DOI: https://doi.org/10.53350/pjmhs221610975

ORIGINAL ARTICLE

Genetic Analysis of K-Casein Gene in Milk Protein of Bos Indicus Cattle

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ABSTRACT

Purpose: K-casein, or kappa casein, is a mammalian milk protein involved in several important physiological processes. Chymosin splits K-casein into an insoluble peptide (para kappa-casein) and a water-soluble glycomacropeptide (GMP). GMP is responsible for increased efficiency of digestion, prevention of neonate hypersensitivity to ingested proteins, and inhibition of gastric pathogens. The gene underlying the production of kappa-casein in cow's Milk shows polymorphisms which affect the amount of protein produced. Higher levels of kappa-casein are associated with increased milk protein and casein content and better cheese yield.

Method: The current study aimed to identify the genetic mutations among three indigenous cattle breeds of Sindh, Pakistan. A particular set of primers amplified the targeted area of the K-CASEIN gene after genomic DNA extraction from blood samples of the three cattle breeds, namely Cholistani cattle breed, Tharri, and Sahiwal.

Findings: The amplified PCR products were sequenced using the ABI Genetic Analyzer 3500; a BioEdit version 7.2 was used to examine the sequencing data. Using the ensemble gene browser, blast analysis was also performed.

Practical Implication: Results revealed 18 mutations in all 03 cattle breeds, including 07 mutations in the Cholistani cattle breed; the Tharri cattle breeds had 06 mutations, and the Sahiwal cattle breeds had 05 mutations. Results of this study of the K-CASEIN gene suggested that Cholistani cattle breeds are more genetically varied than the Sahiwal cattle breeds.

Conclusion: Furthermore, the Sahiwal cattle breed is more diverse than the Thari cattle breed for the said gene.

Keywords: K-Casein gene, Sahiwal cattle breed, Thari cattle breed, Cholistani cattle breed, Polymorphism

INTRODUCTION

West Saxons first used the term "meals" for "milk," which comes from "Old English; Anglians used the term "milk" and "meluks" a term from Proto-Germanic that means Milk. "Milk is a major nutritional resource of required minerals and vital raw materials obtained from farm cattle for the human diet"[1] the ordinary secretions of mammary glands in milk-producing animals"[2] The word "dairy" makes a logical connection with the production of animal milk. It may contain various foods rich in mineral ions as a good source of food and hence be used in dairy industries for dairy products or milk products such as ice-cream, butter, all types of cheese, yoghurt, and many more . Milk from dairy farm animals like water buffaloes, cows, sheep, goats, and camels is a type of farm product. Asia produced nearly 360 million metric tons of Milk in 2019, an increase of 10 million metric tons (or 2.9%) from 2018. India and Pakistan produced over 90% of this total.[3.]

2011 witnessed us getting 730 million metric tons of Milk from 260 million dairy cows across various farms [4]. Before 2016, Russia and China were known worldwide as the countries that made and bought the most Milk. Now that it is payday, both countries are providing a glut of self-sufficient Milk throughout the world [5]. About 852 metric tons of Milk were produced globally in 2019, an increase of 1.4 percent above the 852 million tonnes produced in 2018; India, Pakistan, the European Union, and the USA have all seen an increase in milk output [6].

Around 800 domestic cattle breeds have been reported around the world, including species of two types: Bos indicus (indicin or zebu) humped cattle and Bos taurus (taurine) non-humped cattle, which are morphologically differentiated into many characters as zebu cattle contain. Indian cattle have a few physiological benefits over bulls used in bullfights, which include a hump on the shoulder and neck region, heat endurance, less susceptibility to intestinal system worms and pathogens, and decreased metabolic output and nutritional needs[7].

Livestock is a significant industry in Pakistan. It increased at a pace of 4% in 2018–19 and contributed 11.2 percent of the country'scountry's GDP, and around 60.5% of the value added to agriculture (Economic Survey of Pakistan 2018-19). The current study focuses on the quantity and quality of milk protein genetic polymorphism in the K-CSN gene in three different Zebu cattle breeds based on the genetic code of two different districts: District Khairpur and Sukkur, Sindh, Pakistan, which includes the Sahiwal cattle breed, the Cholistani cattle breed, and the Thari cattle breed. As we know, cow's Milk contains various essential amino acids coded by various genes located at different positions on different chromosomes. k-CSN is also a gene that codes for different amino acids for our body's development.

Bovine Milk contains 12% or more of the casein that is made up of the kappa-casein (K-CN) gene. The location of this gene is on chromosome 6. K-CN has a molecular weight of 19800 Dalton, 169 amino acids, five exons, and four introns. Most K-CN coding regions are in the fourth exon, which is close to 13 kb long. The Kappa Casein gene is helpful because it can be chosen to improve milk quality or quantity, such as fat and protein levels[8]. While acting as a stabilizing agent during the development of muscle structure in milk curdling, k-CN has a structure and other characteristics that are very different from other caseins. The casein protein complex comprises four different casein types: beta, kappa, alpha S1, and alpha S2.[1] found that the chromosome 06 locus has four casein genes (CSN1S1, CSN1S2, CSN2, and CSN3) that code for proteins with different amino acid sequences.

MATERIAL AND METHODS

Collection of Samples and Extraction of DNA: The research involved 45 blood samples, roughly. The samples were collected from three cattle breeds: Cholistani, Sahiwal, and Thari. The jugular vein of cattle served as the source of the blood samples. Cattle are being cared for on farms and ranches in Pakistan's Khairpur Mir, Goushala Shaikh Chock Gambat district. Mubeen Ahmed Phulpoto cow farm with Visrio wahan and Naheed Qazi cattle farm, near dargah Loung Fakir, the Fakir Milk and Cow Farm were on the Shadi Shaheed Road, Khairpur, Sindh, Pakistan.

Using the Gen JET genomic DNA purification micro kit K0781 from Thermo Scientific, genomic DNA was extracted from blood cells called leucocytes. It takes about twenty to thirty minutes from the time of cell lysis until the time when the very pure DNA is released. Nanodrop machines assessed the DNA concentration of the extracted sample at the Genome Research Centre (HEJ, University of Karachi) to ensure adequate amplification. A 260/280 ratio was also used to evaluate the purity of the DNA.

Primer design and PCR amplification: Oligonucleotide primers for beta-lactoglobulin and the Kappa Casein gene were initially designed through Primer Premier 3 software and synthesized by Macrogen of Korea. The target region of interest is complemented by PCR primers that are 20–24 nucleotides long. Primers showed the best performance at a temperature of 58 °C.

Primer sequences are:

K-CN F 5ACGCAAGACACTAACACCCTT3 Forward Primer

K-CN R Reverse Primer 5CCTTGTGTGACCGTCAGCTCTT3
PCR Amplification: A PCR reaction mixture was composed of 12.5 l of Taq DNA polymerase (Green Master Mix, Promega, Madison, WI, USA), ten pmol of each primer, 50 mg of template DNA, and any remaining nuclease-free water [9]**.** PCR is increased in a thermal cycler (Applied Biosystems 2720, USA).
Gel Electrophoresis: Electrophoresis of gel EDTA, Tris base, and acetic acid are all components of a solution known as TAE buffer. The bands were seen under a UV transilluminator (Gel Doc system) to analyze the fragment measured using a one kB ladder. The PCR-amplified product was processed on a 1.5% Agarose gel (AG) and afterwards subjected to 2 l of ethidium bromide (EtBr) in TAE as the buffer was added for 60 minutes at 70 V current.
Bioinformatics analysis: The Macrogen Company of Korea (https://dna.macrogen.com/) offered the purified and sequenced PCR product obtained commercially. Subsequently, oligonucleotide blasting, online sequencing, alignment, and evaluation of mutations were applied to the sequencing data, and natural mutation percentages were counted using MS Excel.

RESULTS

Quantified DNA samples: Using a nanodrop spectrophotometer to measure how much DNA was in the extracted DNA samples, the amount of DNA was found to be between 30-103ng/l, which is enough for PCR amplification. Below is a list of the DNA quantification results.

The current study's findings provide complete genomic information and explain how the mutation rates and percentages in the three cow breeds can be compared. There were determined to be seven mutations in the Cholistani breed, five in the Sahiwal breed, and six in the Thari breed. These modifications are called "point mutations" since they are based on the genetic code. Threonine is converted to Lysine in the Cholistani breed. The type of mutation was a missense one in which the essential amino acid ACA changed to the essential amino acid AAA at 20 bp. Threonine converted into isoléusine, and at position 35 bp, the essential amino acid ACT was altered into the essential amino acid ATT via a missense mutation. At 53 base pairs, TGC and TAC, two non-essential amino acids, underwent missense mutations. The essential amino acid ACT changed to the essential amino acid ATT at position 39 in the transition from threonine to isoléusine, and the mutation was missense. At position 44 bp, threonine converted into asparagine; the missense mutation caused the amino acid ACC to change into the non-essential amino acid AAC. The non-essential amino acid CCA changed into the non-essential amino acid CAA at a position of 56 base pairs, and the mutation was a missense one, turning proline into glutamine. At 62 BP, the non-essential amino acid GCT transformed into the non-essential amino acid GAT, converting alanine into aspartic acid.

In the Sahiwal cattle breed, non-essential amino acid serine TCT transformed into TCG serine, a non-essential amino acid at 34bp kind of mutation, was silent. GGT non-essential amino acid changed into deletion amino acid where the glycine changes into deletion GG- at 14 bp, the kind of mutation was missense. Leucine transforms into the non-essential amino acid CTA, which transforms into the amino acid used for deletion. Missense was the type of mutation in CT 41bp. The essential amino acid TTT changes from phenylalanine to serine, a non-essential amino acid. Missense was the type of mutation in TCT at 269bp. When Lysine was changed into glutamic acid, the non-essential amino acid AAG changed into the non-essential amino acid GAG over 271bp. This was a missense mutation.

Serine changed into deletion in the Thari cattle breed, while non-essential amino acid TCA changed into -GA at 35 and 36 bp, respectively. Proline changed into proline, in which the non-essential amino acid CCA changed into CCC at 35 bp. This type of mutation was missense. Threonine was deleted, and the non-essential amino acid ACT was changed into -CT AAT in 35 and 36 bp missense mutations. The non-essential amino acid ACT transformed into TCT at 39 bp as threonine turned into serine; the type of mutation was missense. Alanine changed into deletion, in which non-essential amino acid GCA changed into G-A at 43 bp. This type of mutation was missense. Histidine changed into deletion, in which the essential amino acid TCA changed into -GA at bp 35 and bp 36. This type of mutation was missense.

Table 1: Details of the mutation among all 03 cattle breeds for K-Casein gene

Samples	N-Base Number	Original Codon	Modified Codon	Original Amino Acid	Changed Amino Acid	Type of Mutation
Cholistani (C1)	20	ACA	AAA	Thrionine (E)	Lysine (E)	Missense
	35	ACT	ATT	Thrionine (E)	Isoleusine (E)	Missense
Cholistani (C2)	53	TGC	TAC	Cysteine (N)	Tyrosine (N)	Missense
Cholistani (C3)	39	ACT	ATT	Thrionine (E)	Isoleusine (E)	Missense
	44	ACC	AAC	Thrionine (E)	Aspergine (N)	
	56	CCA	CAA	Proline (N)	Glutamine (N)	
	62	GCT	GAT	Alanine (N)	Aspartic Acid (N)	
Sahiwali (S1)	34	TCT	TCG	Serinne (N)	Serine (N)	Silent
Sahiwali (S2)	14	GG-	GGT	--	Glycine (N)	Missense
	41	CT-	CTA	--	Leucine (E	
Sahiwali (S3)	269	TTT	TCT	Phenylalanine (E)	Serine (N)	Missense
	271	AAG	GAG	Lysine (N)	Glutamic Acid (N)	
Thari (T1)	35 & 36	-GA	TCA	--	Serine (N)	Missense
Thari (T2)	35	CCA	CCC	Proline (N)	Proline (N)	Missense
	36	ACT	-CT	Thrionine (E)	--	Nonsense
Thari(T3)	39	ACT	TCT	Thrionine (E)	Serine (N)	Missense
	43	G-A	GCA	--	Alanine (N)	Missense
	73	CAC	CCC	Histidine (E)	Proline (N)	

SNPs identification: The positions of the nucleotide changes were noted from the PCR product of the CSN3 gene following blasting on Emseble.org was used to analyze all polymorphisms. Figure 1 provides the size of the PCR result; Table 1 provides specific information on the mutations.

DISCUSSION

Understanding how farm animal behaviours react to casein and milk protein estimating genetic and phenotypic correlations here between amount, ratio, and daily production of components of bovine milk. As stated by[10] the same degree of environmental factors and a genetic factor might be responsible for the similar direction of phenotype and genetic relationship. The same biological mechanism regulates the production of casein and whey proteins [11] In truth, the genetic loci that determine how whey protein and casein are expressed have a lot of linkage disequilibrium[12]

Through this work, the use of genomic PCR and DNA sequence analysis successfully found Variations within kappa-casein (CSN3) genes. In remote and urban areas, dairy cattle are regarded as one of the most important livestock species, even more so than a dairy cows. In reality, they could serve as a

traditional small-scale dairy sector that promotes the health of individuals, food security, and poverty reduction [13]**.** For the dairy processing industries, particularly in fermented foods, cow milk is a great raw material[14]. As a result, milk production is now the primary goal of cattle breeding[15]**.**

CONCLUSION

Results revealed 18 mutations in all 03 cattle breeds, including 07 mutations in the Cholistani cattle breed; the Tharri cattle breeds had 06 mutations, and the Sahiwal cattle breeds had 05 mutations. Based on obtained results of this study of the K-CASEIN gene, it is concluded that Cholistani cattle breeds are more genetically varied than the Sahiwal cattle breed. Furthermore, the Sahiwal cattle breed is more diverse than the Thari cattle breed for the said gene.

REFERENCES

1 Rashaydeh F. Genetic Polymorphisms of Milk Genes (β-Lactoglobulin and κ-Casien) in Palestinian Awwasi Sheep Breeds (Doctoral dissertation, Palestine Technical University-Kadoorie), (2018).

2 Neves RC, Leno BM, Epidemiology of subclinical hypocalcemia in early-lactation Holstein dairy cows: The temporal associations of plasma calcium concentration in the first 4 days in milk with disease and milk production. Journal of dairy science, (2018). 101(10), 9321-9331.

3 Akdemir F, & Sahin K. The effect of dietary colostrum powder on performance, carcass yields and serum lipid peroxidation levels in Japanese quails (Coturnix coturnix japonica). Journal of Applied Animal Research, (2018). 46(1), 39-43.

4 Van W, Velde SV, De Bruyne R, Van Biervliet, S "Clinical Practice". European Journal of Pediatrics. (2011).170 (12):1489–1494. doi:10.1007/s00431-011-1547-x. PMID 21912895. S2CID 26852044

5 Dudukalova GN, Tkach AV, & Nechitaylov AS. The development of the dairy market in Russia. In Complex systems: innovation and sustainability in the digital age (2020). (pp. 437-447). Springer, Cham.

6 Kapaj A. Factors that influence milk consumption world trends and facts. European Journal of Business, Economics and Accountancy, (2018). 6(2), 14-18.

7 Bradley DG, MacHugh DE, Cunningham P, & Loftus RT. Mitochondrial diversity and the origins of African and European cattle. Proceedings of the National Academy of Sciences, (1996). 93(10), 5131-5135.

8 Grădinaru AC, Ilie DE, & Creangă S. Milk protein genetic variants in Romanian Spotted, Holstein Friesian and Montbéliarde cows and some correlations with milk parameters. Research Journal of Biotechnology, (2013).8, 3-9.

9 Zhang C, Xing D. Miniaturized PCR chips for nucleic acid amplification and analysis: latest advances and future trends. Nucleic acids research. (2007) ;35(13):4223-37.

10 Sodini SM, Kemper KE, Wray NR, Trzaskowski M. Comparison of genotypic and phenotypic correlations: Cheverud's conjecture in humans. Genetics. (2018) ;209(3):941-8.

11 Pegolo Sara, "Structural equation modeling for unraveling the multivariate genomic architecture of milk proteins in dairy cattle." Journal of Dairy Science (2021): 104.5 5705-5718.

12 Huang W, & Khatib H. Association between milk protein gene variants and protein composition traits in dairy cattle. Journal of dairy science, (2012). 95(1), 440-449.

13 Altschul SF & Lipman, DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research, (1997). 25(17), 3389-3402.

14 Barbosa SBP, Batista Â, MV & Silva. MVBD Genetic association of variations in the kappa-casein and β-lactoglobulin genes with milk traits in girolando cattle. Brazilian Journal of Health and Animal Production , (2019). 20.

15 Gallinat JL, Tetens J. DNA-based identification of novel bovine casein gene variants. Journal of dairy science, (2013). 96(1), 699-709