Milk protein genetic variation in Butana cattle

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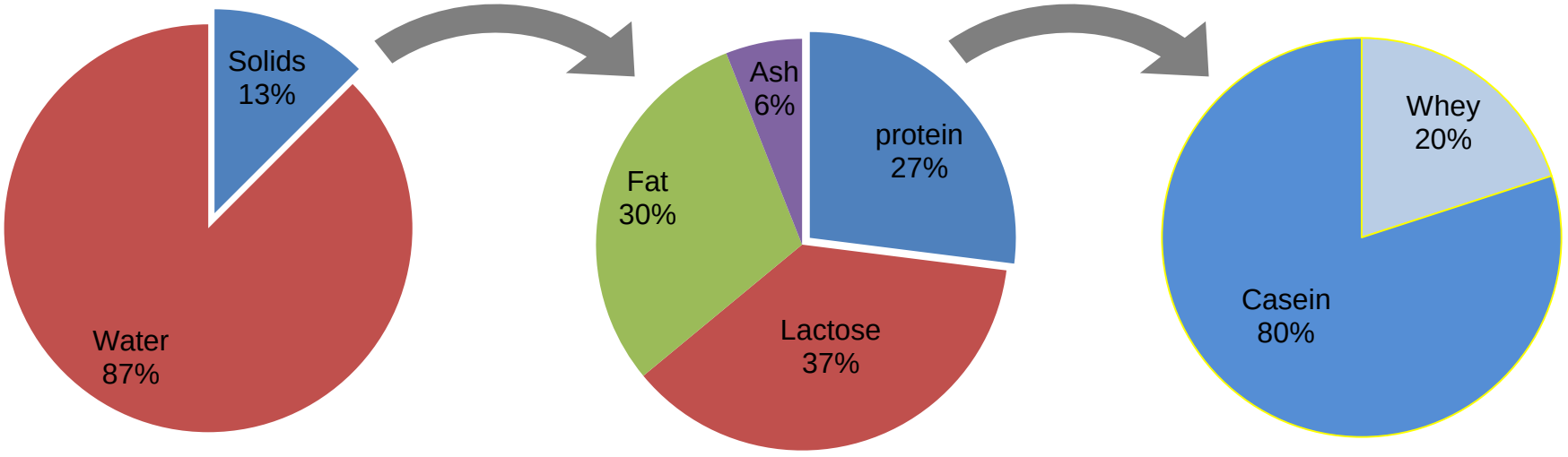
Composition of milk protein

➢Mainly divided into two fractions:

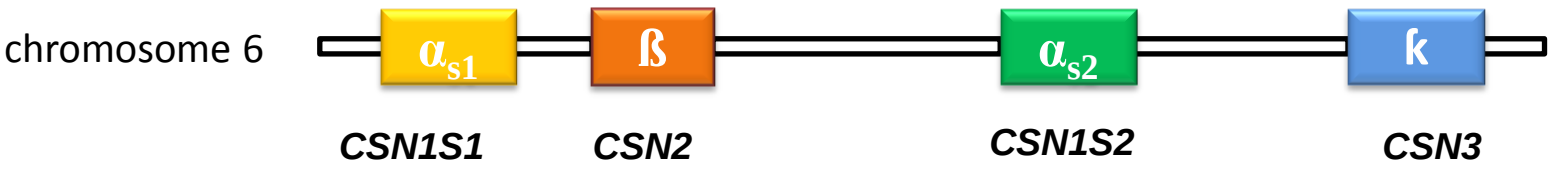
- Soluble → **whey protein (**α-LA) and (β-LG)
  coded by: *LALBA* and *LGB* genes.
- Insoluble → **casein** (αs1, β, αs2, and κ-caseins)
  coded by: *CSN1S1, CSN2, CSN1S2* and *CSN3* genes.



Milk protein genes structural organization

- Casein genes cluster

The four casein genes mapped to chromosome 6 in a tightly linked 250-kb cluster.



- *LALBA* and *LGB*, were mapped on chromosome 5 and 11.

Why milk protein variants are of interest?

MP variants = SNPs at encoding genes ⟹ Amino acid exchange or deletion

- Influences on milk performance traits
- Breed characterization, biodiversity and phylogeny studies
- Evolutionary histories of cattle breeds
- Influences on human nutrition

Butana breed characteristics

- Introduced into Africa from South Asia (around 670 AD)
- The second best zebu dairy breeds in the world (DAGRIS, 2007)
- Well adapted to the harsh environment
- Deep red coat color
- Longevity
- Disease resistance
- Milk yield (2.264 ± 131 liters in 283 ±11 days)

This work aims at:

Assessing allelic variation of the main milk protein genes that are characteristic of Butana dairy cattle

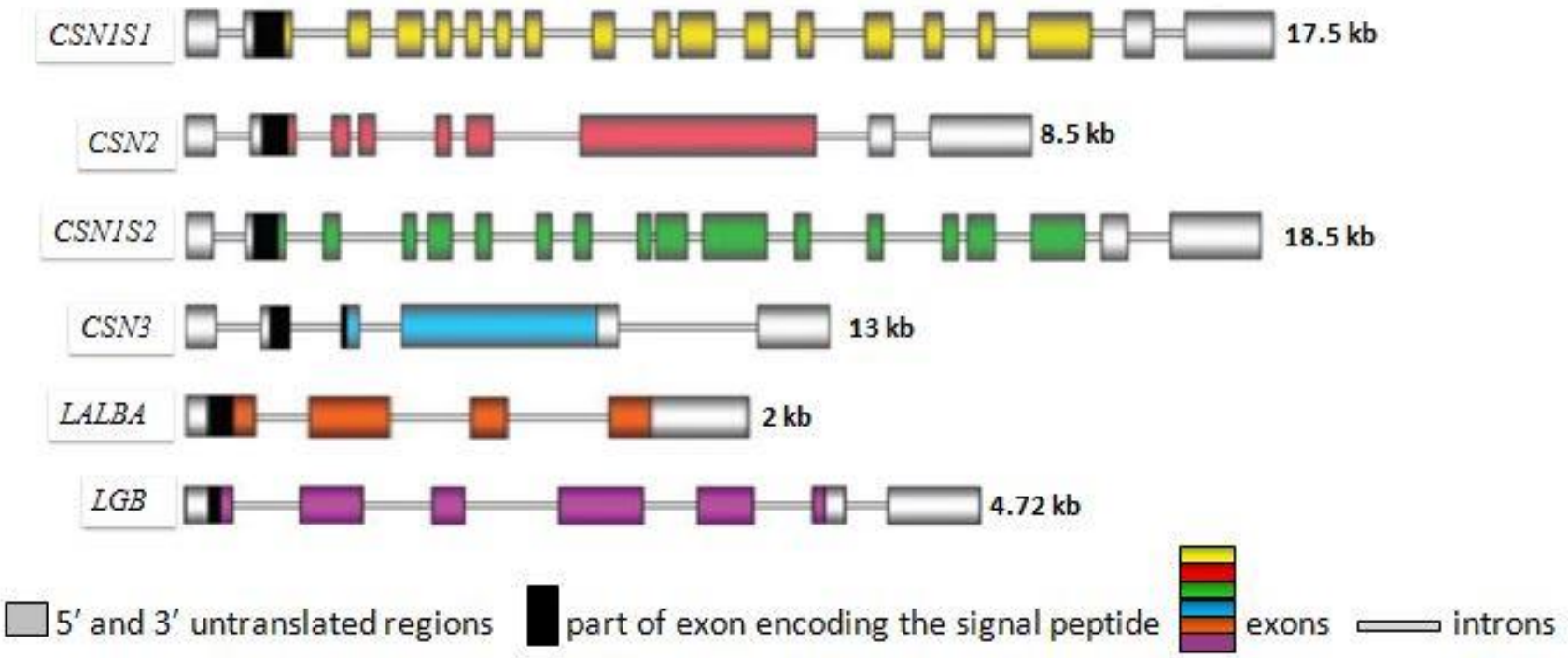
Samples

Blood samples from **50** unrelated individuals from *Butana area* in the north of Sudan.

Area of the study

Sequencing

- Sequencing of the six bovine MP genes was carried out using genomic DNA of **five** Butana cattle.



Structural organization of the transcription units encoding the 6 milk protein genes.

Sequence analysis

- Editing and alignment = DNA Baser v3 and Clustalw2
- Comparative analysis = reference sequence (UMD3.1 genome database)

Genotyping of SNPs at milk protein loci

- Allele specific primers
- Genotyping = KASP assays

Statistical analysis

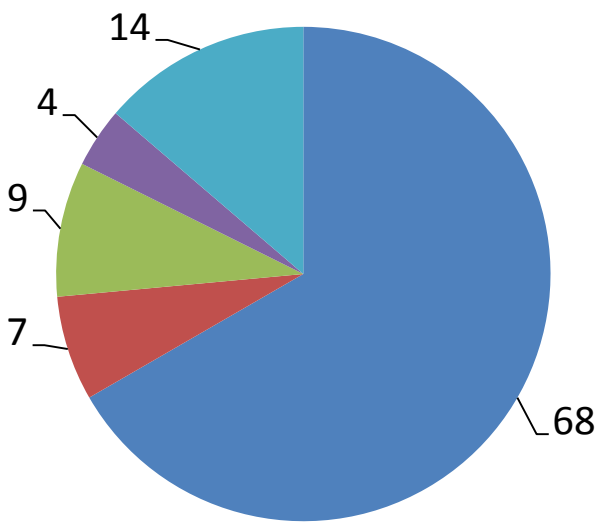
Estimation of allele and haplotype frequencies

Gene	Exon	SNP position
CSN1S1	17	6:87157262 A>G
CSN1S2	3	6:87266177 C>T
CSN3	4	6:87390573 C>T
		6:87390576 T>C
		6:87390612 C>A
LALBA	1	5:31347973 G>A
	2	5:31348386 G>A
LGB	3	11:103303475 G>A
	4	11:103304757 T>C

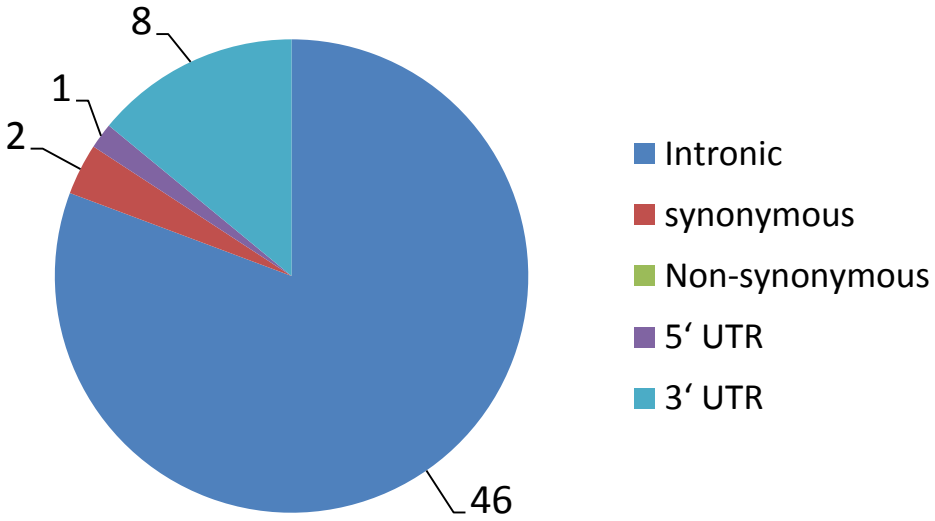
Sequence variants in exon and exon flanking regions of bovine *CSN1S1, CSN2, CSN1S2, CSN3, LALBA and LGB* genes

Total number of SNPs (n = 98)

3.3 SNPs per 100 bp



Novel SNPs (n = 57)



Distribution of identified SNPs and novel SNPs in Butana cattle

Sequence variants in exons of analyzed MP genes in Butana cattle

Milk Protein gene	Protein variant	Location within gene	[1]BTA chromosomal position	Amino acid exchange	[2]AF
CSN1S1	***B***	Reference	-	-	0.34
	C	Exon 17	6:87157262A>G	Glu207Gly	0.66
CSN1S2	***A***	Reference	-	-	0.61
	B	Exon 3	6:87266177C>T	Ser23Phe	0.39
CSN3	***A***	Reference	-	-	0.59
	H	Exon 4	6:87390573C>T	Thr156Ile	0.25
	B		6:87390576T>C 6:87390612C>A	Thr157Ile Asp169Ala	0.16
LALBA	***B***	Reference	-	-	0.46
	A	Exon 1	5:31347973G>A	Arg29Gln	0.19
	E	Exon 2	5:31348386A>G	Ile60Val	0.35
LGB	***B***	Reference	-	-	0.88
	A	Exon 3 Exon 4	11:103303475G>A 11:103304757T>C	Gly80Asp Val134Ala	0.12

[1]Bos taurus autosome (BTA) position relative to reference sequence (UMD3.1)
[2]AF: allele frequencies are given for 50 genotyped Butana cattle.

Haplotypes in Butana cattle comprising five DNA polymorphisms in the genes CSN1S1, CSN1S2, and CSN3 of the casein gene cluster

	Casein protein variant			
HT	***CSN1S1***	***CSN1S2***	***CSN3***	**Haplotype frequency**
1	*B*	*A*	*A*	0.25
2	*B*	*A*	*B*	0.05
3	*B*	*A*	*H*	0.08
4	*B*	*B*	*A*	0.03
5	*C*	*A*	*A*	0.29
6	*C*	*A*	*B*	0.04
7	*C*	*A*	*H*	0.04
8	*C*	*B*	*A*	0.21
9	*C*	*B*	*H*	0.01
10	*C*	*B*	*B*	0.01

HT, Haplotype number

Conclusion

- The current study identified a high number of polymorphisms in the bovine milk protein genes in Butana cattle.
- Nine SNPs were detected in the coding regions of the examined genes with an influence on the amino acid sequence of the encoded milk proteins.
- The numerous non-coding SNPs do not influence the amino acid sequence of milk protein genes; some of them may be located in functionally important sequence regions.
- The high variability of the milk protein genes in Butana dairy cattle provides a resource for the development, utilization, and conservation of the local breed.

Thank you for your attention!

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