



# Genetic Variants at Exon 3 in AAT Gene in Relation to Milk Production Traits in Sahiwal and Karan Fries Cattle

Alok Kumar Yadav<sup>1</sup>, Anupama Mukherjee<sup>1</sup>

10.18805/ajdfr.DR-2202

## ABSTRACT

**Background:** The present study pertained 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, Karnal Haryana.

**Methods:** Good quality genomic DNA was used for amplification of exon 3 of AAT gene (474bp) by polymerase chain reaction under optimized conditions and Sequenced data analysis for SNPs detection. The analysis was carried out with appropriate Statistical method using software's in the computer centre of the institute under the Restricted Maximum Likelihood Method (REML), Estimation of breeding value, Association Estimation, Effect of genotypes on Breeding Value.

**Result:** In Sahiwal SNP at position G6997C was highly associated for FL305DMY, FLTMY, and FL305DSNFY. The mean  $\pm$  SE of GG genotype for FL305DMY, FLTMY, FL305DFY, FL305DSNFY and FL305DPY were found to be 1748.45 $\pm$ 6.47, 1962.30 $\pm$ 8.47, 100.33 $\pm$ 0.44, 154.41 $\pm$ 0.09 and 43.99 $\pm$ 0.10 respectively and for GC genotype 1860.17 $\pm$ 5.86, 2050.44 $\pm$ 7.66, 100.35 $\pm$ 0.39, 155.28 $\pm$ 0.08 and 43.82 $\pm$ 0.10 respectively. GG genotype was superior for FL305DPY and Heterozygous GC genotype was superior for rest of other traits. In Karan Fries cattle SNP at position G6997C was highly associated for FL305DMY, FLTMY, FL305DSNFY and FL305DPY. The mean  $\pm$  SE of GG genotype for FL305DMY, FLTMY, FL305DFY, FL305DSNFY, FL305DPY were found to be 3564.74 $\pm$ 6.0, 4584.50 $\pm$ 6.00, 135.05 $\pm$ 4.76, 278.53 $\pm$ 0.06 and 113.80 $\pm$ 0.06 respectively and for GC genotypes were 3459.23 $\pm$ 7.48, 4478.76 $\pm$ 7.48, 136.19 $\pm$ 5.96, 277.50 $\pm$ 0.07, 112.75 $\pm$ 0.08 respectively. Heterozygous (GC) genotype was superior for FL305DFY and GG genotype was superior for FL305DMY, FLTMY, FL305DSNFY and FL305DPY.

**Key words:** FABP3 gene, Karan fries cattle, Milk constituents traits, Sahiwal.

## INTRODUCTION

India has a rich genetic diversity in cattle with 53 recognized breeds. Country's total cattle population is 192.49 million, out of which indigenous cattle population comprises of 142.11 million (Livestock Census, 2019). Milk is an important source of essential nutrients for calves and a key raw material for human food (Reinhardt *et al.*, 2012). India ranks first in the world in terms of milk production with production of 198.4 million tons in 2019-20 (BAHS, 2019). Sahiwal is the best dairy breed of the Indian subcontinent. It is a comparatively heavy breed with a symmetrical body and loose skin (Nivsarkar *et al.*, 2000). The Karan Fries breed has been evolved from crossbreeding between Tharparkar and Holstein Friesian at the ICAR-National Dairy Research Institute, Karnal, Haryana. The breed has 50 % inheritance from Friesian. The breed carries black patches and sometime is completely dark with white patches on the forehead and the switch of the poll. The udder is also dark with white patches in teats. The animals are extremely docile and very good yielders.

Alpha 1-antitrypsin (AAT), a strong protease inhibitor, also known as  $\alpha$ 1-protease inhibitor ( $\alpha$ 1PI), belongs to the super family of serpins or serine proteinase inhibitors that include in addition to others C1 esterase, antithrombin and  $\alpha$ 1-antichymotrypsin. AAT is a glycoprotein which forms a sodium dodecyl sulfate (SDS) stable complex with

<sup>1</sup>Division of Animal Genetics and Breeding, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India.

**Corresponding Author:** Alok Kumar Yadav, Division of Animal Genetics and Breeding, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India.

Email: alokvet1000@gmail.com

**How to cite this article:** Yadav, A.K. and Mukherjee, A. (2024). Genetic Variants at Exon 3 in AAT Gene in Relation to Milk Production Traits in Sahiwal and Karan Fries Cattle. Asian Journal of Dairy and Food Research. DOI: 10.18805/ajdfr.DR-2202.

**Submitted:** 08-02-2024 **Accepted:** 08-05-2024 **Online:** 23-07-2024

elastase. The molecular mass of AAT is about 52 kDa and carbohydrates account for 15% of its mass (Carrell *et al.*, 1982). The bovine AAT gene consists of five exons, spanning about 9 kb of genomic DNA and encoding a 416-AA protein. Alpha 1-antitrypsin (AAT) can protect vulnerable elastic tissues from degradation by neutrophil elastase, this is an important issue as protein degradation in bovine milk affects the quality of dairy products.

## MATERIALS AND METHODS

### Experimental animals and genomic DNA isolation

The data for present study pertained to various milk production and milk constituent's traits were collected from

history sheets and milk constituent's registers, of 100 Sahiwal and 115 Karan Fries cattle spread over a period of 13 years from 2004 to 2016 in Animal Genetics and Breeding division of ICAR-National Dairy Research Institute, Karnal, Haryana. Blood samples were collected from the selected population. About 10 ml of venous blood was collected aseptically from the jugular vein of the animals in a 15ml polypropylene centrifuge tube under sterile condition using 0.5 ml of EDTA as an anticoagulant. The tube was shaken gently to facilitate thorough mixing of blood with the anticoagulant. The tubes containing blood samples were transported to the laboratory in an icebox containing ice

packs and were stored in the refrigerator at -20°C temperature until the isolation of DNA was done. 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. The 6 µl of genomic DNA of each sample was dissolved in 294 µl of triple distilled water and spectrophotometer readings at OD<sub>260</sub> and OD<sub>280</sub> was recorded against 300µl double distilled water as a blank. Genomic DNA samples showing the OD ratio in the range of 1.7 to 1.9 was used further in the study.

**Table 1:** The standard programme for PCR Reaction mix (25 µl).

| Components              | Concentration | Volume (µl) |
|-------------------------|---------------|-------------|
| 10X Buffer              | 10 X          | 2.5         |
| 15 mM MgCl <sub>2</sub> | 15 mM         | 1.5         |
| dNTP's                  | 200 µM        | 0.5         |
| F-primer                | 30 pm         | 3.0         |
| R-primer                | 30 pm         | 3.0         |
| Taq (5 units/µl)        | 1 unit        | 0.2         |
| Template                | 100 ng        | 1.0         |
| Distilled water         | -             | 14.3        |

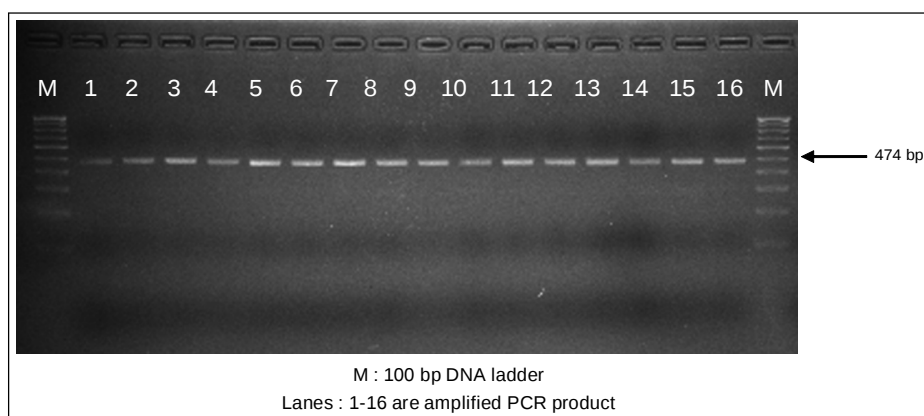
#### Amplification of targeted region of AAT genes

Only good quality genomic DNA was used for amplification of exon 3 of AAT gene (474bp) by polymerase chain reaction under optimized conditions.

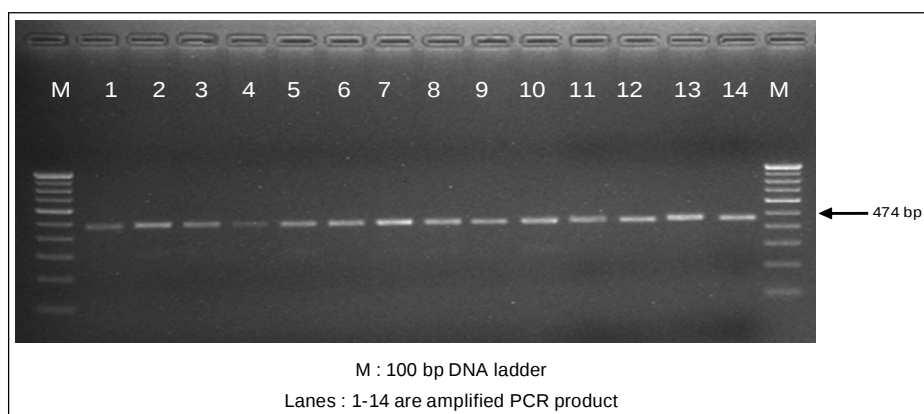
F: 5'- ACACACGCAAACCTGAGGC -3'

R: 5'- CGTGGTCGGTGGGTCTATCC -3'

Primers (F) and (R) was used to amplify exon 3 region of AAT gene, which were designed to amplify genes 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



**Plate 1:** Amplified PCR product of 474 bp of AAT Gene electrophoresed on 2% agarose in Sahiwal Cattle.



**Plate 2:** Amplified PCR product of 474 bp of AAT Gene electrophoresed on 2% agarose in Karan Fries Cattle.

checked for specificity by BLAST (version 1.2.0). Primers were designed and synthesized from Sigma Aldrich Chemicals Pvt. Ltd (USA), the best amplification of the desired fragment was taken for further analysis. The standard programme is given as under (Table 1).

#### Polymerase chain reaction amplification of exon 3 region of AAT genes

Various annealing temperatures were tried for PCR amplification of AAT genes. Total volume of 25 µl for each sample was used to set up the PCR reactions. The set of primer was used to amplify target regions of AAT genes in Sahiwal and Karan Fries cattle. The best results were obtained when amplification was performed in PCR thermal cycler (Eppendorf Germany) programmed for 32 cycles with an initial denaturation at 94°C for 05 minutes, final denaturation at 94°C for 30 second, annealing at 62°C for 30 second and extension at 72°C for 30 second with a final extension at 72°C for 10 minutes.

#### Agarose gel electrophoresis of polymerase chain reaction product

The amplified product was checked for quality and quantity by agarose gel electrophoresis as described by Sambrook and Russell (2001).

Total 5 µl of amplified PCR product of each sample was mixed with 1 µl of 6X gel loading dye buffer from each tube. The samples were loaded on 2% agarose gel containing ethidium bromide (1% solution @ 5 µl/100 ml) 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 on agarose gel was visualized as a single compact band under UV transilluminator and documented by photograph through gel documentation system (Bio-Rad, USA) (Plate 1 and 2).

#### 4.5.13 Sequenced data analysis for SNPs detection

For custom sequencing ten amplicons of different sizes were sent to 1<sup>st</sup> BASE 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 Bio edit software. Amplified PCR products were sequenced and after Clustal W analysis ([www.ebi.ac.uk/tools/msa/clustalw](http://www.ebi.ac.uk/tools/msa/clustalw) 2) (Larkin *et al.*, 2007). Clustal W 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 (Fig 1 and 2).

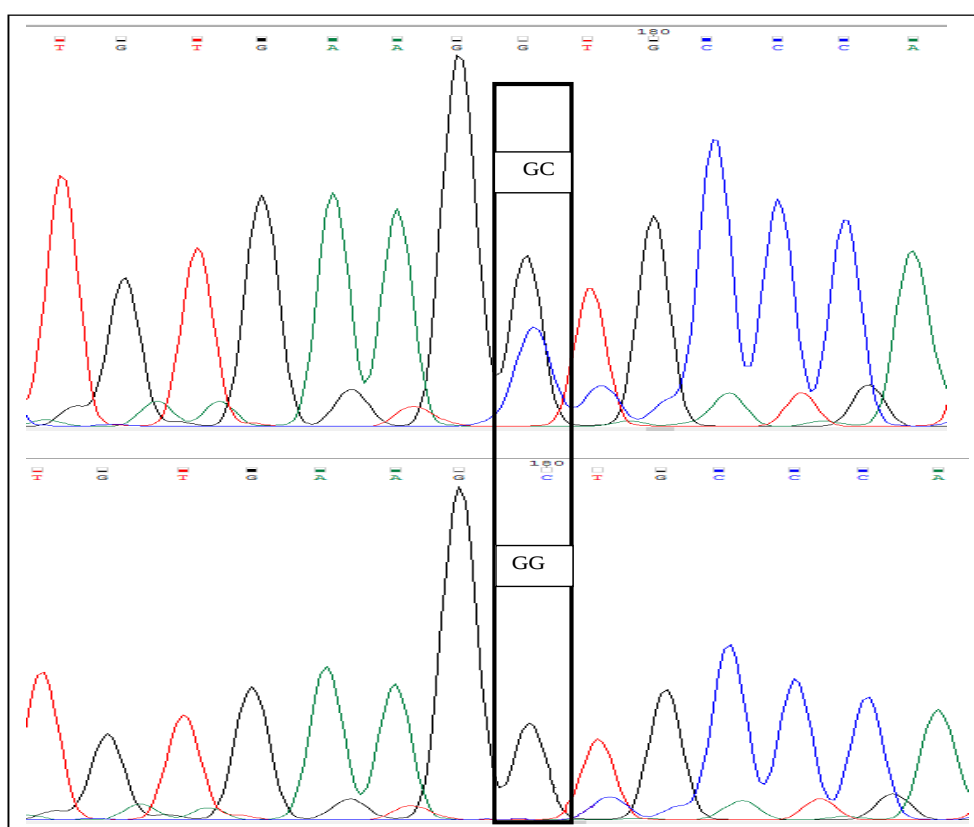
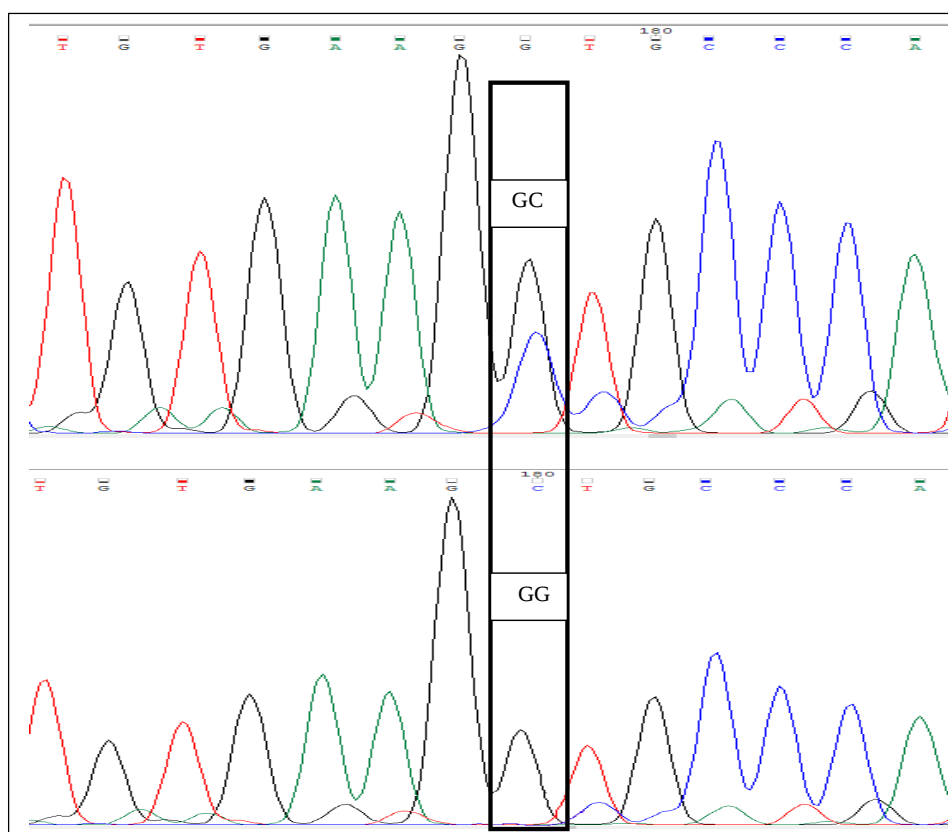


Fig 1: Chromatogram showing change at G6997C of AAT gene (474bp) in Sahiwal cattle



**Fig 2:** Chromatogram showing change at G6997C of AAT Gene (474 bp) in Karan Fries cattle.

**Table 2:** ANOVA for SNP G6997C in AAT Gene in Sahiwal.

| Source            | df | SS     | MSS    | F-value  |
|-------------------|----|--------|--------|----------|
| <b>FL305D MY</b>  |    |        |        |          |
| G6997C            | 1  | 308929 | 308929 | 163.76** |
| Error             | 98 | 184872 | 1886   |          |
| Total             | 99 | 493801 |        |          |
| <b>FLTMY</b>      |    |        |        |          |
| G6997C            | 1  | 192279 | 192279 | 59.62**  |
| Error             | 98 | 316076 | 3225   |          |
| Total             | 99 | 508355 |        |          |
| <b>FL305DFY</b>   |    |        |        |          |
| G6997C            | 1  | 0.01   | 0.01   | 0.00     |
| Error             | 98 | 857.08 | 8.74   |          |
| Total             | 99 | 857.09 |        |          |
| <b>FL305DSNFY</b> |    |        |        |          |
| G6997C            | 1  | 18.65  | 18.65  | 45.42**  |
| Error             | 98 | 40.24  | 0.41   |          |
| Total             | 99 | 58.89  |        |          |
| <b>FL305DPY</b>   |    |        |        |          |
| G6997C            | 1  | 0.68   | 0.68   | 1.46     |
| Error             | 98 | 46.26  | 0.47   |          |
| Total             | 99 | 46.95  |        |          |

\*\*Highly significant ( $p < 0.01$ ).

**Statistical analysis of PCR-RFLP data**

The analysis was carried out with appropriate statistical method using softwares 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:

$$Y_{ijk} = X b_i + Z u_j + e_{ijk}$$

Where:

$Y_{ijk}$  =  $k^{th}$  observation of  $j^{th}$  random effect of  $i^{th}$  fixed effect.

$b_i$  = Vector of observation of fixed effect.

$X$  = Incidence matrix of fixed effect.

$u_j$  = Vector of additive genetic effect (animal effect).

$Z$  = Incidence matrix of random effect.

$e_{ijk}$  = 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.

$$Y_{ijk} = a + b_1 \text{SNP}_i + b_2 \text{SNP}_j + \dots + b_n \text{SNP}_n + e_{ijk}$$

Where,

$Y_{ijk}$  = Adjusted observation on  $k^{th}$  animal of  $i^{th}$ ,  $j^{th}$ , ...,  $n^{th}$  SNPs.

$a$  = Intercept.

$b_1, \dots, b_n$  = Partial regression coefficient for SNPs considered.

$\text{SNP}_{i,j,\dots,n}$  = Effect of SNPs taken as independent variable.

$e_{ijk}$  = Random error NID (0,  $\sigma_e^2$ ).

**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

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

Where:

$Y_{ij}$  = Breeding value of  $j^{th}$  animal of  $i^{th}$  genotype.

$\mu$  = Overall mean.

$G_i$  = Effect of  $i^{th}$  genotypes (SNPs/ haplotypes).

$e_{ij}$  = Residual error NID (0,  $\sigma_e^2$ ).

**RESULTS AND DISCUSSION****Genetic variant (SNP) at G6997C at Exon 3 in AAT Gene (474bp) in Sahiwal**

In Sahiwal SNP at position G6997C was highly associated for FL305DMY, FLTMY, FL305DSNFY and non significant for FL305DFY and FL305DPY. The mean $\pm$ SE of GG genotype for FL305DMY, FLTMY, FL305DFY, FL305DSNFY and FL305DPY were found to be 1748.45 $\pm$ 6.47, 1962.30 $\pm$ 8.47, 100.33 $\pm$ 0.44, 154.4 $\pm$ 0.09 and 43.99 $\pm$ 0.10 respectively and for GC genotype 1860.17 $\pm$ 5.86, 2050.44 $\pm$ 7.66, 100.35 $\pm$ 0.39, 155.28 $\pm$ 0.08 and 43.82 $\pm$ 0.10 respectively in the present study. GG genotype was superior for FL305DPY and Heterozygous GC genotype was superior for rest of other traits (Table 2 and Table 3).

The result is in line with the findings of Li *et al.* (2010) in Chinese Holstein who reported significant effect of genotype on milk fat percentage, milk protein percentage and 305-day milk yield. They concluded that AAT is a potential candidate gene influencing milk production traits.

The result is also in agreement with Kheiripour *et al.* (2014) in Holstein dairy cows who reported that the cows of AB genotype had higher milk fat percentage than those of genotype AA. It was concluded that the association value could be implemented as a marker in breeding programmes for these traits.

The result is also in agreement with Yadav and Mukherjee (2019) in Sahiwal and Karan Fries Cattle where AB genotype was superior for FL305DMY, FLTMY, FL305DSNFY traits and AA genotype was superior for

**Table 3:** Least Square Mean and Standard Error for milk production traits.

| G6997C            | N  | Mean (Kg) $\pm$ SE              |
|-------------------|----|---------------------------------|
| <b>FL305DMY</b>   |    |                                 |
| GC                | 55 | 1860.17 $\pm$ 5.86 <sup>a</sup> |
| GG                | 45 | 1748.45 $\pm$ 6.47 <sup>b</sup> |
| <b>FLTMY</b>      |    |                                 |
| GC                | 55 | 2050.44 $\pm$ 7.66 <sup>a</sup> |
| GG                | 45 | 1962.30 $\pm$ 8.47 <sup>b</sup> |
| <b>FL305DFY</b>   |    |                                 |
| GC                | 55 | 100.35 $\pm$ 3.99               |
| GG                | 45 | 100.33 $\pm$ 4.41               |
| <b>FL305DSNFY</b> |    |                                 |
| GC                | 55 | 155.28 $\pm$ 8.60 <sup>a</sup>  |
| GG                | 45 | 154.41 $\pm$ 9.60 <sup>b</sup>  |
| <b>FL305DPY</b>   |    |                                 |
| GC                | 55 | 43.8 $\pm$ 9.26                 |
| GG                | 45 | 43.99 $\pm$ 10.20               |

FL305DFY and BB genotype was superior for FL305DPY trait.

#### 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:

$$\text{FL305DMY} = 1804.31 + 55.86 \text{ SNP\_GC} - 55.86 \text{ SNP\_GG} \quad (R^2=62.56)$$

$$\text{FLTMY} = 2006.37 + 44.07 \text{ SNP\_GC} - 44.07 \text{ SNP\_GG} \quad (R^2=37.82)$$

$$\text{FL305DFY} = 100.34 + 0.01 \text{ SNP\_GC} - 0.01 \text{ SNP\_GG} \quad (R^2=0.00)$$

$$\text{FL305DSNFY} = 154.84 + 0.43 \text{ SNP\_GC} - 0.43 \text{ SNP\_GG} \quad (R^2=31.67)$$

$$\text{FL305DPY} = 43.91 - 0.08 \text{ SNP\_GC} + 0.08 \text{ SNP\_GG} \quad (R^2=1.47)$$

**Table 4:** ANOVA for SNP G6997C of AAT Gene in Karan Fries.

| Source            | df  | SS        | MSS     | F-value  |
|-------------------|-----|-----------|---------|----------|
| <b>FL305D MY</b>  |     |           |         |          |
| G6997C            | 1   | 306260    | 306260  | 121.54** |
| Error             | 113 | 284752    | 2520    |          |
| Total             | 114 | 591011    |         |          |
| <b>FLTMY</b>      |     |           |         |          |
| G6997C            | 1   | 306260    | 306260  | 121.54** |
| Error             | 113 | 284752    | 2520    |          |
| Total             | 114 | 591011    |         |          |
| <b>FL305DFY</b>   |     |           |         |          |
| G6997C            | 1   | 36.00     | 36.00   | 0.02     |
| Error             | 113 | 178979.80 | 1583.89 |          |
| Total             | 114 | 179015.81 |         |          |
| <b>FL305DSNFY</b> |     |           |         |          |
| G6997C            | 1   | 28.92     | 28.92   | 116.57** |
| Error             | 113 | 28.03     | 0.24    |          |
| Total             | 114 | 56.95     |         |          |
| <b>FL305DPY</b>   |     |           |         |          |
| G6997C            | 1   | 30.01     | 30.01   | 106.12** |
| Error             | 113 | 31.95     | 0.28    |          |
| Total             | 114 | 61.97     |         |          |

\*\*Highly significant ( $p < 0.01$ )

**Table 5:** Least square mean and standard error for milk production traits.

| G6997C            | N  | Mean (Kg)± SE              |
|-------------------|----|----------------------------|
| <b>FL305 DMY</b>  |    |                            |
| GC                | 45 | 3459.00± 7.48 <sup>a</sup> |
| GG                | 70 | 3564.74± 6.00 <sup>b</sup> |
| <b>FLTMY</b>      |    |                            |
| GC                | 45 | 4478.76± 7.40 <sup>a</sup> |
| GG                | 70 | 4584.50± 6.00 <sup>b</sup> |
| <b>FL305DFY</b>   |    |                            |
| GC                | 45 | 136.19± 5.90               |
| GG                | 70 | 135.05± 4.80               |
| <b>FL305DSNFY</b> |    |                            |
| GC                | 45 | 277.50± 7.40 <sup>a</sup>  |
| GG                | 70 | 278.53± 6.00 <sup>b</sup>  |
| <b>FL305DPY</b>   |    |                            |
| GC                | 45 | 112.75± 7.90 <sup>a</sup>  |
| GG                | 70 | 113.80± 6.40 <sup>b</sup>  |

### Genetic variant (SNP) at G6997C at Exon 3 of AAT Gene (474 bp) in Karan Fries

SNP at position G6997C was highly associated for FL305DMY, FLTMY, FL305DSNFY and FL305DPY and non significant for FL305DFY. The mean $\pm$  SE of GG genotype for FL305DMY, FLTMY, FL305DFY, FL305DSNFY, FL305DPY were found to be 3564.74 $\pm$ 6.0, 4584.50 $\pm$ 6.00, 135.05 $\pm$ 4.76, 278.53 $\pm$ 0.06 and 113.80 $\pm$ 0.06 respectively and for GC genotypes were 3459.23 $\pm$ 7.48, 4478.76 $\pm$ 7.48, 136.19 $\pm$ 5.96, 277.50 $\pm$ 0.07, 112.75 $\pm$ 0.08 respectively in the present study. Heterozygous (GC) genotype was superior for FL305DFY and GG genotype was superior for FL305DMY, FLTDMY, FL305DSNFY and FL305DPY (Table 4 and Table 5).

The result is in line with the findings of Li *et al.* (2010) in Chinese Holstein who reported significant effect of genotype on milk fat percentage, milk protein percentage and 305-day milk yield. They concluded that AAT is a potential candidate gene influencing milk production traits.

The result is also in agreement with Kheiripour *et al.* (2014) in Holstein dairy cows who reported that the cows of AB genotype had higher milk fat percentage than those of genotype AA. It was concluded that the association value could be implemented as a marker in breeding programmes for these traits.

The result is also in agreement with Yadav and Mukherjee (2019) in Sahiwal and Karan Fries Cattle where AB genotype was superior for FL305DMY, FLTDMY, FL305DSNFY traits and AA genotype was superior for FL305DFY and BB genotype was superior for FL305DPY trait.

### 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:

$$\text{FL305DMY} = 3511.87 - 52.87 \text{ SNP\_GC} + 52.87 \text{ SNP\_GG} (R^2 = 51.82)$$

$$\text{FLTMY} = 4531.63 - 52.87 \text{ SNP\_GC} + 52.87 \text{ SNP\_GG} (R^2 = 51.82)$$

$$\text{FL305DFY} = 135.62 + 0.57 \text{ SNP\_GC} - 0.57 \text{ SNP\_GG} (R^2 = 0.02)$$

$$\text{FL305DSNFY} = 278.02 - 0.51 \text{ SNP\_GC} + 0.51 \text{ SNP\_GG} (R^2 = 50.78)$$

$$\text{FLDPY} = 113.28 - 0.52 \text{ SNP\_GC} + 0.52 \text{ SNP\_GG} (R^2 = 48.43)$$

### SUMMARY AND CONCLUSION

In Sahiwal SNP at position G6997C is highly significant for FL305DMY, FLTMY, FL305DSNFY and non significant for FL305 DFY and FL305DPY. The mean $\pm$ SE of GG genotype for FL305DMY, FLTDMY, FL305DFY, FL305DSNFY, FL305DPY were 1748.45 $\pm$ 6.47, 1962.30 $\pm$ 8.47, 100.33 $\pm$ 0.44, 154.41 $\pm$ 0.09 and 43.99 $\pm$ 0.10 respectively and for GC

genotype were 1860.17 $\pm$ 5.86, 2050.44 $\pm$ 7.66, 100.35 $\pm$ 0.39, 155.28 $\pm$ 0.08 and 43.82 $\pm$ 0.10 respectively. GG genotype was superior for FL305DPY and GC genotype was superior for all other traits.

In Karan Fries SNP at position G6997C was highly significant for FL305DMY, FLTMY, FL305DSNFY and FL305DPY and non significant for FL305 DFY. The mean $\pm$  SE of GG genotype for FL305DMY, FLTDMY, FL305DFY, FL305DSNFY and FL305DPY were 3564.74 $\pm$ 6.0, 4584.50 $\pm$ 6.00, 135.05 $\pm$ 4.76, 278.53 $\pm$ 0.06 and 113.80 $\pm$ 0.06 respectively and for GC genotype were 3459.23 $\pm$ 7.48, 4478.76 $\pm$ 7.48, 136.19 $\pm$ 5.96, 277.50 $\pm$ 0.07, 112.75 $\pm$ 0.08 respectively. GC genotype was superior for FL305DFY and GG genotype was superior for FL305DMY, FLTDMY, FL305DSNFY and FL305DPY traits.

The result obtained for the study conducted on Sahiwal and Karan fries cattle reveals that heterozygous genotype of AAT genes can be applied as a potential genetic marker for milk production traits after validation on large population.

### Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

### REFERENCES

- BAHS (2019). Basic Animal Husbandry Statistics. Department of Animal Husbandry, Dairying and Fisheries. Ministry of Agriculture, Government of India.
- Carrell, R.W., Jeppsson, J.O., Laurell, C.B., Brennan, S.O., Owen, M.C., Vaughan, L., Boswell, D.R. (1982). Structure and variation of human alpha-1-antitrypsin. *Nature*, 298: 329-334.
- Kheiripour, M.H., Mahdavi, A.H., Rahmani, H.R., Soltani-Ghombavani, M., Edriss, M.A. (2014). Association of polymorphism in the alpha-1-antitrypsin gene with milk production traits in Holstein dairy cows. *South African Journal of Animal Science*. 44(2): 155-160.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F. and Wallace, I.M. (2007). Clustal W and Clustal X version 2.0. *Bioinform*, 23: 2947-2948.
- Li, Q.L., Zhang, Z.F., Wang, C.F., Yang, H., Wang, H.M., Li, J.B., Huang, J.M., Zhong, J.F. (2010). Association of polymorphism of the alpha 1 antitrypsin gene with milk production traits in Chinese Holstein. *South African Journal of Animal Science*. 40: 113-120.
- Livestock Census, (2019). 20<sup>th</sup> All India Livestock Census. Department of Animal husbandry, Dairying and Fisheries. Ministry of agriculture. Government of India.
- Meyer, K. (2010). WOMBAT-A Tool for Mixed Model Analyses in Quantitative Genetics by Restricted Maximum Likelihood (REML). *Journal of Zhejiang University Science*. 8(11). 815-821.
- Nivsarkar, A.E., Vij, P.K., Tandia, M.S. (2000). *Animal Genetic Resources of India: Cattle and Buffalo*. ICAR publication, New Delhi.
- Reinhardt, T.A., Lippolis, J.D., Nonnecke, B.J., Sacco, R.E. (2012). Bovine milk exosome proteome. *Journal of Proteomics*. 75(5): 1486-92.

- Sambrook, J., Russell, D.W. (2001). Molecular cloning. A laboratory manual 3<sup>rd</sup> Edn. Cold Spring Harbor Laboratory Press, New York.
- Reinhardt, T.A., Lippolis, J.D., Nonnecke, B.J., Sacco, R.E. (2012). Bovine milk exosome proteome. *Journal of Proteomics*. 75(5): 1486-1492.
- Untergrasser, A., Cutcutache, I., Koressaar, T.Y.J., Faircloth, B.C. (2012). Primer 3 new capabilities and interfaces. *Nucleic Acids Research*. 40(15): 115-117.
- Yadav, A. K. and Mukherjee, A. (2019). Association of polymorphism at Exon 1 of the alpha 1 antitrypsin gene with milk production traits in Sahiwal and Karan Fries Cattle. *Indian Journal of Dairy Science*. 72(2): 186-193.