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Allele and haplotype polymorphism of the myostatin gene (*MSTN*) microsatellite containing region in *Latvian Blue* and *Latvian Brown* cattle breeds

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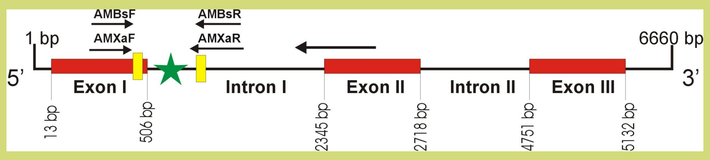
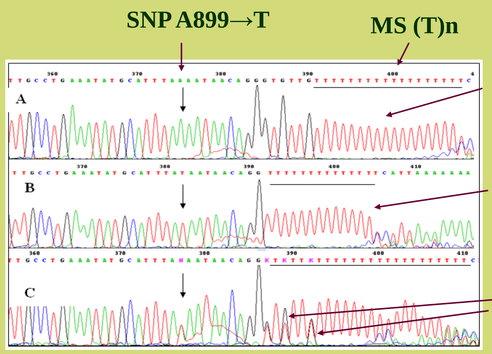
Myostatin (MSTN), a member of the transforming growth factor-beta (TGF-beta) superfamily, is a highly conserved, potent negative regulator of skeletal muscle growth in many species from rodents to humans . *MSTN* is highly conserved across species. The bovine *MSTN* gene is located at 2q11 and consists of three exons and two introns. Several *MSTN* gene structural variations especially in exon II and exon III have been reported as potentially significant in cattle phenotype performance. In many species belonging to the *Artiodactyls* (pig, goat, sheep, cattle), first intron of the *MSTN* gene is characterized by the presence of the T-mononucleotide microsatellite (MS). High variability of the MS 5' flanking sequence and of the number of T- units in the repeat was identified within and between bovine breeds (De la Rosa-Reyna et al., 2006) .

STRATEGY OF THE EXPERIMENT

***Bos taurus* (GeneBank:AB076403.1) *MSTN* gene structure**



Mazversite J., Grislis Z., Sugoka O., Sokolovska J. & Sjakste T. (2008) Evaluation of the microsatellite polymorphism in intron I of the myostatine gene (MSTN) in Latvian Blue cattle breed. *Latvian Journal of Agronomy* 10:267 – 270.



$A^{773}(G)_2(GT)_2TG(T)_nC^{798}$ (AB076403-like or L allele)

$A^{773}(G)_2(T)_nC^{798}$ (AY850105-like or M allele)

LM heterozygote

MATERIALS AND METHODS

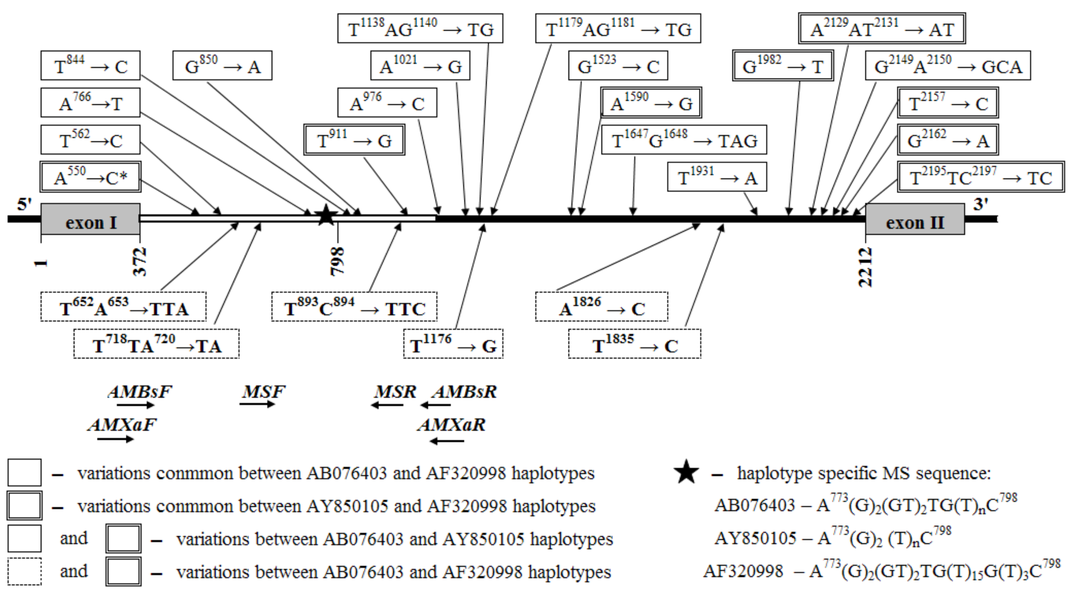
- **18 animals of *Latvian Brown* cattle breed**
- **14 animals of *Latvian Blue* cattle**
- **Genotyping:**
  - **MS region sizing (amplified fragment size analysis)**
  - **Sequencing of the MSTN gene intron I 5' region encompassing MS**

Results

Animals description and genotypes presented as the alleles of the MS sequence motif, MS length polymorphism and $(T)_n$ repeat number.

MS region genotype				Number of animals	
No	Motif	Fragment length (bp)	Repeat number	*Latvian Blue*	*Latvian Brown*
I	LL	285/285	T_{17}/T_{17}		4
II	LL	286/286	T_{18}/T_{18}		1
III	LL	287/287	T_{19}/T_{19}		1
IV	LL	284/285	T_{16}/T_{17}	1	1
V	LL	285/286	T_{17}/T_{18}	5	5
VI	MM	276/276	T_{14}/T_{14}	1	1
VII	MM	277/277	T_{15}/T_{15}	1	
VIII	MM	275/276	T_{13}/T_{14}	1	
IX	LM	285/275	T_{17}/T_{13}	3	1
X	LM	285/276	T_{17}/T_{14}	1	1
XI	LM	286/276	T_{18}/T_{14}		1
XII	LM	285/277	T_{17}/T_{15}	1	
XIII	LM	286/277	T_{18}/T_{15}		1
XIV	LM	286/278	T_{18}/T_{16}		1
Total animals of breed				14	18

Haplotype variations of the intron I of *MSTN* gene



□ – variations conmmon between AB076403 and AF320998 haplotypes

□ – variations conmmon between AY850105 and AF320998 haplotypes

□ and □ – variations between AB076403 and AY850105 haplotypes

□ and □ – variations between AB076403 and AF320998 haplotypes

★ – haplotype specific MS sequence:

AB076403 – $A^{773}(G)_2(GT)_2TG(T)_nC^{798}$

AY850105 – $A^{773}(G)_2(T)_nC^{798}$

AF320998 – $A^{773}(G)_2(GT)_2TG(T)_{15}G(T)_3C^{798}$

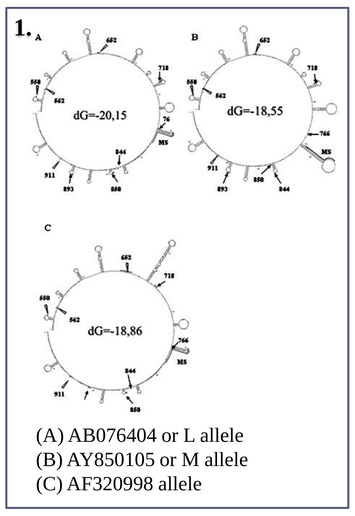
Haplotypes and eventual functional significance of polymorphisms evaluated on the perturbations of the TFBSs, miRNA targets, and DNA secondary structures. Function of variation was scored as generation (G), loss (L) or absence (N) of perturbation. Strand is indicated in brackets (+/-). Numbering of polymorphic loci was calculated from the first ATG of the genomic sequence reported for *MSTN* gene (GenBank AB076403).

Variation	Haplotype					Eventual functional significance of mutation		
	AB076403	L	AY850105	M	AF320998	Function & Family/Matrix TFBSs (+/-)	Function & Family/Matrix miRNA target	DNA SS
SNP	A^{550}	- *C^{550}	-	-	-	N	N	N
SNP	T^{562}	-	C^{562}	-	-	L & NFAT/NFAT.01 (-)	N	N
InDel	$T^{652}A^{653}$	-	-	-	$T^{652}TA^{653}$	N	N	N
InDel	$T^{718}TA^{720}$	-	-	-	$T^{718}A^{720}$	L & SORY/HMGIY.01 (-)	N	G
SNP	A^{766}	-	T^{766}	T^{766}	-	G & TBPF/TATA.01 (-)	N	G
MS	n = 16 19		n = 13 16			N	N	
SNP	T^{844}	- *C^{844}	C^{844}	C^{844}	-	G & GATA/GATA1.06 (+) G & AP4R/PARAXIS.01 (-)	N	G
SNP	G^{850}	-	A^{850}	A^{850}	-	G & HMTB/MTBF.01 (+)	L & has-miR-26a L & has-miR-93	G
InDel	$T^{893}C^{894}$	-	-	-	$T^{893}TC^{894}$	N	G & has-let-7g	G
SNP	T^{911}	- *G^{911}	G^{911}	G^{911}	G^{911}	G & AIRE/AIRE.01 (+)	N	N

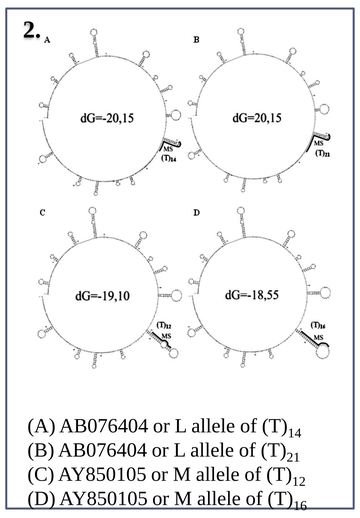
Description of the TFs which possess polymorphism dependent binding sites in 5' region of intron I

Family	Family information	Matrix name	Matrix information
AIRE	Autoimmune regulatory element binding factor	AIRE.01	Autoimmune regulator
AP4R	AP4 and related proteins	PARAXIS.01	Paraxis (TCF15), member of the Twist subfamily of Class B bHLH factors, forms heterodimers with E12
GATA	GATA binding factors	GATA1.06	Complex of Lmo2 bound to Tal-1, E2A proteins, and GATA-1, half-site 2
HMTB	Human muscle-specific Mt binding site	MTBF.01	Muscle-specific Mt binding site
NFAT	Nuclear factor of activated T-cells	NFAT	Nuclear factor of activated T-cells
SORY	SOx/sRY-sex/testis determinig and related HMG box factors	HMGIY.01	HMGI(Y) high-mobility-group protein I (Y), architectural transcription factor organizing the framework of a nuclear protein-DNA transcriptional complex
TBPF	TATA-binding protein factor	TATA.01	Cellular and viral TATA box elements

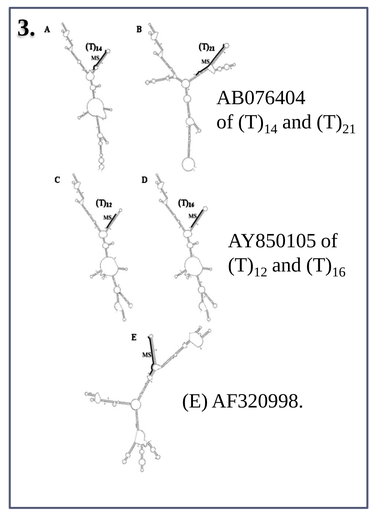
Predicted DNA secondary structures (1), impact of MS repeat number on DNA secondary structures (2) and RNA secondary structures (3). White and black arrows indicate correspondingly mutations neutral and active for reorganization of the DNA secondary structure.



(A) AB076404 or L allele
(B) AY850105 or M allele
(C) AF320998 allele



(A) AB076404 or L allele of $(T)_{14}$
(B) AB076404 or L allele of $(T)_{21}$
(C) AY850105 or M allele of $(T)_{12}$
(D) AY850105 or M allele of $(T)_{16}$



Conclusions

- Two MS sequence motifs: $A^{773}(G)_2(GT)_2TG(T)_nC^{798}$ (AB076403-like or L allele) and $A^{773}(G)_2(T)_nC^{798}$ (AY850105-like or M allele) were identified in gene portion in animals of both breeds in homozygote and heterozygote state
- High MS length variability was revealed within of the each L and M MS motifs
- MS motif and MS (T)n repeat polymorphism appears to posses functional significance in *MSTN* gene function