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**Research Article**

# GENETIC VARIANTS IN EXON 2 REGION OF FABP3 GENE IN RELATION TO MILK PRODUCTION TRAITS IN SAHIWAL AND KARAN FRIES CATTLE

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## ABSTRACT

The present study pertains to records on milk production and milk constituents of 100 Sahiwal cattle and 115 Karan Fries cattle, the data collected over a period of 2004 to 2016 from Animal Genetics and Breeding division from ICAR-National Dairy Research Institute (NDRI), Karnal Haryana. In Sahiwal, SNP at position G4312C of FABP3 gene was highly associated with FL305DFY and however, non-significant for FL305DMY, FLTMY, FL305DSNFY and FL305DPY. GG genotype was superior for FLTMY traits and GC genotype was superior for all other traits. In Karan Fries, SNP at position G4363T was highly associated for FL305DFY and however non-significant for FL305DMY, FLTMY, FL305DSNFY and FL305DPY.TT genotype was superior for all traits in present study.

**Keywords:** FABP3 gene, Karan Fries cattle, Milk Constituents traits, Sahiwal

India is a rich reservoir of genetic diversity in cattle with 41 recognized cattle breeds. As far as milk production is concerned, Sahiwal is the best dairy breed of the Indian subcontinent. Karan Fries breed has been evolved by crossbreeding between Tharparkar and Holstein Friesian at the ICAR-NDRI Karnal, Haryana. FABPs (Fatty Acid Binding Proteins) are 14-15 kDa proteins that reversibly bind non-esterified saturated and unsaturated long-chain fatty acids, eicosanoids and other lipids with the purpose of transporting or storing them inside a cell (Shimano *et al.*, 1996). There are nine members of the FABPs protein family that are expressed differentially in multiple tissues. The most abundant isoforms of FABPs expressed in the lactating bovine mammary gland are FABP3, FABP4 and FABP5 (Jensen, 2002). The main function of FABP3 is to channel fatty acids inside a cell towards mitochondrial α-oxidation in heart and skeletal muscles. The discovery of FABP3 protein in mammary gland was related to the identification of a Mammary Derived Growth Inhibitor (MDGI) that turned out to be a mixture of FABP3 and FABP4 proteins (Yang *et al.*, 2002; German and Dillard, 2006). The FABP3 protein is highly expressed in the mammary gland during cell differentiation and formation of ductal structures at the onset of lactation (Binas *et al.*,1992). The polymorphisms in FABP3 genes affect the selectivity of fatty acid uptake from the blood and fatty acid transport inside the mammary epithelial cells, resulting in differences in milk fatty acid composition (Jensen, 2002; Soyeurt *et al.*, 2006), but the requirement of FABP3 for mammary tissue development and function is not well established (Schaefer, 2002; Stoop *et al.*, 2008).

## MATERIAL AND METHODS

**Experimental animals and genomic DNA isolation:** The data for present study pertained to various milk production and milk constituents traits gathered from history sheets and milk constituents registers maintained at Animal Genetics and Breeding division of ICAR-NDRI, Karnal, Haryana. Data of 100 Sahiwal and 115 Karan Fries cattle over a period of 13 years from 2004 to 2016 was collected. About 10 ml of venous blood was collected aseptically from the jugular vein of the selected animals in a 15ml polypropylene centrifuge tube under sterile condition using 0.5 ml of EDTA as anticoagulant. The tubes containing blood samples were transported to the laboratory as soon as possible on ice and were stored in refrigerator at –20 °C until isolation of DNA. Phenol extraction method as described by Sambrook and Russell (2001) was used for isolation of genomic DNA. Horizontal submarine agarose gel electrophoresis was used to check the quality of genomic DNA. The purity of genomic DNA was checked by spectrophotometer. Genomic DNA samples showing the OD ratio in the range of 1.7 to 1.9 was used for the study.

**Amplification of targeted region of FABP3gene:** Only good quality genomic DNA was used for amplification of exon 2 of FABP3 gene (497bp) by polymerase chain reaction under optimized conditions using primer pair-F: 5' -CGCTCCAGCTCATGCTCATA -3' and R: 5'-CATGCCATGTGCAAGGTCAC -3'. The primer pair to amplify exon 2 region of FABP3 gene was designed using Primer 3 software (http://www.primer3.ut.ee) (Untergrasser *et al.*, 2012) and gene sequence available at NCBI database (http://www.ncbi.nlm.nih.gov).The primers designed were checked for specificity by BLAST (version 1.2.0). Primers were got synthesized from Sigma Aldrich Chemicals Pvt. Ltd. (USA).

The PCR reaction (25µl) composition was 2X PCR

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Master Mix (Fermentas)-12.5µl, Deionised water (DNAase free water)-7.5 µl,Forward and Reverse primers (10 pmol)- 1µl each and Genomic DNA (30ng/µl)-3.0µl. The PCR was carried out in PCR thermal cycler (Eppendorf Germany) with protocol: initial denaturation at 94 °C for 05 min. followed by 32 cycles of denaturation at 94 °C for 30 sec., annealing at 62 °C for 30 sec. and extension at 72 °C for 30 sec. and final extension at 72 °C for 10 min.The amplified product was checked for quality and quantity by 2% agarose gel electrophoresis as described by Sambrook and Russell (2001) along with 100bp DNA ladder (O'GeneRuler™-Fermentas) at a constant voltage of 70V for 45 minutes in 0.5X TBE buffer. The amplified PCR product was visualised under UV transilluminator and documented by photograph through gel documentation system (Bio-Rad, USA) (Fig. 1 and 2).

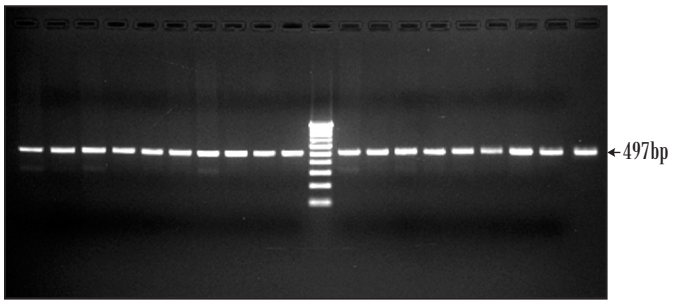


Fig. 1. Amplified PCR product of 497 bp of FABP3 Gene electrophoresed on 2% agarose in Sahiwal Cattle; M-100bp DNA ladder; Lanes1 to 19- amplified PCR products

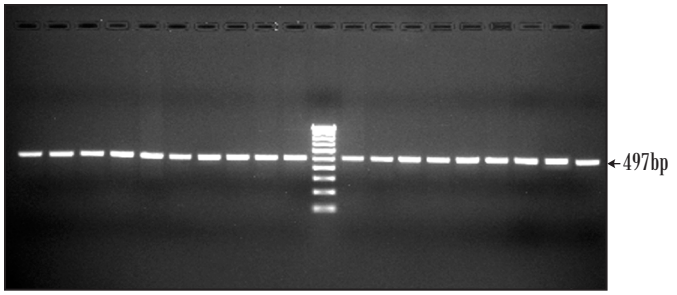


Fig. 2. Amplified PCR product of 497 bp of FABP3 Gene electrophoresed on 2% agarose in Karan Fries Cattle. M-100bp DNA ladder; Lanes1 to 18-amplified PCR products

**Sequencing of PCR product and analysis of sequenced data for SNPs detection:** Ten amplicons of different sizes were sent to 1stBASE Sequencing INT by using forward and reverse primers and the final sequences of each contig for Sahiwal and Karan Fries cattle were deduced from the raw sequences by using Bioedit software. ClustalW software was used for determining the SNPs in which complete coding sequence of animal were compared and aligned with the edited sequences of other Sahiwal and Karan Fries cattle (www.ebi.ac.uk/tools/msa/clustalw2) (Larkin *et al.*, 2007).

**Statistical analysis of PCR-RFLP data:** The analysis was carried out with Statistical method using software's in the computer centre of the institute under the following headings:

**Restricted Maximum Likelihood Method (REML)**

**Estimation of breeding value:** The single trait animal model was considered for estimation of breeding value using WOMBAT software (Meyer, 2010). The following animal model was considered:

$$Y_{ijk} = Xb_i + Zu_j + e_{ijk}$$

Where, $Y_{ijk}$ is $k^{th}$ observation of $j^{th}$ random effect of $i^{th}$ fixed effect; $b_i$ isVector of observation of fixed effect; X is Incidence matrix of fixed effect; $u_j$ is Vector of additive genetic effect (animal effect); Z is Incidence matrix of random effect and $e_{ijk}$ is Vector of residual errors.

**Association Estimation:** Based on the adjusted records, pertaining to milk yield and its constituents on Sahiwal and Karan Fries cattle maintained at ICAR-NDRI, Karnal, regression analysis was carried out to identify SNPs contributing significantly to the variation in milk and its constituents.

$$Y_{ijk} = a + b_i\, SNP_i + b_j\, SNP_j + \ldots\ldots b_n\, SNP_n + e_{ijk}$$

Where, $Y_{ijk}$ is Adjusted observation on $k^{th}$ animal of $i^{th}$, $j^{th}$….$n^{th}$ SNPs; A is Intercept; $b_i$ …n is partial regression coefficient for SNPs considered; $SNP_{i,j}$ ...n is effect of SNPs taken as independent variable and $e_{ijk}$ is Random error NID (0, σ2e)

**Effect of genotypes on Breeding Value:** The relative contribution of Genotypes to breeding value of the animal for milk yield and milk constituents was assessed using the following model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where,$Y_{ij}$ is Breeding value of $j^{th}$ animal of $i^{th}$ genotype; m is Overall mean; $G_i$ is Effect of $i^{th}$ genotypes (SNPs/ haplotypes) and $e_{ij}$ is Residual error NID (0, σ2e).

## RESULTS AND DISCUSSION

**Genetic variant at SNP G4312C in Exon 2 of FABP3 gene (497 bp) in Sahiwal:** In Sahiwal, SNP at positionG4312C was highly associated with FL305DFY and however non-significant for FL305DMY, FLTMY, FL305DSNFY and FL305DPY. The mean±SE of GG genotype for FL305DMY, FLTMY, FL305DFY, FL305DSNFY, FL305DPY were found to be 1803.3±10.5, 2011.9±10.7, 98.45±0.35, 154.81±0.11 and 43.79±0.10 Kg, respectively and for GC genotype were 1815.29±9.54, 2009.89±9.71, 101.88±0.32, 154.95±0.10 and 43.99±0.09

Kg, respectively in the present study (Table 1 and 2). GG genotype was superior for FLTMY traits and GC genotype was superior for all other traits. The result is in agreement with Nafikov *et al.* (2013), who reported that the significant association of overall haplotype effect of FABP3 with the concentrations of pentadecylic (15:0) acid and elongation index. FABP3 gene can be used to select for cattle producing milk with healthier fatty acid composition and with higher percentage of milk fat.

**Regression Equation:** The significance of association of SNP with different performance traits were estimated by constructing the regression equation and the best fit equation for each of them is given below:

FL305DMY=1809.29+5.99 SNP_CG–5.99 SNP_GG (R2=0.72)

FLTMY = 2010.88 – 0.98 SNP_CG + 0.98 SNP_GG (R2=0.02)

FL305DFY=100.17+1.71 SNP_CG–1.71 SNP_GG (R2=33.90)

FL305DSNFY=154.88+6.88 SNP_CG–6.88 SNP_GG (R2=0.79)

FL305DPY = 43.89 + 9.64 SNP_CG – 9.64 SNP_GG (R2=1.96)

**Genetic Variant (SNP) at G4363T at Exon 2 of FABP3 Gene (497 bp) in Karan Fries:** SNP at position G4363T was highly associated for FL305DFY and however non

**Table 1**
**ANOVA for SNP G4312C of FABP3 Gene in Sahiwal**

| Source | df | SS | MSS | F-value |
|---|---|---|---|---|
| **FL305D MY** | | | | |
| G4312C | 1 | 3557 | 3557 | 0.71 |
| Error | 98 | 490244 | 5002 | |
| Total | 99 | 493801 | | |
| **FLTMY** | | | | |
| G4312C | 1 | 96 | 95.92 | 0.02 |
| Error | 98 | 508259 | 5186.31 | |
| Total | 99 | 508355 | | |
| **FL305DFY** | | | | |
| G4312C | 1 | 290.57 | 290.57 | 50.27** |
| Error | 98 | 566.51 | 5.78 | |
| Total | 99 | 857.09 | | |
| **FL305DSNFY** | | | | |
| G4312C | 1 | 0.46 | 0.46 | 0.79 |
| Error | 98 | 58.42 | 0.59 | |
| Total | 99 | 58.89 | | |
| **FL305DPY** | | | | |
| G4312C | 1 | 0.92 | 0.92 | 1.96 |
| Error | 98 | 46.03 | 0.46 | |
| Total | 99 | 46.95 | | |

**Highly significant (p<0.01)

**Table 2**
**Least Square Mean and Standard Error for milk production traits**

| G4312C | N | Mean (Kg) ± SE |
|---|---|---|
| **FL305 DMY** | | |
| CG | 55 | 1815.29 ± 9.54 |
| GG | 45 | 1803.30 ± 10.50 |
| **FLTMY** | | |
| CG | 55 | 2009.89 ± 9.71 |
| GG | 45 | 2011.90 ± 10.70 |
| **FL305DFY** | | |
| CG | 55 | 101.88 ± 3.24[a] |
| GG | 45 | 98.45 ± 3.58[b] |
| **FL305DSNFY** | | |
| CG | 55 | 154.95 ± 10.40 |
| GG | 45 | 154.81 ± 11.50 |
| **FL305DPY** | | |
| CG | 55 | 43.99 ± 9.24 |
| GG | 45 | 43.79 ± 10.20 |

significant for FL305DMY, FLTMY, FL305DSNFY and FL305DPY. The mean±SE of GG genotype for FL305DMY, FLTMY, FL305DFY, FL305DSNFY and FL305DPY were found to be 3514.50±10.6, 4534.30± 10.6, 98.37±3.76, 278.05±0.10 and 113.26±0.10, respectively and for TT genotype were 3529.28± 8.66, 4549.04±8.66, 160.25±3.07, 278.19±0.08 and 113.47± 0.08, respectively in the present study (Table 3 and 4). TT genotype was superior for all traits. The result is in agreement with Nafikov *et al.* (2013), who reported significant association of overall haplotype effect of FABP3 with concentrations of pentadecylic (15:0) acid and elongation index. FABP3 gene can be used to select for cattle producing milk with healthier fatty acid composition and with higher percentage of milk fat.

**Regression Equation:** The significance of association of SNP with different performance traits were estimated by constructing the regression equation and the best fit equation for each of them is given below:

FL305DMY=3521.89–7.39 SNP_GG+7.39 SNP_TT (R2=1.02)

FLTMY = 4541.65 – 7.39 SNP_GG + 7.39 SNP_TT (R2=1.02)

FL305DFY=129.31–30.94 SNP_GG+30.94 SNP_TT (R2=59.00)

FL305DSNFY=278.12–0.0 6 SNP_GG+0.06 SNP_TT (R2=0.93)

FL305DPY=113.37–0.10 SNP_GG+0.10 SNP_TT (R2=1.92)

## CONCLUSIONS

In Sahiwal, SNP at position G4312C was highly associated with FL305DFY. GG genotype was superior for FLTMY traits and GC genotype was superior for all other traits. In Karan Fries, SNP at position G4363T was highly

**Table 3**

**ANOVA for SNP G4363TofFABP3 Gene in Karan Fries**

| Source | df | SS | MSS | F-value |
|---|---|---|---|---|
| **FL305D MY** | | | | |
| G4363T | 1 | 6030 | 6030 | 1.16 |
| Error | 113 | 584981 | 5177 | |
| Total | 114 | 591011 | | |
| **FLTMY** | | | | |
| G4363T | 1 | 6030 | 6030 | 1.16 |
| Error | 113 | 584981 | 5177 | |
| Total | 114 | 591011 | | |
| **FL305DFY** | | | | |
| G4363T | 1 | 105680.25 | 105680.25 | 162.84** |
| Error | 113 | 73335.55 | 648.98 | |
| Total | 114 | 179015.81 | | |
| **FL305DSNFY** | | | | |
| G4363T | 1 | 0.52 | 0.52 | 1.06 |
| Error | 113 | 56.42 | 0.49 | |
| Total | 114 | 56.95 | | |
| **FL305DPY** | | | | |
| G4363T | 1 | 1.18 | 1.18 | 2.21 |
| Error | 113 | 60.78 | 0.53 | |
| Total | 114 | 61.97 | | |

**Highly significant ($p<0.01$)

**Table 4**

**Least Square Mean and Standard Error for milk production traits**

| G4363T | N | Mean (Kg) ± SE |
|---|---|---|
| **FL305 DMY** | | |
| GG | 46 | 3514.5 ± 10.6 |
| TT | 69 | 3529.28 ± 8.6 |
| **FLTMY** | | |
| GG | 46 | 4535.3 ± 10.6 |
| GT | 69 | 4549.04 ± 8.6 |
| **FL305DFY** | | |
| GG | 46 | 98.37 ± 3.76[a] |
| GT | 69 | 160.25 ± 3.07[b] |
| **FL305DSNFY** | | |
| GG | 46 | 278.05 ± 10.4 |
| GT | 69 | 278.19 ± 8.50 |
| **FL305DPY** | | |
| GG | 46 | 113.26 ± 10.8 |
| GT | 69 | 113.47 ± 8.80 |

associated for FL305DFY. TT genotype was superior for all traits. FABP3 gene can be used to select for cattle producing milk with healthier fatty acid composition and with higher percentage of milk fat.


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