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Polymorphism of bovine STAT5A gene and its association with milk production traits in crossbred cattle of Kerala, India

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ABSTRACT

Signal transducer and activator of transcription 5A (STAT5A) is a transcription factor that mediates signals from prolactin and growth hormone, the corresponding main regulators of lactation and growth of mammals. STAT5A is suggested to be candidate marker gene for quantitative traits in dairy cattle with respect to milk production traits. The objectives of present study were to investigate polymorphism of bovine STAT5A gene and its probable association with milk yield and milk-constituent traits in crossbred cattle. Crossbred cattle (250) in different farms of Kerala Veterinary and Animal Sciences University and different field centres of ICAR-Field progeny testing scheme, Thrissur were screened for polymorphism in exon-8 region of STAT5A gene using PCR-single-strand conformation polymorphism analysis. A synonymous polymorphism (C/T) was found at position 1071 in exon 8. All three possible genotypes were identified with estimated frequencies of 0.584 (CC), 0.332 (CT) and 0.084 (TT); the estimated allele frequencies were 0.75 (C) and 0.25 (T). The C/T genotypes demonstrated significant impact on milk fat per cent being significantly higher in CC genotype cattle in comparison to other genotypes. It is evident by this investigation that STAT5A polymorphism could be a promising indirect marker to improve milk production in crossbred cattle of Kerala.

Keywords: Crossbred cattle, Production traits, Single-nucleotide polymorphism, STAT5A gene

The signal transducer and activator of transcription (STAT) family are a group of transcription factors that mediate actions of a variety of peptide hormones and cytokines within target cells (Schindler *et al.* 1995). They act as signal transducers in the cytoplasm and transcription activators in the nucleus and are thought to play a central role in signal transmitting from prolactin to milk protein genes (Bole-Feysot *et al.* 1998). In mammals, the STAT family comprises of 7 structurally and functionally related proteins including STAT1, 2, 3, 4, 5A, 5B and 6. STAT5 also known as mammary gland factor (MGF), contains two closely related subtypes, STAT5A gene and STAT5B, which can form heterodimers after phosphorylation while they are encoded by two separate genes (Darnell 1997). In cattle, STAT5A gene has been mapped to chromosome 19 within 40 kb STAT locus which consists of 19 exons encoding 794 amino acid chains [Goldammer *et al*.1997, Seyfert *et al*. 2000). STAT5A gene are promising candidate markers for quantitative traits in farm animals because of its mandatory role for mammary gland development, lactogenesis and mediatory function in prolactin and GH actions (Teglund *et al*.1998). Previous studies in different cattle populations have reported several nucleotide sequence polymorphisms of bovine STAT5A gene (Khatib *et al.* 2008, Schennink *et al*. 2009, He *et al*. 2012, Selvaggi *et al.* 2013, Michel-Regalado *et al*. 2020).

Despite the importance of the STAT5A gene in milk production, association studies between STAT5A gene polymorphisms and production traits in cattle are limited. Moreover, to improve genetic gain of a herd, molecular marker assisted selection would be used as it envisage selection of animals at early age and enhances reliability in predicting the favourable phenotypes of the individual (Selvaggi *et al.* 2009). Crossbred cattle of Kerala state of India is developed by crossing local non-descript indigenous breeds with exotic breeds mainly Holstein Friesian (exotic inheritance limited to 50–62.5%). Kerala ranks 14th among the milk producing states with a share of just 1.5% of the total milk production in the country (Ashmi 2019). This calls for reorientation in the approach for future development in the selection methods of crossbred cattle of Kerala. Hence, present study was carried out with the objective to identify genetic polymorphism in STAT5A gene and to explore association of this region with production traits of crossbred cattle of Kerala state, India.

MATERIALS AND METHODS

Experimental animals and blood sample collection: The study was conducted on a random sample of 250 crossbred

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cows maintained at University Livestock Farm, Mannuthy; Livestock Research Station, Thiruvzahamkunnu; Cattle Breeding Farm, Thumburmuzhy and different field centres of ICAR-Field progeny testing scheme, Thrissur, Kerala. Thrissur district of Kerala is located between the Arabian Sea to the west and the Western Ghats to the east, lies between north latitudes 10.5276º N and 76.2144º E. Crossbred cattle included in this study had exotic inheritance of 50–62.5% mainly from Holstein Friesian crosses. Blood samples were obtained by jugular venipuncture using EDTA (1 mg/ml of blood) as an anticoagulant and stored at −40°C until DNA extraction.

DNA isolation and PCR amplification: Genomic DNA was isolated by the phenol–chloroform extraction method with modifications (Sambrook and Russell 2001). The concentration and purity of DNA were checked by NanoDrop spectrophotometer. Those samples with an optical density (OD) ratios 260/280 ratio between 1.7 and 1.8 were used and further assessed by 1% agarose gel electrophoresis and visualized with gel documentation system. Genomic DNA of crossbred cattle was successfully amplified using one pair of primer for exon 8 region of STAT5A gene. Polymerase chain reaction (PCR) was carried out in a final reaction volume of 20 µl. PCR mixture consisting of 50 ng of bovine genomic DNA, 1× PCR buffer, 1.8 mM $MgCl_2$, 0.2 mM each dNTP, 10 pM of each primer and 1 U of Taq DNA polymerase were used for each reaction. The primers used for amplification of exon 8 of STAT5A gene, the size of the amplicon and the annealing temperature are shown in Table 1. The PCR programme was made with the following conditions—initial denaturation of 3 min at 95°C, and 35 cycles of denaturing at 94°C for 30 sec, annealing for 45 sec at 61°C for exon 8, extension at 72°C for 45 sec with a final extension at 72°C for 5 min. The PCR products were separated on 1.5% agarose gel.

The PCR-single-strand conformation polymorphism (SSCP) method was used to scan for mutations within the amplified regions. Aliquots (10 µl) of the PCR products were mixed with equal amount of denaturing solution (95% formamide, 25 mm EDTA, 0.025% xylene cyanol and 0.025% bromophenol blue) heated at 95˚C for 10 min and immediately chilled on ice. The denatured DNA was subjected to 12% polyacrylamide gel (acrylamide: bisacrylamide = 29:1) electrophoresis (PAGE) in 1×TBE buffer with a constant voltage of 120 V for 3 h at a constant temperature. The gel was stained with 0.1% silver nitrate, photographed and analysed to identify any possible single-nucleotide polymorphism at the locus. Individual genotypes were defined according to band patterns. Representative samples of each PCR-SSCP pattern were sequenced by automated sequencer using Sanger's dideoxy chain termination method at Sci Genome Labs Pvt. Ltd., Cochin, India, and aligned with other sequences in GenBank employing BLASTn to identify the SNPs responsible for mobility shift, which resulted in different banding patterns.

Data collection and statistical analysis: To find association of milk yield and constituent traits with genetic variants of STAT5A gene, the following traits of 250 crossbred cattle were considered: (a) 305 days milk yield (MY), (b) monthly test day fat per cent, (c) monthly test day SNF per cent, (d) monthly test day lactose per cent, and (e) monthly test day protein per cent. MY data were collected from milk record registers of University Livestock Farms, Livestock Research Station, Thiruvzahamkunnu and different field centres of ICAR-Field Progeny Testing Scheme, Mannuthy, Thrissur, Kerala. All milk samples were analyzed using milk analyzer with frequent standardization using Gerber's method for fat, Kjeldahl method for protein and Lane-Eynon method (lactose) for lactose, respectively.

Popgene 32 Version 1.32 was used to estimate the allele and genotype frequencies, observed heterozygosity, expected heterozygosity, polymorphism information content, effective number of alleles and Hardy Weinberg equilibrium.

Association analysis of genotypes with milk production traits were analyzed using fixed General Linear Model (GLM) of SPSS V.21 (SPSS Inc., Chicago, IL, USA) as follow:

$$Y_{ijklm} = \mu + S_i + P_j + C_k + G_l + R_m + e_{ijklm}$$

where, Y_{ijkl}, Observation on each milk production trait of ijklmth animal; µ, Overall mean; S_i, Effect of season of calving: four seasons were considered, viz. winter (January–February), summer (March–May), rainy (June–September), and autumn (October–December); P_j, Effect of year of calving from 2006 to 2019 with (1–11); C_k, Effect of centre (1–4); G_l, Effect of genotype (1,2,3); R_m, Effect of sire (1–95); e_{ijklm}, Random residual error associated with each observation assumed normally and independently distributed with mean zero and unit variance.

RESULTS AND DISCUSSION

PCR products amplified are shown in Fig. 1. The results showed that amplicon sizes were consistent with the target ones and had a good specificity, which could be directly analysed by SSCP. Analysis by SSCP indicated that the PCR products displayed three different banding patterns which indicate polymorphisms. Three genotypes (CC, CT, and TT) were detected (Fig. 2). On sequencing PCR products from each pattern, one SNP (T → C) transition was revealed at 85th position of 245 bp fragment as shown in Fig. 3. The observed SNP was located in nucleotide sequences at positions 1071th in exon 8 and at 1456th position of ORF of STAT5A gene. Further analysis revealed c.1456T>C was a synonymous mutation and it resulted in a codon change of GGT to GGC. Hence, STAT5A gene was found to be polymorphic in crossbred cattle of Kerala. The polymorphism studies of STAT5A gene in bovines were reported intensively by several other researchers. However, very few research reports were available on polymorphism studies in the exon 8 region of STAT5A gene which was explored in the current study. Bao *et al*. (2010) identified a silent SNP, C/T transition in exon 16 of STAT5A gene in Chinese Holstein cattle. Dario and Selvaggi (2011) and

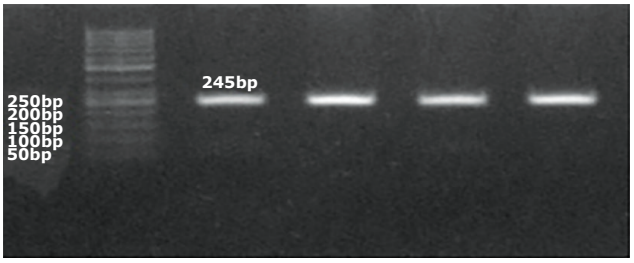

Fig. 1. PCR amplification of 245 bp fragment of STAT5A gene (exon 8).

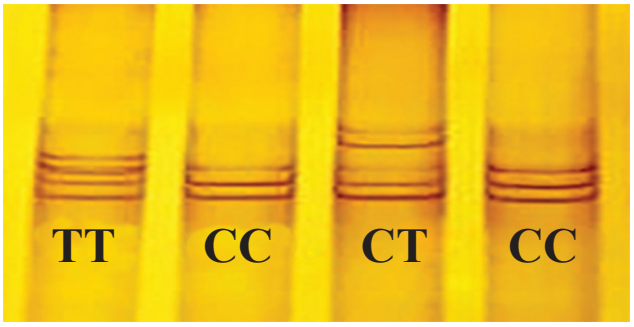

Fig. 2. SSCP pattern of 245 bp fragment of exon 8 of STAT5A gene (CC, CT and TT genotype).

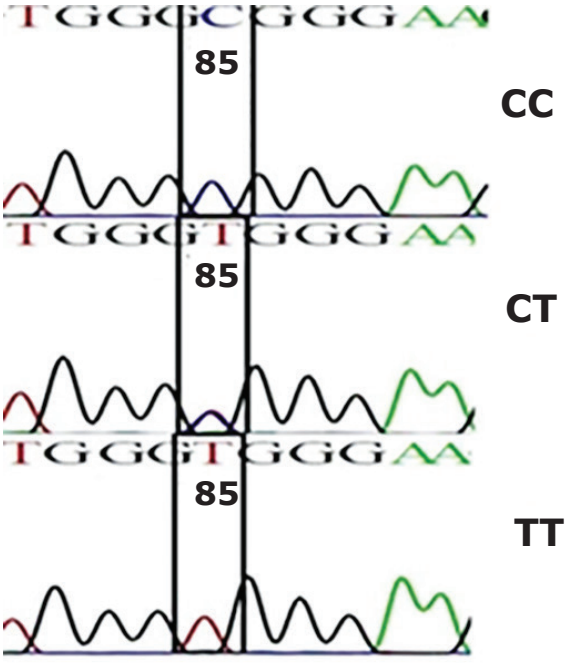

Fig. 3. Sequence map of exon 8 of STAT5A gene (CC, CT and TT genotype).

Selvaggi *et al*. (2009) detected C /T transition at position 6853 within the exon 7 of STAT5A gene in Jersey cattle and in Italian Brown cattle respectively. Khatib *et al*. (2008) recognized 1 exonic and 11 intronic single nucleotide polymorphisms (SNP) in the STAT5A gene of Holstein cows using the DNA-pooling sequencing approach. A point mutation in position 12743 (T/C) which plays a key role in the phosphorylation, activation and dimerisation of STAT5A gene was reported in Holstein cattle by Flisikowski and Oprządek (2003).

Genotype frequencies and allele frequencies were estimated (Table 2). Frequencies of genotypes were 0.584 (CC), 0.332 (CT) and 0.084 (TT) and those of alleles C and T were 0.75 and 0.25, respectively (Table 2). Genetic characteristics of STAT5A gene in crossbred cattle are presented in Table 3. The genotype distributions of the polymorphisms analysed by chi-square test were in Hardy–Weinberg equilibrium for the studied population of crossbred cattle. The genotype frequencies of the polymorphic site were not affected by selection, mutation or migration and other factors. In crossbred cattle of Kerala, C allele of STAT5A gene had higher frequency than T allele and correspondingly genotypic frequency of CC was higher than CT and TT. The STAT5A genotypes and allele frequencies obtained in this study were similar to those reported in Jersey cattle by Dario and Selvaggi (2011). They also identified all the possible genotypes for the C/T polymorphism and reported that overall frequencies of alleles C and T were 0.75 and 0.25, respectively. He *et al*. (2012) also detected three genotypes (CC, CT, and TT) in Holstein cows, and the frequency of genotype were 0.751 (CC), 0.234 (CT) and 0.015 (TT). The effective allele number (Ne) was 1.6 in the current study. The effective number of alleles is the number of equally frequent alleles that would take to achieve the same expected heterozygosity as in studied population. Sharma *et al*. (2013) suggested that lower values of expected number of alleles in the populations indicate there were many low frequency alleles in the population. Meanwhile, value of the polymorphism information content (PIC) was 0.305, indicating that the investigated population belonged to intermediate-degree PIC ($0.25 < PIC < 0.5$) in the studied locus. The statistical assessment of informativeness of a marker, which is often used to measure the informativeness of a genetic marker for linkage studies is referred to as PIC (Elston 2005). In conformity with the present results, He *et al*. (2012) also noticed PIC as 0.375 for the identified polymorphisms of the STAT5A gene in Chinese Holstein cows. Observed heterozygosity (0.625) was higher than expected heterozygosity (0.375) in the studied population. Genetic variability is also measured as the amount of actual or potential heterozygosity. High heterozygosity means lots of genetic variability (Sharma *et al.* 2016).

Association of the STAT5A gene polymorphisms with milk production traits: The least squares means and standard errors for the milk production traits (namely 305 days milk yield, monthly test day fat per cent, monthly test day SNF per cent, monthly test day lactose per cent, monthly test day protein per cent) of the animals under STAT5A genotypes in crossbred cattle are given in Table 4. It shows that, the differences of the least squares means for four milk production traits of the animals between CC, CT and TT genotypes were not significant ($P>0.05$). Different genotypes of STAT5A gene significantly ($P<0.05$) affects only monthly test day fat per cent. Least squares mean of

Table 1. Primer sequence, exon number, amplicon size and annealing temperatures for the primer used for amplification of Bovine STAT5A gene

Name	Sequence	Exon	Amplicon size in bp	Annealing temperature (°C)
STAT5A	5'-GCAGCACCTTCATCATCGAG-3' 5'-ACCCCAGCTACAGAGCAGAG-3'	Exon 8	245	61°C

Table 2. Allele and genotype frequencies of SNP of STAT5A gene (exon 8)

Genotype	Number of samples	Genotype frequency	Allele	Allele frequency
CC	146	0.584	C	0.75
CT	83	0.332		
TT	21	0.084	T	0.25

Table 3. Genetic characteristics of polymorphic sites in crossbred cattle

$\chi 2$	Polymorphism information content	Observed heterozygosity	Expected heterozygosity	Effective number of alleles
3.55	0.305	0.625	0.375	1.6

Table 4. Association of the STAT5A genotypes on milk production traits of crossbred cattle given as least square means with standard error (LSM±SE)

Milk production traits	Genotypes (Means±Standard Error of Means)		
	STAT5A (Exon-8)		
	CC	CT	TT
305 days milk yield	2734.679±57.41	2510.84±6 8.93	2486.31±118.13
Monthly test day fat %	3.98±0.01[a]	3.69±0.02[b]	3.79±0.03[c]
Monthly test day SNF %	7.93±0.04	7.87±0.05	7.95±0.08
Monthly test day protein %	3.01±0.02	2.99±0.02	3.03±0.05
Monthly test day lactose %	4.21 ± 0.04	4.17 ± 0.05	4.23 ± 0.10

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

fat per cent for CC was significantly higher than that for CT and TT (P<0.05). Fat per cent was significantly affected also by period of calving (P<0.05) and 305 days milk yield was significantly affected by period of calving and centre of calving (P<0.05). The genotypes of bovine STAT5A gene had significant association with milk production traits especially fat percent in crossbred cattle of Kerala. Brym *et al*. (2004) suggested that SNPs occurring within STAT5A genes may influence the chemical content of milk or at least be an effective DNA marker of the dairy cattle genome. Although polymorphism found in the present study is a synonymous mutation, Selvaggi *et al.* (2009) indicated that owing to the localization of the STAT5A gene on BTA19, it is possible to assume that the effect of the synonymous mutation on the phenotype results from linkage disequilibrium with other closely linked genes on BTA19. The results showed that genotypes were significantly associated with fat per cent in crossbred cattle where CC genotype showed increased fat per cent assuming a superiority of the C allele. This finding was in agreement with He *et al.*(2012) who reported that the mutation A9501G of bovine STAT5A gene was associated with fat per cent in Chinese Holstein cows. They also found that least squares mean of fat per cent for CC or CT was significantly higher than that for TT. In a previous study conducted on Jersey cows, Selvaggi *et al.* (2013) investigated the association among the STAT5A gene polymorphism and some milk production traits found that difference concerning the fat content of milk belonging to heterozygous and homozygous groups was found significant and in particular, milk from homozygous animals had a higher fat content in comparison with that of heterozygous ones. On the other side, present results were quite different with other literatures by Brym *et al.*(2004) in Black-and-White cows and Bao *et al.* (2010) in Chinese Holstein cows. They reported no relationship between the studied SNP and fat content of milk. This was probably related to the various external factors such as dairy cattle feeding and management level.

In conclusion, crossbred cattle genotyped in the present study revealed a synonymous mutation located within exon 8 of STAT5A gene. Frequency of CC, CT and TT genotypes were 0.584, 0.332 and 0.084 respectively. Association of each SNP genotype revealed significant associations of STAT5A genotypes with fat per cent in crossbred cattle. CC homozygous genotype showed higher fat per cent compare to CT and TT. The polymorphism identified in the present study and its association with milk production traits confirms the genetic variability of STAT5A gene and emphasized its role as a promising candidate marker for

milk production traits for higher productivity in crossbred cattle of Kerala. However, taking into account the relatively small size of the studied population, further investigations are necessary to better clarify the role of STAT5A gene variants on production traits in cattle.

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REFERENCES

Ashmi V G. 2019. Livestock sector development and implications for rural poverty alleviation in Kerala. *International Journal of Recent Scientific Research* **10**(8): 34484&87.

Bao B, Zhang C, Fang X, Zhang R, Gu C, Lei C and Chen H. 2010. Association between polymorphism in STAT5A gene and milk production traits in Chinese Holstein cattle. *Animal Science Papers and Reports* **28**(1): 5&11.

Bole-Feysot C, Goffin V, Edery M, Binart N and Kelly P A. 1998. Prolactin (PRL) and its receptor: Actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocrine Reviews* **19**(3): 225&68.

Brym P, Kamiński S and Rusc A. 2004. New SSCP polymorphism within bovine STAT5A gene and its associations with milk performance traits in Black-and-White and Jersey cattle. *Journal of Applied Genetics* **45**(4): 445&52.

Dario C and Selvaggi M. 2011. Study on the STAT5A/AvaI polymorphism in Jersey cows and association with milk production traits. *Molecular Biology Reports* **38**(8): 5387&92.

Darnell J E. 1997. STATs and gene regulation. *Science* **277**(5332): 1630&35.

Elston R C. 2005. Polymorphism information content. *Encyclopedia of Biostatistics*. (Eds) Armitage P and Colton T. John Wiley & Sons, Ltd., NY, USA.

Flisikowski K and Oprządek J. 2003. New polymorphism in the bovine STAT54 gene and its. *Animal Science Papers and Reports* **21**(3): 147&57.

Goldammer T, Meyer L, Seyfert H M, Brunner R M and Schwerin M. 1997. STAT5A encoding gene maps to chromosome 19 in cattle and goat and to chromosome 11 in sheep. *Mammalian Genome* **8**(9): 705.

He X, Chu M X, Qiao L, He J N, Wang P Q, Feng T, Di R, Cao G L, Fang L and An Y F.2012. Polymorphisms of STAT5A gene and their association with milk production traits in Holstein cows. *Molecular Biology Reports* **39**(3): 2901&07.

Khatib H, Monson R L, Schutzkus V, Kohl D M, Rosa G J and Rutledge J J. 2008. Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle. *Journal of Dairy Science* **91**(2): 784&93.

Michel-Regalado N G, Ayala-Valdovinos M Á, Galindo-García J, Duifhuis-Rivera T, Sánchez-Chiprés D R and Valencia-Posadas M. 2020. Effect of COQ9 and STAT5A polymorphisms on reproductive performance in a Holstein cow herd in Mexico. *Animal Reproduction* **17**(3).

Sambrook J and Russell D W. 2001. *Molecular Cloning: A Laboratory Manual*. 3rd edn. Cold Spring Harbor Laboratory Press, New York, NY, USA.

Schennink A, Bovenhuis H, Léon-Kloosterziel K M, Van Arendonk J A and Visker M H.2009. Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. *Animal Genetics* **40**(6): 909&16.

Schindler C and Darnell Jr J E. 1995. Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annual Review of Biochemistry* **64**(1): 621&52.

Selvaggi M, Dario C, Normanno G, Celano G V and Dario M. 2009. Genetic polymorphism of STAT5A protein: relationships with production traits and milk composition in Italian Brown cattle. *Journal of Dairy Research* **76**(4): 441&45.

Selvaggi M, Tufarelli V, Pinto F, Centoducati G, Dambrosio A, Santacroce M P and Dario C. 2013. Bovine STAT5A gene polymorphism analysis and its association with milk composition traits in Jersey cows. *International Journal of Bioscience, Biochemistry and Bioinformatics* **3**(4): 341.

Seyfert H M, Pitra C, Meyer L, Brunner R M, Wheeler T T, Molenaar A and Schwerin M. 2000. Molecular characterization of STAT5A-and STAT5B-encoding genes reveals extended intragenic sequence homogeneity in cattle and mouse and different degrees of divergent evolution of various domains. *Journal of Molecular Evolution* **50**(6): 550&61.

Sharma R, Maitra A, Singh P K and Tantia M S. 2013. Genetic diversity and relationship of cattle populations of East India: Distinguishing lesser known cattle populations and established breeds based on STR markers. *Springer Plus* **2**(1): 1&10.

Sharma R, Kumar B, Arora R, Ahlawat S, Mishra A K and Tantia M S. 2016. Genetic diversity estimates point to immediate efforts for conserving the endangered Tibetan sheep of India. *Meta Gene* **8**: 14&20.

Teglund S, McKay C, Schuetz E, Van Deursen J M, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G and Ihle J N. 1998. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* **93**(5): 841&50.