



ORIGINAL ARTICLE

Association of a *Cac8I* polymorphism in the *IGF1* gene with growth traits in Indian goats



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Abstract The *Insulin-like Growth Factor 1 (IGF1)* gene is a member of somatotrophic axis and plays a key role in proliferation of cells, mitosis, myogenesis, meiosis, differentiation in foetal development and post natal growth. The objectives of this study were to verify the single nucleotide polymorphisms (SNPs) in *IGF1* gene and their association with growth traits in two indigenous native goat genetic groups of Kerala, viz., Malabari and Attappady Black. A total of 277 goats were genotyped using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the restriction enzyme *Cac8I*. One SNP, *A224G* was detected in the 5' non-coding region of the *IGF1* gene, and accordingly two genotypes were revealed, GG and AG. This SNP was significantly associated with growth traits in Attappady Black goats, which is maintained as meat breed in Kerala. Results from this study demonstrated higher performance of GG animals for growth traits. The association of *IGF1* gene with these traits emphasizes the importance of caprine *IGF1* as a candidate gene for growth traits in goat breeding.

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1. Introduction

In Kerala, a southern state of India, goats are mainly reared in small holder farming community and goat production is mainly centred on two indigenous goat breeds, Attappady Black and Malabari goats. In the small ruminant livestock industry, growth traits determine the economic value of

animals and are of primary importance in breeding. Validation of the novel genetic markers of growth traits is the primary step to establish a marker assisted selection programme [1]. Candidate genes for most of the growth traits are located in the growth hormone (GH) axis. The GH gene pathway contains various interdependent genes, such as *GH*, *IGF1*, *pituitary specific transcription factor1 (PIT1)*, *growth hormone releasing hormone (GHRH)*, *growth hormone receptor (GHR)*, and others. Growth hormone influences the growth of bones and muscles which is mediated by IGF1, which is the key factor for the postnatal growth [2]. The IGF1 has an important

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role in major biological functions as key regulator molecules that affect cell proliferation, mitosis, myogenesis, meiosis, differentiation and reproduction [3–9]. It also stimulates glucose absorption, lipid synthesis, myogenesis and progesterone synthesis in ovarian cells, inhibition of cell death, activation of cell cycle genes, etc. [10]. The growth retardation in both mice and humans due to mutations in *IGF1* gene [3,11] indicates its central role in growth. In cattle, two *IGF1* gene polymorphisms have been reported to be located in the promoter region, a CA_n microsatellite [12], and a T/C transition, detected as *IGF1/SnaBI* [13,14]. The microsatellite has been associated with birth and weaning weight in Hereford cattle, but these associations were not found in breeds such as Nellore, Canchim and Simmental/Angus crosses studied by Curi et al. [15]. After its evaluation with growth characteristics in Angus cattle, the SNP *IGF1/SnaBI* was considered to be a potential molecular marker associated with weight gain during the first 20 days after weaning [14]. Zhang et al. [16] reported a SNP in intron 4 of the caprine *IGF1* gene associated with birth weight, body weight at six months and twelve months, heart girth at two months, body length at six months, six and twelve months wither height and heart girth at twelve months. Recent researches suggest that IGF1 mediates stimulation of embryonic skeletal muscle development, which could have important implications for the improvement of meat production [17].

Since the previous studies suggested that the polymorphisms in the promoter region of *IGF1* gene have significant effect on growth traits, the present study was designed to analyse the genetic variations of *IGF1* gene and their association with body conformation traits in 277 goats of Attappady Black and Malabari breeds of Kerala, India. The economic traits included for the association study in the current research are body weight, body length, body height, trunk and chest circumferences, body length index, chest circumference index, and trunk index. The genetic association analysis of *IGF1* gene with these traits would benefit the selection of better genotypes in future breeding programs.

2. Materials and methods

2.1. DNA isolation

To study the association between the caprine *IGF1* gene and body conformation traits, 6 mL of venous blood was collected from jugular vein from adult female goats (2–5 years of age) belonging to Malabari ($n = 175$) and Attappady Black breeds ($n = 102$) maintained at University Goat and Sheep Farm, Mannuthy, Thrissur, Kottakkal Aryavaidyasala, Malappuram and Department of Animal Husbandry Goat Farm, Attappady, Palakkad, Kerala. The genomic DNA was extracted by the standard phenol chloroform method and stored at -20°C until use.

2.2. Data collection

To evaluate the meat production related performance traits, the phenotypic traits of goats, body weight, body length, chest circumference, body height and trunk circumference were measured directly. Body length index, chest circumference index and trunk index were estimated as described in a previous association study [18].

2.3. PCR-RFLP and SNP Identification

Primers (forward- 5'TGAGGGGAGCCAATTACAAAGC3', reverse 5'CCGGGCATGAAGACACACACAT 3') for the amplification of a 294 bp fragment 5' flanking region of *IGF1* gene were designed using Primer3 software from the *IGF1* gene sequences of goats available in GenBank (Accession No. HQ731040) and were custom synthesized (Sigma-Aldrich). The PCR was performed in a 25 μL reaction volume contained 50 ng genomic DNA, 2.5 μL 10X reaction buffer, 0.2 mM dNTP, 10 pM of each primer and 0.5 U of Taq DNA polymerase. The cycling protocol was an initial denaturation of 3 min at 95°C , and 35 cycles of denaturing at 94°C for 30 s, annealing at 54°C for 30 s, extension at 72°C for 30 s with a final extension at 72°C for 5 min were conducted in a thermal cycler (Applied Biosystems). Sequencing of the pooled PCR products revealed a SNP, *A224G*. *NEBcutter* tool was used for designing a novel PCR-RFLP for the SNP. PCR-RFLP protocol was formulated using restriction enzymes *CaC8I* (NEB) for simple and quick genotyping the samples. The PCR-amplified DNA fragments of the *IGF1* gene were digested at 37°C for two hours with five units of restriction enzyme *CaC8I* (NEB). The digested products were separated on 8% poly acrylamide gel electrophoresis (Bio-rad) in $1 \times$ TRIS-borate-EDTA (TBE) buffer. The gels were stained with ethidium bromide and visualized and scanned in gel documentation system (Bio-Rad, USA). The PCR products from each genotype were sequenced to detect nucleotide variations and aligned with other sequences in GenBank employing BLASTn from NCBI.

2.4. Statistical analysis

The association between *IGF1* genotypes with different growth traits was analysed using the following fixed effects general linear model (SPSS V.21) and least squares means compared for growth traits among different genotypes.

$$y_{ijk} = \mu + c_i + g_j + e_{ijk}$$

where y_{ijk} is the growth traits measured on ijk th animal (body length, chest circumference, body height, trunk circumference, cannon circumference, body length index, chest circumference index, cannon circumference index and trunk index), μ is the mean for the entire population, c_i is the fixed effect associated with i th centre, g_j is the fixed effect associated with j th genotype and e_{ijk} is the random error.

3. Results

3.1. Identification of polymorphism in the caprine *IGF1* gene

In goats, the *IGF1* gene is located on chromosome 5. A 294 bp fragment of the *IGF1* gene was amplified. Sequencing the PCR products from pooled DNA sample revealed one A \rightarrow G transition at 224th position. PCR-RFLP protocol was formulated using restriction enzyme *CaC8I* (NEB) for *A224G* for simple and quick genotyping of the samples (Fig. 1). Animals with GG homozygotes displayed three bands of 196 bp, 72 bp and 26 bp (26 bp is not visible) while the AG heterozygotes showed four bands *viz.* 268 bp, 196 bp, 72 bp and 26 bp (26 bp is not

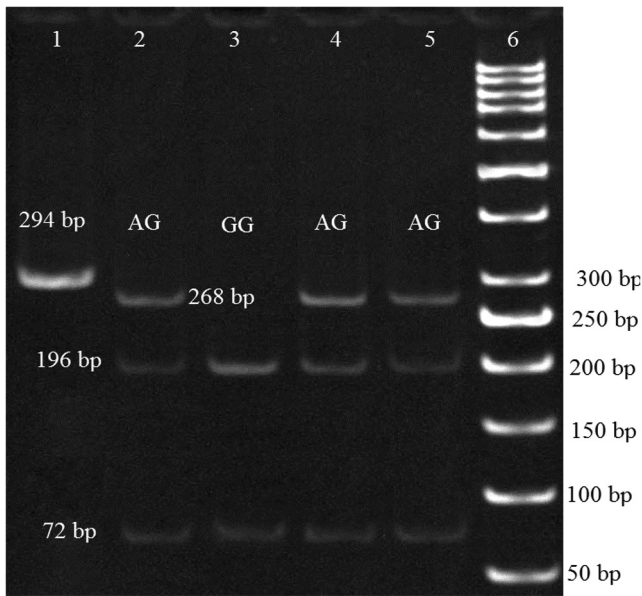


Figure 1 Pattern of *Cac8I* digestion of *A224G* of 294 bp fragment of *IGF1* with AG and GG genotypes. Lane 1: 294 bp PCR product, Lanes 2, 4 & 5: AG genotype, Lane 3: GG genotype, Lane 6: 50 bp marker.

visible). AA genotype (with two band patterns with 268 and 26 bp) was absent in the population. Sequence maps of different genotypes are presented in Fig. 2. The genotype and allele frequencies for *A224G* locus of Malabari and Attappady Black goats are presented in Table 1.

3.2. Association of *IGF1* genotypes with growth traits in goats

Growth traits in goats were significantly affected by *IGF1* genotypes. The least square means with standard error for growth traits for different *IGF1* genotypes are given in Table 2. Almost all the traits considered, viz, body weight, body length, height, trunk and chest circumference, body length index, chest circumference index and trunk index showed higher phenotypic values for GG genotypes. Goats with GG genotypes had significantly higher chest circumference, height and body weight ($P < 0.05$) than AG genotypes in Attappady Black

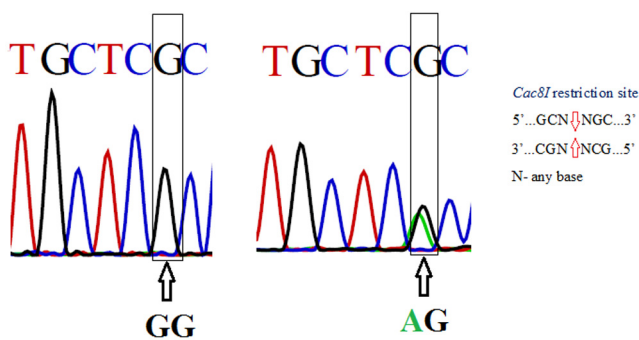


Figure 2 Sequence map of GG and AG genotypes indicating *A224G*, with *Cac8I* restriction site.

goats. The contribution of GG genotype to the total phenotypic variability in growth traits varied from 3 to 6%.

4. Discussion

The physiological regulation of growth in animals is polygenic in nature. Genetic variations in these genes, with associations with economically important traits, are considered as potent markers for marker-assisted selection. SNPs are the most frequently occurring forms of variation in the genetic makeup of individuals and can be used to analyse the associations between them and the economic traits of individuals [19]. Somatotrophic axis plays a crucial role in the animal growth because of its important role in embryonic development, post natal growth and metabolism in mammals; especially in post-natal life, *IGF1* is a key component in linear growth of animals, as a result of its longitudinal bone growth, cartilage growth and muscle growth [20].

In the current research, we detected polymorphisms in the 5' flanking region of the *IGF1* gene among two indigenous goat genetic groups of Kerala, India, by PCR-RFLP and DNA sequencing. We assessed the association between different *IGF1* genotypes and growth traits including the body weight, body length, height, trunk and chest circumference, body length index, chest circumference index and trunk index in Malabari and Attappady Black goats. The effects of genetic variants were estimated. The quantified results showed that caprine *IGF1* is an important growth regulating gene. The differences in the body weight, body height and chest circumference between genotypes GG and AG were significant ($P < 0.05$) in Attappady Black goats. Attappady Black goats, evolved from Attappady region in Palakkad district of Kerala were recognized as a meat type breed with a poor milk production potential, low prolificacy and comparatively more disease resistant than other goats of Kerala [21–23]. The specific relationship of SNP, *A224G* with growth traits suggested that genotype GG and was superior to AG in several growth traits. These results will provide a basis for further studies of the effect of *IGF1* genes on growth traits, and contribute to the development of a better genetic resource of indigenous goats. Attappady Black goats are mainly reared by the tribes of the Attappady region; hence employing the marker assisted selection in the genetic improvement, especially for growth traits in these low prolific meat type goat genetic groups will add to the economics and nutritional security of the tribal community of the specific region. As a member of somatotrophic axis, the role of *IGF1* gene in prenatal and postnatal growth in several livestock species such as sheep, [24,25], caprine [26–28], bovine [29,30], swine [31–33], chicken [34,19,35], Atlantic Salmon [36] and common carp [37] were previously reported. The influence of 5' non-coding region polymorphisms of *IGF1* gene was studied extensively and found to have significant influence on growth and weight gain [38]. Mirzaei et al. [39] reported that the C/T transition in the promoter region of *IGF1* gene can influence the serum concentration of *IGF1* in Holstein cows, suggesting the role of promoter polymorphism in *IGF1* expression. Recent researches report that the mechanism in which the gradational transactivation by the eSTR (expression short tandem repeats) is caused by the interaction of one or more transcriptional complexes located outside the STR of *IGF1* gene, rather than by direct binding to a repeat motif of the STR

Table 1 Genotype and allele frequencies of *Cac8I* locus of *IGF1* gene in native goat genetic groups (Malabari and Attappady Black) based PCR-RFLP.

Breed	Genotype frequency		Allele frequency	
	GG	AG	G	A
Malabari (<i>n</i> = 175)	0.51 (89)	0.49 (86)	0.75 (264)	0.25 (86)
Attappady (<i>n</i> = 102)	0.82 (84)	0.18 (18)	0.91 (186)	0.09 (18)
Total (<i>n</i> = 277)	0.62 (173)	0.38 (104)	0.81 (450)	0.19 (104)

Table 2 Association of the *IGF1* genotypes on growth traits of Malabari and Attappady Black goats given as least square means with standard error (LSM ± SE).

Growth trait	Genotypes (means ± standard error of means)			
	Malabari (<i>n</i> = 175)		Attappady Black (<i>n</i> = 102)	
	GG (<i>n</i> = 89)	AG (<i>n</i> = 86)	GG (<i>n</i> = 84)	AG (<i>n</i> = 18)
Body weight (kg)	32.24 ± 0.53	31.04 ± 0.62	31.86 ± 0.49 ^a	29.56 ± 1.01 ^b
Body length (cm)	62.18 ± 0.82	62.65 ± 0.96	66.52 ± 0.56	65.58 ± 1.17
Body height (cm)	55.56 ± 0.47	54.52 ± 0.55	65.82 ± 0.38 ^a	63.53 ± 0.79 ^b
Chest circumference (cm)	75.10 ± 0.65	75.09 ± 0.75	70.53 ± 0.55 ^a	67.19 ± 1.14 ^b
Trunk circumference (cm)	85.19 ± 0.79	84.81 ± 0.92	75.73 ± 0.72	74.45 ± 1.40
Body length index (%)	112.49 ± 1.78	111.96 ± 2.08	101.13 ± 0.86	102.9 ± 1.17
Chest circumference index (%)	136.75 ± 0.84	136.43 ± 2.98	107.26 ± 0.70	106.83 ± 0.92
Trunk index (%)	119.94 ± 1.93	120.29 ± 2.26	106.4 ± 0.90	104.39 ± 1.01

Means with different superscripts in the same row differ significantly ($P < 0.05$).

[40], validates the role of promoter SNPs in regulation of gene expression.

In conclusion, *IGF1* is one of the important members of somatotrophic axis. Because of its significant role in the postnatal growth, it is considered as an important candidate gene for growth traits in livestock species. The polymorphism we have identified in the *IGF1* gene (*A224G*) could be potential genetic markers for growth traits in goats. The trait association with genetic markers was different among different goat genetic groups. Therefore, further evaluations are needed to confirm the associated effects of the genetic markers on other populations.

References

- [1] M.F. Allan, R.M. Thallman, R.A. Cushman, S.E. Echtenkamp, S.N. White, A. Kueh, E. Casas, T.P.L. Smith, *J. Anim. Sci.* 85 (2007) 341–347.
- [2] P. Sellier, *Domest. Anim. Endocrinol.* 19 (2000) 105–119.
- [3] J.P. Liu, J. Baker, A.S. Perkins, E.J. Robertson, A. Efstratiadis, *Cell* 75 (1993) 59–72.
- [4] D.Z. Ewton, S.L. Roof, K.A. Magri, F.J. McWade, J.R. Florini, *J. Cell. Physiol.* 161 (1994) 277–284.
- [5] J.R. Florini, D.Z. Ewton, S.A. Coolican, *Endocr. Rev.* 17 (1996) 481–517.
- [6] G. Furstenberger, H.J. Senn, *Lancet Oncol.* 3 (2002) 298–302.
- [7] L. Laviola, A. Natalicchio, F. Giorgino, *Curr. Pharm. Des.* 13 (2007) 663–669.
- [8] E.R. Barton, S. Park, J.K. James, C.A. Makarewich, A. Philippou, D. Eletto, H. Lei, B. Brisson, O. Ostrovsky, Z. Li, Y. Argon, *FASEB J.* 26 (2012) 3691–3702.
- [9] N. Thomas, R.T. Venkatachalapathy, T.V. Aravindakshan, K. C. Raghavan, *Anim. Reprod. Sci.* 167 (2016) 8–15.
- [10] X.F. Reyna, H.M. Montoya, V.V. Castrellon, A.M.S. Rincon, M.P. Bracamonte, W.A. Vera, *Genet. Mol. Res.* 9 (2010) 875–883.
- [11] N. Arends, L. Johnston, A. Hokken-Koelega, C. van Duijn, M. de Ridder, M. Savage, A. Clark, *J. Clin. Endocrinol. Metab.* 87 (2002) 2720–2724.
- [12] B.W. Kirkpatrick, *Anim. Genet.* 23 (1992) 543–548.
- [13] W. Ge, M.E. Davis, H.C. Hines, *Anim. Genet.* 28 (1997) 155–156.
- [14] W. Ge, M.E. Davis, H.C. Hines, K.M. Irvin, R.C.M. Simmen, *J. Anim. Sci.* 79 (2001) 1757–1762.
- [15] R.A. Curi, H.N. De Oliveira, A.C. Silveira, C.R. Lopes, *Livest. Prod. Sci.* 94 (2005) 159–167.
- [16] C. Zhang, W. Zhang, H. Luo, W. Yue, M. Gao, Z. Jia, *Asian Aust. J. Anim. Sci.* 21 (2008) 1073–1079.
- [17] M. Yu, H. Wang, Y. Xu, D. Yu, D. Li, X. Liu, W. Du, *Cell Biol. Int.* 39 (2015) 910–922.
- [18] Q.J. Jin, X.T. Fang, C.L. Zhang, L. Yang, J.J. Sun, D.X. Chen, X.Y. Shi, Y. Du, X.Y. Lan, H. Chen, *Small Rumin. Res.* 90 (2010) 150–152.
- [19] W.J. Wang, K. Ouyang, J. Ouyang, H.H. Li, S.M. Lin, H. Sun, *Asian Austral. J. Anim.* 17 (2004) 301–304.
- [20] S. Yakar, C.J. Rosen, W.G. Beamer, C.L. Ackert-Bicknell, Y. Wu, J.L. Liu, et al., *J. Clin. Invest.* 110 (2002) 771–781.
- [21] S. Mathew, T.V. Raja, I. Sosamma, *Anim. Genet. Res. Inf.* 37 (2005) 43–52.
- [22] N. Thomas, S. Joseph, R. Alex, K.C. Raghavan, G. Radhika, L. Anto, S.G. Mohan, *Biotechnol. Anim. Husbandry* 27 (2011) 235–240.
- [23] G. Radhika, S. Ajithkumar, K.C. Raghavan, A Farm study on weakness in goat kids, in: *Proceedings of Kerala Veterinary Science Congress, Thiruvananthapuram, Kerala, 2012.*

- [24] F. Lok, J.A. Owens, L. Mundy, J.S. Robinson, P.C. Owens, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 270 (1996) 1148–1155.
- [25] E.C. Jensen, J.E. Harding, M.K. Bauer, P.D. Gluckman, *J. Endocrinol.* 161 (1999) 485–494.
- [26] C. Deng, R. Ma, X. Yue, X. Lan, H. Chen, C. Lei, *C. Genet, Mol. Biol.* 33 (2010) 266–270.
- [27] W. Qiong, C. Fang, W.J. Liu, Y. Fang, Y.S. Gang, *Asian J. Anim. Vet. Adv.* 6 (2011) 627–635.
- [28] A. Sharma, G. Dutt, S. Jayakumar, V. Saroha, M.K. Singh, O. P. Pathodiya, et al, *Small Rumin. Res.* 115 (2013) 7–14.
- [29] E.R. Chung, W.T. Kim, *Asian Australas. J. Anim.* 18 (2005) 1061–1065.
- [30] A.K. Bennett, P.Y. Hester, D.E. Spurlock, *Anim. Genet.* 37 (2006) 283–286.
- [31] E. Cass, A. Prill, S.G. Price, A.C. Clutter, B.W. Kirkpatrick, *Anim. Genet.* 28 (1997) 88–93.
- [32] J. Estany, M. Tor, D. Villalba, L. Bosch, D. Gallardo, N. Jimenez, et al, *Physiol. Genomics* 31 (2007) 236–243.
- [33] P. Niu, S.W. Kim, B.H. Choi, T.H. Kim, J.J. Kim, K.S. Kim, *Genes Genom.* 35 (2013) 523–528.
- [34] M. Amills, N. Jimenez, D. Villalba, M. Tor, E. Molina, D. Cubilo, et al, *Poultry Sci.* 82 (2003) 1485–1493.
- [35] C. Boschiero, E.C. Jorge, K. Ninov, K. Nones, M.F. do Rosário, L.L. Coutinho, M.C. Ledur, D.W. Burt, A.S.A.M.T. Moura, *J. Appl. Genet.* 54 (2013) 103.
- [36] H.Y. Tsai, A. Hamilton, D.R. Guy, R.D. Houston, *Anim. Genet.* 45 (2014) 709–715.
- [37] X. Feng, X. Yu, J. Tong, *Int. J. Mol. Sci.* 15 (2014) 22471–22482.
- [38] A. Rogberg-Muñoz, R.J.C. Cantet, M.E. Fernández, J.P. Lirón, A. Prando, A.N. Birchmeier, M.V. Ripoli, G. Giovambattista, *Livestock Sci.* 154 (2013) 55–59.
- [39] A. Mirzaei, H. Sharifiyazdi, M. Ahmadi, T. Ararooti, A.R. Ghasrodashti, A. Kadivar, *Asian Pac. J. Trop. Biomed.* 2 (2012) 765–769.
- [40] H.Y. Chen, S.L. Ma, W. Huang, L. Ji, V.H.K. Leung, H. Jiang, X. Yao, N.L.S. Tang, *Sci. Rep.* 6 (2016) 38225.