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Association analysis of novel SNPs in exon 10 of the growth hormone receptor gene with growth traits in Indian sheep

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ABSTRACT

The growth hormone receptor *(GHR)* gene encodes the type I cytokine receptor that helps in joining the growth hormone to this receptor, thus promoting receptor-dimerization, leading to up-regulating growth. The ovine *GHR* gene located on chromosome 16, which consists of 10 exons and 9 introns, along with untranslated regions on either side, comprise a total size of 178.09 kb. However, earlier reports about polymorphism have mainly dealt with exon 10 which is also a larger fragment of this gene comprising 1102 bp. Hence, this study was carried out to detect polymorphism in exon 10 of the *GHR* gene and its association with growth traits. Genomic DNA was isolated from blood samples of Madras Red and Mecheri sheep breeds from India. Part of exon 10 (895 bp) of the *GHR* gene was amplified and sent for sequencing. The sequence analysis revealed transition of nucleotide G>A at loci G177624A and G177878A in both sheep breeds. Populations were screened by Tetra-primer ARMS-PCR. The genotype frequencies of *GG*, *GA* and *AA* were 0.276, 0.519 and 0.205 at 177624 G>A, and 0.307, 0.444 and 0.149 at 177878 G>A in Madras Red sheep; whereas in Mecheri they were 0.476, 0.372 and 0.152 at 177624 G>A, and 0.629, 0.314 and 0.057 at 177878 G>A, respectively. Likewise the estimated allele frequencies of *G* and *A* were 0.5355 and 0.4645 at 177624 G>A, and 0.5790 and 0.4210 at 177878 G>A in Madras Red sheep; whereas in Mecheri they were 0.6620 and 0.3380 at 177624 G>A, and 0.7860 and 0.2140 at 177878 G>A, respectively. The effect of sex was significant for birth, six and nine month weight; but non-significant for three and 12 month weight in Mecheri sheep. However, in the Madras Red breed the effect of sex was significant for all body weights except weaning weight. The effect of variations on growth traits, *viz.*, birth weight, weight at weaning, and weight at six, nine and twelve months in both breeds were analysed for their association, and they were found non-significant. Since these SNPs are salient findings of *GHR* gene polymorphism in Indian sheep breeds, further investigation is required into the significant effects of these novel SNPs, which could be useful for genetic improvement based on marker assisted selection.

Key words: association; body weights; growth hormone receptor gene; native sheep; SNP

Introduction

The sheep population is a major economic and ecological resource, predominantly serving humans in multiple aspects, providing proteinous meat, woolly clothing, raising farmers' income, facilitating rural employment, and above all sheep improve soil fertility (DEVENDRA, 2001). Sheep

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farming is one of the most developed sectors, comprising 12.7% of the total livestock population. India has 43 sheep breeds, numbering 65.06 million heads (LIVESTOCK CENSUS REPORT, 2012). Annual mutton production in India is around 7.12%, and 31,440 tonnes of mutton are produced by Tamil Nadu state of India alone from where the Madras Red and Mecheri sheep breeds studied originate, with 4.47 million sheep (ANON, 2015). The efficiency of sheep enterprises can be improved by increasing litter size, lamb weight, mutton production, and by the improvement of wool quality. DNA based molecular markers are used efficiently for assessment of genetic diversity, selection of animals at an early age, studying population structure, mapping of genes and quantitative trait loci (QTLs), and breeding based on genomic selection (COLLARD et al., 2005). Genomic selection in the livestock industries has been made possible by the availability of high density single nucleotide polymorphism (SNP) marker panels, commonly referred to as "SNP chips" (ZHANG et al., 2012). Genetic analysis of organisms has been revolutionized by recombinant DNA technology, DNA sequencing, and application of marker-assisted selection in contrast to traditional methods of selection (WAKCHAURE et al., 2015). Genome maps contain assignments of markers and genes to specific regions along the chromosomes, which are useful for organizing systematic searches for the chromosome regions containing important genes. Around 200 equally spaced markers are required to entirely scan the genome of a livestock species (MORADI et al., 2012). Due to marker clustering, these markers are required to achieve adequate coverage of a 10 to 20 centimorgan genetic map. Additional markers are very essential to increase the resolution of the map, in order to proceed with the isolation of genes that are economically important and to apply molecular breeding strategies. The significant SNPs are used as molecular markers for genetic analysis, using them as long-term selection markers. These are also prevalent and provide more potential markers near or at any locus of interest, and some SNPs are located in coding regions which directly affect protein function (BEUZEN et al., 2000). The relationship between the economic traits and the genes associated with these have been studied by the candidate gene approach (ANDERSSON, 2001). Marker Assisted Selection enables the unambiguous selection of specific nucleotide variations that are related to differences in growth and meat production traits (DEKKERS AND HOSPITAL, 2002). The growth hormone receptor (*GHR*) is the major candidate gene situated on chromosome 16 in sheep (ARCHIBALD et al., 2010). It consists of 10 exons with 9 intervening regions, comprising exon 10 as the longest (1102 bp) regulatory sequence. Any allelic variation in the regulatory sequences of the growth hormone and its receptor genes affects body growth (MULLIS, 2011). Scanty reports are available on polymorphism of the *GHR* gene in exotic sheep (BASTOS et al., 2001; VALEH et al., 2009; BAHRAMI et al., 2013). In spite of the functional importance of *GHR* in the regulation of growth hormone, there are few findings about the nucleotide variability of indigenous sheep and cattle breeds (SAHU et al., 2017; DEEPIKA ANDSALAR, 2013). Hence, this study was undertaken to find polymorphism in the exonic region and correlate associations with growth traits which may be affected due to single nucleotide polymorphism.

Materials and methods

Experimental animals. Madras Red and Mecheri are meat type breeds native to two different geographical regions of Tamil Nadu, India. Blood was collected from animals of both sexes of Madras Red (n = 127) from the Post Graduate Research Institute in Animal Sciences, Kattupakkam; and Mecheri (n = 105) from the Mecheri Sheep Research Station, Pottaneri, both being constituent units of Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu. The body weights of the animals at birth, pre-weaning, six, nine and twelve months of age were also recorded along with the corresponding dam's weight at lambing. Genomic DNA was isolated using the Phenol-Chloroform extraction method, with a slight change of the DNAzol reagent, instead of SDS and proteinase K (SAMBROOK et al., 1989).

PCR programme. Primer pairs were designed by Fast PCR Primer designing software v. 3 (KALENDAR et al., 2014) to amplify part of exon 10 (895 bp) of the *GHR* gene. The reaction was performed in microfuge tubes (0.2 mL) using thermal cyclers (Eppendorf Mastercycler ep gradient S and Applied Biosystems 2720 models). The total of 20 µL of reaction mixture comprised 10 µL master mix, 0.8 µL each of the forward (CCCTGATGAGAAGACTGAAGGGT) and reverse (TCAATGGGTAGCTCATGGGAA) primers (10 pmol/µL), 1.5 µL template DNA (50 ng/µL) and 6.9 µL of nuclease free water (SAHU et al., 2017). The amplification was performed in a thermal cycler for initial denaturation at 95 °C (5 min), followed by 34 cycles of denaturation at 95 °C (35 sec), annealing at 60 °C (30 sec) and extension at 72 °C (35 sec), with final extension at 72 °C (5 min), and ended at 4 °C. PCR products were confirmed by agarose gel electrophoresis (2% agarose) in 1X TAE, and visualized under UV-illumination using the gel documentation system (Bio-Rad, Laboratories Inc., USA).

Analysis of sequence variations. Sixteen random samples (eight from each breed) were sequenced in both forward and reverse directions for 895 bp amplicon using an ABI PRISM 3730XL Genetic analyzer (Applied Biosystems, USA). Sequence data were analyzed using the SeqMan program of LASERGENE software, version 7.1.0 (44) (DNASTAR Inc., USA). The sequences were assembled and screened for SNPs.

Screening the population. Populations of both breeds were genotyped according to the mutations identified with two pairs of primers (Table 1) designed by the online "Tetra-primer ARMS-PCR" designing software (YE et al., 2001). Amplification was performed for both *GHR*-177624 and *GHR*-177878 primers (Table 2 and Table 3). Agarose electrophoresis was carried out for 60 min with 2.5% agarose in 1X TAE buffer, to confirm the products (AHLAWAT et al., 2013). The gene and genotype frequencies at each locus were calculated which signified population was under the Hardy-Weinberg equilibrium (FALCONER and MACKAY, 1996).

Table 1. Primers used in genotyping the population

Sl. No.	Name of primer	Primers (5'-3' end)	Product size (bp)
1.	*GHR*-177624	Forward inner AGA AGT GGT CAC ACC CAG CCA AGA AA Reverse inner AGA AGT AAG CGC TGT CCA CGA TGA ATT C Forward outer GCC AGC AGC CCA GTG TTA TCC TAG TAG A Reverse outer ACT TGG AAC ATT TTC TGC TGT CCC TGA C	*A* allele = 209 *G* allele = 279 Outer = 434
2.	*GHR*-177878	Forward inner AGT CTC CAC AGG GCC TCG TAC TCA ATT CG Reverse inner CTT TGT CAG GCA AGG GCA GGG CAT TT Forward outer GGT CAC ACC CAG CCA AGC AGA CTT CAT C Reverse outer CAA CTC ATC CCC CTC CCC CAA AAA AGT T	*A* allele = 299 *G* allele = 225 Outer = 469

Table 2. PCR reaction mixture for detected SNPs

Sl. No.	Components	Volume (μL)	
		GHR-177624	*GHR*-177878
1.	Master mix (Ampliqon)	5.0	5.0
2.	Forward Inner (10 pmol/μL)	0.3	0.4
	Reverse Inner (10 pmol/μL)	0.3	0.4
	Forward Outer (10 pmol/μL)	0.3	0.4
	Reverse Outer (10 pmol/μL)	0.3	0.4
3.	Template DNA (50 ng/μL)	1.0	1.2
4.	Nuclease free water	2.8	2.2
	Total	10.0	10.0

Table 3. PCR protocol for genotyping through Tetra-primer ARMS-PCR

Step	Process	*GHR* 177624		*GHR* 177878	
		Temperature	Duration	Temperature	Duration
1	Initial denaturation	95 °C	5 min	95 °C	5 min
2	Denaturation:	95 °C	35 sec	95 °C	35 sec
3	Annealing:	61.5 °C	30 sec	59.5 °C	30 sec
4	Extension:	72 °C	35 sec	72 °C	35 sec
5	Back to steps 2 to 4	34 cycles	35 cycles		
6	Final extension	72 °C	5 min	72 °C	5 min
7	Hold	4 °C	Until samples are removed	4 °C	Until samples are removed

Data analysis. The polymorphisms observed at both loci of the *GHR* gene in Madras Red and Mecheri breeds were analysed for their association with body weights at various ages *viz.*, birth, weaning (three months), six, nine and twelve months weights, using least-squares procedures (HARVEY, 1990). The blood sample was collected for genotyping from 127 animals (27 males and 100 females) of Madras Red, and 105 animals (31 males and 54 females) of Mecheri breed. The number of animals used for association analysis in Madras Red was 22 males and 88 females (n = 110) and in Mecheri 31 males and 54 females (n = 85), which is lower than the number of animals genotyped due to the unavailability of complete sets of data on body weight and deletion of outliers. The outliers in the data set correspond to the animals with extremely high and low body weights, which are definitely due to errors in recording. All data within the range of the body weights reported for the breed average were considered for analysis. The non-availability of grazing land leads to intensive feeding in Madras Red, whereas Mecheri sheep are allowed free ranging along with intensive feeding in their breeding tract.

The period was not partitioned or defined as the annual effect due to the limitation of population size, as only a small number of sheep is kept on the research farms. If the annual effect was assessed, the number would be so small that the least-squares analysis could not be performed. Secondly, the breeding rams were changed once in three or four years depending on the farm management practices. Considering these facts, the data set was classified into period effects. Data were classified into: two periods of birth: *viz.*, 1st (2007 to 2010) and 2nd (2011 to 2014) in Madras Red; and 1st (2008 to 2010) and 2nd (2011 to 2013) in Mecheri sheep; sex as males and females; and three different genotypes each at

loci G177624A and G177878A of *GHR* gene. The weight of the dam at lambing was also considered as a covariable in the given model:

$$Y_{ijkl} = \mu + P_i + S_j + G_k + b\,(WM_{ijk} - WM) + e_{ijkl}$$

where:

Y_{ijkl} = body weight of the l^{th} animal of k^{th} genotype of j^{th} sex born in i^{th} period of lambing,

μ = overall mean,

P_i = fixed effect of i^{th} period of lambing (i = 1 and 2),

S_j = fixed effect of j^{th} sex of the lamb (j = 1 for male and 2 for female),

G_k = fixed effect of k^{th} genotype (k = 1, 2 and 3 for loci G177624A and G177878A),

b (WM_{ijk} - WM) = regression of Y on dam's weight at lambing,

e_{ijkl} = residual random error, NID (0, σ^2).

Results and discussion

The amplified fragment of exon 10 showed transitions of nucleotide G>A at G177624A and G177878A were indicated in the chromatogram (Fig. 1 and Fig. 2) in both the sheep breeds studied (GenBank Accession No. KT757901and KT781164). The nucleotide variability at locus G177624A revealed a change of amino acid aspartic acid (GAC) to aspargine (AAC).

Genotyping was carried out for the locus 177624. G>A yielded amplified products of 209 bp, 279 bp and 434 bp; where: *GG* = 279 bp and 434 bp; *AA* = 209 bp and 434 bp; and *GA* = 209 bp, 279 bp and 434 bp (Fig. 3). All three genotypes were observed in both breeds. Genotypic and allelic frequencies were determined in both breeds and are presented in Table 4. The observed genotype frequencies of *GG*, *GA* and *AA* were 0.276, 0.519 and 0.205 in Madras Red and 0.476, 0.372 and 0.152 in Mecheri breeds. The estimated frequencies of *G* and *A* alleles were 0.5355 and 0.4645; and 0.6620 and 0.3380 in Madras Red and Mecheri, respectively. The population of both breeds was in genetic equilibrium, with a higher frequency of *G* than *A*.

The size of amplified products obtained for locus 177878 G>A was 225 bp, 299 bp and 469 bp; where: *GG* = 225 bp and 469 bp; *AA* = 299 bp and 469 bp; and *GA* = 225 bp, 299 bp and 469 bp (Fig. 4). Genotypic and allelic frequencies at this locus are presented for both sheep breeds (Table 4). The observed genotypic frequencies of *GG*, *GA* and *AA* were 0.307, 0.544 and 0.149; and 0.629, 0.314 and 0.057 in Madras Red and Mecheri breeds, respectively. The estimated frequencies of *G* and *A* alleles were 0.5790 and 0.4210 in Madras Red, while in Mecheri sheep breed they were 0.7860 and 0.2140, respectively, indicating a higher frequency of G than A allele. The populations of both sheep breeds at locus 177878 G>A were in equilibrium due to a non-significant χ^2 value (P>0.05).

The heterozygote frequency of both loci observed was higher in Madras Red sheep and was comparable to the findings of Baluchi sheep (VALEH et al., 2009) and Nilagiri sheep (SAHU et al., 2017). Despite the mutations detected, monomorphism has been reported in exon 10 in Indian sheep breeds (SAHU et al., 2016), as well as in other regions of the *GHR* gene in exotic sheep breeds (BASTOS et al., 2001; BAHRAMI et al., 2013; SHIRI et al., 2006; MAHROUS et al., 2014).

Association of novel mutations with growth traits. The least square means for body weights at various ages, *viz.*, birth, weaning, six, nine and 12 months in both Madras Red and Mecheri sheep breeds, are presented in Table 5 and 6. The effect of sex was significant for birth, six and nine months weight; whereas it was non-significant for three and 12 months weight in Mecheri sheep. However, in the Madras Red breed the effect of sex was significant for all body weights, except weaning weight. The polymorphic effect at both the loci G177624A andG177878A on body weight at various ages, *viz.,* birth, weaning, six, nine and twelve months body weight, were determined to be non-significant in both Madras Red and Mecheri sheep breeds (Table 7 and Table 8).

The non-significant effect of the nucleotide variability of the *GHR* gene on growth traits in both breeds of sheep was similar to the research findings in Baluchi sheep (VALEH et al., 2009) and Nilagiri (SAHU et al., 2017). The non-significant effect may possibly be due to the closed flock and small sample size. Further, the single nucleotide polymorphisms recognized may not be a significant major factor for body weights, as all the quantitative traits are controlled by many genes.

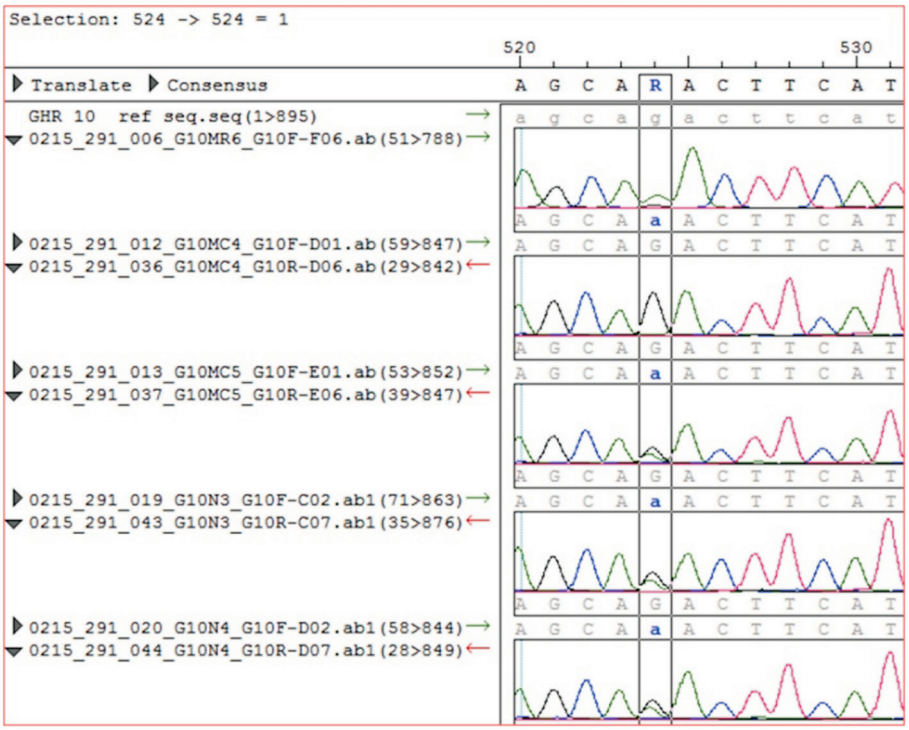

Fig. 1. Polymorphism at locus G177624A in exon 10 of the *GHR* gene in Madras Red and Mecheri sheep

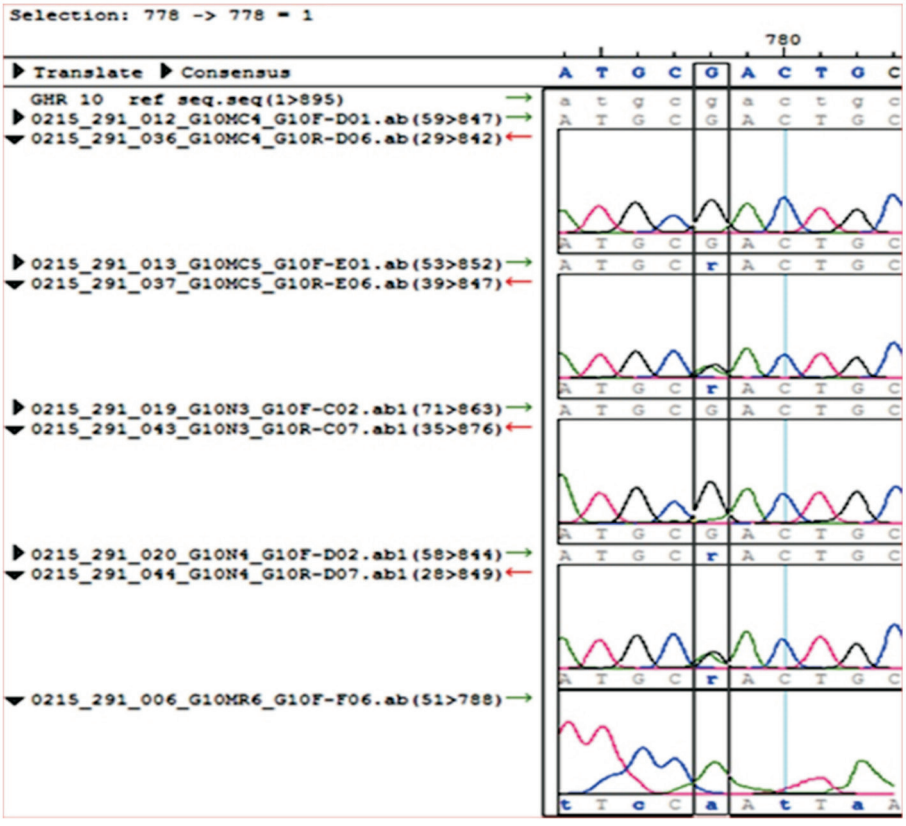

Fig. 2. Polymorphism at locus G177878A in exon 10 of the *GHR* gene in Madras Red and Mecheri sheep

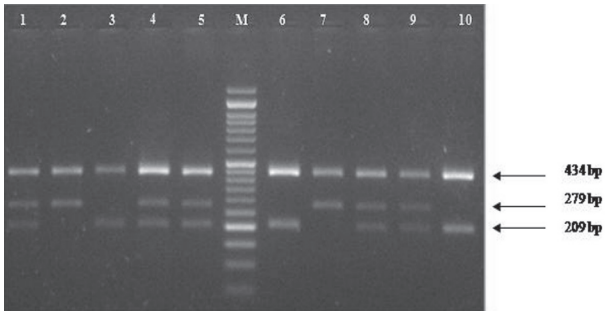

Fig. 3. Genotyping by Tetra-primer ARMS-PCR for locus 177624 G>A. Lane M - 50 bp DNA ladder; Lanes 1, 4, 5, 8 and 9 - GA (209 bp, 279 bp and 434 bp); 3, 6 and 10 - AA (209 bp and 434 bp); and 2 and 7 - GG (279 bp and 434 bp); Lanes 1 to 5 Madras Red; and 6 to 10 Mecheri sheep

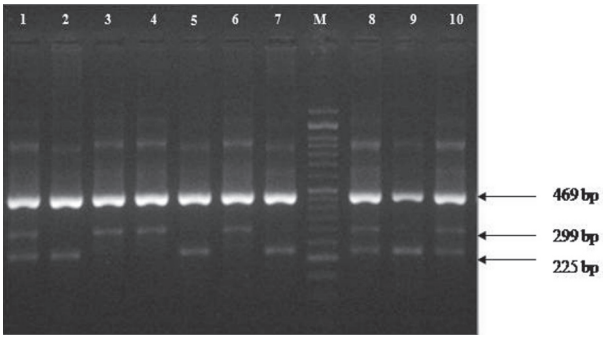

Fig. 4. Genotyping by Tetra-primer ARMS-PCR for locus 177878 G>A. Lane M - 50 bp DNA ladder; Lanes 1, 8 and 10 - GA (225 bp, 299 bp and 469 bp); 2, 5, 7 and 9 - GG (225 bp and 469 bp); and 3, 4 and 6 - AA (299 bp and 469bp); Lanes 1 to 5 Madras Red; and 6 to 10 Mecheri sheep

Table 4. Mutations at loci 177624 G>A and 177878 G>A in exon 10 of *GHR* gene

SNP	Breed	Genotype	Genotypic frequency	Allelic frequency	χ^2value
177624 G>A	Madras Red (127)	GG (35) GA (66) AA (26)	0.276 0.519 0.205	G = 0.5355 A = 0.4645	0.25^{NS}
	Mecheri (105)	GG (50) GA (39) AA (16)	0.476 0.372 0.152	G = 0.6620 A = 0.3380	3.03^{NS}
177878 G>A	Madras Red (127)	GG (39) GA (69) AA (19)	0.307 0.544 0.149	G = 0.5790 A = 0.4210	1.65^{NS}
	Mecheri (105)	GG (66) GA (33) AA (6)	0.629 0.314 0.057	G = 0.7860 A = 0.2140	0.46^{NS}

Figures in parentheses indicate number of observations; ^{NS} - not significant

Table 5. Least-squares means (± SE) of body weights (kg) of Madras Red sheep at different ages

Main effect	Body weight at				
	Birth	3 months	6 months	9 months	12 months
Overall mean	2.728 ± 0.053 (110)	9.158 ± 0.348 (110)	13.410 ± 0.350 (110)	17.014 ± 0.448 (110)	19.052 ± 0.473 (110)
Sex of lamb	*	NS	**	**	**
Male	2.811^{b} ± 0.085 (22)	9.397 ± 0.556 (22)	14.562^{b} ± 0.559 (22)	18.575^{b} ± 0.715 (22)	21.270^{b} ± 0.754 (22)
Female	2.645^{a} ± 0.044 (88)	8.920 ± 0.288 (88)	12.259^{a} ± 0.289 (88)	15.452^{a} ± 0.370 (88)	16.833^{a} ± 0.390 (88)
Prob.	0.0482	0.3836	0.0001	0.0001	0.0001

Figures in parentheses are the number of observations; Means with at least one common superscript within classes do not differ significantly; * - P≥0.05

Table 6. Least-squares means (± SE) of body weights (kg) of Mecheri sheep at different ages

Main effect	Body weight at				
	Birth	3 months	6 months	9 months	12 months
Overall mean	2.531 ± 0.062 (85)	12.623 ± 0.413 (85)	14.791 ± 0.454 (85)	17.411 ± 0.520 (85)	19.671 ± 0.587 (85)
Sex of lamb	*	NS	*	*	NS
Male	2.627^{b} ± 0.083 (31)	13.148 ± 0.554 (31)	15.457^{b} ± 0.609 (31)	18.210^{b} ± 0.697 (31)	20.021 ± 0.787 (31)
Female	2.434^{a} ± 0.065 (54)	12.098 ± 0.434 (54)	14.124^{a} ± 0.477 (54)	16.611^{a} ± 0.546 (54)	19.321 ± 0.616 (54)
Prob.	0.0226	0.0622	0.0320	0.0248	0.3772

Figures in parentheses are the number of observations; Means with at least one common superscript within classes do not differ significantly; * - P≥0.05

Table 7. Least-squares means ± SE (kg) of locus 177624 G>A in *GHR* gene associated with growth traits

Traits	Madras Red (110)				Mecheri (85)			
	AA (22)	*AG* (57)	*GG* (31)	*Prob.*	*AA* (11)	*AG* (32)	*GG* (42)	*Prob.*
Birth weight^{NS}	2.689 ± 0.082	2.743 ± 0.064	2.751 ± 0.072	0.7861	2.569 ± 0.107	2.509 ± 0.092	2.514 ± 0.095	0.9189
Weaning weight^{NS}	8.745 ± 0.535	9.246 ± 0.417	9.484 ± 0.475	0.4946	12.932 ± 0.714	12.345 ± 0.615	12.592 ± 0.633	0.8080
6 months weight^{NS}	13.440 ± 0.538	13.469 ± 0.419	13.321 ± 0.477	0.9561	15.706 ± 0.786	13.900 ± 0.677	14.767 ± 0.696	0.1702
9 months weight^{NS}	17.478 ± 0.689	16.857 ± 0.537	16.706 ± 0.611	0.6068	17.589 ± 0.899	16.885 ± 0.774	17.758 ± 0.797	0.4870
12 months weight^{NS}	18.913 ± 0.726	19.091 ± 0.566	19.151 ± 0.644	0.9597	20.408 ± 1.015	19.240 ± 0.874	19.364 ± 0.899	0.7056

Figures in parentheses indicate number of records used for analysis; ^{NS} - not significant

Table 8. Least-squares means ± SE (kg) of locus 177878 G>A in *GHR* gene associated with growth traits

Traits	Madras Red (110)				Mecheri (85)			
	AA (18)	*AG* (61)	*GG* (31)	*Prob.*	*AA* (5)	*AG* (26)	*GG* (54)	*Prob.*
Birth weight^{NS}	2.711 ± 0.102	2.752 ± 0.075	2.754 ± 0.081	0.9217	2.272 ± 0.176	2.601 ± 0.077	2.719 ± 0.076	0.0950
Weaning weight^{NS}	8.392 ± 0.611	9.355 ± 0.451	9.511 ± 0.483	0.2567	12.596 ± 1.175	12.083 ± 0.514	13.190 ± 0.505	0.2104
6 months weight^{NS}	13.779 ± 0.613	13.412 ± 0.452	13.190 ± 0.485	0.7270	13.241 ± 1.293	15.000 ± 0.566	16.131 ± 0.556	0.1011
9 months weight^{NS}	17.406 ± 0.797	17.131 ± 0.588	16.550 ± 0.631	0.6263	15.493 ± 1.479	18.204 ± 0.647	18.534 ± 0.636	0.2327
12 months weight^{NS}	18.615 ± 0.825	19.552 ± 0.609	18.832 ± 0.653	0.4290	17.574 ± 1.669	20.421 ± 0.730	21.018 ± 0.718	0.2306

Figures in parentheses indicate number of records used for analysis; ^{NS} - not significant.

Conclusions

The growth hormone receptor (*GHR*) gene was investigated to characterize the variations in part of exon 10 and exhibit their allelic status in Madras Red and Mecheri sheep in Tamil Nadu, India. The SNPs177624 G>A and 177878 G>A had no significant association with body weights at birth, weaning (three months), six, nine and twelve months of age. However, since this is the first report of *GHR* polymorphism in Indian sheep breeds, further research on variations and their effect on growth traits is required for them to be used as markers for genetic improvement through molecular breeding.

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SAŽETAK

Gen receptora hormona rasta (GHR) kodira tip I citokinskog receptora koji pomaže u vezanju hormona rasta na ovaj receptor, promovirajući dimerizaciju receptora i time regulirajući rast. Ovčji gen GHR, lociran na kromosomu 16, sadržava 10 egzona i 9 introna s netranslatirajućim regijama s obje strane, tvoreći ukupnu veličinu od 178,09 kb. Dosadašnja istraživanja pokazala su da se polimorfizam nalazi većinom u egzonu 10, koji je ujedno veći fragment ovoga gena i sadržava 1102 bp. Ovo je istraživanje provedeno kako bi se otkrio polimorfizam u egzonu 10 gena GHR i njegova povezanost s pokazateljima rasta. Genomska DNA izolirana je iz uzoraka krvi ovaca pasmina Madras Red i Mecheri iz Indije. Dio egzona 10 (895 bp) gena GHR je umnožen i poslan na sekvenciranje koje je u obje pasmine ovaca pokazalo tranziciju nukleotida G > A na lokusima G177624A i G177878A. Probir populacija učinjen je pomoću Tetra-primer ARMS-PCR-a. Učestalost genotipa GG bila je 0,276, genotipa GA 0,519, a genotipa AA 0,205 na 177624 G>A, te 0,307, 0,444 i 0,149 na 177878 G>A u pasmine Madras Red. U pasmine Mecheri učestalost genotipa GG bila je 0,476, učestalost genotipa GA 0,372, a genotipa AA 0,152 na 177624 G > A, te 0,629, 0,314 i 0,057 na 177878 G > A. Učestalost alela G i A, koja je bila 0,5355 i 0,4645 na 177624 G > A, te 0,5790 i 0,4210 na 177878 G>A u ovaca Madras Red, dok je u pasmine Mecheri bila 0,6620 i 0,3380 na 177624 G>A, te 0,7860 i 0,2140 na 177878 G>A. Spol je znakovito utjecao na tjelesnu masu pri janjenju te u dobi od 6 i 9 mjeseci, no nije bilo znakovitog utjecaja u dobi od 3 i 12 mjeseci u pasmine Mecheri. S druge strane, u pasmine Madras Red spol je znakovito utjecao na tjelesnu masu u svim fazama rasta osim pri odbiću. Analizirana je povezanost varijacija s tjelesnom masom pri janjenju, odbiću te u dobi od 6, 9 i 12 mjeseci u obje pasmine, koja nije bila znakovita. Budući da su otkrića ovih SNP-a važna u proučavanju gena GHR u indijskih pasmina ovaca, potrebna su daljnja istraživanja njihova učinka koja bi mogla biti korisna u genetskom poboljšanju populaciju primjenom markerima potpomognute selekcije.

Ključne riječi: povezanost; tjelesna masa; genski receptor hormona rasta; izvorne pasmine ovaca; SNP
