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CHAPTER 6

Noval Discovery at Noval Place: - A new genetic variant reported in kappa casein gene of cattle breeds in Maharashtra

Tauseef Ahmad,[1]* N.F. Shaikh,[2] Sayyad Shahim[3]

[1]Department of Zoology, Government Vidarbha Institute of Science and Humanities, Amravati, Maharashtra, India

[2]Department of Botany, Government College of Arts and Science Aurangabad Maharashtra, India

[3]Department of Botany, Dr. Rafique Zakariya College for Women's Aurangabad, Maharashtra, India

Corresponding author Email: tauseef.bioinfo@gmail.com

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Abstract: The study of functional gene variations in Indigenous cow breeds is scarce, with only a few breeds being investigated using PCR-RFLP. In the Maharashtra state of India, remote villages were visited to collect 32 samples of cattle belonging to eight breeds as part of this study. By resequencing the 403 bp of the Exon IV CSN3 allele, haplotypes were derived. Fourteen genotypes

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(G1 to G14) defined by seven SNPs were found in a small panel of thirty-two samples. Three haplotypes (H01, H02, and H03) were reconstructed from these 14 genotypes, and their ratio were calculated. Two of the three haplotypes (H01, H03) match the CSN3*B4, CSN3*B, CSN3*H, and CSN3*G alleles, while the third haplotype (H02) was discovered to be a novel allele because it varies from the CSN3*B4, CSN3*B, and CSN3*H, CSN3*G alleles by mutation at location C5306T-Ile100Thr. The initial report in this study cluster cited three haplotypes, which contained A1, B2, B4, B, G, and H alleles that were previously documented in both subspecies of Bos taurus, The Neighbor Joining (NJ) tree building shows that there might be some sharing of genetic traits or mixing between Bos taurus indicus and Bos taurus taurus. Finding a new allele highlights the importance of looking at local breeds for different gene traits that can have significant economic benefits to stop the loss of genetic quality, this project was designed to record the native cow breeds.

Keywords: k-casein, SNP, Maharashtra, CSN3, nonsynonymous.

Genetic variants that influence milk-protein composition have gained increased research interest because of their economic relevance to modern dairy improvement programs. Such genetic differences can alter both the yield and quality-related characteristics of milk, including traits important for processing. (Gustavssaon et al.,2014) Multiple studies have reported considerable diversity within the four major casein proteins, with numerous variants documented across cattle populations. Many different factors affect how milk is produced, how well it can be turned into cheese, and what nutrients it contains. (Gallint et al., 2013). Kappa-casein plays a key structural role in stabilizing casein micelles, which in turn influences the efficiency of dairy product formation, particularly in cheese making. (Prinzenberg et al., 2005)

Previous work using PCR-SSCP has identified several kappa-casein variants across both Bos indicus and Bos taurus breeds. (Prinzenberg et al., 2008). Understanding how functional genes differ among local breeds is essential for evaluating their adaptive potential and long-term sustainability. (FAO, United Nations, 2007). Mitochondrial DNA analyses conducted by our team and others have contributed to clarifying the domestication history of Bos taurus indicus in South Asia (Chen et al., 2010). But few studies target functional important genes like Kappa casein in cattle from India using Molecular and genetic approach (Deb et al., 2014). Most of the cows in India are of mixed types, and past studies about the kappa casein gene focused mainly on the two most common breeds, Sahiwal and Tharparkar, which are located in the northwest part of India. (Deb et al., 2014). Maharashtra is the state in India with the second highest population living in cities, and it is found in the western region of the nation. Because of where it is on the map, this state is where the northern and southern parts of India come together. Furthermore, there is no information on functional gene variants of the primary native cattle breeds in Maharashtra, which include the nondescript Gaolo, Deoni, Dangi, Khillari, and Lal-khandari. The casein family shows a lot of different shapes at the molecular level because it creates many genetic differences, even with strong selection pressure. In light of these, the study's primary goals were (i) to look for novel CSN3 alleles in Maharashtra's native cow breeds and (ii) to verify the theory that local cattle breeds are important livestock's for development.

Materials and Methods:

By using ear-pluckers, tissue samples were collected from local cattle breeds in remote villages of Maharashtra. (Figure 1). These 32 samples were taken from eight indigenous cattle breeds. We also sampled one exotic breed, Jersey (Bos taurus taurus), for comparison. Precautions were taken during the sample process to prevent sampling of related persons. Genomic DNA was extracted using a commercial purification kit following the manufacturer's recommendations. PCR amplification was carried out using equal volumes of forward and reverse primers along with the standard reaction components., which was conducted in a total of 17 microliters of reaction mixture described by Prinzenberg et al., (1999) was used to amplify, kappa casein Exon IV in thermal cycler and immediately sequence the PCR results Haplotype reconstruction was performed using the DnaSP software package. (Librado et al., 2009) was used to construct haplotypes, and deposited in the GenBank (KY368689-KY368691) A phylogenetic tree was created by adding new haplotypes to the alleles that were reported before. The CSN3 (X14908) reference sequence was included as an outgroup, using water buffalo. This phylogenetic information was assembled using the P-distance model and the MEGA7 method. (Kumar et al., 2016).

Results and Discussion:

In our panel of study 14 different genotype (G1 to G14) were defined by 7 SNP (single nucleotide polymorphism) and three haplotype (H01 to H03) out of three one newly (H02) haplotype were investigated in all breeds. Which is revealed in Table No 1, from these 14 genotypes and 3 haplotypes were constructed and their frequencies were estimated. Among the three haplotype two (H01, H03) are correspond to the most commonly known cattle allele A & B respectively. New H02 haplotype which is differ by one mutation (T5309 C- Thr 101 Ile) while haplotype H01, H03 (C5306T, A5345C, G5376T), (Ile **100** Thr), (Asp **113** Aln), (Glu **123** Asp) respectively. The findings indicate that the level of variation within exon IV of the kappa-casein gene may be greater than previously recognized in domestic cattle. The third haplotype H03, which developed in the exotic breed with the B allele (KR149429) of Bos taurus and was reported from Kerala State, India, is likewise clustered in the tree. (Figure 1).

Table 1: Observed genotypes (G01 to G14) with their distribution and inferred haplotypes (H01 to H03) with estimated frequencies.

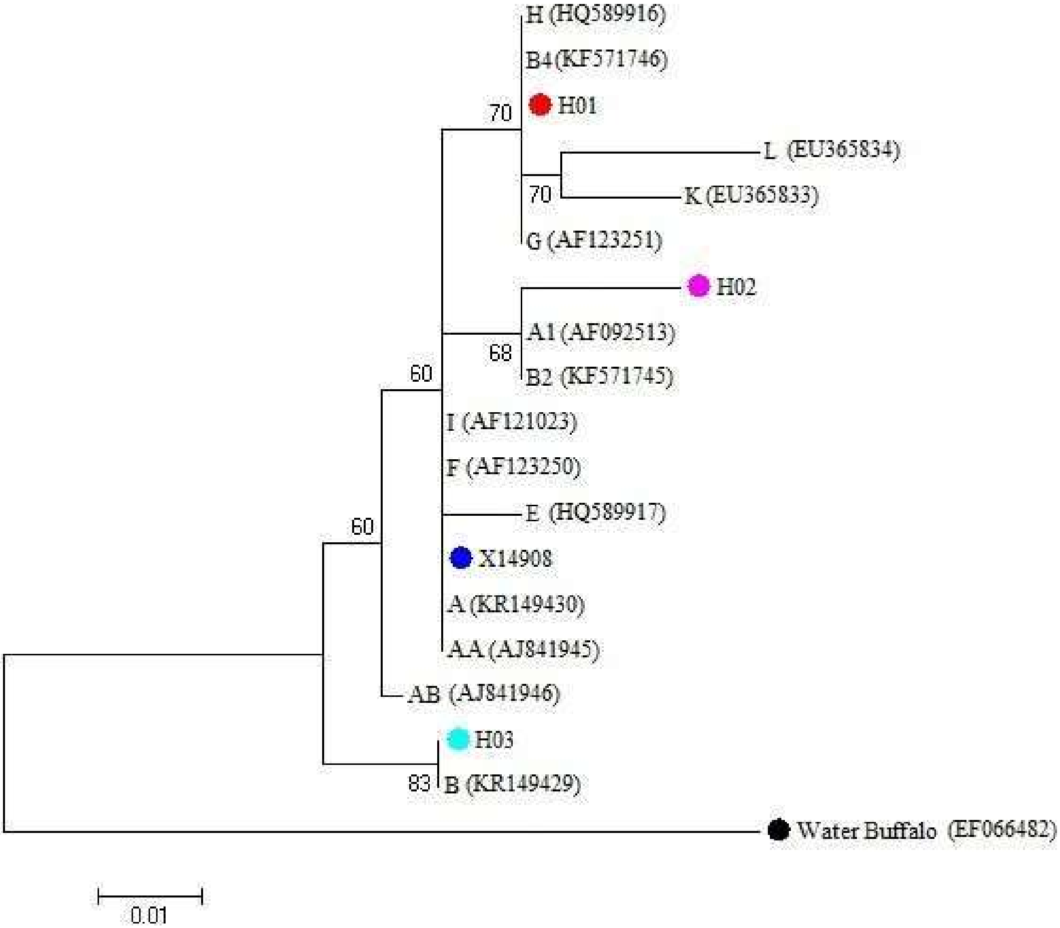
		SNP POSITION[3]							DISTIRIBUTION IN DIFFERENT BREEDS[4]								
ITEM[1]	N[2]	5306	5309	5345	5352	5370	5376	5406	GAL	GIR	JER	DEO	KLR	DAN	LKD	HAR	Haplotype Frequency
REF_SEQ[5]		C	C	A	A	A	G	A									
G1	2	C	C	A	A	C	T		1			1					
G2	2	T	C	A	A	A	G	A	1				1				
G3	2	T	C	A	G	C	T			1					1		
G4	8	C	C	A	G	A	G	A		1		1		1	2	3	
G5	4	C	C	A	A	A	G	G			1		2			1	
G6	1	C	T	C	A	A	G	G			1						
G7	2	C	C	A	G	C	T	A			1	1					
G8	4	T	C	A	G	A	T	A					1	1	1	1	
G9	2	C	C	A	G	C	T	A					1	1			
G10	1	T	C	A	A	C	T	A						1			
G11	1	C	C	A	G	C	T				1						
G12	1	C	C	A	G	A	G			1							
G13	1	T	C	A	A	A					1						
G14	1	C	C	A	G	C	G					1					
H01	10						T		1	1	1		2	2	2	1	0.3125
H02	21	C							2	2	2	4	3	2	2	4	0.6562
H03	1		T	C							1						0.0312

The detection of matching kappa-casein alleles across phenotypically distinct cattle implies either shared ancestry or ongoing genetic mixing among local populations. In order to comprehend the evolution of the CSN3 gene in regional cow breeds, our study highlights the significance of resequencing and haplotype inference analysis. The identification of a previously unreported variant within a small sample set highlights the likely rich functional genetic diversity present in regional cattle breeds.

1-Observed genotype **2**-Indicate the number of observed individual **3**-Single Nucleotide Polymorphism (SNP) position **4**-Represented by an abbreviation of various cattle breeds. GAL–

Gaolao, GIR-Gir, JER-Jersey, DEO-Deoni, KLR Khillar, DAN-Dangi, LKD-Lal-Khandari, HAR-Hariana. **5**-Reference sequence of Kappa casein gene (X14908). H01, H02 & H03 are identified haplotype and their position.

Figure 1: Neighbour joining tree constructed out of newly generated and retrieved haplotypes. Water buffalo was used as outgroup.



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