

Analysis of the ovine homolog of the T-locus as a candidate gene for short tail phenotype

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Genetic background of short tail phenotypes

- Phenotypic variation of tail length between sheep breeds worldwide
- Different heritabilities of tail length were estimated
- Several candidate genes are discussed



Tail phenotypes in domestic sheep

- Fat tailed
- Fat rumped
- Short tailed
- Long tailed
- Thin tailed



Fat tailed, Horro sheep Ethiopia (FAO)



Fat rumped; Hissar sheep (vieh-ev.de)



Short tailed, German Grey Heath



Long tailed, Shugor sheep Sudan (FAO)



Thin tailed, Merinoland sheep

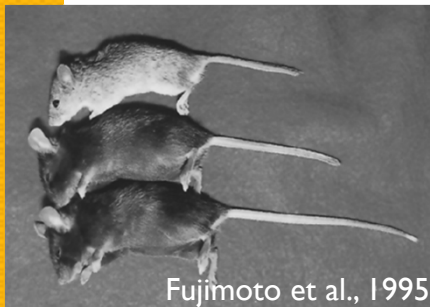
according to Mason (1991)

Heritability (h^2) of tail length in sheep

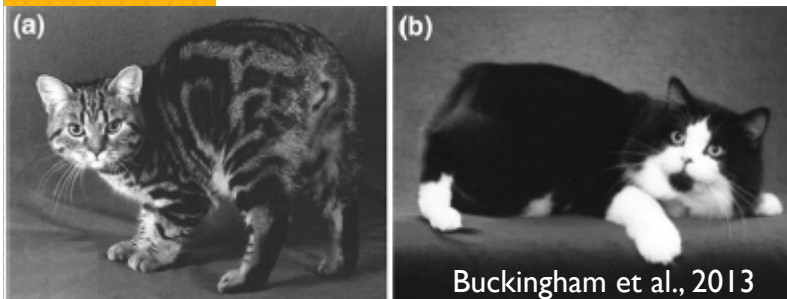
Breed	h^2	Study
Finnish landrace	0.77	Branford Oltenacu & Boylan, 1974
Rambouillet	0.39	Shelton, 1977
Mouflon crosses	0.71	Shelton, 1977
Finnish landrace – Cheviot crosses	0.82	Scobie & O'Connell, 2002
Suffolk	0.41	De Haas & Verkamp, 2004
Hampshire Down	0.2	Bohte Wilhelmus et al., 2010
Clun Forest	0.27	Bohte Wilhelmus et al., 2010

Short tail phenotypes in other species

- Short tail phenotype in mice
- Variable tail length in Manx cats
- Tail length variation in several dog breeds



T-Gene



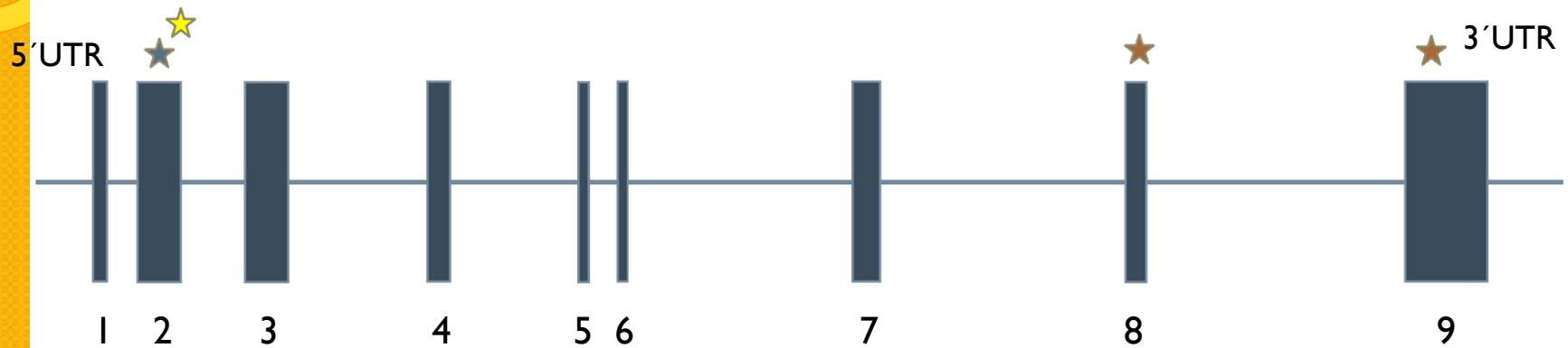
The T-gene (brachyury gene)

- Founding member of the T-box family of transcription factors
- Brachyury encodes a transcription factor essential for the genesis and maintenance of mesoderm and notochord
- Important for the axial development of all vertebrates
- Polymorphisms in the T-gene lead to short tail phenotypes in several species



Is the T-gene also responsible for tail-length variation in sheep?

Structure of the T-gene including SNPs for short tail phenotypes in different species



- ★ SNP in canine Exon 2
- ★ SNP in murine Exon 2
- ★ SNPs in feline Exon 8 and 9

Aims of the study

- Characterization of the T-gene in sheep
- Identification of sequence variations within the coding regions of the gene
- Association between sequence variations and short tail phenotype



Animals

Research Station of the Justus-Liebig-University in Gießen

Long tailed



Merinoland Sheep (n = 10)



Rhoensheep (n = 5)

Short tailed



Romanov Sheep (n = 6)



German Grey Heath (n = 5)

Molecular Genetic Methods

- BLAST analysis using the feline and canine sequence of the T-gene
- Primer design based on the ovine genomic reference sequence (GenBank NC_019465) in region 87796143 – 87805552 of chromosome 8
- Sequencing of coding regions and part of untranslated regions

SNPs in Exon 3, 4 and 9

Exon 3

AGG AGG ATG TTC CCG GTG CTG AAG GTG AA^TC GTA TCC GGC CTG GAC CCC AAC GCC ATG TAC TCC TTC
 CTG CTG GAC TTC GTG GC^GC^TGC^CGAC AAC CAC CGC TGG AAG TAC GTG AAC GGG GAG TGG GTG CCG
 GGG GGC AAG CC^AG^GGAG CCG CAG GCG CCC AGC TGC GTC TAC ATC CAC CCC GAC TCC CCC AAC TTC
 GGG GCG CAC TGG ATG AAG GCA CCT GTC TCC TTC AGC AAA GTC AAG CTC ACC AAC AAG CTC AAT
 GGA GGG GGC CAG

Exon 4

ATC ATG TTG AAC TCC TTA CAT AAG TAT GAG CCT CGG ATC CAC ATC GTG AGA GTT GGG GGT CCA CAG
 CGT ATG ATC ACC AGC CAC TGC TTC CCC GAG ACC CAG TTC ATC GC^GT^TGTG AC^CT^TGCT TAC CAA AA^TC^CGAG
 GAG

Exon 9

CAG TAC CCC AGC CTG TGG TC^TC^CGTG AGC AGC GGT GCC GTC GCC CCG GGC GCC CAG GCG GCG GGT
 GTG CCC AGC GGG CTG GGA GCC CAG TTC TTC CGA GGC TCC CCT GCC CAC TCT ACC CCC CTC GCC CAC
 CCG GTC TCA GCG TCC TCC TCG TCG GGG TCC CCA CTG TAC GAG GGG GCC^AG^GCC ACG GCC ACA GAC
 GTA GCT GAC AGC CAG TAT GAT GCC TCC GCC CAG GCC CGC CTC CTG GCC TCG TGG ACG GCC GTG TCG
 CCC CCG TCC ATG TGA

SNPs in Exon 3, 4 and 9

Exon 3

AGG AGG ATG TTC CCG GTG CTG AAG GTG AA**T**C GTA TCC GGC CTG GAC CCC AAC GCC ATG TAC TCC TTC
 CTG CTG GAC TTC GTG GC**G**GC**T**C GAC AAC CAC CGC TGG AAG TAC GTG AAC GGG GAG TGG GTG CCG
 GGG GGC AAG CC**A**G GAG CCG CAG GCG CCC AGC TGC GTC TAC ATC CAC CCC GAC TCC CCC AAC TTC
 GGG GCG CAC TGG ATG AAG GCA CCT GTC TCC TTC AGC AAA GTC AAG CTC ACC AAC AAG CTC AAT
 GGA GGG GGC CAG

Exon 4





ATC ATG TTG AAC TCC TTA CAT AAG TAT GAG CCT CGG ATC
 CGT ATG ATC ACC AGC CAC TGC TTC CCC GAG ACC CAG TT
 GAG

Ala Ala Thr
 GCC **GCC** ACG
 GCC **ACC** ACG
 Ala Thr Thr





Exon 9

CAG TAC CCC AGC CTG TGG TC**T**C GTG AGC AGC GGT GCC G
 GTG CCC AGC GGG CTG GGA GCC CAG TTC TTC CGA GGC TCC CCT GCC CAC TCT ACC CCC CTC GCC CAC
 CCG GTC TCA GCG TCC TCC TCG TCG GGG TCC CCA CTG TAC GAG GGG GSC **A**GC ACG GCC ACA GAC
 GTA GCT GAC AGC CAG TAT GAT GCC TCC GCC CAG GCC CGC CTC CTG G**G**CC TCG TCG ACG GCC GTG TCG
 CCC CCG TCC ATG TGA

Typing results for the non synonymous SNP in Exon 9

Breed	Tail	Animal No.	SNP	Amino acid
German Grey Heath	short	919	A/A	Thr
		920	G/G	Ala
		922	A/A	Thr
		923	G/G	Ala
Romanov	short	400	G/G	Ala
		404	G/G	Ala
		405	G/G	Ala
Rhoensheep	long	346	A/A	Thr
		347	A/A	Thr
		351	G/G	Ala
		345	G/G	Ala
Merinoland	long	543	G/G	Ala
		1976	G/G	Ala
		2644	G/G	Ala
		844	G/G	Ala

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		347	A/A	Thr
		351	G/G	Ala
		345	G/G	Ala
Merinoland	long	543	G/G	Ala
		1976	G/G	Ala
		2644	G/G	Ala
		844	G/G	Ala

Exon skipping

Exon 6

R S D H K E M M E E A G D S Q Q P
 AGA AGC GAT CAC AAA GAG ATG ATG GAA GAA GCG GGA GAC AGC CAG CAG CCT
 G Y S Q
 GGG TAC AGC CAA T >>>>>

Exon 7

W G W L I P G T S S L C P P A T P
 >>>>>GG GGC TGG CTG ATC CCC GGA ACC AGT AGC CTG TGT CCA CCC GCC ACC CCC
 H P Q F Q G P L S L P S T H G C E R
 CAC CCC CAG TTT CAG GGC CCC CTC TCG CTC CCC TCC ACG CAC GGC TGC GAA AGG
 F P A L R S H R P A P Y P S P Y A H
 TTC CCG GCC CTG AGG AGC CAC CGG CCA GCC CCC TAC CCC AGC CCG TAC GCG CAT
 R N S S P S K
 CGC AAC AGC TCT CCA AGT AAG

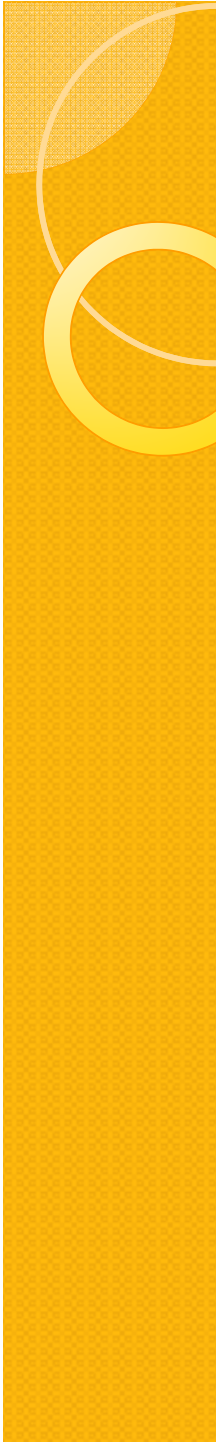
- Splicing variants in other species described
- No polymorphism at the donor/acceptor sites in the present study

Conclusions

- High conservation of the T-gene between cat, dog and sheep
- Polymorphisms causing the short tail phenotype in mice, cats and dogs were not identified in sheep
- The non synonymous SNP in Exon 9 of the gene is not the causal mutation for short tail phenotype in sheep



Thank you for your attention



Position of the detected SNPs and coded amino acids

Location	SNP	Amino acid	Exchange
Exon 3	c.234C>T	Asn	
Exon 3	c.288C>G	Ala	
Exon 3	c.291C>T	Ala	
Exon 3	c.345G>A	Pro	
Exon 4	c.582T>G	Ala	
Exon 4	c.588T>C	Thr	
Exon 4	c.600T>C	Asn	
Exon 9	c.1060C>T	Ser	
Exon 9	c.1217G>A	Ala	Thr

SNPs in the coding regions of the ovine T – gene

ATG ACC TCC CCG GGC ACC GAC AGC CCG GGG AAG AGC CTG CAG TAC CGC GTG GAC CAT CTG CTG AGC GCC GTG GAG AGC GAG
CTG CAG GCG GGC AGC GAG AAG GGC GAC CCC ACG GAG CGC GAG CTG CGC GTG GGC CTG GAG GAG AGC GAG CTG TGG CTG CGC
TTC AAG GAG CTC ACC AAC GAG ATG ATC GTC ACC AAG AAC GGC AGG AGG ATG TTC CCG GTG CTG AAG GTG AAC GTA TCC GGC CTG
GAC CCC AAC GCC ATG TAC TCC TTC CTG CTG GAC TTC GTG GCC GCC GAC AAC CAC CGC TGG AAG TAC GTG AAC GGG GAG TGG
GTG CCG GGG GGC AAG CCG GAG CCG CAG GCG CCC AGC TGC GTC TAC ATC CAC CCC GAC TCC CCC AAC TTC GGG GCG CAC TGG
ATG AAG GCA CCT GTC TCC TTC AGC AAA GTC AAG CTC ACC AAC AAG CTC AAT GGA GGG GGC CAG ATC ATG TTG AAC TCC TTA CAT
AAG TAT GAG CCT CGG ATC CAC ATC GTG AGA GTT GGG GGT CCA CAG CGT ATG ATC ACC AGC CAC TGC TTC CCC GAG ACC CAG TTC
ATC GCT GTG ACT GCT TAC CAA AAT GAG GAG ATC ACA GCT CTT AAA ATT AAA TAC AAT CCG TTT GCA AAA GCT TTC CTC GAC GCA
AAG GAA AGA AGC GAT CAC AAA GAG ATG ATG GAA GAA GCG GGA GAC AGC CAG CAG CCT GGG TAC AGC CAA T>>>>GG GGC TGG
CTG ATC CCC GGA ACC AGT AGC CTG TGT CCA CCC GCC ACC CCC CAC CCC CAG TTT CAG GGC CCC CTC TCG CTC CCC TCC ACG CAC
GGC TGC GAA AGG TTC CCG GCC CTG AGG AGC CAC CGG CCA GCC CCC TAC CCC AGC CCG TAC GCG CAT CGC AAC AGC TCT CCA
AGT AAG TAT TCC GAC AGT TCA TCT GCA TGT CTG TCC ATG CTC CAG CCC CAT GAC AAC TGG TCC AGC CTT GGA ATG CCT GCC CAC
ACC AGC ATG CTG CCC ATG GGT CCG AAC GCT GGT CCT CCT GCG GGC TCC CAG TAC CCC AGC CTG TGG TCG GTG AGC AGC GGT
GCC GTC GCC CCG GGC GCC CAG GCG GCG GGT GTG CCC AGC GGG CTG GGA GCC CAG TTC TTC CGA GGC TCC CCT GCC CAC TCT
ACC CCC CTC GCC CAC CCG GTC TCA GCG TCC TCC TCG TCG GGG TCC CCA CTG TAC GAG GGG GCC GCC ACG GCC ACA GAC GTA
GCT GAC AGC CAG TAT GAT GCC TCC GCC CAG GCC CGC CTC CTG GCC TCG TGG ACG GCC GTG TCG CCC CCG TCC ATG TGA