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Session GPh1.5 Abstract No. 621

Sequence Polymorphisms in the 5‘ Region of the a_{S1}-Casein Gene in Goat – A new Perspective to explain quantitative Variability in a_{S1}-Casein Content?

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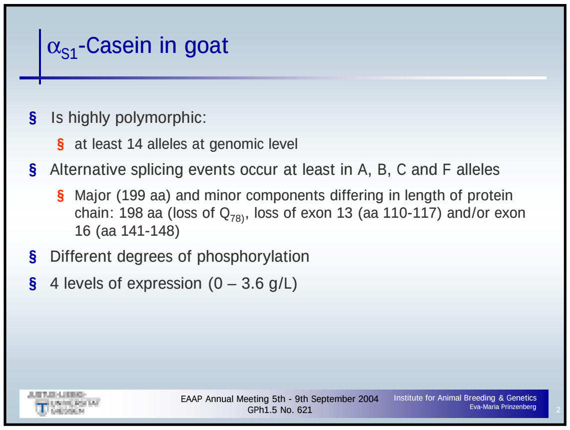
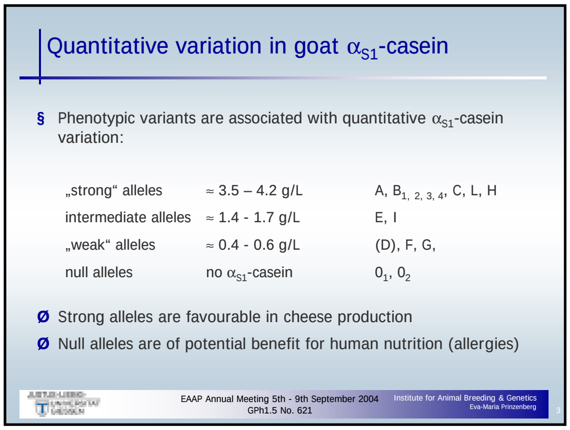


α_{S1}-Casein in goat

- Is highly polymorphic:
  - at least 14 alleles at genomic level
- Alternative splicing events occur at least in A, B, C and F alleles
  - Major (199 aa) and minor components differing in length of protein chain: 198 aa (loss of $Q_{78)}$, loss of exon 13 (aa 110-117) and/or exon 16 (aa 141-148)
- Different degrees of phosphorylation
- 4 levels of expression (0 – 3.6 g/L)

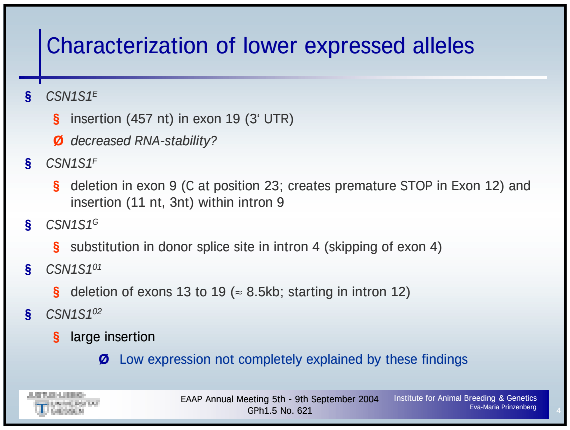


Quantitative variation in goat α_{S1}-casein

- Phenotypic variants are associated with quantitative α_{S1}-casein variation:

„strong“ alleles	≈ 3.5 – 4.2 g/L	A, $B_{1, 2, 3, 4}$, C, L, H
intermediate alleles	≈ 1.4 - 1.7 g/L	E, I
„weak“ alleles	≈ 0.4 - 0.6 g/L	(D), F, G,
null alleles	no α_{S1}-casein	0_1, 0_2

- Ø Strong alleles are favourable in cheese production
- Ø Null alleles are of potential benefit for human nutrition (allergies)



Characterization of lower expressed alleles

- *CSN1S1^E*
  - insertion (457 nt) in exon 19 (3‘ UTR)
  - Ø *decreased RNA-stability?*
- *CSN1S1^F*
  - deletion in exon 9 (C at position 23; creates premature STOP in Exon 12) and insertion (11 nt, 3nt) within intron 9
- *CSN1S1^G*
  - substitution in donor splice site in intron 4 (skipping of exon 4)
- *CSN1S1^01*
  - deletion of exons 13 to 19 (≈ 8.5kb; starting in intron 12)
- *CSN1S1^02*
  - large insertion
    - Ø Low expression not completely explained by these findings

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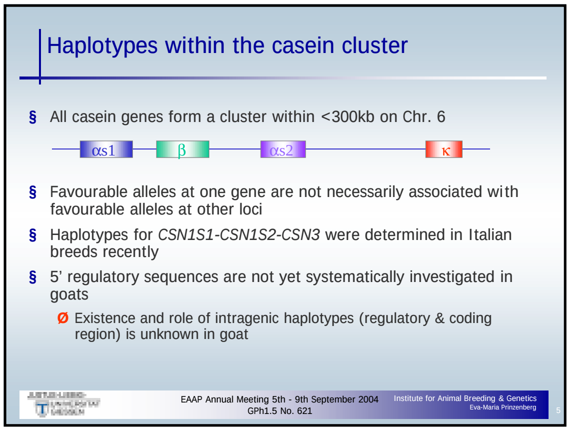

Haplotypes within the casein cluster

- All casein genes form a cluster within <300kb on Chr. 6

- Favourable alleles at one gene are not necessarily associated with favourable alleles at other loci
- Haplotypes for *CSN1S1-CSN1S2-CSN3* were determined in Italian breeds recently
- 5’ regulatory sequences are not yet systematically investigated in goats
  - Ø Existence and role of intragenic haplotypes (regulatory & coding region) is unknown in goat

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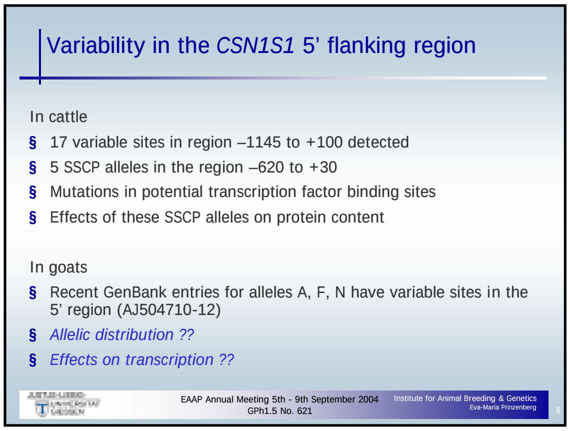


Variability in the *CSN1S1* 5’ flanking region

In cattle

- 17 variable sites in region –1145 to +100 detected
- 5 SSCP alleles in the region –620 to +30
- Mutations in potential transcription factor binding sites
- Effects of these SSCP alleles on protein content

In goats

- Recent GenBank entries for alleles A, F, N have variable sites in the 5’ region (AJ504710-12)
- *Allelic distribution ??*
- *Effects on transcription ??*

Aims

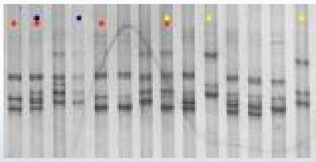
- Assess variability in the goat *CSN1S1-5'* region
- Determine sequence variation, especially variable transcription factor binding sites
- Examine allele frequencies for 5' SNPs
- Check intragenic haplotypes of promoter and coding region
  - especially for low expressed *CSN1S1*^{F}

Material and Methods

- DNA of unrelated German and Turkish goats
  - German Improved Fawn (BDE) and White (WDE), Thuringian Forest Goat (TWZ)
  - Anatolian Hair Goat (THG) and Turkish Angora Goat (TAG)
- PCR-SSCP for polymorphism screening
- Sequence analyses of *CSN1S1-5'* region
- Search for restriction enzyme sites and transcription factor binding motifs (DNASIS^{TM})
- Design of goat specific primers for PCR-RFLP typing *CSN1S1-5'*
- Modification of PCR-RFLP typing exon/intron 9
- Calculation of allele frequencies and genotypic disequilibrium using CONVERT, GENEPOP and FSTAT

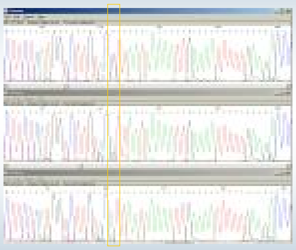
Screening of the *CSN1S1* 5' region in goats

- German Improved Fawn (Bunte Dt. Edelziege)
- PCR amplification with primers derived from the bovine sequence
  - Fragment corresponds to pos. 1513 – 2167 in X59856



Ø Selection of DNA with different patterns for sequencing

Sequencing



- PCR primers for sequencing include pos 943-2167 of the bovine sequence (X59856)

Ø ~1kb 5' *CSN1S1*

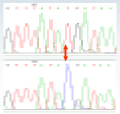
- Bi-directional sequencing of PCR products (BigDye Terminator Kit 1.1, Applied Biosystems), ABI 377

Sequencing results

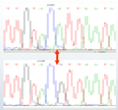
- 65 variable sites within position – 1100 and –100
  - 47 SNPs between goat/cattle (27 transitions, 20 transversions)
  - 3 insertions + 3 deletions goat/cattle
  - 2 deletions discriminate goat sequences
  - 10 SNPs within goat sequences (7:4) => *CSN1S1-5'* alleles
- Compared to the sequences in GenBank for *CSN1S1* $^{A, F, N}$
  - Oligo-T stretch around position – 222 is $(T)_{12}$ in our sequences, $(T)_{14}$ in those available => *re-sequencing of cloned fragments is in progress*
  - 2 new sequence variants were detected (2 transversions)

Mutations affecting transcription factor binding motifs

Search with DNASIS^{TM} 7.0



- GATA-motif: consensus WGATAR
  - present (TGATAAtta) in sequence type 3
  - missing (<u>C</u>GATAAtta) in other sequences

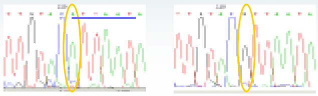


- GMCSF-motif: consensus CATTW
  - gtaCATTAat in sequence type 2 and 3
  - gtaC<u>G</u>TTAat in type 1

Ø Type 1 is lacking both sites
  - Ø discrimination by PCR-RFLP

PCR-RFLP typing: *CSN1S1-5'*

- A/G SNP affects *Ase*I (*Vsp*I) recognition sequence: ATTAAT



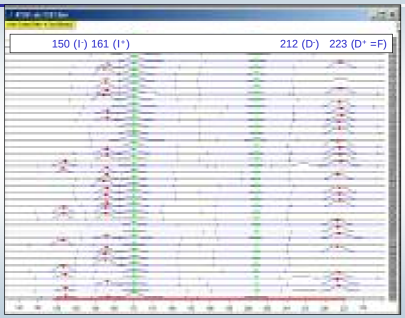
- Amplification of 577bp, " *VspI*
  - G ⇒ 577 bp
  - A ⇒ 387 + 190 bp
  - AG ⇒ 577 + 387 + 190 bp

PCR-RFLP typing: Exon/Intron 9

- *Xmn*I restriction analysis for detection of C deletion (nt23) within exon 9 (*CSN1S1*^{F})
- Simultaneous detection of insertion (11nt) within intron 9

- Modification of procedure described by Rammuno et al. 2000
  - Cy5-labelled reverse primer => fluorescent detection (A.L.F.*express*)

PCR-RFLP typing: Exon/Intron 9

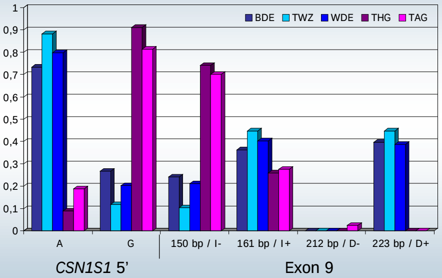


150 (I^-) 161 (I^+) 212 (D^-) 223 (D^+ =F)

RFLP allele frequencies *CSN1S1* 5' and Exon 9

Breed		RFLP 5' region			RFLP Exon9			
	n	A	G	n	I^-	I^+	D^-	D^+
BDE	30	0.733	0.267	29	0.241	0.362	-	0.397
TWZ	30	0.883	0.117	29	0.103	0.448	-	0.448
WDE	32	0.797	0.203	31	0.210	0.403	-	0.387
THG	50	0.090	0.910	50	0.740	0.260	-	-
TAG	40	0.188	0.813	40	0.700	0.275	0.025	-
total	182			179				

RFLP allele frequencies *CSN1S1* 5' and Exon 9

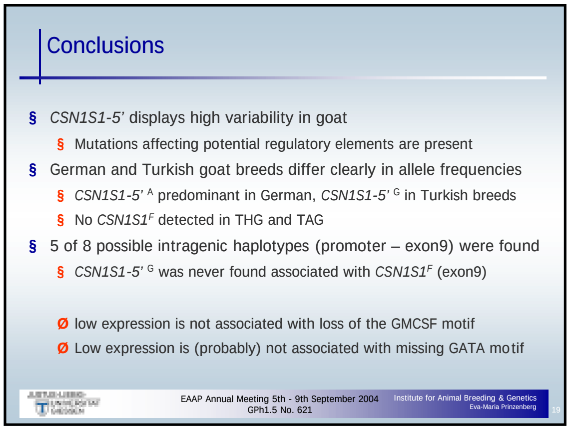


Intragenic haplotypes

- Linkage disequilibrium between RFLP in *CSN1S1* 5' region and exon 9
  - high significant in BDE, WDE, THG and TAG (after Bonferroni correction)

- A in the 5' region is found with I^+ *and* D^+ in the exon 9 region
  - 2 haplotypes: A-D^+ (47%), A-I^+ (53%)

- G in the 5' region RFLP is found preferentially with I^+ and I^-
  - G-D^- is specific in Turkish Angora Goat (two goats)
  - 3 haplotypes: G-I+(~20%), G-I^- (~80%), G-D^-



Conclusions

- *CSN1S1-5′* displays high variability in goat
  - Mutations affecting potential regulatory elements are present
- German and Turkish goat breeds differ clearly in allele frequencies
  - *CSN1S1-5′* ^{A} predominant in German, *CSN1S1-5′* ^{G} in Turkish breeds
  - No *CSN1S1* ^{F} detected in THG and TAG
- 5 of 8 possible intragenic haplotypes (promoter – exon9) were found
  - *CSN1S1-5′* ^{G} was never found associated with *CSN1S1* ^{F} (exon9)

Ø low expression is not associated with loss of the GMCSF motif

Ø Low expression is (probably) not associated with missing GATA motif

Thanks!

To

- Christine Förster, Sandra Stein
- German and Turkish goat breeders