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Identification of genetic marker for CSN3 gene in Karan Fries (Holstein Friesian crossbred) population

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ABSTRACT

The study was conducted to identify genetic marker for CSN3 gene related to higher casein yield in 78 pedigreed Karan Fries (HF crossbred) cattle population at ICAR-NDRI, Karnal. PCR-RFLP of exon 4 region of CSN3 gene, carried out using *HindIII* restriction endonuclease, revealed three genotypes—CC, CA and AA with frequencies as 0.18, 0.67 and 0.15 and frequency of C and A allele as 0.51 and 0.49. Eight SNPs (C296A, C380T, C383T, G394T, C419A, G480A, A488T and G529T) were revealed through sequencing. Using Regression model, SNP (C419A) in the targeted region was found to contribute an increase of 1.8 g in κ-casein yield (CY) which was 2.81% of CY (63.62 g) in average test day milk yield (ATDMY). After screening progenies for SNPs based on Daughter-Dam Design, SNP G480A was found to be a potential genetic marker, contributing an increase of 7.5 g in CY (11.79% of CY in ATDMY).

Key words: Casein yield, CSN3 gene, Genetic marker, Holstein Friesian, Karan Fries

Traditionally, buffalo milk has been the main source of cheese production in India. The crossbred/exotic animals which comprise only 21% of the total cattle population but still contribute as much as 52.17% in the total milk production from cattle, present a unique opportunity to diversify our source of milk for cheese production. Many countries are using cattle milk as the main source for producing cheese. About 80% of the milk proteins component is contributed by Casein and it comprises α-, β–, γ- and κ-casein. κ-casein (CSN3 gene) constitutes approximately 13% of milk casein (Farrell *et al*. 2004). The integration of molecular genetics approach in conventional selection methods could help to improve protein component in the milk.

Many countries have adopted the use of genomic information in their genetic evaluations of dairy animals. Canada collaborated with the United States in developing genomic evaluations based on Bovine SNP50 genotypes and released official genomic evaluations in 2009. New Zealand, Australia, Germany, Italy and Switzerland also implemented a genomic evaluation system by 2011 (Wiggans *et al.* 2011).

Marker assisted selection (MAS) is a strategy to improve the accuracy of selection and genetic evaluation by combining the genetic information at markers with the phenotypic information thereby, reducing the generation interval. During the last few decades, MAS programmes related to milk protein genes, particularly CSN3 and LGB genes, have attracted the dairy industry (Neamt *et al*. 2017). The present study was undertaken with the objective to identify the genetic markers for CSN3 gene related to higher casein yield in pedigreed Karan Fries (HF Crossbred cattle developed by crossing Holstein Friesian males and Tharparkar females with 50–75% exotic level of inheritance and maintained by inter-se mating) population so that the information could be further used to develop MAS strategy for selection of young Karan Fries bulls to improve casein yield in milk.

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MATERIALS AND METHODS

Sample collection: The study was conducted by using the blood samples of 78 pedigreed Karan Fries (HF Crossbred) cattle (22 male calves, 17 female calves and 39 dams), maintained at Livestock Research Centre (LRC) of ICAR-National Dairy Research Institute, Karnal. The geographical location of the farm is at an altitude of 250 metres above the mean sea level in the Indo-Gangetic alluvial plains on 29°42′ N latitude and 72°02′ E longitude. The climate of the farm is subtropical in nature.

DNA isolation and quality checking: Phenol-chloroform method, as suggested by Sambrook and Russel (2001) with minor modifications was used for DNA isolation from blood of Karan Fries (HF Crossbred) cattle. The quality and the quantity of DNA was assessed using agarose gel electrophoresis and nanodrop spectrophotometer, respectively.

Primer designing: The targeted region of CSN3 gene was exon 4 and the primers, both forward (P1) and reverse (P2) covering complete targeted region were designed using primer3 software. The primers designed were checked for specificity by BLAST programme. The sequence of primers and their nucleotide numbers were as follows: F-CAGCGCTGTGAGAAAGATGA (20) and R-CCCA-TTTCGCCTTCTCTGTA (20).

PCR amplification of targeted region: Targeted region of CSN3 gene was amplified by standardizing PCR program with the help of thermal cycler. The reaction mixture for PCR comprised of 2.5 µl of 10× buffer, 0.5 µl each of dNTPs, forward and reverse primer, 0.25 µl of Taq Polymerase, 18.75 µl of distilled water and 2 µl of template DNA. The final volume was made to be 25 µl. The amplified product was checked using agarose gel electrophoresis using 1.7% agarose.

DNA sequencing of samples: The dams and progenies of Karan Fries (HF Crossbred) cattle were screened for presence of SNPs by sequencing of samples. DNA sequencing results for the respective region of bovine CSN3 gene were visualized and edited using BioEdit software. Each edited sequence was aligned with corresponding reference sequence using ClustalW multiple sequence alignment program for DNA (Larkin *et al*. 2007) to identify SNPs.

Restriction fragment length polymorphism: The amplified PCR product was subjected to Restriction Fragment Length Polymorphism (RFLP) with a selected restriction endonuclease enzyme to generate a unique restriction polymorphic profile. To search the restriction enzyme site for typing SNPs and develop PCR-RFLP test, two bioinformatics software were utilized, viz. NEB cutter and cleaver. The 633 bp amplified product was digested with *HindIII*. The restricted PCR products were checked on 2.5% agarose gel. The agarose gels were photographed in the gel documentation system under the UV light and were then scored for their respective genotypes.

Estimated genotype and gene frequency: The frequency of genotype and gene were calculated by Gene Counting method, as suggested by Falconer and Mackay (1996).

Screening for genetic marker: The animals were screened for the genetic marker following Daughter-Dam Design. The inheritance of marker was evaluated, considering each family.

Phenotypic traits of Karan Fries cattle: The phenotypic data of Karan Fries animals', viz. first and second lactation 305 day milk yield (kg) and total milk yield (kg) were collected from the Livestock Record Cell of Animal Genetics and Breeding Division. The data regarding ten monthly test day protein percentage of first and second lactation were collected from the record room of Livestock Production and Management Section at ICAR-NDRI, Karnal. The test day protein yield (kg) and κ-casein yield was generated for both first and second lactation.

Effect of SNPs on κ-casein yield: Each trait was normalized using mean and standard deviation of the traits estimated by standard statistical procedures (Snedecor and Cochran 1994). The effect of genetic marker (SNP) of CSN3 gene (exon 4) on test day κ-casein yield was assessed with the help of following model.

$$Y_{ij} = a + b_1SNP_1 + e_{ij}$$

where Y_{ij}, test day κ-casein yield; a, intercept; b_1, regression coefficients for SNPs; SNP_1, effect of SNP_1 and e_{ij}, random residual, NID (0, σ^2_e) as suggested by Wang *et al*. (2011).

RESULTS AND DISCUSSION

Polymerase chain reaction revealed amplified product of 633 bp fragment of exon 4 of CSN3 gene at the annealing temperature of 58.2°C for 40 sec.

Eight SNPs-C296A, C380T, C383T, G394T, C419A, G480A, A488T and G529T were revealed in the targeted region on sequencing.

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for 633 bp fragment of CSN3 gene (exon 4) using *HindIII* digestion was designed to screen all Karan Fries animals for genotyping. Three genotypes namely, CC, CA and AA were identified in the 633 bp fragment. The genotype CC had 633 bp fragment, the genotype CA had 633, 423 and 210 bp fragments whereas the genotype AA had 423 and 210 bp fragments.The chromatogram of the targeted region also revealed the same.

The frequency of C and A allele was 0.51 and 0.49 whereas the frequencies of CC, CA and AA genotypes were estimated as 0.18, 0.67 and 0.15.

Deb *et al*. (2014) carried out PCR-RFLP of the same region with *Hinf1* enzyme in Frieswal cattle and reported two genotypes AA and AB with frequency of A and B allele as 0.58 and 0.42, respectively. Awad *et al*. (2016) also found two genotypes- AA (633 bp) and AB (633, 416 and 217 bp) on PCR-RFLP with *HindIII* in exon 4 region of CSN3 gene in Holstein Friesian cattle with frequency of A and B allele as 0.80 and 0.20, respectively.

Eleven families of Karan Fries cattle were analysed for the identification of genetic markers for CSN3 gene after sequencing for test day κ-casein yield. The genotypes of dams, males and female progenies as well as the probable genotypes of sires were identified. It was found that SNP G480A was present in all of the heterozygous individuals and may be considered as a potential genetic marker. The pedigree wise inheritance of SNPs in male and female families has been illustrated in Figs 1 and 2, respectively.

The average test day milk yield (ATDMY) of Karan Fries animals was 12.69±0.49 kg. The average test day κ-casein yield (ATDCY) was 63.62±2.90 g with a C.V. of 21.88%. The maximum test day κ-casein yield was observed on TD2 (74.93±4.97 g) with a C.V. of 28.12% and the minimum test day κ-casein yield was observed on TD8 (56.66±5.78 g) and TD10 (56.98±3.89 g) with a C.V. of 32.25% and 19.29% respectively. Though the literature on test day protein yield are available, the literature on test day κ-casein yield is not available in Karan Fries (KF) and HF crossbred cattle. Tripathy (2015) found average test day protein yield

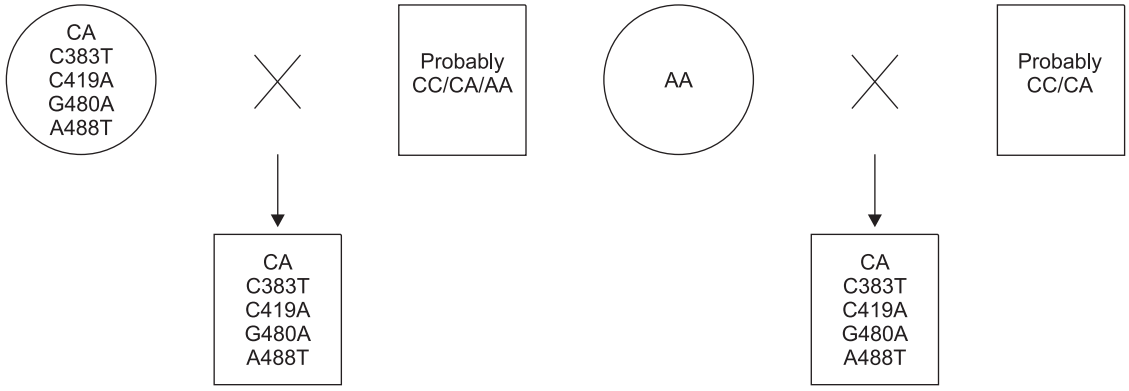

Fig. 1. Inheritance of SNPs of CSN3 gene under Daughter Dam Design (Male families)

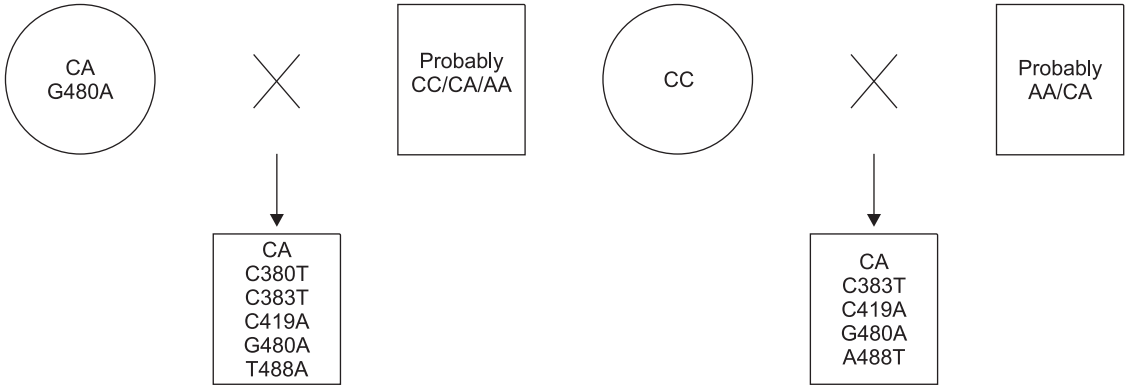

Fig. 2. Inheritance of SNPs of CSN3 gene under Daughter Dam Design (Female families).

in KF cattle as 421.34±7.30 g.

The effect of change of SNP (C419A) of CSN3 gene (exon 4) on test day κ-casein yield (TDCY) in Karan Fries cattle was assessed using the regression analysis, as presented in Table 1. On an average, the change of SNP increased the TDCY by about 1.8 g which is about 2.81% of the ATDCY (63.62 g) in Karan Fries cattle. The perusal of Table 1 further shows that the highest effect of SNPs on TDCY was observed in TD8 (b=0.0193) which indicated that, on an average, there was 19.3 g increase in TD8CY which was 34.06% of the average TD8CY (56.66 g).

Table 1. Effect of SNP of CSN3 gene (exon 4) on test day κ-casein yield of pooled first and second lactations in Karan Fries cattle

Test day	Intercept (a)	Regression coefficients (b)	R^2 (%)
TD1	0.0781	–0.0080	3.8132
TD2	0.0789	–0.0039	0.8358
TD3	0.0611	0.0119	7.0439
TD4	0.0599	0.0060	3.6377
TD5	0.0868	–0.0181	15.5582
TD6	0.0561	0.0031	1.4557
TD7	0.0489	0.0137	22.8607
TD8	0.0373	0.0193	24.8161
TD9	0.0516	0.0073	2.4035
TD10	0.0673	–0.0082	12.0159

In order to find the early test days for selection of animals for higher κ-casein yield, the correlation analysis was carried out between test day κ-casein yield and lactation milk yield (LMY) of pooled first and second lactations in Karan Fries (HF Crossbred) cattle. The correlation between TD6CY and LMY (r= 0.75) and TD3CY and LMY (r=0.50) was high. This indicates that KF animals can be selected at an early age based on TD3MY for higher casein yield.

Twenty two Karan Fries animals (eleven families) under Daughter Dam Design were screened and it was observed that one SNP (G480A) was present in all of the six families having heterozygous genotype (CA). The genetic marker, G480A was quantified for its effect on average test day κ-casein yield by regression analysis. The SNP (G480A) contributed an increase of 7.5 g in κ-casein yield which was around 11.79% of κ-casein yield (63.62 g) in average test day milk. Thus, it could be considered as the most important genetic marker.

The study identified the genetic marker of CSN3 gene in relation to monthly test day κ-casein yield in pedigreed Karan Fries cattle. PCR-RFLP method using *HindIII* restriction endonuclease revealed three genotypes– CC, CA and AA in the population with the frequency of C and A

allele as 0.51 and 0.49 and genotype frequencies were 0.18, 0.67 and 0.15, respectively. The effect of SNP of CSN3 gene (exon 4) increased the average test day κ-casein yield by 1.8 g. High association between test day κ-casein yield and average lactation milk yield was observed on TD3 and TD6, indicating that the early selection of animals for higher casein yield can be done on these test days. The progenies were screened for the presence of SNPs based on Daughter-Dam Design. SNP G480A was present in all of the six families having heterozygous genotype (CA) and it contributed an increase of 7.5 g in κ-casein yield which was around 11.79% of κ-casein yield (63.62 g) in average test day milk. It could be further explored as a potential genetic marker for developing marker assisted selection strategy for improving κ-casein yield in Karan Fries cattle.

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