

## CRH Gene Polymorphism Associated with Milk and Its Constituents in Karan Fries Cattle

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### ABSTRACT

The aim of this study was to estimate the relations between the CRH-A787G polymorphism and milk production traits ( first lactation 305 days milk yield, first lactation total milk yield, first lactation 305 days protein and first lactation fat yield , as well as first lactation 305 days lactose and first lactation 305 days SNF yield) in 60 Karan Fries cows. The genotype and allele frequencies were estimated and they were as follows: AG – 0.63; AA – 0.37; A – 0.69; G – 0.31. Statistical analysis revealed that studied polymorphism significantly affected the first lactation 305 days milk yield, total milk yield, 305 days fat and SNF yield ( $P \leq 0.01$ ). The results indicate that selection for the CRH-A787G, AG animals might contribute to increase the value of these traits in Karan Fries cattle. However, further studies are necessary to verify the results of our study.

**Key words:** Karan Fries cattle, Milk Yield, Milk Constituents traits.

### INTRODUCTION

Agriculture is the main stay of Indian economy as agriculture and allied sectors contribute nearly 14 % of Gross Domestic Production. Livestock sector alone contributes nearly 25.6% of value of output in Agriculture, Fishing and Forestry sector<sup>1</sup>. About 58% of population is engaged in agriculture and rearing of livestock in the country. As per All India Livestock Census, 2012. India has 190.90 million (37.28%) cattle and 108.7 million (21.23%) buffaloes. Out of total cattle population, about 39.73 million are crossbred in our country. The decade wise trend in livestock population (1997 to 2012) shows a distinct shift in composition of dairy animal

stock in favor of buffaloes and crossbred cattle, as their numbers increased by 3.19% and 20.18%, respectively, while that of indigenous cattle declined by 8.94% (19<sup>th</sup> Livestock Census). Milk productivity in the country remains one of the lowest as compared to many leading countries of the world. In India, average milk productivity of crossbred cows, indigenous cows and buffaloes is about 7.02, 2.36 and 4.8 kg/day, respectively (Ministry of Agriculture, GOI, 2013-14). India has always been 100% self sufficient in milk, with total imports/exports of about 0.3 million tonnes per annum and thus it may be considered as almost unconnected with the world dairy market.

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The economic survey 2011 analyzed the dairy situation in India, considering that the requirement of milk in 2021 to 2022 is expected to be 180 million tones as against the current level of milk production of 137.7 million tonnes (BAHS,2015).

One of the major constrains in genetic progress of dairy animal is that milk production traits are controlled by several genes which are expressed later in life. Recent advances of molecular genetics in identification of QTLs affecting production traits of domestic animals have opened new vistas for genetic improvement of economic traits. . It is noteworthy that QTLs for milk performance traits have been mapped to all the bovine autosomes, mostly to autosome 6, 14 and 20<sup>9</sup>. Moreover, mutations in candidate genes for the above-mentioned traits are identified and analyzed<sup>7,12,13</sup>. The positional and functional candidate gene approach have been applied to different genes in dairy animals .GH, DGAT1, SCD1, PRL, STAT5A, OLR1, LEP, LGB, ABCG2, CSN3 are some of the important candidate genes for milk production traits. First positional clone of QTL in cattle was done for DGAT1 that is associated with fat yield<sup>5</sup>.

Corticotropin-releasing hormone (CRH), also called corticoliberin or corticotropin-releasing factor (CRF), is a 41-amino acid peptide deriving from a 191-amino acid precursor. It is synthesized mainly in the hypothalamus, but also in other brain areas. The highest level of the *CRH* gene expression was found in the hypothalamus, but this gene is also expressed in many other places, such as placenta, uterus, ovaries, testes, liver, stomach, skin, immune system. CRH functions as a neuropeptide hormone participating, among others, in the stress response in vertebrates. Corticotropin-releasing hormone is also involved in controlling the energy balance of an organism, and thus can affect body weight. In addition, it participates in modulating immune and reproductive systems<sup>15</sup>. The gene encoding corticoliberin has been mapped to the bovine chromosome 14<sup>2</sup>, where QTLs for the postnatal growth have also been identified.

Therefore, it has been considered a candidate gene for growth traits in cattle<sup>3</sup>. Taking into account the position of this gene, in the proximity of the QTL for milk performance traits, it can be considered a positional candidate gene for the abovementioned traits. Within the bovine *CRH* gene, several single nucleotide polymorphic (SNP) sites have been identified: *C22G* causing substitution of amino acids in the signal sequence, *A145G* and *C240G*, leading to changes of amino acids in a propeptide<sup>3</sup> as well as two SNPs in exon 2<sup>14</sup>. The undertaken study aimed at determining the frequencies of alleles and genotypes with regard to the polymorphism in the gene encoding corticotropin-releasing hormone (*CRH-A787G*) and establishing association between the genotypes and some milk production traits of Karan Fries cows.

#### MATERIALAND METHODS

The study involved a total of 60 Karan Fries cows from the herd kept in the Livestock Research Center (LRC) of ICAR-National Dairy Research Institute, Karnal (Haryana), India. DNA isolation was done by collecting 10 ml blood aseptically by jugular vein puncture in a sterile Vacutainer (Beckton-Dickinson vacutainer containing 0.5% EDTA solution (10µl/ml of blood).The samples were transported to the laboratory in an ice box and stored at -20°C till further processing for DNA isolation. DNA Extraction from blood was done by Phenol chloroform method ,as described by Sambrook and Russel with minor modifications was used for DNA isolation from blood of Karan Fries Cattle .

In investigated SNP adenine to guanine at position 787 in exon 1 – *CRH-A787G* has been observed. DNA sequencing (First Base Laboratories) was used for genotyping and the sequenced data was analysed by BLAST (version 1.2.0) and Clustal W. A set of forward and reverse gene – specific oligonucleotide primer was designed to amplify CRH gene 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

primer designed was checked for specificity by BLAST (version 1.2.0). Primer was designed and synthesized from Sigma Aldrich Chemicals Pvt. Ltd (USA). The sequence of primer from 5' to 3' end and its length is 353 bp respectively was designed.

F 5'-CTGCGTGGTTTCTGAAGAGG-3'

R 3'CGCGTTCACACACAAACAC-5'

The gene fragment of 353 bp was amplified. The amplification reaction thermal program was as follows: initial denaturation at 95°C for 3 min, followed by 30 cycles: 94°C for 30 s (denaturation), 55°C for 30 s (primer hybridization), 72°C for 30 sec (product synthesis), and final extension at 72°C for 10 min. After PCR amplification, the PCR product was checked on 0.8% agarose gel to verify the amplification of target region.

At the end of run, power supply was turned off and the gel was carefully removed from the chamber and placed on trans illuminator for visualization of Gel and viewed under UV light and photographs were taken with the help of gel documentation system. A single sharp band indicated proper amplification of the target DNA.

The amplified PCR products from the set of primer was sent for DNA sequencing to 1<sup>st</sup> Base sequencing INT (Singapore) for purification and custom sequencing from both ends (5' and 3' ends). DNA sequence was aligned with corresponding reference sequence using Clustal W multiple sequence alignment program for DNA

(www.ebi.ac.uk/tools/msa/clustalw2)<sup>10</sup> to identify SNPs.

The data on various economic traits during last 8 years (2007-2014) were collected from the history-cum pedigree sheet and milk constituents register maintained under AGB division and LPM Section, NDRI, Karnal

The following model will be used for all lactation traits:

$$Y_{ijklm} = \mu + P_i + S_j + A_k + e_{ijklm}$$

Where,

$Y_{ijklm}$  =  $m^{\text{th}}$  observation in  $i^{\text{th}}$  period,  $j^{\text{th}}$  season,  $k^{\text{th}}$  age at first calving

$\mu$  = Overall mean

$P_i$  = Effect of  $i^{\text{th}}$  period ( $i = 1$  to 3)

$S_j$  = Effect of  $j^{\text{th}}$  season ( $j = 1$  to 4)

$A_k$  = Effect of  $k^{\text{th}}$  age at first calving ( $k = 1$  to 5)

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

(Haryana), India respectively. The following information was collected: Animal number, Date of birth, Sire number, Dam number, Date of calving, Lactation number. Traits considered (Parity, Period of calving, Season of calving, first lactation 305 days milk yield, first lactation total lactation milk yield, first lactation test day fat yield, fat content (%), first lactation test day protein yield, protein content(%), first lactation test day lactose yield, first lactation test day SNF yield), traits generated (first lactation 305 day fat yield, first lactation 305 day protein yield, first lactation 305 day lactose yield, first lactation 305 day SNF yield, age at first calving).

As a part of the characterization of the genetic structure of examined herd, the frequencies

of the *CRH-A787G* genotypes and alleles were estimated and 2 out of the 3 possible genotypes were identified. The next stage was the analysis of the association between genotypes and the values of the following milk production traits in the first lactation: first lactation 305 days milk yield, first lactation total lactation milk yield, first lactation 305 day fat yield, first lactation 305 day protein yield, first lactation 305 day lactose yield, first lactation 305 day SNF yield (kg). The production records of the animals were collected from the breeding documentation carried out for herd as a part of milk recording. Statistical analysis of the data was done by Least squares analysis of variance for unequal and non-orthogonal data using the technique described by Harvey<sup>6</sup> will be used to study effect of non-genetic factors. The model will be used with assumptions that different components being fitted into the model are linear, independent and additive.

For association Analysis mixed effect model of multifactor analysis of variance (ANOVA), using the GLM (General Linear Model) procedure was used which was based on the available records pertaining to milk yield and its constituents on Karan Fries cattle

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

Where,

$$Y_{ijk} = \text{Observation on } k^{\text{th}} \text{ animal of } i^{\text{th}}, j^{\text{th}}, \dots, n^{\text{th}} \text{ SNP}$$

$a$  = intercept

$b_{i..n}$  = Partial regression coefficient for the SNP considered

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

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

Allelic and genotypic frequencies and their accordance with or deviation from Hardy-Weinberg law will be studied using POPGENE software package. Effect of SNP, found significantly contributing towards milk yield and constituents, will be analyzed as under:

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

Where,

$Y_{ij}$  = Observation on  $j^{\text{th}}$  animal having  $i^{\text{th}}$  genotype

$\mu$  = Overall mean

$G_i$  = Fixed effect of  $i^{\text{th}}$  genotype

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

## RESULTS AND DISCUSSION

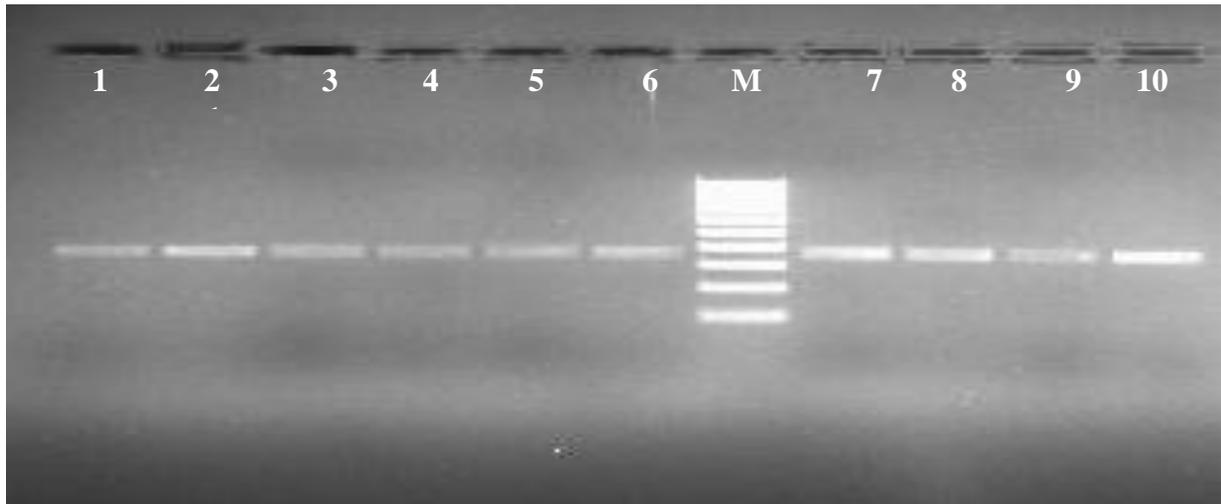
DNA sequencing analysis of the amplified fragment of the *CRH* gene enabled distinguishing two genotypes: *AG* and *GG*, determined by the presence of alleles and genotype amounted to: *AA* – 0.37; *AG* – 0.63; *A* – 0.69; *G* – 0.39. In the Table 01 and 02 shows effect of genetic and non genetic factors on milk production traits in the First lactation in Karan Fries cattle. The results of the study showed statistically significant differences in the milk production traits for different genotypes and period of calving (First lactation 305 days milk yield, First lactation total lactation milk yield, First lactation 305 day fat yield, First lactation 305 day SNF yield for genotypes, First lactation 305 day protein yield, First lactation 305 day lactose yield). Cows with the *AG* genotype were characterized by significantly higher values for the above-mentioned traits compared with the *AA* cows.

maintained at National Dairy Research Institute, Karnal (Haryana), India an attempt will be made to find the association of different allelic variants of *CRH* gene with the milk and its constituents.

The approach aimed at identifying mutations in candidate genes facilitates discovering and locating major-effect genes (in particular functional mutations within these genes) for quantitative traits. The candidate genes strategy is used for various genes whose products may affect production traits of cattle. In dairy cattle, genes encoding, among others, milk proteins (e.g.  $\alpha$ S1-casein), enzymes involved in the metabolism of fatty acids (e.g. SCD, DGAT1), some hormones (e.g. leptin, growth hormone) or transcription factors (e.g. PIT-1) are intensively analyzed. Significant associations between polymorphisms in these genes and milk performance traits have been found<sup>4,8,11,17</sup>.

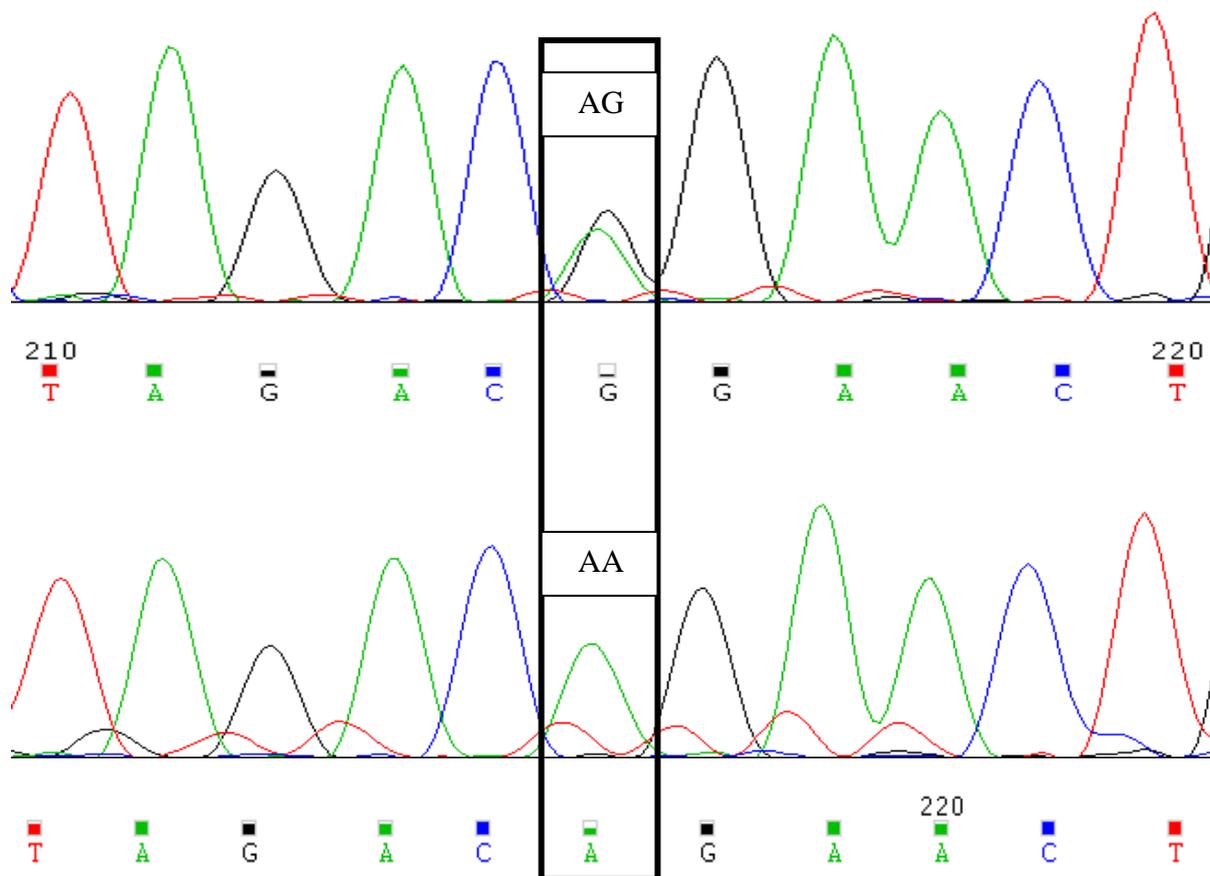
The results of the conducted study indicate that the polymorphism within the corticotropin-releasing hormone gene (*CRH-A787G*) is associated with an increased first lactation 305 days milk yield, first lactation total lactation milk yield, first lactation 305 day fat yield, first lactation 305 day SNF yield in Karan Fries cattle. However, due to the lack of accessible literature data that would concern the analysis of the association between the above-mentioned polymorphism and milk production traits of cattle, the verification of the obtained results is impossible. The research on the association between the *CRH* gene polymorphism and production traits in cattle has only been conducted in beef cattle herds. Three polymorphisms within the *CRH* gene have been analyzed in association with meat related traits<sup>3</sup>.

**Fig 01:** Gel Electrophoresis with 2 % agarose and stained with ethidium bromide showing the PCR amplification of exon 1 region using primer 1 for CRH gene in Karan Fries cattle



Lane1-10 : PCR product ( 353 bp )

Lane M : 100 bp ladder



**Fig 02:** Chromatogram showing change of A787G in Karan Fries cattle

**Table 1: Least square mean and standard error of milk production traits in Karan Fries cattle**

FACTORS	N	FL305DMY	FLTMY	FL305DFY	FL305DPY	FL305DLY	FL305DSNFY
<b>Season of calving</b>							
Dec - Mar	27	3590.99 ± 97.31	5298.34 ± 426.87	155.16 ± 4.05	86.94 ± 10.21	132.13 ± 15.22	299.35 ± 9.19
April - June	21	3432.77 ± 106.81	4479.86 ± 468.56	148.08 ± 4.45	105.51 ± 15.80	161.01 ± 23.55	310.26 ± 14.23
July - Sept	8	3581.17 ± 165.30	4700.53 ± 725.17	155.10 ± 6.89	109.46 ± 20.72	164.51 ± 30.89	319.56 ± 18.66
Oct - Nov	4	3663.04 ± 216.76	5189.03 ± 950.91	161.67 ± 9.03	130.16 ± 14.08 <sup>a</sup>	196.68 ± 20.98 <sup>a</sup>	329.61 ± 12.68 <sup>a</sup>
<b>Period of calving</b>							
2007-10	12	3808.74 ± 147.27 <sup>a</sup>	4984.94 ± 646.06	166.48 ± 6.14 <sup>a</sup>	86.61 ± 10.16 <sup>b</sup>	129.61 ± 15.15 <sup>b</sup>	309.14 ± 9.15 <sup>ab</sup>
2011-12	33	3541.11 ± 106.35 <sup>ac</sup>	4686.62 ± 466.53	152.17 ± 4.43 <sup>b</sup>	79.79 ± 11.49 <sup>b</sup>	122.27 ± 17.14 <sup>b</sup>	292.66 ± 10.35 <sup>b</sup>
2013-14	15	3351.13 ± 120.27 <sup>bc</sup>	5079.02 ± 527.62	146.37 ± 5.01 <sup>b</sup>	111.67 ± 14.48	168.94 ± 23.55	320.18 ± 13.04
<b>Group of age at first calving</b>							
<947	9	3674.99 ± 151.51	5503.20 ± 664.65	161.91 ± 6.31	111.77 ± 13.05	167.86 ± 19.46	317.29 ± 11.76
948-1065	12	3647.15 ± 136.58	4489.99 ± 599.15	156.20 ± 5.69	86.23 ± 12.50	130.61 ± 18.64	297.47 ± 11.26
1066-1183	16	3416.22 ± 130.84	4412.28 ± 573.98	147.74 ± 5.45	81.18 ± 11.43	123.54 ± 17.04	305.14 ± 10.29
1184-1301	15	3503.53 ± 119.57	5168.87 ± 524.56	153.20 ± 4.98	103.39 ± 17.39	156.64 ± 25.92	312.26 ± 15.66
>1302	8	3593.07 ± 181.91	5006.95 ± 798.03	155.97 ± 7.58	98.85 ± 7.89	149.52 ± 11.77	310.47 ± 7.11

The mean values with different superscript alphabet indicate highly significant difference ( $p < 0.01$ ) among themselves

**Table 2: Generalised linear model (GLM) of milk production traits in cows with different *CRH-A787G* genotypes**

Trait	SNP/Genotype	N	Breeding Value ± SE	CV%
<b>FL305DMY</b>				
	AA	22	3129.10 <sup>a</sup> ± 88.0	13.19
	AG	38	4237.80 <sup>b</sup> ± 77.9	11.33
<b>FLTMY</b>				
	AA	22	4191 <sup>a</sup> ± 235	26.33
	AG	38	6279 <sup>b</sup> ± 325	31.93
<b>FL305DFY</b>				
	AA	22	13.47 <sup>a</sup> ± 3.89	13.53
	AG	38	183.04 <sup>b</sup> ± 3.31	11.15
<b>FL305DPY</b>				
	AA	22	77.70 ± 7.30	44.08
	AG	38	98.70 ± 7.74	48.36
<b>FL305DLY</b>				
	AA	22	117.40 ± 10.92	43.63
	AG	38	148.52 ± 11.60	48.14
<b>FL305DSNFY</b>				
	AA	22	273.07 <sup>a</sup> ± 7.50	12.89
	AG	38	369.20 <sup>b</sup> ± 6.63	11.06

N – Number of individuals with the genotype

### CONCLUSION

In order to use the obtained results for the improvement of First lactation 305 days milk yield, First lactation total lactation milk yield, First lactation 305 days fat yield, First lactation 305 days SNF yield in Karan Fries cattle, the inclusion of individuals with the AG genotype in the breeding work could be considered. However, due to the absence of the individuals with the AA genotype in the examined herd, further research involving much larger herd as well as herds of other dairy cattle breeds is necessary. It would allow to verify the obtained results prior to their potential application in selection programs of dairy cattle.

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