

# Genetic Variability and Significance of *STAT* Gene in Dairy Animals

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## Abstract

Importance in the bovine signal transducer and activator of transcription (*STAT*) gene has increased due to involvement of *STAT1* in the development of the mammary gland and regulating the transcription of genes involved in milk protein synthesis and fat metabolism in lactating dairy animals. Signal transducer and activator of transcription gene are a family of cytoplasmic proteins that are stimulated in response to a large number of growth factors, hormones and cytokines. *STAT5* gene is associated to milk composition, survival of embryo, conception rate, fertilization rate, service period and calving interval of dairy animals. Presently, seven bovine *STAT* genes have been identified and it has been found that *STAT1*, *STAT5A*, and *STAT5B* were not completely regulated by individual effectors of differentiation but their expression was tightly interrelated with lipid accumulation in dairy animals. Studies on the expression of *STAT* in different tissues and at different developmental stages have shown that *STAT1* and *STAT3* are constitutively expressed at constant levels during pregnancy, involution, lactation but *STAT4* and *STAT5* are developmentally regulated. *STAT5A* is a potential candidate or main gene because it is a member of interferon and placental lactogen (*PL*) signal transduction pathways and plays vital role in reproduction and milk production traits. In dairy animals, a number of association studies with milk yield, fat yield, protein yield, embryonic survival, conception rate, fertilization rate and calving interval have been carried out. These associations will be helpful to understand the underlying mechanism of *STAT* gene and genetic variability or polymorphisms that can be used for selection purposes in superior dairy animals.

**Keywords:** Candidate gene, fertility, milk content, polymorphism

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## INTRODUCTION

During the last decade there has been increasing scientific interest in the use of genomic information as an additional tool in conventional dairy cows' breeding schemes [1, 2]. Advancement in molecular genetics has led to discovery of potential candidate genes or genetic markers which exert significant effects on economic traits such as production reproduction, growth and disease resistant traits. It may be possible to select a superior breeding animal for a wide range of economic important traits with the use of molecular technologies and to increase reliability in predicting the phenotype or external makeup of individual dairy animal. To make dairying a successful and profitable business, the animals should not only be high producing, but also reproductively efficient. Therefore, *STAT* gene is important candidate gene which is associated with both production and

reproduction of dairy animals. In dairy animals, it is important to explore genetic variations at DNA level in *STAT* gene and subsequently utilize them in breeding programmes for genetic improvement of dairy animals. Recent studies carried by Khatib *et al.* reported that polymorphisms or genetic variation in the *FGF2* and *STAT5A* genes are associated with male fertility [3]; and Stoop *et al.* in exotic cattle have discovered large genetic variation or polymorphism in bovine milk fat composition and many SNPs in these potential candidate genes which play a role in fat synthesis in lactating dairy animals [4]. Several such potential candidate genes have been identified for fat synthesis and fertility in dairy animals such as fatty acid synthase (*FASN*) gene [5–8], Diacylglycerol-acyl-transferase1 (*DGAT1*) gene [9, 10], Signal transducer and activator of transcription 1 (*STAT1*) gene [11], and *STAT5A* showed the

highest significant association with both fertilization rate and embryonic survival at the blastocyst stage [12]. Complete genome scans have shown significant associations between production traits of dairy animals and microsatellite markers in the vicinity of *STAT1* gene [14, 15]. Asadollahpour *et al.* found that genetic research in farm animals focuses mainly on the identification of genes or molecular markers affecting economically important traits like production and fertility traits that could be useful in breeding programmes for genetic improvement of dairy animals [16]. Many studies reported that *STAT* gene is the potential candidate gene that influences milk composition (fat and protein percentage or yield), embryonic survival, conception rate, fertilization rate, service period and calving interval of dairy animals.

Signal transducer and activator of transcription (*STAT*) factors are a family of cytoplasmic proteins that are stimulated activated in response to a large number of cytokines, growth factors, and hormones in animals [17]. *STAT* proteins are activated through cascade of phosphorylation events in which Janus protein tyrosine kinases are first phosphorylated. The activated Janus protein tyrosine kinases then phosphorylate *STAT* proteins at their tyrosine residues. In turn, *STAT* separated from the receptor complex, form homo- or heterodimers, and translocate from the cytoplasm to the nucleus, where they interact with specific promoter regions and regulate gene expression [17]. At present, seven bovine *STAT* genes have been identified. *STAT1* and *STAT4* map to chromosome number 2, *STAT3*, *STAT5A* and *STAT5B* map to chromosome 19. *STAT6* maps to chromosome no. 5, whereas *STAT2* has not yet been mapped. Physiological functions of *STAT* proteins were originally defined by knockout experiments in mice. *STAT4*- deficient mice gave abnormalities in IL-12-mediated functions, comprising increases in IFN- $\gamma$  production and signaling, cellular proliferation, and T helper-1 cell differentiation in experimental mice [18].

*STAT5* consists of two highly related genes which are 96% similar at the AA genotype level. *STAT5* and *STAT3* activation may play major roles in the regulation of growth, differentiation and apoptosis in animals [19].

Some evidence exists that *STAT1* is involved in the development and differentiation of the mammary glands. Bovine *STAT1* maps to chromosome number 2 at interval 60 to 63 cM [20]. Boutinaud and Jammes reported that expression levels of *STAT1*, *STAT3*, and *STAT5* in the mammary gland of lactating goats and found that the expression of these genes were regulated by growth hormone in goat [21]. Stewart *et al.* studied the regulation of *STAT* expression by effectors of adipocyte differentiation in animals [22]. They found that *STAT1*, *STAT5A* and *STAT5B* were not exclusively regulated by individual effectors of differentiation, but their expression was tightly correlated with lipid accumulation. Studies on the expression of *STAT* in different tissues and at different developmental stages have shown that *STAT1* and *STAT3* are constitutively expressed at constant levels throughout pregnancy, lactation, and involution, whereas *STAT4* and *STAT5* are developmentally regulated [23]. Moreover, Watson found that *STAT1* is regulated during mammary gland development and apoptosis and that this constitutively active gene is an oncogene in the mammary glands [23]. The positional and functional potential candidate gene approaches have been applied to different genes in dairy animals.

### Genetic Variability of *STAT1* Gene

Signal transducer and activator of transcription 1 (*STAT1*) gene involved in the development of the mammary gland and regulating the transcription of genes involved in milk protein synthesis and fat metabolism lactation in dairy animals. Genetic research in dairy farm animals mostly focuses mainly on the identification of genes influencing economically important traits (production, reproduction and growth) that could be useful in breeding programs for genetic improvement [16]. The *STAT1* gene was important candidate gene because of its involvement in the development and differentiation of the mammary glands [24]. Also results from some genomic studies on dairy cattle showed that quantitative trait loci (QTL) with major effect on milk composition traits located in the region of *STAT1*, interval 60 to 63 cM [13–15]. The pooled DNA sequencing approach has been used in several studies both, for polymorphism identification and for

estimation of allele frequencies in pooled DNA or RNA samples. Kwok *et al.* studied the identification of SNP with >20% allele frequency for the minor allele [25]. In previous studies, pooled DNA and RNA samples were used to identify SNP in coding and noncoding regions in candidate genes affecting milk production traits [26, 27]. In this study, using the PCR-RFLP based method; monomorphic pattern was detected in a total length of 314 bp. Kumar *et al.* also reported monomorphic pattern in 3' UTR region of *STAT1* gene in Murrah, Gojri and Chhattisgarhi buffaloes by using PCR-RFLP based method [28].

Mosig *et al.* described a quantitative trait loci affecting the milk composition (protein percentage) in linkage with microsatellite marker at position 61.7 cM from the centromere [13]. Ashwell *et al.* reported a QTL affecting milk composition (fat percentage) in linkage with microsatellites markers at the interval 38.0 to 60.3 cM [14]. Ron *et al.* studied a QTL affecting milk composition (protein percentage) at the interval 61.7 to 70 cM from the centromere [15]. Observed associations of bovine *STAT1* gene with milk production traits were not surprising due to following three reasons:

- Expression of *STAT1* gene is controlled by prolactin hormone. Binding of prolactin to its receptor leads to a cascade of events that results in activation of the *STAT1*, *STAT3* and *STAT5* proteins which in turn control the transcription of genes involved in secretion of milk proteins and milk constituents like fat yield [29, 30].
- There is evidence that *STAT* genes might be important for fat metabolism regulation and milk protein synthesis probably through the prolactin signal transduction pathway operating in the mammary glands of lactating dairy animals [31]. Results show that *STAT1* gene was associated with milk composition traits (fat and protein percentages and yields).
- Interferon controls cellular anti-proliferative, antiviral and immunological responses in animals. The *STAT1* gene has been shown to be essential for cell growth suppression in response to IFN- $\gamma$  [18]. Moreover, mice deficient with

*STAT1*-gene were reported to be highly sensitive to infection by pathogens as compared to normal mice [18, 23]. These studies indicate that *STAT1* gene may have roles in the immune response of animals. The results of the effect of *STAT1* gene on somatic cells in milk (indicator for udder or mammary gland health in cows) are consistent with reported functions of this gene in the immune response of the human and mouse. They used positional and functional candidate gene analysis to search for potential candidate genes affecting milk production traits. A significant association was found between different haplotypes of the protease inhibitor gene and many production traits including milk yield, milk fat yield and SCS in Holstein Fries dairy cattle [26].

#### Genetic Variability of *STAT5* Gene

*STAT5* is the primary transcription factor responsible for signaling by prolactin in the mammary glands of lactating animals. *STAT5* was initially identified as a binding activity in the lactating mammary gland known as mammary gland factor. Mammary gland factor was cloned initially from sheep mammary glands and was found to be a new *STAT* protein family member and designated *STAT5*. *STAT5* protein is also known as mammary gland factor and is a main mediator of growth hormone action on target genes [32, 33]. Polymorphisms or genetic variability in the *STAT5A* gene have been associated with cows' production traits (milk yield and its composition traits) [34] and particularly a SNP or genetic variability in exon8 of the *STAT5A* gene was found to influence milk protein and fat percentage as well as in vitro embryonic survival of fetus and fertilization rate [35, 36]. Numerous SNPs of the bovine *STAT5A* gene have been reported by McCracken *et al.* [37], who found TG repeats of different lengths within intron12; Antoniou *et al.* described two SSCP variants of the gene fragment that encodes the SH2 domain in bovine *STAT5A* protein [38]; and Brym *et al.* reported a new SNP9501 (A/G) located in intron9 [34].

*STAT5* exists in two closely related forms, A and B, encoded by two separate genes.

*STAT5A* and *STAT5B* show differences both in their DNA binding specificities and with respect to their tissue distribution [39, 40]. The bovine *STAT5A* and *STAT5B* genes were mapped on chromosome 19 [41]. *STAT5A* is a crucial mediator in the lactogenic hormone response being a candidate marker for milk traits in dairy animals. Studies in mouse have shown that *STAT5A* is involved in both, milk production and fertility and *STAT5A* knockout female mice fail to lactate [42]. Disruption of *STAT5A* leads to infertility in females as a result of small-sized or absent corpora lutea [43]. Expression analysis revealed that *STAT5A* is primarily monoallelically expressed in early embryonic stages but biallelically expressed in later fetal stages, and that monoallelic maternal expression of *STAT5A* was significantly higher in blastocysts compared with degenerate embryos [12]. *In-vitro* fertilization (IVF) study reported that *STAT5A* to be significantly associated with fertilization rate and early embryonic survival [36]. Khatib *et al.* states that *STAT5A* is a candidate gene because it is a member of interferon- $\tau$  (IFN- $\tau$ ) and placental lactogen (PL) signal transduction pathways, which play important roles in reproduction and milk production traits [35]. Khatib *et al.* reported that polymorphisms in the *FGF2* and *STAT5A* genes are associated with male fertility [3]. Single SNP analysis described that single nucleotide polymorphism in exon 8 of *STAT5A* gene showed the maximum significant association with embryonic survival at the blastocyst stage and fertilization rate in cattle [12].

## CONCLUSIONS

Genetic variability or polymorphism of signal transducer and activator of transcription (*STAT*) gene is associated with milk production as well as its composition traits and fertility traits. Therefore, *STAT* gene acts as potential candidate gene for higher production and efficient reproduction in dairy animals. So, it can facilitate selecting superior germplasm of dairy animals.

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