	Kamieniec, Pomeranian	200	BW, ADG	PCR-SSCP
Osman <i>et al.</i>	2021	Egypt	Barki, Ossimi, Rahmani, Nadji	75	BW, ADG	PCR Assay
Sahu <i>et al.</i>	2017	India	Madras Red, Mecheri	232	BW, WW	PCR-RFLP
Sahu <i>et al.</i>	2018	India	Nilagiri	103	BW, WW	PCR-RFLP
Wang <i>et al.</i>	2015	China	New Zealand Romney	300	BW, WW, ADG	PCR-SSCP

BW, Body weight; ADG, average daily gain; WW, weaning weight; PCR, polymerase chain reaction; SSCP, single strand conformation polymorphism; RFLP, restriction fragment length polymorphism.

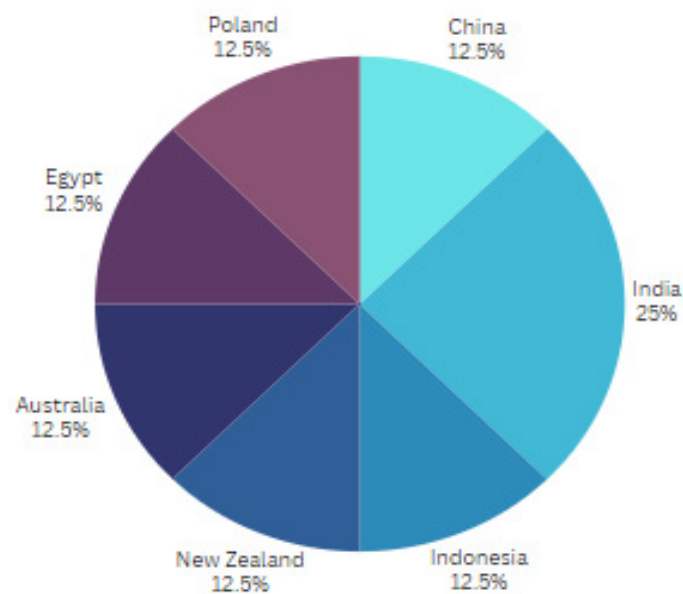


Figure 2: Publication by country pie chart.

the occurrence of 13 unique MSTN SNPs with multiple occurring amongst different breeds. The identified unique SNPs emanated primarily from Intron 1 (46,15%), 3'UTR (23,08%), and the remaining articles (30.77%) were split equally between exon 3 and the promoter region of the MSTN gene. There occurred 13 unique MSTN SNP's, with a total of 25 SNP observations within the included breeds, with multiple SNP's occurring multiple times. In exon 1's observed SNPs 18G>T, 159A>T, 173T>G, 241T>C, 243 G>A, 259G>T accounted for 16.67% each. In the 3'UTR 50% of the observed SNP's were 1232G>A and the remaining 50% was split equally between 6223G>A and 6723G>A. In exon 3's SNP's, 5622G>C accounted for 75% and the remaining 25% by 121G>C. 2449G>C and 2379G>T accounted for 50% of the observed SNPs in the promoter region.

GENOTYPIC FREQUENCIES

The included articles all detailed the genotypic frequencies

that occur as shown in Table 2. The results showed that 78 genotypes were observed throughout all the reviewed articles in total. The included articles observed genotypic frequencies ranged from 0.00 to 1.00.

ALLELIC FREQUENCIES

The frequencies with which alleles were observed as a result of SNP occurrence are detailed in Table 2. Throughout the analysed papers 56 alleles were observed in total. The lowest allelic frequencies ranged from 0 to 1.

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) AND THEIR ASSOCIATION WITH GROWTH TRAITS

The association of growth traits and the occurring SNPs are shown in Table 3. In the 8 reviewed articles, 5 traits were studied including body weight (BW) (and its variation), average daily gain (ADG) (and its variation), weaning weight (WW), full weight (FW), and growth rate (GR) including their association to observed SNP's. 3 articles out of the systematically reviewed 8 have observed significant differences in at least 1 trait and the associated SNP. Of the 5 investigated traits, 3 were observed to have a significant association to at least one of the SNPs to which they're associated with. In the 3 papers that observed significant association between genotypes and growth traits, 100% observed it with BW, and GR and ADG were observed in only 33.33% of the papers.

The Myostatin (MSTN) gene and its variations have been observed to phenotypically impact animal musculature significantly across multiple species, as such it had become a gene of interest in animal breeding due to its potential to improve production characteristics (Wang *et al.*, 2016). The Myostatin (MSTN) gene and its variations have been observed to phenotypically impact animal musculature significantly across multiple species, as such it had become a gene of interest in animal breeding due to its potential to improve production characteristics (Wang *et al.*, 2016).

Table 2: Single nucleotide polymorphisms (SNPs), genotypic and allelic frequencies of goat MSTN gene.

Breed	SNP	Region	Genotypic frequencies	Gene frequencies	Author
New Zealand Romney	6223G>A	3'-UTR	GG (0.54), AG (0.46)	G (0.772), A (0.228)	Han <i>et al.</i> (2010)
Batur	121G>C	Exon 3	GG (0.55), GC (0.38) AC (0.07)	G (0.74), C (0.26)	Haren <i>et al.</i> (2020)
(Poll Dorset × White Suffolk ram) × (White Suffolk × (Border Leicester × Merino) dam)	6723G>A	3'-UTR	AA (0.29), AG (0.33), GG (0.38)	A (0.46), G (0.54)	Hope <i>et al.</i> (2013)
Kamieniec	1232G > A	3'-UTR	AA (0.06), AG (0.21), GG (0.73)	A (0.165), G (0.835)	Kolenda <i>et al.</i> (2019)
Pomeranian	1232G > A	3'-UTR	AA (0.06), AG (0.56), GG (0.38)	A (0.34), G (0.66)	Kolenda <i>et al.</i> (2019)
Barki (Egyptian Sheep)	18 G>T	Intron 1	GG (0.39), GT (0.47), TT (0.14)	G (0.63), T (0.37)	Osman <i>et al.</i> (2021)
	159 A>T	Intron 1	AA (0.69), AT (0.28), TT (0.03)	A (0.83), T (0.17)	Osman <i>et al.</i> (2021)
	173 T>G	Intron 1	TT (0.69), TG (0.28), GG (0.03)	T (0.83), G (0.17)	Osman <i>et al.</i> (2021)
	241 T>C	Intron 1	TT (0.39), TC (0.47), CC (0.14)	T (0.63), C (0.37)	Osman <i>et al.</i> (2021)
	243 G>A	Intron 1	GG (0.39), GA (0.47), AA (0.14)	G (0.63), A (0.37)	Osman <i>et al.</i> (2021)
	259 G>T	Intron 1	GG (0.39), GT (0.47), TT (0.14)	G (0.63), T (0.37)	Osman <i>et al.</i> (2021)
Ossimi (Egyptian Sheep)	18 G>T	Intron 1	GG (0.39), GT (0.47), TT (0.14)	G (0.63), T (0.37)	Osman <i>et al.</i> (2021)
	159 A>T	Intron 1	AA (1), AT (0), TT (0)	A (1), T (0)	Osman <i>et al.</i> (2021)
	173 T>G	Intron 1	TT (1), TG (0), GG (0)	T (1), G (0)	Osman <i>et al.</i> (2021)
	241 T>C	Intron 1	TT (0.55), TC (0.36), CC (0.9)	T (0.63), C (0.37)	Osman <i>et al.</i> (2021)
	243 G>A	Intron 1	GG (0.55), GA (0.36), AA (0.9)	G (0.76), A (0.24)	Osman <i>et al.</i> (2021)
	259 G>T	Intron 1	GG (0.39), GT (0.47), TT (0.14)	G (0.63), T (0.37)	Osman <i>et al.</i> (2021)
Rahmani (Egyptian Sheep)	18 G>T	Intron 1	GG (0.39), GT (0.47), TT (0.14)	G (0.63), T (0.37)	Osman <i>et al.</i> (2021)
	159 A>T	Intron 1	AA (1), AT (0), AA (0)	A (1), T (0)	Osman <i>et al.</i> (2021)
	173 T>G	Intron 1	TT (1), TG (0), GG (0)	T (1), G (0)	Osman <i>et al.</i> (2021)
	241 T>C	Intron 1	TT (0.55), TC (0.36), CC (0.9)	T (0.63), C (0.37)	Osman <i>et al.</i> (2021)
	243 G>A	Intron 1	GG (0.55), GA (0.36), AA (0.9)	G (0.76), A (0.24)	Osman <i>et al.</i> (2021)
	259 G>T	Intron 1	GG (0.39), GT (0.47), TT (0.14)	G (0.63), T (0.37)	Osman <i>et al.</i> (2021)
Madras Red	5622G > C	Exon 3	MM (0.42), Mm (0.58)	M (0.71), m (0.29)	Sahu <i>et al.</i> (2017)
Mecheri	5622G > C	Exon 3	MM (0.49), Mm (0.51)	M (0.74), m (0.26)	Sahu <i>et al.</i> (2017)
Nilagiri	5622G > C	Exon 3	MM (0.69), Mm (0.31)	M (0.84), m (0.16)	Sahu <i>et al.</i> (2018)
New Zealand Romney	2449G>C	Promoter region	GG (0.70), GC (0.30)	G (0.83), C (0.17)	Wang <i>et al.</i> (2015)
	2379C>T	Promoter region	CC (0.086), CT (0.14)	C (0.92), T (0.08)	Wang <i>et al.</i> (2015)

The objective of the systematic review was to evaluate the effects of the ovine MSTN gene's single nucleotide polymorphisms (SNPs) on the species growth parameters. The variations of the MSTN gene, that arise due to SNP's, which showcase a significant association to the growth traits of sheep have the potential to be utilised in animals breeding programs as genetic markers which can be utilized. The results of the systematic review indicated that there was 13 unique SNP's (18G>T, 159A>T, 173T>G, 241T>C, 243 G>A, 259G>T, 1232G>A 6223G>A, 6723G>A, 5622G>C, 121G>C. 2449G>C and 2379G>T) in the identified in the ovine MSTN gene and it was found that 4 SNP (6223G>A, 5622G>C, 2449G>C and 2379G>T) of them exhibited a relationship with growth

traits in sheep across multiple breeds. The weight of an animal and the rate at which it attains it are important economic attributes and the study by Sahu *et al.* (2017) investigated multiple variants of body weight and observed a significant difference between the SNP and the body weight at both 9 and 12 months in Madras Red, Mecheri and Nilagri breeds and these findings relative to weight are further supported by the studies of Han *et al.* (2010) and Wang *et al.* (2016), who not only observed a significant association between the relevant SNPs 6223G>A and 2449G>C, respectively, and the birth weight, but Wang *et al.* (2016) also identified an association with growth rate in the New Zealand Romney. On the contrary to these multiple other studies including those by

Table 3: SNPs association with growth traits.

Author	Breed	SNP	Growth traits	Genotypes	Sig
Han <i>et al.</i> (2010)	New Zealand Romney	6223G>A	BW	GG AG -	*
Haren <i>et al.</i> (2020)	Batur	121G>C	BW	GG GC CC	ns
Hope <i>et al.</i> (2013)	(Poll Dorset × White Suffolk ram) × (White Suffolk × (Border Leicester × Merino) dam)	6723G>A	BW	AA AG GG	ns
Kolenda <i>et al.</i> (2019)	Kamieniec	1232G>A	BW (0) (2)	AA AG GG	ns
		1232G>A	BW (14)	AA AG GG	ns
		1232G>A	ADG	AA AG GG	ns
Kolenda <i>et al.</i> (2019)	Pomeranian	1232G>A	BW (0) (2)	AA AG GG	ns
		1232G>A	BW (14)	AA AG GG	ns
		1232G>A	ADG	AA AG GG	ns
Osman <i>et al.</i> (2021)	Egyptian sheep	18 G>T	BW (0) (0)	GG GT TT	ns
		18 G>T	FW	GG GT TT	ns
		18 G>T	ADG	GG GT TT	ns
Osman <i>et al.</i> (2021)	Egyptian sheep	241 T>C	BW (0) (0)	CC CT TT	ns
		241 T>C	FW	CC CT TT	ns
		241 T>C	ADG	CC CT TT	ns
Osman <i>et al.</i> (2021)	Egyptian sheep	243 G>A	BW (0) (0)	GG AG AA	ns
		243 G>A	FW	GG AG AA	ns
		243 G>A	ADG	GG AG AA	ns
Osman <i>et al.</i> (2021)	Egyptian sheep	259 G>T	BW (0) (0)	GG GT TT	ns
		259 G>T	FW	GG GT TT	ns
		259 G>T	ADG	GG GT TT	ns
Osman <i>et al.</i> (2021)	Egyptian sheep	159 A>T	BW (0) (0)	GG GT TT	ns
		159 A>T	FW	GG GT TT	ns
		159 A>T	ADG	GG GT TT	ns
Osman <i>et al.</i> (2021)	Egyptian sheep	173 T>G	BW (0) (0)	GG GT TT	ns
		173 T>G	FW	GG GT TT	ns
		173 T>G	ADG	GG GT TT	ns
Sahu <i>et al.</i> (2017)	Madras Red	5622G > C	BW (0) (0)	MM Mm -	ns
		5622G > C	WW	MM Mm -	ns
		5622G > C	BW (6)	MM Mm -	ns
		5622G > C	BW (9)	MM Mm -	*
		5622G > C	BW (12)	MM Mm -	*
Sahu <i>et al.</i> (2017)	Mecheri	5622G > C	BW (0) (0)	MM Mm -	ns
		5622G > C	WW	MM Mm -	ns
		5622G > C	BW (6)	MM Mm -	ns
		5622G > C	BW (9)	MM Mm -	ns
		5622G > C	BW (12)	MM Mm -	ns
Sahu <i>et al.</i> (2018)	Nilagri	5622G > C	BW (0) (0)	MM Mm -	ns
		5622G > C	WW	MM Mm -	ns
		5622G > C	BW (6)	MM Mm -	ns
		5622G > C	BW (9)	MM Mm -	ns
		5622G > C	BW (12)	MM Mm -	ns
Wang <i>et al.</i> (2015)	New Zealand Romney	2449G>C	BW (0) (0)	GG GC -	*
		2449G>C	WW	GG GC -	ns
		2449G>C	PREW-ADG	GG GC -	ns
		2449G>C	PSTW-ADG	GG GC -	ns
		2449G>C	GR	GG GC -	*
Wang <i>et al.</i> (2015)	New Zealand Romney	2379C>T	BW (0) (0)	GG GC -	*
		2379C>T	WW	GG GC -	*
		2379C>T	PREW-ADG	GG GC -	*
		2379C>T	PSTW-ADG	GG GC -	*
		2379C>T	GR	GG GC -	ns

BW, Body weight; FW, full weight; ADG, average daily gain; PREW-ADG, pre-weaning weight average daily gain; PSTW-ADG, post-weaning weight average daily gain; WW, weaning weight; GE, growth rate; ns, non-significant, *, significant.

Haren *et al.* (2020), Hope *et al.* (2013), Kolenda *et al.* (2019) identifies the SNPs 121G>C, 6723G>A and the 1232G>A SNP respectively, and the analysis performance against them and the body weight across multiple breeds and yielded relationships that were not significant. To our knowledge, this systematic review is the first of its kind to discuss the relationship between growth traits and the single nucleotide polymorphisms of the MSTN gene in sheep and from this arises its novelty. Therefore, there is no basis for comparison of the results with those of another systematic review. The results of this study imply that the identified SNP 2379C>T is a candidate genetic marker for multiple growth traits including the birth weight, weaning weight, pre-weaning weight average daily gain, post-weaning weight average daily gain and the results can be used to design marker assisted breeding program for the improvement of growth traits in sheep using the MSTN genes SNP's. The strength of this review was that no similar study has been conducted that amalgamates the relevant topics and their results in sheep. This systematic review brings together the findings from this field of research and identifies the MSTN gene SNPs that could be further considered for their use in research and for marker assisted selection. The vast majority of papers 62.5% (n=5) in regards to growth traits looked at weight (body weight at different ages, weaning weight, full weight) with only 37.5% (n=3) of the articles having at least one additional parameter (average daily gain and variations or growth rate) and there is no paper investigating linear body measurements, which is limiting in the attempt to understand the overall effect that these MSTN SNPs have on growth traits as only a limited few growth parameters have been assessed to their relationship with MSTN variations. As such, it is recommended that more studies be conducted that are inclusive of a greater number of growth parameters and that SNPs 6223G>A, 5622G>C which could potentially be genetic markers of merit should be further investigated within larger populations to further supports the reported results.

CONCLUSIONS AND RECOMMENDATIONS

The *myostatin* (MSTN) gene's polymorphisms have been observed to significantly impact the phenotypic characteristics of goats and other livestock and has great economic consequence in the rearing of animals. The use of marker assisted selection (MAS) using single nucleotide polymorphisms (SNPs) is one of the most efficient selection methods available in which there can occur targeted and efficient trait selection in the attempt to improve animal growth traits. It is concluded that the ovine MSTN gene does have an apparent influence on the growth traits of sheep and as such the SNPs 2449G>C and that further

investigation of the SNPs 6223G>A and 5622G > C in larger populations should be considered as they could potentially be used as genetic markers in the improvement of growth traits in marker assisted breeding programs.

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NOVELTY STATEMENT

This is the first systematic review on single nucleotide polymorphisms (SNPs) of myostatin (MSTN) gene and their associations with growth traits in sheep. This systematic review identified SNPs of MSTN gene discovered in different studies that might be the candidate gene for improvement of growth traits in sheep.

AUTHOR'S CONTRIBUTION

All the authors wrote, read, and approved the final manuscript.

DATA AVAILABILITY

All data is available through a request to the corresponding author.

LIST OF ABBREVIATIONS

ADG, Average daily gain; BW, Body weight; GDF-8, Growth differentiation factor 8; GR, Growth rate; MSTN, Myostatin; PCR, Polymerase chain reaction; PREW-ADG, Pre-weaning weight average daily gain; PSTW-ADG, Post-weaning weight average daily gain; RFLP, Restriction fragment length polymorphism; SNP, Single nucleotide polymorphism; SNPs, Single nucleotide polymorphisms; SSCP, Single strand conformation polymorphism; WW, Weaning weight.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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