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GENETIC VARIATION OF THE MITOCHONDRIAL D-LOOP REGION CONTAINING MITOCHONDRIAL TRANSCRIPTION FACTOR A (*TFAM*) BINDING SITES IS NOT ASSOCIATED WITH MARBLING IN WAGYU X LIMOUSIN F_2 CROSSES

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ABSTRACT

Mitochondrial transcription factor A (*TFAM*) is a nucleus-encoded protein that is essential for initiation of transcription and replication of mitochondrial DNA (mtDNA). It has been shown that TFAM binds the entire length of the mtDNA, but with higher affinity to sequences around both mitochondrial promoters located in 3' domain of the D-loop region. The objectives of this study were to detect genetic polymorphisms in the sequence flanking TFAM binding sites of the mtDNA D-loop region and investigate their associations with marbling in Wagyu x Limousin F_2 crosses. We identified six polymorphis sites in the TFAM binding sites of the D-loop mtDNA (G8A, T106C, A169G, A173G and C190T and one insertion/deletion at position 221 with one or two cytosines) with no significant differences in frequencies of polymorphic sites between extreme high/low marbling pools.

Key words: cattle / breeds / Limousin / Wagyu / crosses / molecular genetics / mitochondrial transcription factor / mtDNA / muscles / marbling

GENETSKA RAZNOLIKOST MITOHONDRIJSKE D-LOOP REGIJE, KI VSEBUJE VEZAVNO MESTO ZA MITOHONDRIJSKI TRANSKRIPCIJSKI FAKTOR A (TFAM), NI POVEZANA Z MARMORIRANOSTJO PRI F_2 WAGYU X LIMOUSIN KRIŽANCIH

IZVLEČEK

Mitohondrijski transkripcijski factor A (*TFAM*) je v jedru kodiran protein, udeležen v iniciaciji transkripcije in replikacije mitohondrijske DNA (mtDNA). Predhodne raziskave so pokazale, da se TFAM veže po vsej dolžini mtDNA, z večjo afiniteto pa na zaporedja v področju mitohondrijskih promotorjev, ki se nahajata na 3' območju D-loop regije. Namen naše študije je bil identificirati genetske polimorfizme v območju vezave TFAM v D-loop regiji mtDNA in raziskati njihovo povezavo z marmoriranostjo mišic pri F_2 Wagyu x Limousin križancih. V območju vezave TFAM smo v D-loop regiji mtDNA identificirali šest polimorfnih mest (G8A, T106C, A169G, A173G, C190T, insercija/delecija enega ali dveh C na poziciji 221), razporeditev alelov pa se ni statistično značilno razlikovala med skupinama živali z visokimi in nizkimi vrednostmi marmoriranosti.

Ključne besede: govedo / pasme / limuzin / wagyu / križanci / molekularna genetika / mitohondrijski transkripcijski faktor / mtDNK / mišice / marmoriranost

INTRODUCTION

Mitochondrial transcription factor A (*TFAM*) is a nucleus-encoded protein that is essential for initiation of transcription and replication of mitochondrial DNA (mtDNA). Decreased mitochondrial gene expression has been associated with onset of obesity in ob/ob mice (Wilson-Fritch *et al*., 2004). Therefore, genetic variants in the mtDNA flanking the TFAM binding sites might have influence on mitochondrial gene expression and biogenesis of mitochondria, and consequently affect energy metabolism and body fat deposition. Additionally, it has been shown that mtDNA substitution G2232A affect beef marbling score (Mannen *et al.*, 2003). The complete bovine mitochondrial DNA contains 16338 bp (GenBank accession No. V00654) (Anderson *at al*., 1982). Evidence has shown that TFAM binds the entire length of the mtDNA, but with higher affinity to sequences around both mitochondrial promoters located in 3' domain of the D-loop region (Kang *et al.*, 2002; Garrido *et al*., 2003). The objectives of this study were to detect genetic polymorphisms in the sequence flanking TFAM binding sites of the mtDNA D-loop region and investigate their associations with marbling in Wagyu x Limousin F_2 crosses.

MATERIAL AND METHODS

Primer sequences and PCR amplification

Primers were designed to amplify a fragment of 459 bp from positions 16 292 to 412 in the bovine mitochondrial DNA (Anderson *et al*., 1982). The primer sequences were: forward, 5'-CATCTAAAACGGTCCATTCTTTCCTC-3' and reverse, 5'-ACTCATCTAGGCATTTTCA-GT-3'. Genomic DNA (50 ng) was amplified in a final volume of 10 µl that contained 1 x PCR buffer, 1 mM $MgCl_2$, 200 µM dNTPs, 0.5 U *Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA) and 5 pmol of each primer. PCR was performed in an Applied Biosystems Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) under the conditions: initial denaturation at 94 °C for 2 min, followed by touchdown amplification (1 °C temperature decrease per cycle for eight cycles from 66 °C to 59 °C) and followed by 28 cycles of denaturation at 94 °C (30 sec), annealing at 59 °C (30 sec) and extension at 72 °C (30 sec) with a final extension step at 72 °C for 7 min. The PCR products were examined by electrophoresis on a 1.5% agarose gel with 1 X TBE buffer. The gels were stained with ethidium bromide and photographed.

PCR-RFLP analysis

The 459 bp PCR products were digested with 2U of *Nla*III at 37 °C overnight and analysed on 3% agarose gels. The PCR product included two *Nla*III sites (recognition motif CATG↓) on positions 22 and A169G yielding the products of 69 bp and 390 bp in case of no *Nla*III recognition on 169 site, or products of 69 bp, 149 bp and 241 bp in case of *Nla*III recognition. In case of heteroplasmy at 227 site, we expected the appearance of additional two *Nla*III-digestion products of 205 bp and 185 bp in case of no recognition on 169 site or additional *Nla*III-digestion products of 56 bp and 185 bp in case of *Nla*III recognition at 169 site.

RESULTS

We used 31 animals at the two tails of marbling score distribution for mutation detection and initial association screening, including 15 samples with low marbling scores (ranging from 4 to 4.5) and 16 samples with high marbling scores (ranging from 7.5 to 9.5). Sequencing was done

directly and individually on PCR products using the BigDye Terminator Cycle Sequencing method (Applied Biosystems, Foster City, CA, USA). A total of six polymorphic sits were identified, including five substitutions at positions G8A, T106C, A169G, A173G and C190T and one insertion/deletion at position 221 with one or two cytosines (Table 1). Fisher's exact test revealed no significant differences in frequencies of all polymorphic sites between extreme high/low marbling pools ($P > 0.05$) (Table 1).

Table 1. Variants identified in the 3' mtDNA D-loop region that contains two TFAM binding sites

Preglednica 1. Polimorfizmi, identificirani v 3' D-loop področju mtDNA, ki vsebuje dve vezavni mesti za TFAM

Position*	Mutation	Low marbling	High marbling	Significance
8	G	15	15	P = 0.516129
	A	0	1	
106	T	13	15	P = 0.373748
	C	2	1	
169	G	13	11	P = 0.174416
	A	2	5	
173	A	14	12	P = 0.160672
	G	1	4	
190	C	13	16	P = 0.225806
	T	2	0	
221**	CC	0	1	P = 0.205381
	C -	9	11	
	- -	6	4	

* Nucleotide positions were based on GenBank accession number V00654.

**No insertion of cytosine (- -), insertion of one cytosine (C -) or two cytosines (CC) on the nucleotide position 221.

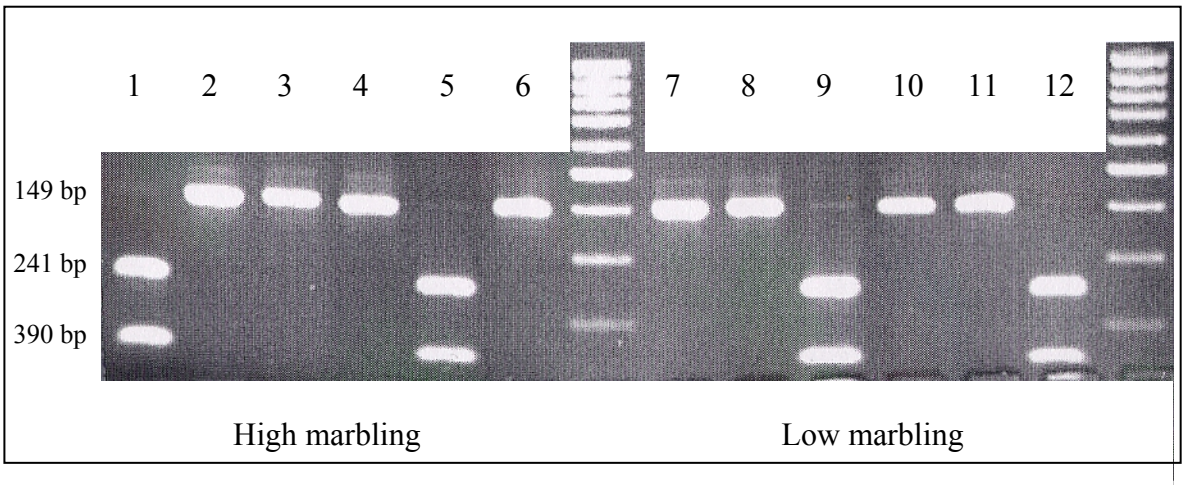

Figure 1. Restriction patterns of samples representing high (1–6) and low (7–12) marbling score. On the right hand side of each block is molecular marker.

Slika 1. Restrikcijska analiza vzorcev živali z visoko (1–6) in nizko (7–12) oceno za marmoriranost. Na desni strani vsake skupine vzorcev je molekularni marker.

DISSCUSION

Previous studies showed the relationships between mtDNA sequence variations and economic traits as milk yield, milk fat yield and milk fat percentage (Schutz *et al*, 1994; Boettcher *et al*., 1996) and beef marbling score (Mannen *et al*., 2003). Identified six polymorphic sites in the bovine mtDNA were also reported previously by different groups (Steinborn *et al*., 1998; Mannen *et al*., 1998; Mannen *et al*., 2003). In the present study, none of these polymorphic sites showed any significant association with marbling in the Wagyu x Limousin cross population, because there were no significant genetic differences observed between high and low marbling animals (Table 1). In dairy cattle, it was also found that there were no significant relationships established between D-loop polymorphism and any traits, such as mature equivalent yield of milk, fat, SNF, and milk energy as well as concentrations of fat, SNF, and milk energy (Boettcher *et al.,* 1996). The major reason might be that none of these polymorphisms occurs within the TFAM binding sites.

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REFERENCES

Anderson, S./ de Bruijn, M.H./ Coulson, A.R./ Eperon, I.C./ Sanger, F./ Young, I.G. Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. J. Mol. Biol., 156(1982)4, 683–717.

Boettcher, P.J./ Freeman, A.E./ Johnston, S.D./ Smith, R.K./ Beitz, D.C./ McDaniel, B.T. Relationships between polymorphism for mitochondrial deoxyribonucleic acid and yield traits of Holstein cows. J. Dairy Sci., 79(1996)4, 647–54.

Garrido, N./ Griparic, L./ Jokitalo, E./ Wartiovaara, J./ van der Bliek, A.M./ Spelbrink, J.N. Composition and dynamics of human mitochondrial nucleoids. Mol. Biol. Cell., 14(2003)4, 1583–1596.

Kang, D./ Hamasaki, N. Maintenance of mitochondrial DNA integrity: repair and degradation. Curr. Genet., 41(2002)5, 311–322.

Mannen, H./ Kojima, T./ Oyama, K./ Mukai, F./ Ishida, T./ Tsuji, S. Effect of mitochondrial DNA variation on carcass traits of Japanese Black cattle. J. Anim. Sci., 76(1998), 36–41.

Mannen, H./ Morimoto M.L./ Oyamat, K./ Mukai, F./ Tsuji, S. Identification of mitochondrial DNA substitutions related to meat quality in Japanese Black cattle. J. Anim. Sci., 81(2003)1, 68–73.

Steinborn, R/ Muller, M./ Brem, G. Genetic variation in functionally important domains of the bovine mtDNA control region. Biochim. Biophys. Acta., 1397(1998)3, 295–304.

Wilson-Fritch, L./ Nicoloro, S./ Chouinard, M./ Lazar, M.A./ Chui, P.C./ Leszyk, J./ Straubhaar, J./ Czech, M.P./ Corvera, S. Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. J. Clin. Invest., 114(2004)9, 1281–1289.