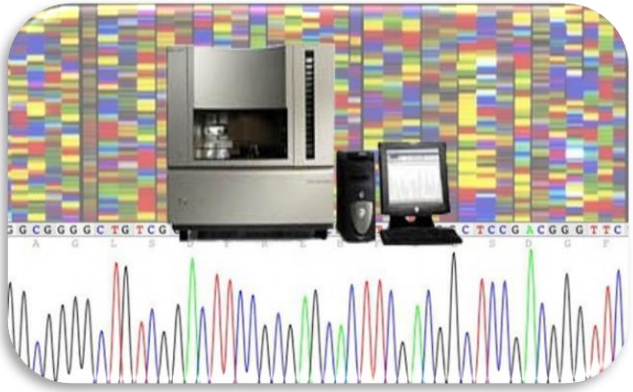
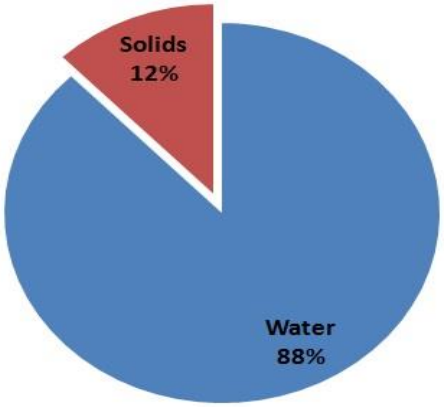
Casein milk proteins - novel genetic variation and haplotype structure

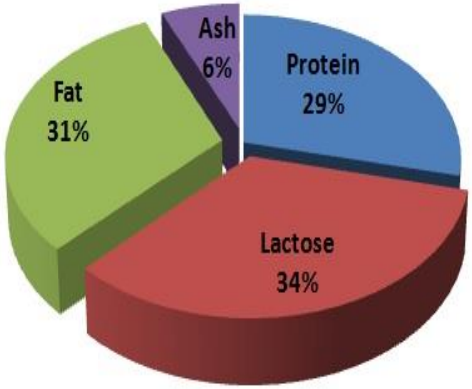
Siham A. Rahmatalla, *Danny Arends, Monika Reissmann, Stefan Krebs, Gudrun A. Brockmann*

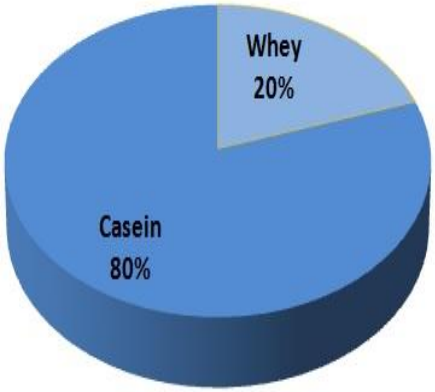


Animal Breeding Biology and Molecular Genetics, Humboldt-Universität zu Berlin

Composition of goat milk



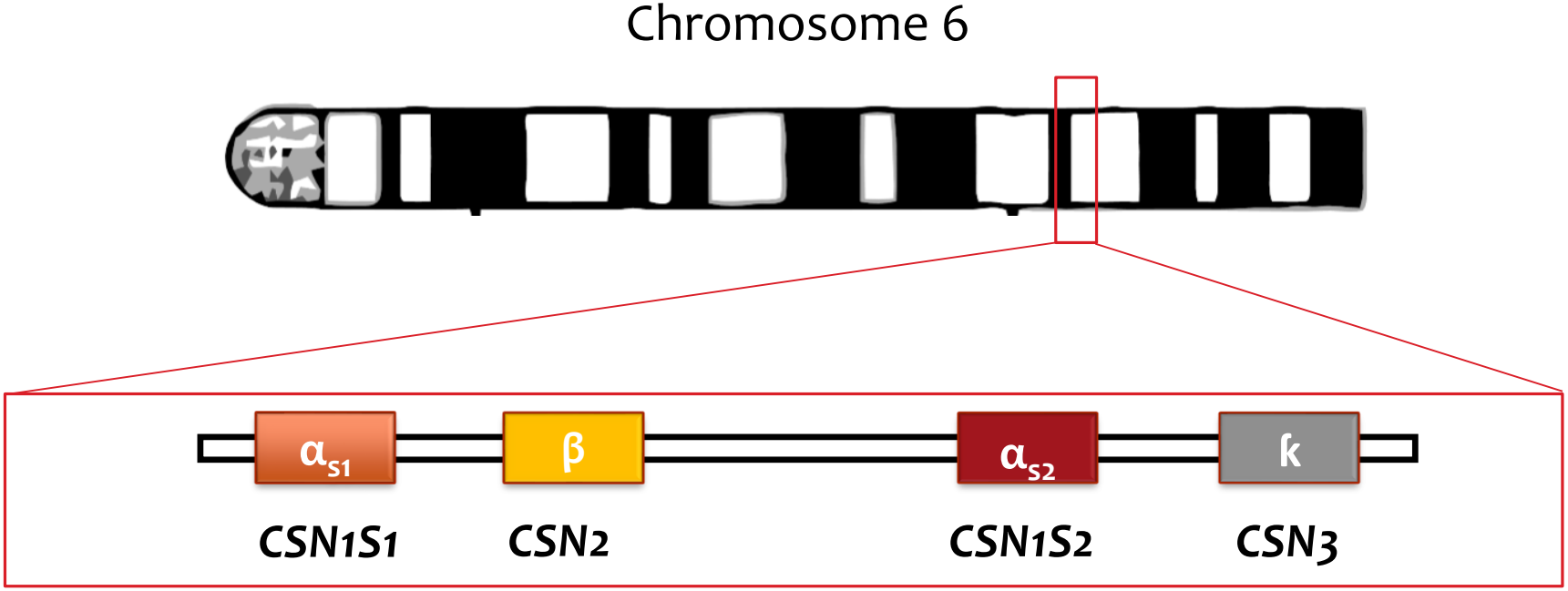




* Protein fraction can be divided into:
  * Insoluble:
    Casein (α_{s1}-casein, α_{s2}-casein, β-casein and κ-casein)
    encoded by *CSN1S1*, *CSN1S2, CSN2* and *CSN3* genes
  * Soluble:
    Whey (α-lactalbumin and β-lactoglobulin)
    encoded by *LALBA* and *LGB* genes

Structural organization

* Casein gene cluster
  * The four casein genes mapped to chromosome 6 in a tightly linked 250-kb cluster at around 86 Mb



Importance of goat milk proteins

- Proteins from goat milk are easier digestible[1]
- α_{S_1}-casein is one of the major milk allergens[2]
- Goat milk has higher levels of ß-casein and a lower level of α_{S_1}-casein[3]
  - More similar to human breast milk
- Cheese from goat milk is softer and more fragile compared to cheese made from cow milk[4]

[1] Park (2010) *Encyclopedia of Animal Science*
[2] Ballabio et al. (2004) *Dairy Science*
[3] Wang et al.(2017) *Scientific Reports*
[4] Yangilar (2013) *Food and Nutrition Research*

Objective

- Assess the allelic variation in casein genes from different rarely studied breeds by using high density capture sequencing
  - Focus on indigenous Sudanese goat breeds
  - Compared to commonly studied breeds

Identification of genetic variation is necessary for association analysis, breed improvement, and breed conservation

Different protein variants could affect human digestion and properties of goat cheese

M&M: Animals and samples

Nubian (7)

Desert (5)

Taggar (7)

Nilotic (7)

Nubian ibex (2)

Saanen (2)

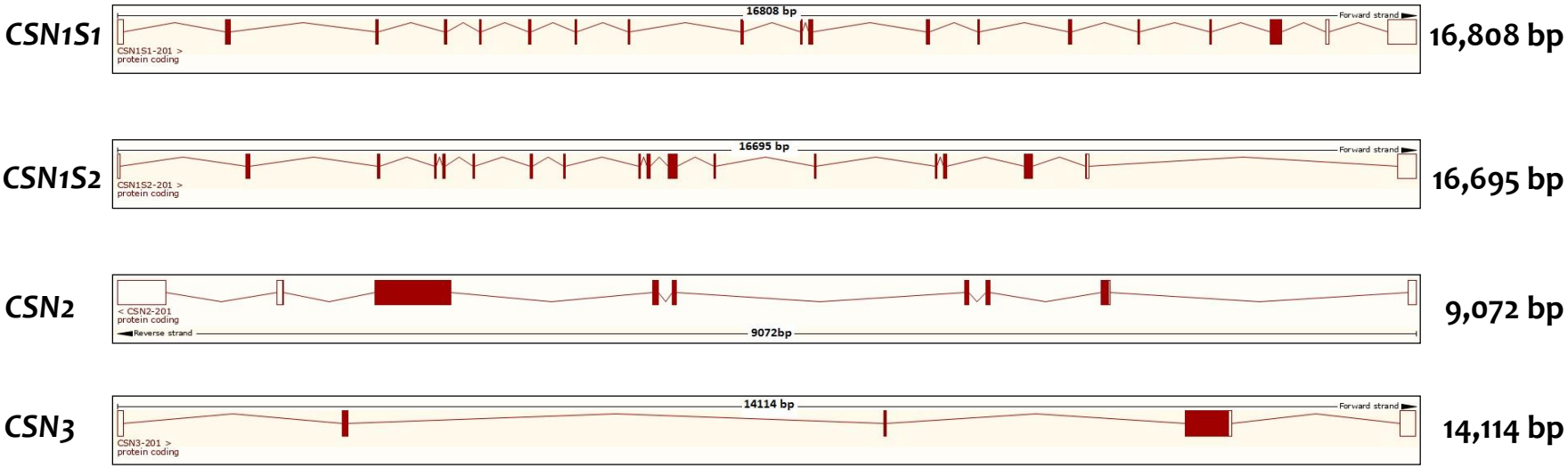
Alpine ibex* (1)

Bezoar ibex* (2)

* Kindly provided by Leibniz institute for Zoo and Wildlife research, Berlin, Germany

M&M: Sequencing casein genes

* Goat reference sequences were obtained from the *Capra hircus* LWT01 genome version (NCBI)
* Array capture was used to obtain the casein cluster
* Sequencing using high density capture sequencing

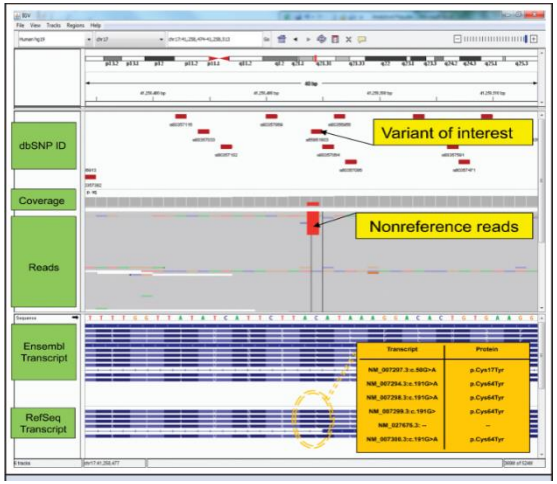


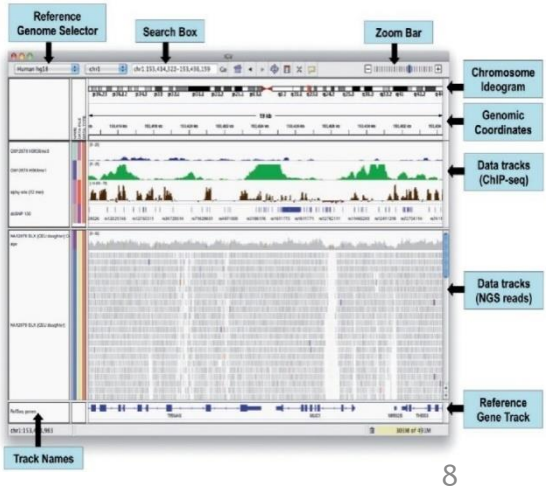
M&M: Data analysis

* Variants were called using VarScan[1], optimized to detect SNPs

* Polymorphisms were identified manually using the Integrative Genomics Viewer (IGV)[2]

* Haplotypes were reconstructed from nonsynonmous SNPs using the haplo.stats package[3] for R



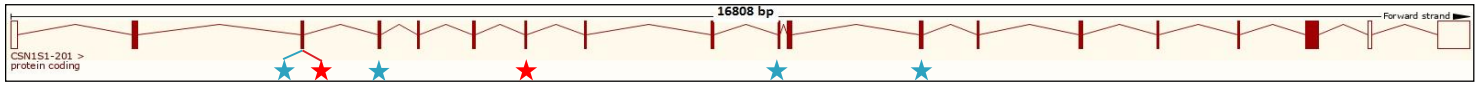


[1] Koboldt et al. (2012) *Genome Research*
[2] Robinson et al. (2011) *Nature Biotechnology*
[3] Sinnwell et al. (2013) *CRAN*

Results: Novel nonsynonymous SNPs

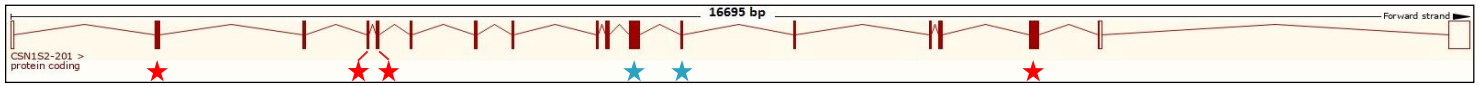
* *CSN1S1* - 6 SNPs, **2** Novel

CHR6:85981710_C>A (Saanen), Histidine to Asparagine (-1)
CHR6:85984154_A>G (Alpine ibex), Isoleucine to Valine (3)



* *CSN1S2* - 6 SNPs, **4** Novel

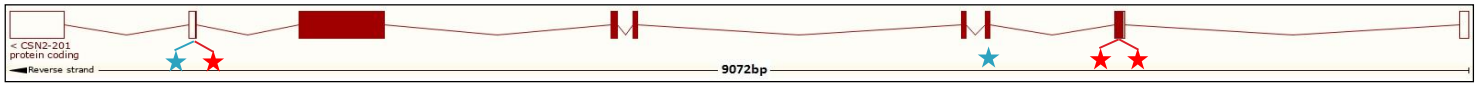
CHR6:86079098_T>C (Saanen & Taggar), Phenylalanine to Serine (-2)
CHR6:86081790_T>C (Nubian ibex), Phenylalanine to Serine (-2)
CHR6:86081887_T>C (Nubian ibex), Isoleucine to Threonine (-1)
CHR6:86089407_G>A (Nubian ibex), Serine to Asparagine (0)



Results: Novel nonsynonymous SNPs

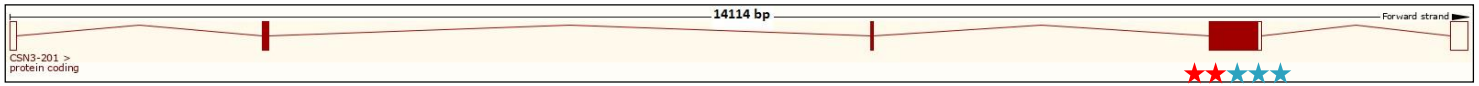
* *CSN2* - 5 SNPs, **3** Novel

CHR6:86008103_G>A (Alpine ibex), Proline to Leucine (-3)
CHR6:86015259_T>C (Alpine & Nubian ibex), Histidine to Arginine (0)
CHR6:86015278_G>C (Alpine & Nubian ibex), Leucine to Valine (1)



* *CSN3* - 5 SNPs, **2** Novel

CHR6:86208927_G>A (Alpine ibex), Serine to Asparagine (0)
CHR6:86208939_G>C (Alpine ibex), Serine to Threonine (1)



A large number of **novel nonsynonymous SNPs** are **only** found in the **Alpine/Nubian Ibex**

Results: Constructed haplotypes

	CHR6:85981710_C>A	rs652562211	rs64418353	CHR6:85984154_A>G	rs155505536	rs268293072
Haplotype A	A	T	C	A	G	A
Haplotype B	C	A	C	A	G	A
Haplotype C	C	A	C	A	G	G
Haplotype D	C	A	T	A	C	G
Haplotype E	C	A	T	A	G	A
Haplotype F	C	A	T	G	C	G

	Saanen	Desert	Nubian	Nilotic	Tagger	Ibex	Bezoar
Haplotype A	0,25						
Haplotype B	0,50	0,50	0,57	0,57	0,64		
Haplotype C	0,25						
Haplotype D		0,50	0,43	0,43	0,36	0,83	0,50
Haplotype E							0,50
Haplotype F						0,17	

CSN1S1:	6 haplotypes
CSN1S2:	6 haplotypes
CSN2:	6 haplotypes
CSN3:	4 haplotypes

Results: Constructed haplotypes

	CHR6:86208927_G>A	CHR6:86208939_G>C	rs268293109	rs268293113	rs651045868
Haplotype A	A	C	A	G	T
Haplotype B	G	G	A	A	C
Haplotype C	G	G	A	G	T
Haplotype D	G	G	G	G	T

	Saanen	Desert	Nubian	Nilotic	Tagger	Ibex	Bezoar
Haplotype A						0.50	
Haplotype B		0.10	0.14				
Haplotype C	0.50	0.90	0.86	1.00	0.93	0.50	1.00
Haplotype D	0.50				0.07		

CSN1S1: 6 haplotypes
CSN1S2: 6 haplotypes
CSN2: 6 haplotypes
CSN3: 4 haplotypes

Summary

Novel nonsynonymous SNPs in the casein genes were found in **Alpine ibex** and **critically endangered Nubian ibex**

This highlights the importance of
studying and preservation
these rare and/or endangered breeds

Acknowledgments

Humboldt university, Berlin

Prof. Dr. Gudrun Brockmann

Dr. Danny Arends

Dr. Monika Reissmann

Special thanks for the efforts of the goat owners and Research stations in Sudan. Leibniz institute for Zoo and Wildlife research in Berlin.

Unterstützt von / Supported by

Alexander von Humboldt
Stiftung/Foundation

Thank you for your attention