Original Research

Association of *IGF*1 Gene Polymorphism with Growth Rates in Madras Red Sheep

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Abstract

Insulin-like growth factors (IGFs), formerly called somatomedin, are members of a family of insulin related peptides. Insulin-like growth factors 1 (IGF-1) plays a key role in mammalian growth, lactation and metabolism, by stimulating anabolic processes such as cell proliferation, skeleton and hair growth and protein synthesis. The aim of this study was to investigate the association of IGF1 (exon1) gene polymorphism with growth rates in Madras Red sheep. Blood samples were collected from 66 animals maintained at Post Graduate Research Institute in Animal Sciences, Kattupakkam and 70 animals from farmers' flock in the breeding tract of Madras Red sheep and DNA was isolated using high salt method. The animals were genotyped by Polymerase Chain Reaction – Single Strand Conformation Polymorphism (PCR-SSCP). In the PCR reaction the primers used were 5' ATTACAGCTGCCTGCCCCTT 3' and 5' CACATCTGCTTACACCTTACCCG 3'. A 265 bp long IGF1 (exon1) gene PCR product was genotyped for polymorphic pattern using SSCP method. The PCR-SSCP analysis of exon1 IGF-I revealed three distinct patterns viz., AA, AG and GG and the genotype frequencies were in the order of 0.963, 0.022 and 0.015. In the present study, no significant effect (P>0.05) was observed between genotypes on all age groups in Madras Red sheep.

Key words: Exon 1, Genetic Polymorphism, *IGF*1 gene, Madras Red Sheep, PCR-SSCP

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Introduction

Madras red sheep named after the name of breeding tract, Chennai (Madras) and the skin colour is red and hence the breed name. Madras Red sheep are generally maintained on grazing alone, their meat yield is good as compared to that of other superior meat breeds of sheep of the country. Madras red sheep population is 1.15 million numbers based on 18th Livestock and Poultry Census and has a share of 25.73

per cent of total sheep population in Tamil Nadu. They represent a unique genetic resource by virtue of their adaptability, resistance to many infectious diseases in the humid tropics of Tamil Nadu. They also exhibit considerable variation in individual performance in meat production and growth rate. Insulin-like growth factors one and two (somatomedins - *IGF*-1 and *IGF*-2) are structurally related proteins, playing a key role in cell differentiation, embryogenesis, growth and regulation of metabolism (Siadkowska *et al*., 2006). IGF-1 is a hormone that functions as the major mediator of growth hormone (GH) stimulated somatic growth, as well as a mediator of GH-independent anabolic responses in many cells and tissues. IGF-1 plays a key role in mammalian growth, lactation and metabolism (Zhang *et al*., 2008) by stimulating anabolic processes such as cell proliferation, skeleton and hair growth and protein synthesis. Due to their role in regulation of cell proliferation and animal growth, the *IGF-I* and its gene are considered as candidate markers for growth rate and meat production traits in mammalian species (Zhang *et al*., 2008).

Effect of *IGF*-1gene variants on growth traits were reported in Makoei *sheep* by Hajihosseinlo *et al.,* 2013; Moradian *et al.,* 2013; Chelongar *et al.,* 2014; Negahdary *et al.,* 2013 and Negahdary *et al*., 2014 and in the indigenous Iranian Baluchi breed by Tahmoorespur *et al.,* 2009 and Gholibeikifard *et al*., 2013. Realising the importance of sheep in Indian economy particularly in rural economy, the present study of association of *IGF*1 *(*exon1) gene polymorphism with growth rates in Madras red sheep.

Materials and Methods

A random sample from 136 genetically unrelated Madras Red sheep formed the material for this study. The blood samples of Madras Red sheep breed were collected from 66 animals maintained at Post Graduate Research Institute in Animal Sciences, Kattupakkam and 70 animals from farmers' flock in the Kancheepuram district, the breeding tract. Blood samples (5 ml each) were collected from jugular vein aseptically in the vaccutainer containing EDTA as anticoagulant using sterile disposable needle. The samples were brought in ice to the laboratory and stored at -40 ºC till processed. Genomic DNA from whole blood was extracted by using the standard high salt method as described by Miller *et al.* (1988). The isolated DNA was checked for quality, purity and concentration by Nanodrop and agarose gel electrophoresis and only the DNA samples of good quality and concentration were used for further analysis.

Primers and PCR Amplification

Template DNA for PCR was prepared by diluting the DNA stock solution in 1 X TE buffer (pH 8.0) to a concentration of 100 ng / µl and was stored at –20 ºC. The primers for the present study were selected

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from published reports (Chung and Davis, 2012) and were custom synthesised (Merck Millipore Pvt. Ltd., Bangaluru). The details of primers are as follows-

Sequence (5' to 3')	Tm Values	Expected PCR Product Size (bp)
F: ATTACAGCTGCCTGCCCCTT	59.4	265
R: CACATCTGCTTACACCTTACCCG	62.4	

Tm - Melting temperature

The PCR cycling program for amplification is as follows-

Steps					
Initial Denaturation (1)	**Denaturation (2)**	**Annealing (3)**	**Extension (4)**	**No. of cycles from step 2-4 (5)**	**Final Extension (6)**
95 °C/3min	95 °C/45sec	62 °C/4 sec	72 °C/50 sec	35	72 °C/10min

Single Strand Confirmation Polymorphism (SSCP)

The PCR products were checked by agarose gel electrophoresis to confirm the amplification before analysing for polymorphism. To 5 µl of PCR Amplicon 10 µl of SSCP gel loading dye (0.05% bromophenol blue, 0.05% xylene cyanol, 95% formamide, 20 mM EDTA), were mixed and denatured at 95°C for 10 min, followed by a rapid chilled on ice. The final denatured PCR products were loaded on 12% polyacryamide gels to observe the polymorphic patterns in the *IGF*-1gene. The electrophoresis was performed in 0.5 × TBE buffer at 12 °C for 17 hours at 8 V/cm. The gel was staining with silver staining to visualize the ssDNA bands.

Statistical Analysis

Information about each experimental animal and data on body weights at different ages were collected and recorded in the data-recording sheet. Birth weight, weight at one month, two months, three months, six months, nine months and twelve months of age or up to sale or slaughter were collected. Weight gains for all age groups were calculated from birth weight (Morris, 1999; Behzadi *et al*., 2014).

Result and Discussion

All the DNAs samples yielded 265 bp long fragments as single band PCR product without any nonspecific band (Fig. 1). Hence the amplified products were consistent with the target fragments and could be directly analyzed by SSCP. In the present study, analysis of exon1 *IGF*-I revealed three distinct patterns (Fig. 2) *viz. AA, AG* and *GG* and their frequencies were 0.963, 0.022 and 0.015 respectively in Madras Red sheep.

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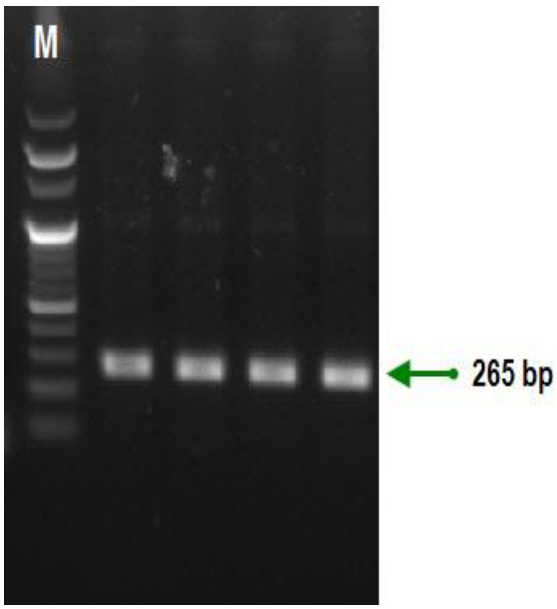

Fig. 1: *IGF* -1 (exon1) PCR products

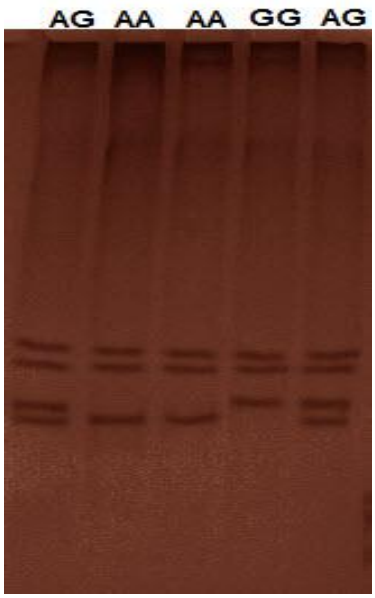

Fig. 2: SSCP pattern of *IGF*-1 gene

Similar genotypes were observed previously by PCR-SSCP by Yilmaz *et al.* (2005) in purebred sheep Polypays and crossbreds consisting of the Hampshire, Targhee, Rambioullet, Dorset and Suffolk breeds; Tahmoorespur *et al.* (2009) in the indigenous Iranian Baluchi breed; Honarvar *et al.* (2012) in native Iranian tailed Zel sheep; Bahrami *et al.* (2013); Gholibeikifard *et al*. (2013) in Baluchi sheep; Moradian *et al.* (2013) in Makoei Sheep; Negahdary *et al.* (2013) in Makoei sheep; Chelongar *et al.* (2014) in Makoei sheep and (Negahdary *et al.,* 2014) in Makoei sheep breeds which are fat-tailed sheep with medium body size. However, comparative studies of *IGF*1 / SSCP polymorphism in Indian sheep breed could not be traced in the literature. Allele frequencies were 0.974 and 0.026 for *A, G* allele respectively. The expected heterozygosity and polymorphic information content value were 0.0719 and 0.0706 respectively. Lower PIC value in Madras Red sheep population studied suggests that this group is in homozygous state.

In the present study, no significant effect (P>0.05) was observed between genotypes on all age groups and weight gain and birth weight, when the breed, management and sex effects were excluded (Table 1). Similar to the present findings, Gholibeikifard *et al*. (2013) reported no significant association between the polymorphism of *IGF*-I and body weights. The present findings are contrary to the reports of several authors for different sheep breeds, including indigenous Iranian Baluchi (Tahmoorespur *et al.*, 2009) and Makoei (Hajihosseinlo *et al*., 2013, Negahdary *et al*., 2013, Chelongar *et al*., 2014, Negahdary *et al*., 2014).

The *IGF*-I gene polymorphism as three banding patterns (genotypes) named as *AA*, *AB* and *BB* was reported by Tahmoorespur *et al.* (2009) in the indigenous Iranian Baluchi breed. The evaluation of an association effect between these SSCP patterns with birth weight (BW), weaning weight (WW) and

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average daily gain from birth to weaning (GBW), weaning to six month (GWS) and from six month to yearling age (GSY) suggested a positive effect of pattern (genotype) *AB* with GBW and weaning weight (WW). Pattern (genotype) *BB* had a superior birth weight when compared to those of individuals with other patterns. Moreover, the *AA* pattern (genotype) appeared favourable for live weight at yearling age. Individuals with the *GG* genotype of *IGF*-I gene had lower WW and SW when compared to those of individuals with other genotypes (P <0.05). In addition the results demonstrated superiority of the heterozygous *AB* genotype for weaning weight, and of the *AA* genotype for birth weight. Hajihosseinlo *et al.* (2013) reported an association between these SSCP patterns with birth weight (BW), weaning weight (WW), six month weight (SW), nine month weight (9W), average daily gain from birth to weaning (GBW), weaning to six months (GWS), from six months to nine months (GSN), from nine months to yearling weight (GNY) and development traits in one year demonstrated a positive effect of the pattern (Genotype) *AG* with GBW and weaning weight (WW) and six month weight (SW). The pattern (Genotype) *AA* demonstrated superior birth weight when compared to those with other gene patterns. GBW, GNY and biometric or development traits were influenced significantly by sex (p<0.01) except RL: rump length trait. Also type of birth effect influenced early weight changes but had no significant effect on GSN, GNY traits (p>0.05) in Makoei sheep.

Negahdary *et al*. (2013) observed that the genotype *AG* had the highest additive estimated breeding value for the trait pre-six month weight (SW). The effect of the *IGF*-I gene was significant ($P < 0.01$) for average daily gain from birth to weaning (GBW), birth weight (BW), weaning weight (WW), six-month weight (SW), and average daily gain from six months to nine months (GSN). In the tested Makoei sheep population, mean body weight of the genotype *AG* at 6MW was about 31.12 kg, and this was 7.13 kg higher than those of *AA* (28.43 kg) and *GG* (23.99 kg) SSCP patterns, respectively. Also mean wool weights of genotype *AG* at one year was about 0.517 kg and 0.172kg higher than that of *AA* (0.486) and GG (0.345) SSCP patterns, respectively. Higher performance of *AA* animals in BW and GBW, also *AG* animal in *WW* and *W*6 (pre-puberty ages) may be related to the *IGF*-1 role in pre-puberty ages. Chelongar *et al.* (2014) reported that no significant effect (P>0.05) was found between *IGF*1 genotypes with tail length (Rump length) and tail width (Rump width). In the studied population although, fat thickness (The thick rump) was influenced significantly by *IGF*1 genotypes. Also, the analysis of fat-tail measurement traits showed that *AA* and *AG* conformational patterns of *IGF*1 gene have a significant effect on fat thickness (the thick rump) and this patterns of *IGF*1 gene (*AA*, *AG*) had the highest fat thickness (the thick rump) (P<0.05). *IGF*1 gene with *AG* genotype had a dominance wool weight in one year when contrasted to those of individuals with difference genotypes (Negahdary *et al*., 2014).

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Conclusion

The *IGF*1 gene is considered to be a factor that regulates growth, differentiation and the maintenance of differentiated function in numerous tissues and in specific cell types of mammals through binding to a family of specific membrane associated glycoprotein receptors (Werner *et al*., 1994). The PCR-SSCP analysis of exon1 *IGF*-I revealed three distinct patterns viz., *AA*, *AG* and *GG* and the genotype frequencies were in the order of 0.963, 0.022 and 0.015 in Madras Red sheep with very low *G* allele frequency of 0.026. Lower PIC value (0.0706) in the sheep population studied suggests that this group is in homozygous state. In the present study in Madras red sheep, no significant effect (P>0.05) was observed between genotypes on growth rates on all age groups.

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