RELATIONSHIPS BETWEEN MYOSTATIN GENE SINGLE NUCLEOTIDE POLYMORPHISMS AND GROWTH TRAITS IN GOATS: A SYSTEMATIC REVIEW

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Abstract. A member of the transforming growth factor-β superfamily, myostatin (MSTN) functions in the regulation and growth of muscle mass in animals. The MSTN gene’s single nucleotide polymorphisms (SNPs) are implicit in their relationship with growth traits in multiple agricultural species, although the research has been limited with regards to goats. As such, the objective of the paper was to conduct a systematic review of the relationship between the SNPs of the MSTN gene and growth traits in goats. 4 databases were systematically evaluated, and 6 studies were found to be eligible. The 6 included articles observed 24 SNPs of the MSTN gene across several breeds, and 5 articles identified 7 SNPs with a significant association to at least one growth trait. All identified SNPs were unique to the studies, except 368A>C of exon 1 which was identified in 2 articles and can potentially be used as a candidate genetic marker. The systematic review concludes that there is an existing relationship between the MSTN gene’s SNPs and growth traits in goats and further studies need to be done to confirm the validity of the identified MSTN gene SNPs so that they might be used as potential genetic markers in goats.

Keywords: genetic variation, genetic marker, marker-assisted selection, breeding, PCR

Introduction

Growth traits are significant as they influence the economic viability and profitability of industry-related enterprises (Khani et al., 2017). The complexity of growth traits is made evident in their control and ability of a trait to be influenced by multiple genes and their concurrent epistasis, as such many genes have been implicated in their effect on growth traits, one of which is the Myostatin (MSTN) gene (Khani et al., 2017). The myostatin protein, which is encoded by the MSTN gene, a constituent of the transforming growth factor β superfamily, expresses itself phenotypically to limit skeletal muscle accumulation through its regulatory role of muscle fiber numbers and growth (An et al., 2011). The type of genetic variation that occurs the most frequently in the genome is that of single nucleotide polymorphisms (SNPs) and variations of the MSTN gene can limit gene expression, or produce a Myostatin protein that is non-functional, resulting in considerable muscle development or the “double muscling” phenomenon observed in multiple species of livestock having significant associations with growth and related traits, however, studies of such a nature have been limited in goats (Zhang et al., 2012; Khani et al., 2017; Ahad et al., 2017). Multiple MSTN gene variants have been discovered in goats that have phenotypic consequences amongst multiple breeds, but there is the minimal occurrence of studies that associate SNPs with growth traits (An et al., 2011). The generation of DNA markers through partial sequencing using restriction enzymes to target specific identified genomic regions of importance is a viable method...
of marker-assisted selection (MAS), although greater sequence variability can be identified from full genome sequencing, it is still too expensive to genotype numerous individuals this way. In excess of 90% of differences between individuals are accounted for by single nucleotide polymorphisms (SNPs), this prevalence offers more potential markers close to the locus of interest and as their inheritance is more stable than that of other DNA markers, they are more applicable as long term selection markers. SNPs studies assist in the identification of gene variants and their possible relations to the phenotypic expression of functional traits and are an important aspect of the development of breeding systems that make use of MAS of traits that, impact the economic value of the animal in that breeding program (Zhang et al., 2013). The studies detailing the caprine MSTN gene have detailed the protein coding regions of exon1 and exon 3’s occurring SNPs and their association to growth, but there is no study investigating this in exon 2. To the best of our knowledge, there is no systematic review that looked into the discovered single nucleotide polymorphisms of the goat Myostatin gene and their associations with growth traits. Hence, the objective of the study was to systematically review the literature published on the influence of single nucleotide polymorphisms of the goat Myostatin gene on growth traits. The study will help in describing and detailing the current progress and findings about the SNPs of the MSTN gene and its potential as a genetic marker during MAS in goats.

Materials and methods

Eligibility criteria

Identification of Population, Exposure, and Outcomes (PEO) components of the research question as explained by Bettany-Saltikov (2010) was performed before conducting the systematic review. The population was defined as “Goats”, with an exposure of “Polymorphisms” and outcomes of “Growth traits”. 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 research publications was performed by the two authors (Tebogo Letsukulo Percy Thepa and Thobela Louis Tyasi) through the use of the database of Google Scholar, PubMed, ScienceDirect, and Web of Science up to February 20, 2023 where the following keywords were made use of: ‘myostatin (MSTN)’, ‘polymorphisms/genetic variations’, ‘growth traits’, and ‘goat’.

Inclusion criteria

The eligibility of all the acquired articles was set to the extent where the following was fulfilled by the studies: (1) have the MSTN gene under investigation; (2) include the polymorphisms of the MSTN gene; (3) include the species of interest, goats.

Exclusion criteria

The criteria for exclusion involved: (1) duplicated records; (2) lacking the association of MSTN polymorphisms to growth traits; (3) and studies that inferred association without association analysis with growth traits.
Data extraction

The content that was extracted was done independently by the two authors (Tebogo Letsukulo Percy Thepa and Thobela Louis Tyasi). The contents extracted from the articles include the name of the 1st author, the year the article was published, country, species, breed, population size and observed genotypes.

Ethical considerations

Ethical issues including Plagiarism, misconduct, informed consent, data falsification, and fabrication were considered by all authors.

Results

Search results

As shown in Figure 1, the articles retrieved for the systematic review amounted to a total of 60 (n = 60) from the following databases: Google Scholar (n = 34), PubMed (n = 10), ScienceDirect (n = 6), and Web of Science (n = 10). The duplicates (n = 19) that occurred in between the search databases were removed and the remaining articles were further analyzed for inclusion and exclusion criteria. The remaining documents (n = 41) were screened for their title and twenty-seven (n = 27) were excluded, followed by the screening for the abstract, and five (n = 5) were excluded, the whole article search excluded three (n = 3) articles for a total remainder of six (n = 6) articles to be included in the systematic review.

Characterization of included studies

About Six (n = 6) studies of the sixty (n = 60) analyzed were retained for inclusion in the literature review (Table 1). The retained articles had a range from the year 2011 to 2019. Of the six papers, 83.33% originated from Asia (China = 3, India = 1, Iran = 1) and 16.67% originated from Africa (Nigeria = 1). The included papers are all investigating MSTN association with traits in goats with the Boer breed probed most, being investigated in 66.67% of the included studies.

Table 1. General characteristics of studies included in the review

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Breed</th>
<th>N</th>
<th>Trait</th>
<th>Genotyping method</th>
</tr>
</thead>
<tbody>
<tr>
<td>An et al.</td>
<td>2011</td>
<td>China</td>
<td>Xinong Saanen, Boer, F1 Boer x Guanzhong dairy, F2 Boer x Guanzhong dairy</td>
<td>664</td>
<td>BW, WH, BL, CG</td>
<td>PCR-SSCP</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>2012</td>
<td>China</td>
<td>Boer</td>
<td>482</td>
<td>BW, BL, BH, CG</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>2013</td>
<td>China</td>
<td>Anhui white and Boer</td>
<td>288</td>
<td>BW, BH, BL, CC</td>
<td>PCR-SSCP</td>
</tr>
<tr>
<td>Khani et al.</td>
<td>2017</td>
<td>Iran</td>
<td>Iranian Markhoz</td>
<td>150</td>
<td>BW, ADG, WW</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Ahad et al.</td>
<td>2017</td>
<td>India</td>
<td>Boer and Bakerwal</td>
<td>600</td>
<td>BW, BL, BH, CG</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>Sanni et al.</td>
<td>2019</td>
<td>Nigeria</td>
<td>Red Sokoto</td>
<td>55</td>
<td>BW, WH, BL, CD</td>
<td>PCR assay</td>
</tr>
</tbody>
</table>

The results showed that all the reviewed articles (6/6) targeted the exon 1 region. Three articles out of six reviewed target exon 1 and exon 3 regions (An et al., 2011; Khani et al., 2017 and Sanni et al., 2019). Only one article out of six reviewed target exon 1 and 5'UTR regions (Zhang et al., 2013) (Fig. 2).

**Figure 1. Study selection flow diagram**

**Targeted genomic region**

The results showed that all the reviewed articles (6/6) targeted the exon 1 region. Three articles out of six reviewed target exon 1 and exon 3 regions (An et al., 2011; Khani et al., 2017 and Sanni et al., 2019). Only one article out of six reviewed target exon 1 and 5'UTR regions (Zhang et al., 2013) (Fig. 2).

**Figure 2. Genomic map of MSTN gene. Black dots indicate the study conducted in a genomic region**
Identified single nucleotide polymorphisms (SNPs) and regions

The SNPs described in the six reviewed articles are detailed in Table 2. The articles detailing SNPs described the occurrence of 24 SNPs across multiple breeds. The observed MSTN polymorphisms were found primarily in Exon 1 (62.5%), followed by Exon 3 (25%) and 5’UTR (12.5%). In exon 1’s SNPs, 40% were 368A>C, 13.33% were 345A>T and the remaining 46.67% was split equally between 339T>A, 32A>T, 80A>G, 84C>G, 85C>G, 473T>G and 1388T>A. In exon 3’s SNPs, 66.67% were 4911C>T, 169T>G, and 49C>A equal account for 33.33%. In the 5’UTR, 66.67% of the polymorphisms were 197G>A, and the remaining 33.33% was accounted for by 368A>C.

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

<table>
<thead>
<tr>
<th>Breed</th>
<th>SNP</th>
<th>Region</th>
<th>Genotypic frequencies</th>
<th>Gene frequencies</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boer</td>
<td>368A&gt;C</td>
<td>Exon 1</td>
<td>AA (0.70-0.80), AC (0.20-0.30)</td>
<td>A (0.70-0.80), C (0.20-0.30)</td>
<td>Ahad et al. (2017)</td>
</tr>
<tr>
<td>Bakerwal</td>
<td>368A&gt;C</td>
<td>Exon 1</td>
<td>AA (0.70-0.80), AC (0.20-0.30)</td>
<td>A (0.60-0.80), AC (0.20-0.40)</td>
<td>Ahad et al. (2017)</td>
</tr>
<tr>
<td>Xinong Saanen</td>
<td>368A&gt;C</td>
<td>Exon 1</td>
<td>AA (0.61), AC (0.39)</td>
<td>A (0.81), C (0.19)</td>
<td>An et al. (2011)</td>
</tr>
<tr>
<td>Boer</td>
<td>368A&gt;C</td>
<td>Exon 1</td>
<td>AA (0.56), AC (0.43)</td>
<td>A (0.78), C (0.22)</td>
<td>An et al. (2011)</td>
</tr>
<tr>
<td>F1</td>
<td>368A&gt;C</td>
<td>Exon 1</td>
<td>AA (0.496), AC (0.504)</td>
<td>A (0.75), C (0.25)</td>
<td>An et al. (2011)</td>
</tr>
<tr>
<td>F2</td>
<td>368A&gt;C</td>
<td>Exon 1</td>
<td>AA (0.54), AC (0.46)</td>
<td>A (0.77), C (0.23)</td>
<td>An et al. (2011)</td>
</tr>
<tr>
<td>Xinong Saanen</td>
<td>4911C&gt;T</td>
<td>Exon 3</td>
<td>CC (0.52), CT (0.48)</td>
<td>C (0.76), T (0.24)</td>
<td>An et al. (2011)</td>
</tr>
<tr>
<td>Boer</td>
<td>4911C&gt;T</td>
<td>Exon 3</td>
<td>CC (0.60), CT (0.40)</td>
<td>C (0.80), T (0.20)</td>
<td>An et al. (2011)</td>
</tr>
<tr>
<td>F1</td>
<td>4911C&gt;T</td>
<td>Exon 3</td>
<td>CC (0.63), CT (0.37)</td>
<td>C (0.82), T (0.18)</td>
<td>An et al. (2011)</td>
</tr>
<tr>
<td>F2</td>
<td>4911C&gt;T</td>
<td>Exon 3</td>
<td>CC (0.51), CT (0.49)</td>
<td>C (0.76), T (0.24)</td>
<td>An et al. (2011)</td>
</tr>
<tr>
<td>Iranian Markhoz</td>
<td>339T&gt;A</td>
<td>Exon 1</td>
<td>AA (0.90), AT (0.07), TT (0.03)</td>
<td>A (0.93), T (0.07)</td>
<td>Khani et al. (2017)</td>
</tr>
<tr>
<td>Iranian Markhoz</td>
<td>169T&gt;G</td>
<td>Exon 3</td>
<td>TT (0.81), TG (0.12), GG (0.07)</td>
<td>T (0.88), G (0.12)</td>
<td>Khani et al. (2017)</td>
</tr>
<tr>
<td>Red Sokoto</td>
<td>32A&gt;T</td>
<td>Exon 1</td>
<td>KK (0.925), MM (0.075)</td>
<td>M (0.075), K (0.925)</td>
<td>Sanni et al. (2019)</td>
</tr>
<tr>
<td>Red Sokoto</td>
<td>80A&gt;G</td>
<td>Exon 1</td>
<td>QQ (0.85), RR (0.15)</td>
<td>R (0.15), Q (0.85)</td>
<td>Sanni et al. (2019)</td>
</tr>
<tr>
<td>Red Sokoto</td>
<td>84C&gt;G</td>
<td>Exon 1</td>
<td>II (0.975), MM (0.025)</td>
<td>M (0.025), I (0.975)</td>
<td>Sanni et al. (2019)</td>
</tr>
<tr>
<td>Red Sokoto</td>
<td>85C&gt;G</td>
<td>Exon 1</td>
<td>FF (0.975), VV (0.025)</td>
<td>V (0.025), F (0.975)</td>
<td>Sanni et al. (2019)</td>
</tr>
<tr>
<td>Red Sokoto</td>
<td>473T&gt;G</td>
<td>Exon 1</td>
<td>LL (0.975), RR (0.025)</td>
<td>L (0.025), L (0.975)</td>
<td>Sanni et al. (2019)</td>
</tr>
</tbody>
</table>
Genotypic frequencies

All the reviewed articles detailed the genotypic frequencies as presented in Table 2. A total of 51 genotypes were recognized throughout the reviewed articles. The genotypic frequencies of the studied articles ranged from 0.02 to 0.98 with Sanni et al. (2019) had the highest genotype (0.98) and the lowest genotype (0.02).

Allelic frequencies

The alleles and their frequencies observed as a result of the occurrence of SNPs are detailed in Table 2. A total of 46 alleles were observed throughout all the papers that were analyzed. The lowest allelic frequency (0.07) was observed in a study by Khani et al. (2017) while the highest allelic frequency (0.977) was observed by Sanni et al. (2019).

Single nucleotide polymorphisms (SNPs) and their association with growth traits

The SNPs and their association with growth traits are detailed in Table 3. Out of a total of 6 articles reviewed, 5 articles observed a significant difference in more than one trait of interest as a result of the SNPs that occur in the MSTN gene of the goat. From the 6 papers that studied 8 traits including body weight (BW), body length (BL), body height (BH), chest girth (CG), withers height (WH), chest depth (CD), average daily gain (ADG) and weaning weight (WW), their association with SNPs identified was also investigated. Of the 8 traits of interest, the genotypes identified in 7 SNPs were found significantly associated with traits. Of the papers that observed significant association between identified genotypes and traits, 5 papers observed significant association between genotypes and BW followed by BL in 4, BH and CG each in 3, WH and CD observed in 1 paper each.

Discussion

The myostatin (MSTN) gene’s polymorphisms have been observed to significantly impact the phenotypic characteristics of livestock and have a great economic consequence in the rearing of animals (Zhang et al., 2012; Ahad et al., 2017; Sanni et al., 2019). The current systematic review was conducted to assess the overall effects identified of the MSTN gene on the growth traits of goats. The allelic variations in the caprine MSTN gene that display significant association with economically important
traits might be used as genetic markers in selective animal breeding efforts, more so in the use of MAS.

### Table 3. SNPs association with growth traits

<table>
<thead>
<tr>
<th>Author</th>
<th>Breed</th>
<th>SNP</th>
<th>Growth traits</th>
<th>Genotypes</th>
<th>Sig</th>
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<tr>
<td>Ahad et al. (2017)</td>
<td>Boer</td>
<td>368A&gt;C</td>
<td>BW</td>
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<td>AA</td>
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<tr>
<td></td>
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<td>368A&gt;C</td>
<td>BL</td>
<td>AC</td>
<td>AA</td>
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<tr>
<td></td>
<td></td>
<td>368A&gt;C</td>
<td>BH</td>
<td>AC</td>
<td>AA</td>
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<tr>
<td></td>
<td></td>
<td>368A&gt;C</td>
<td>CG</td>
<td>AC</td>
<td>AA</td>
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<td>Ahad et al. (2017)</td>
<td>Bakerwal</td>
<td>368A&gt;C</td>
<td>BW</td>
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<td>AC</td>
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<td>AC</td>
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<td>BW</td>
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<td>MM</td>
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<td>KK</td>
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<td>BW</td>
<td>QQ</td>
<td>RR</td>
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<td>BL</td>
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<tr>
<td>Sanni et al. (2019)</td>
<td>Red Sokoto</td>
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<td>FF</td>
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<td>BL</td>
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The results retrieved in the study indicated that five of the six studies described a significant association between the observed SNPs and growth traits in goats. The included studies recognized 11 single nucleotide polymorphisms (SNPs) (368A>C, 339T>A, 32A>T, 80A>G, 84C>G, 85C>G, 473T>G, 49C>A, 1388T>A, 197G>A, 345A>T) where some occur across multiple breeds and articles with the 368A>C being the most commonly described of the identified SNPs. Ahad et al. (2017) and An et al. (2011) described the 368A>C SNP and found it to be significantly associated with five traits (body weight, body height, body length, chest girth, withers height) across the Boer and the Bakerwal breeds. The SNP 339T>A described by Khani et al. (2017) in Iranian Markhoz, showed no significance association with studied traits and also SNPs 84C>G, 85C>G, 473T>G described by Sanni et al. (2019) in Red Sokoto. Sanni et al. (2019) further identified and observed significant correlation between the traits and SNPs 32A>T (body weight body length, withers height, chest depth), 80A>G (wither height, chest depth), 49C>A (chest depth, withers height, body weight) all of which were unique and not observed in any other reviewed articles. The 1388T>A SNP investigated by Zhang et al. (2012) showcased a significant association with growth traits (body weight, body height) in the Boer breed. Zhang et al. (2013) reported two SNPs (197G>A, 345A>T) in the Boer and Anhui White breeds, the 197G>A displayed no association across both breeds while the 345A>T showed a significant relationship.

<table>
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<tr>
<th>Sanni et al. (2019)</th>
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with all investigated traits (body weight, body length, body height, chest girth chest girth) in Anhui White and the same SNP showed significant association with in three growth traits (body weight, body length, body height) of the four investigated in the Boer breed. The systematic review confirms the influence of the MSTN gene on the growth traits of goats.

According to our knowledge, this systematic review is the first of its nature reporting on the association between the SNPs of the caprine MSTN gene and growth traits. As such, there is no basis for comparison of the findings with other systematic review findings. The findings of the current study imply that the frequently identified 368A>C SNP may be utilized as a genetic marker for body weight, body length, body height, chest girth, and withers height. The review combines the results investigated in this field of research and highlights the MSTN genes SNPs of importance which have potential and should be considered for further research and for use in MAS. The establishment of a MAS system generally has the identification of genetic markers for growth traits as the initial and critical condition (Zhang et al., 2013). The genetic improvement of traits of economic importance in small ruminants has produced higher production records in the last decades (Ahad et al., 2017). In the recent years there has been tremendous progress in the genetic basis of MAS through genome mapping which is important in distinguishing genetic markers and multiple quantitative trait loci that influence traits of economic importance have been identified (Ahad et al., 2017). As a result, both in developed and developing nations, the use of MAS and the credentials of genetic markers linked to QTLs have emerged as cutting-edge and economical breeding program strategies. Continentally, Asia is the leading producer of goats worldwide, this is evident being that 83.33% (n=5) of the included articles emanate from Asia whereas only 16.67% (n=1) of articles emanate from Africa which has the second largest goat population, this disproportionality is limiting. This, along with the association of growth traits with indels instead of individual SNPs and only 2 articles describing a common SNP disenabled the conduction of a meta-analysis, which limited the results. As such, it is recommended that more studies need to be conducted African continent to bridge the research disparity and it is also recommended that further research on the SNP 368A>C of exon 1 of the MSTN gene and their association with growth traits be investigated.

**Conclusion**

It is concluded that the MSTN gene influences the growth traits of goats and as such 368A>C SNP might be used as potentially viable genetic markers for the improvement of growth traits in goats during breeding programs.

**Acknowledgements.** This systematic review has been supported and approved by the University of Limpopo.

**REFERENCES**

Thepa - Tyasi: Relationships between myostatin gene single nucleotide polymorphisms and growth traits in goats: a systematic review


