Potential candidate gene markers for milk fat in bovines: A review

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ABSTRACT

In dairy animals, the principal goal of selection is to improve quality and quantity of milk. Genetic information inferred from single nucleotide polymorphism (SNP) primarily linked to Quantitative Trait Loci (QTL) can be used to improve selection for milk and milk constituent traits in bovines. Selection for a marker allele known to be associated with a beneficial QTL increases the frequency of that allele and hence, dairy performance can be enhanced. One of the potential benefit of selection based on molecular marker is that the marker genotypes can be determined in a dairy animal just after birth. Thus, marker information can be used to predict an animal’s genotype before its actual performance recording for a trait is available, which considerably reduces generation interval and thus improves genetic gain in a herd for milk and its constituent traits. This review article is an attempt to comprehend the idea behind marker based selection for milk fat and genes regulating milk fat with significant effects that can be targeted specifically in selection of superior dairy animals. Once an association is established, it can be utilized in a marker assisted breeding program for improvement of bovines.

Keywords: Fat associated genes, Marker assisted selection, Milk fat, Polymorphism

India is endowed with vast genetic resources of bovines with an estimated number of 302.79 million including 192.49 million cattle and 109.85 million buffalo population (Livestock Census 2019) and 50 recognized breeds of cattle and 17 recognized breeds of buffaloes present in various states of the country (NBAGR 2020). Milk production of India during 2016–17 was 165.4 million tonnes and in 2017–18, it reached 176.3 million tonnes showing an annual growth rate of 6.62% (DAHD 2018–19).

In India, breeding strategies for dairy animals must have emphasis not only on increasing production performance (milk yield), but also on milk composition and its quality like fat percent in milk. This has an economic importance because in organized sectors and dairy co-operatives, livestock owners are remunerated on the basis of fat percentage or fat corrected milk (FCM). Additionally, many milk processing companies are willing to pay the premium for milk rich in fat content, thus encouraging livestock keepers to improve the fat content in milk. However, in dairy animal’s, fat percent and milk quantity are negatively correlated to each other, but genetic selection for higher fat yield could improve the dairy economics. Genetic gain is proportional to the amount of genetic variation present in a population and more genetic variation exists for fat yield as compared to fat percentage. Thus, in order to get more genetic gain, selection of dairy animals should be done based on fat yield as compared to fat percentage. Kumar et al. (2016a) reported that in 5th monthly test day estimates of phenotypic and genetic correlations of monthly test day fat yield with 305-day fat yield was generally higher suggesting that this test day yields could be used as selection criteria for early evaluation and selection of Murrah buffaloes.

It is important to balance molecular genetic techniques with conventional animal breeding methodologies to improve the animal breeding program (Barillet et al. 2007). Development in the field of molecular biology involves the use of molecular markers for improvement of production traits including fat yield or fat percentage in bovines. Markers are mainly of 4 types, viz. morphological, biochemical, cytological and molecular or DNA markers. Molecular marker is defined as a DNA fragment or small portion of DNA which is present at a certain location in the genome and is associated with particular traits. Molecular markers have umpteen number of applications like parentage and twin zygosity determination, estimation of genetic distance, identification of disease carrier individuals, marker-assisted selection, gene mapping etc. Soyeurt et al. (2007) and Stoop et al. (2008) reported extensive genetic variations in bovine milk and milk fat genes and stated that many single nucleotide variations (SNP) among potential candidate genes play a specific role in fat synthesis or have been associated with fat percentage or fat yield in milk.

Marker-assisted selection for quantitative traits like fat yield followed with appropriate breeding methods can enable fast genetic gains under various bovine breed improvement programmes (Erhardt and Weimann 2007). In India, dairy animals are not selected based on molecular markers related to fat percentage or fat yield. Present review...
deals with the numerous molecular markers associated with fat percentage or fat yield in the selection of superior animals for better milk fat production in bovine (cattle and buffaloes).

Numbers of single nucleotide polymorphisms (SNPs) have shown positive and significant associations with milk fat secretion among different breeds of cattle and buffaloes. Information from SNPs linked to QTL can be used to improve selection for milk and milk constituent (fat) traits. Candidate genes studied for the association with milk fat in cattle and buffaloes, their location, SNPs and their association are given in Table 1.

Table 1. Important milk fat associated potential candidate gene markers in bovines

| Gene          | *Chr. | Function                       | SNP                        | Association                                      | References                                      |
|---------------|-------|--------------------------------|----------------------------|--------------------------------------------------|------------------------------------------------|
| FASN          | 19    | Fatty acid synthase            | Exon 1 and 34              | Significantly associated in HF cattle            | Ciecierska et al. 2013, Roy et al. 2001         |
|               |       |                                | Exon 40                    | Significantly associated in Murrah buffaloes     | Kumar et al. 2017a                              |
| OLR1          | 5     | Oxidized low density           | Exon 4 and Intron 4        | Significantly associated in HF cattle            | Soltani Ghomdavani et al. 2013, Wang et al. 2012 |
|               |       | lipoprotein receptor 1         | 3’ UTR                     | Significantly associated in HF cattle            | Schennink et al. 2009, Khatib et al. 2006       |
|               |       |                                | Intron 1 and               | Monomorphic pattern in Mehsana buffaloes        | Deshpande et al. 2013, Shahir et al. 2011       |
|               |       |                                | 3’ UTR                     |                                                   |                                                 |
| ANXA9         | 3     | Annexin-9 gene                 | Exon 5                     | Significantly associated in Spanish HF cattle    | Martinez-Royo et al. 2009                        |
|               |       |                                | Intron 3, 4, 5             |                                                   |                                                 |
| PRLR          | 20    | Prolactin receptor             | Exon 2, 4, 6, 9            | Associated in riverine buffaloes                 | Javed et al. 2011                               |
| SCD           | 26    | Stearoi-CoA desaturase         | Exon 3 and 5               | Significantly associated in Italian HF cattle    | Moioli et al. 2007                              |
|               |       |                                | Exon 5                     | Monomorphic pattern in Khuzestan buffaloes       | Taghizadeh et al. 2014                          |
| DGAT1         | 14    | Diacylglycerol- O-acyl transferase1 | Exon 8                    | Significantly associated in HF cattle            | Gautier et al. 2007, Grisart et al. 2002        |
| Leptin        | 8     | Leptin gene                    | Exon 2 and 3               | Significantly associated in Murrah buffaloes     | Datta et al. 2013                               |
|               |       |                                | Exon 2                     | Significantly associated in Murrah buffaloes     |                                                 |
| Ghrelin       | 22    | Ghrelin gene                   | Intron 3                   | Significantly associated in water buffaloes      | Gil et al. 2013                                 |
| PPARGC1A      | 6     | Peroxisome proliferator        | Intron 1 and 9             | Significantly associated in HF cattle            | Khatib et al. 2007, Weikard et al. 2005, Javed et al. 2011 |
|               |       | activated receptor Gamma        | 3’ UTR                     | Associated in Riverine buffaloes                 |                                                 |
|               |       | Co-activator1 alpha            | Intron 3 and 6             |                                                   |                                                 |
|               |       |                                | Exon 6 and 8               |                                                   |                                                 |
| PRL           | 23    | Prolactin                      | Intron 1                   | Significantly associated in Holstein dairy cattle| He et al. 2006                                  |
| ALG and BLG   | 11    | Alpha and beta-Lactalbumin     | Exon 1 and 2               | Significantly associated with fat % and fat yield in different buffalo breeds | Vohra et al. 2012 |
|               |       |                                | Exon 1                     | Significantly associated with milk production in water buffalo | Dayal et al. 2006 |
|               |       |                                | Exon 1 and 4               | Significantly associated with fat yield in riverine buffalo | Vohra et al. 2006 |
| STAT1         | 2     | Signal transducer and          | 3’ UTR                     | Significantly associated in HF cattle            | Cobanoglu et al. 2006                           |
|               |       | activator of transcription 1   |                            | Not-significantly associated in Murrah buffaloes | Kumar et al. 2015                              |
| OPN/SPP1      | 6     | Osteopontin/ Secreted          | Intron 4                   | Significantly associated with milk fat in cattle  | Leonard et al. 2005                             |
|               |       | Phosphoprotein 1               |                            |                                                   |                                                 |
| GHR           | 20    | Growth hormone receptor        | Intron 3                   | Significantly associated in Beijing Holstein cattle | Guoli et al. 2005 |

*Chr., chromosome location.
DGAT1 gene: Diacylglycerol O-acyl transferase 1 (DGAT1) is one of the main enzymes in controlling the rate of triglyceride synthesis in adipocytes and plays an important role in metabolism of cellular glycerolipid. DGAT gene catalyzes the conversion of diacylglycerol and fatty acyl CoA to triacylglycerol in triglyceride synthesis. Gautier et al. (2007) and Grisart et al. (2002) reported that DGAT gene is a potential candidate gene for milk fat percentage in bovines. Grisart et al. (2002) and Winter et al. (2002) identified a variation in DGAT gene (exon 8) in Bos taurus cattle and found that AA converted to GC genotype resulting in variation in DGAT gene (exon 8) in Bos taurus. Grisart (2007) and Grisart (2002) identified a potential candidate gene for milk fat percentage in bovines.

FASN gene: Fatty acid synthase (FASN) gene is a complex homodimeric enzyme that catalyzes the formation of palmitate in presence of NADPH from acetyl-coenzyme A and malonyl-coenzyme A. Chirala et al. (2003) reported that mammalian FASN is an important enzyme in fatty acid synthesis during adult stage as well as plays crucial role during embryonic development of individual. Mapping studies revealed the location of bovine FASN gene on 19th chromosome (Roy et al. 2001). Thioesterase (TE) domain has crucial role in determining the product chain length of FASN gene. Abe et al. (2009) reported that TE domain is a part between exons 39 to 41 of FASN gene. Vohra et al. (2015) reported that polymorphism is present in exon 40 region of FASN gene in Gojri and Chhattisgarhi buffaloes. Results suggest that exon 40 region of FASN gene in Gojri and Chhattisgarhi buffaloes is highly polymorphic and sufficient genetic variability is present in this fat related gene. Kumar (2015) evaluated milk fat yield trait and found its significant association with FASN exon 40 in Murrah buffaloes. Kumar et al. (2016b) reported that polymorphism is present in coding region of TE domain in FASN gene with guanine to adenine transition and suggested that exon 40 region of FASN gene in Murrah buffaloes is polymorphic. This genetic variation can be used as potential candidate gene marker, which could be beneficial to animal breeders for further studies. Kumar et al. (2017a) reported that exon 40 of FASN gene is highly polymorphic in Murrah buffaloes and significant association of FASN gene was observed with lactation fat average, lactation total solid average and peak milk yield and this variation could be helpful to breeders for carrying out future association studies and selecting superior Murrah buffaloes for higher milk fat.

Leptin gene: Leptin is synthesized and secreted by white adipocytes of the body and travels to brain where it causes release of factors that ultimately result in increased energy expenditure, reduction of food intake as well as increased physical activity (Banks et al. 1996). Leptin gene is considered a potential QTL influencing different production traits including milk production in cattle (Buchanan et al. 2002, Soita et al. 2004). Dudey et al. (2007) reported in their review that leptin gene is a chief candidate gene which can be used to increase production and reproduction performance of dairy as well as beef animals and genetic information related to polymorphism of leptin gene can be used efficiently in bovine breed improvement programme. Jamuna et al. (2016) reported that Murrah buffaloes with TC genotype of leptin gene produced more milk yield than TT and CC genotypes of leptin gene and their results revealed that C allele can be used as a potential marker for higher milk production in marker assisted selection of Murrah buffaloes.

OLR1 gene: Oxidized low-density lipoprotein receptor 1 (OLR1) plays a crucial role in degradation of oxidized low-density lipoprotein which causes damage to arterial endothelium (Imanishi et al. 2002). In cattle, large number of QTL affecting milk production traits had been reported in OLR1 gene including milk yield (DeKoning et al. 2001) and milk fat yield (Olsen et al. 2002). Khatib et al. (2006) reported significant associations of a SNP in 3’ UTR region and haplotypes of OLR1 gene with fat percentage and milk fat yield in a granddaughter-design Holstein bull population and association between OLR1 haplotypes and milk production traits was further confirmed in a daughter-design study in cattle population (Khatib et al. 2007). Schennink et al. (2009) also reported a significant association between OLR1 gene polymorphism and milk fat percentage in HF cattle.

STAT1 gene: Signal transducer and activator of transcription1 (STAT1) gene is involved in the development of mammary glands and regulation of transcription of genes involved in milk protein synthesis and fat metabolism. STAT1 gene is located on chromosome number 2 (Band et al. 2000). In different studies, it was found that STAT1 and STAT3 are expressed at constant levels throughout lactation period, pregnancy period as well as involution period, whereas STAT4 and STAT5 are developmentally regulated (Watson 2001). Kumar et al. (2015) reported that 3’UTR region of STAT1 gene in riverine buffaloes is fixed or monomorphic and there were no variations at this locus whereas STAT4 and STAT5 are developmentally regulated. In different studies, it was found that STAT1 and STAT3 are expressed at constant levels throughout lactation period, pregnancy period as well as involution period, whereas STAT4 and STAT5 are developmentally regulated. In different studies, it was found that STAT1 and STAT3 are expressed at constant levels throughout lactation period, pregnancy period as well as involution period, whereas STAT4 and STAT5 are developmentally regulated. However, Ashwell et al. (2004) and Cobanoglu et al. (2006) reported that polymorphism in 3’UTR region in STAT1 was associated with milk fat yield and milk percentage.

Butyrophilin gene: Butyrophilin (BTN1A1) is a candidate gene related to milk yield and milk composition (fat) in dairy animals. Bovine butyrophilin gene is located on the 23rd chromosome (Ashwell et al. 1996). Jack and Mather (1990) reported that BTN1A1 is directly involved in secretion of fat globules at apical surface of mammary epithelial cells and is maintained throughout lactation. BTN1A1 is the most abundant protein in milk fat globule membrane and is partially expressed in lactating mammary tissue. Ogg et al. (2004) also found that BTN1A1 is produced at the end of pregnancy and is maintained throughout lactation. Komisarek and Dorynek (2003) reported that BTN1A1 is a candidate gene affecting milk
yield and milk composition in bovine. Kumar et al. (2017b) also reported that BTN1A1 is a candidate gene affecting fat percent in dairy bovines and found that BTN1A1 gene has polymorphism in cattle breeds but monomorphic across buffalo breeds in India.

Conclusion

Molecular markers for improving milk production as well as fat yield in bovines have proved to be beneficial to animal breeders for designing suitable breeding programme as well as to dairy producers for gaining more profit. In India, the breeding strategies in bovines should focus not only on increasing the milk yield, but also on the milk composition and its quality including fat yield. Conventional selection methodologies must be assisted by use of genetic markers for early selection as well as for more genetic gain. Polymorphism outlined in potential candidate genes significantly associated with milk fat percentage and fatty acid composition can be utilized as SNPs markers for selecting superior germplasm of cattle and buffaloes having quality milk production.

REFERENCES

20th Livestock Census. 2019. All India Report. Ministry of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India (www.dahd.nic.in).

Abe T, Saburi J, Hasebe H, Nakagawa T, Misumi S, Nade T, Nakajima H, Shoji N, Kobayashi M and Kobayashi E. 2009. Novel mutations of the FASN gene and their effect on fatty acid composition in Japanese Black beef. Biochemical Genetics 47: 397–411.

Ashwell M S, Heyen D W, Sonstegard T S, VanTassell C P, Da Y, Vanraden P M, Rom M, Weller J I and Lewin H A. 2004. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. Journal of Animal Sciences 82: 468–75.

Ashwell M S, Ogg S L and Mather J H. 1996. The bovine butyrophilin gene maps to chromosome 23. Animal Genetics 27(3): 171–73.

Band M R, Larson J H, Rebeiz M, Green C A, Heyen D W, Donovan J, Windish R, Steinig C, Mahyuddin P, Womack J E and Lewin H A. 2000. An ordered comparative map of the cattle and human genomes. Genome Research 10: 1359–68.

Banks A, Kastin A J, Huang W, Jaspan J B and Maness L M. 1996. Leptin enters the brain by a saturable system independent of insulin. Peptides 17: 305–11.

Barillet F. 2007. Genetic improvement for dairy production in sheep and goats. Small Ruminant Research 70: 60–75.

Buchanan F C, Fitzsimmons C J, Van Kessel A G, Thue T D, Winkelman-Sim D C and Schmutz S M. 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genetics Selection Evolution 34: 105–16.

Chirala S S, Chang H, Matzuk M, Abu-Elheiga L, Mao J, Mahon K, Finegold M and Waki S J. 2003. Fatty acid synthesis is essential in embryonic development: fatty acid synthase null mutants and most of the heterozygotes die in utero. Proceedings of National Academy of Sciences 100: 6358–63.

Ciecierska D, Frost A, Grzesiak W, Proska C W, Dybus A and Olszewski A. 2013. The influence of fatty acid synthase polymorphism on milk production traits in polish holstein-friesian cattle. Journal of Animal and Plant Sciences 23: 376–79.

Cobanoglu O, Zaitoun I, Chang Y M, Shook G E and Khatib H. 2006. Effects of the signal transducer and activator of transcription 1 (stat1) gene on milk production traits in Holstein dairy cattle. Journal of Animal Sciences 89: 4433–37.

Datta S, Verma A, Chatterjee P and Pal A. 2013. Molecular characterization of the leptin gene in riverine buffaloes. Buffalo Bulletin 32: 196–211.

Dayal S, Bhattacharya T K, Vohra V, Kumar P and Sharma A. 2006. Effect of Alpha-lactalbumin gene polymorphism on milk production traits in water buffalo. Asian Australasian Journal of Animal Sciences 19: 305–08.

De Koning D J, Schulman N F, Ely K, Moisio S, Kinos R, Vilki J and Maki-Tanila A. 2001. Mapping of multiple quantitative trait loci by simple regression in half-sib designs. Journal of Animal Sciences 79: 616–22.

Department of Animal Husbandry, Dairying & Fisheries (DAHDC). 2018–19. Annual report.

Deshpande D, Rank D N, Vataliya P H and Joshi C G. 2013. Oxidized low density lipoprotein receptor 1 (OLR1) gene polymorphism in Mehsana buffaloes (Bubalus bubalis). Buffalo Bulletin 32: 260–64.

Dubey P P, Sharma A and Kumar D. 2007. Leptin gene and its role in cattle- A review. Agricultural Review 28(3): 207–15.

Erhardt G and Weimann C. 2007. Use of molecular markers for evaluation of genetic diversity and in animal production. Archivos Latinoamericanos de Produccion Animal 15: 63–66.

Gautier M, Capitan A, Fritz S, Eggan A, Boichard D and Druet T. 2007. Characterization of the DGAT1K232A and variable number of tandem repeat polymorphisms in French dairy cattle. Journal of Dairy Sciences 90: 2980–88.

Gil F M M, de Camargo G M F, Pablos de Souza F R, Cardoso D F, Fonseca P D S, Zetouni L, Bzaz C U, Aspilcueta-Borquis R R and Tonhati H. 2013. Polymorphisms in the ghrelin gene and their associations with milk yield and quality in water buffaloes. Journal of Dairy Sciences 96: 3326–31.

Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Cambisano N, Mni M, Reid S, Spelman R, George M and Snell R. 2002. Positional candidate cloning of a QTL in dairy cattle; Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Research 12: 222–31.

Guoli Z, Qi Z and Shanli G. 2005. Analysis on polymorphism of HFABP gene of Luxi cattle and its relation to meat quality traits. Northwestern Agricultural Journal 14: 5–7.

He F, Sun D, Yu Y, Wang Y and Zhang Y. 2006. Association between SNPs within prolactin gene and milk performance traits in Holstein dairy cattle. Asian Australasian Journal of Animal Sciences 19: 1384–89.

Imanishi T, Hano T, Sawamura T, Takarada S and Nishio I. 2002. Oxidized low density lipoprotein potentiation of Fas-induced apoptosis through lectin-like oxidized-low density lipoprotein receptor-1 in human umbilical vascular endothelial cells. Circulation 106: 1060–64.

Jack L J and Mather H. 1990. Cloning and analysis of cDNA encoding bovine butyrophilin, an apical glycoprotein expressed in mammary tissue and secreted in association with the milk-fat globule membrane during lactation. Journal of Biological Chemistry 265(24): 14481–86.

Jamuna V, Gupta A K, Chakravarty A K, Singh A, Patil C S, Kumar M and Vohra V. 2016. Leptin gene polymorphism in association with lactation milk yield in Murrah buffaloes.
Khatib H, Leonard S, Schutzkus V, Luo W and Chang Y M. 2006. Association of the OLR1 gene with milk composition in Holstein dairy cattle. *Journal of Dairy Sciences* 89: 1753–60.

Khatib H, Zaitoun I, Wiebelhaus-Finger J, Chang Y M and Rosa G J M. 2007. The association of bovine PPARGCA1 and OPN genes with milk composition in two independent Holstein cattle populations. *Journal of Dairy Sciences* 90: 2966–70.

Komisarek J and Dorynek Z. 2003. Polymorphism of BTN and GHR genes and its impact on bulls’ breeding value for milk production traits. *Journal of Animal and Feed Sciences* 12(4): 681–88.

Kumar M, Ratwan P, Das R, Chopra A and Vohra V. 2017b. Allelic diversity of butyrophilin (BTN1A1) gene in Indian bovines. *Indian Journal of Biotechnology* 22(2): 92–97.

Kumar M, Vohra V, Ratwan P and Chakravarty A K. 2015. Exploring polymorphism in 3' UTR region of STAT1 gene in different Buffalo breeds. *Indian Journal of Dairy Science* 68: 473–76.

Kumar M, Vohra V, Ratwan P and Chakravarty A K. 2016b. SNP identification in thioesterase domain of fatty acid synthase gene in Murrah buffaloes. *Journal of Animal and Plant Sciences* 26(6): 182–83.

Kumar M, Vohra V, Ratwan P, Chopra A and Chakravarty A K. 2017a. Influence of FASN gene polymorphism on milk composition traits in Murrah buffaloes. *Indian Journal of Animal Research* 51: 640–43.

Kumar M, Vohra V, Ratwan P, Valsalan J, Patil C S and Chakravarty A K. 2016a. Estimates of genetic parameters for fat yield in Murrah buffaloes. *Veterinary World* 9(3): 295–98.

Kumar M. 2015. ‘Genetic screening of fat associated genes in relation to breeding value in Murrah buffalo’. M.V.Sc. Thesis, Karnal, NDAI (Deemed University).

Leonard S, Khatib H, Schutzkus V, Chang Y M and Maltecca C. 2005. Effects of the osteopontin gene variants on milk composition traits in riverine buffalo. *Indian Journal of Animal Sciences* 42(8): 884–88.

Vohra V, Kumar M, Chopra A, Niranjani S K, Mishra A K and Kataria R S. 2015. Polymorphism in Exon-40 of FASN gene in lesser known buffalo breeds of India. *Journal of Animal Research* 5(2): 325–28.

Vohra V, Bhattacharya T K, Dayal S and Bhattacharya T K. 2012. SSCP typing of alpha-lactalbamin and beta-lactoglobulin gene and its association with milk production and constituent traits in Indian riverine buffalo. *Indian Journal of Animal Sciences* 52(6): 884–88.

Watson C J. 2001. Stat transcription factors in mammary gland development and tumorigenesis. *Journal of Mammary Gland Biology Neoplasia* 6: 115–27.

Weikard R, Kuhn C, Goldammer T, Freyer G and Schwerin M. 2005. The bovine PPARGCA1 gene: molecular characterization and association of an SNP with variation of milk fat synthesis. *Physiological Genomics* 21: 1–13.

Winter A, Kramer W, Werner F A O, Kollers S, Kata S, Durstewitz G, Buitink J, Womack J E, Thaller G and Fries R. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. *Proceedings of the National Academy of Sciences* 99: 9300–05.