Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

Identification of polymorphism in the SCL24A5 gene of cattle

Letizia Nicoloso, Riccardo Negrini, Elisabetta Milanesi & Paola Crepaldi

To cite this article: Letizia Nicoloso, Riccardo Negrini, Elisabetta Milanesi & Paola Crepaldi (2008) Identification of polymorphism in the SCL24A5 gene of cattle, Italian Journal of Animal Science, 7:4, 505-512, DOI: 10.4081/ijas.2008.505

To link to this article: http://dx.doi.org/10.4081/ijas.2008.505

Short Communication

Identification of polymorphism in the *SCL24A5* gene of cattle

Letizia Nicoloso[1], Riccardo Negrini[2], Elisabetta Milanesi[1], Paola Crepaldi[1]

[1]Dipartimento di Scienze Animali. Università di Milano, Italy

[2]Istituto di Zootecnica. Università Cattolica del S. Cuore, Piacenza, Italy

Corresponding author: Dr. Letizia Nicoloso. Dipartimento di Scienze Animali, Sezione di Zootecnica Agraria. Università degli Studi di Milano. Via Celoria 2, 20133 Milano, Italy - Tel. +39 02 50316448 - Fax: +39 02 50316434 - Email: letizia.nicoloso@unimi.it

Paper received February 15, 2008; accepted June 15, 2008

ABSTRACT

The *SLC24A5* (Solute Carrier family 24, member 5) gene is implicated in skin pigmentation in zebrafish and humans as it regulates the morphogenesis of melanosomes, specialized lysosomes involved in melanin deposit. In humans, the ancestral allele predominates in African and East Asian populations, while the allelic variant is nearly fixed in European populations and correlates with lighter pigmentation. Considering the role of melanin in the protecting of DNA from ultraviolet radiation, the lack of information in cattle and the importance of polymorphisms associated with pigmentation phenotypes, we investigated the *SLC24A5* gene in cattle with light and dark skin pigmentation. To identify SNPs (Single Nucleotide Polymorphisms) in this gene and their association to dark skin pigmentation in cattle, each of the nine *SLC24A5* exons, three introns (1, 3 and 8) and a portion of intron 5, were sequenced in a set of sixteen animals belonging to four Italian cattle breeds, two African zebu breeds and two African sanga breeds. The region spanning exons 3 and 4 was sequenced in fifteen animals belonging to seven additional breeds. A total of sixteen SNPs were identified: eleven positioned in introns (six in intron 1, one in intron 5 and four in intron 8) and five in exons (one in exon 1, two in exon 6 and two in exon 7). Three SNPs (located in exons 1, 6 and 7) were non synonymous, determining Pro19Leu, Ala238Val, and Met341Ile amino acid changes, respectively. All the SNPs identified were polymorphic between *Bos taurus*, *Bos indicus* and Sanga, while none of them resulted associated with the studied phenotype and discriminated the three breeds (Chianina, Mucubal and Goudali) characterized by dark pigmented skin from the others.

Key words: SNPs, Pigmentation, *SLC24A5*, Cattle.

RIASSUNTO

IDENTIFICAZIONE DI POLIMORFISMI NEL GENE BOVINO *SLC24A5*

Il gene SLC24A5 (Solute Carrier family 24, member 5) è coinvolto nella pigmentazione della cute in zebrafish ed in uomo, regolando la morfogenesi dei melanosomi, lisosomi specializzati nel deposito di melanina. Nella specie umana l'allele ancestrale è diffuso nelle popolazioni africane e dell'Asia orientale,

inoltre è noto un polimorfismo quasi fissato nelle popolazioni europee che è correlato alla cute più chiara. Vista l'importanza della melanina nella protezione del DNA dai danni delle radiazioni ultraviolette, la mancanza di informazioni nella specie bovina e l'utilità di polimorfismi genetici associati a fenotipi legati alla pigmentazione, abbiamo studiato il gene SLC24A5 nei bovini per individuare polimorfismi associati a caratteristiche fenotipiche della pigmentazione della cute. Per identificare SNPs (Polimorfismi di Singolo Nucleotide) in questo gene e per indagare la sua associazione con la pigmentazione della cute nei bovini, i nove esoni, gli introni 1, 3 e 8 e parte dell'introne 5, sono stati sequenziali in sedici animali appartenenti a quattro razze bovine italiane, due razze zebuine africane e due razze sanga africane. La regione compresa tra l'esone 3 e 4 è stata sequenziata anche in quindici animali appartenenti a sette ulteriori razze. Sono stati identificati un totale di sedici SNPs: undici SNP sono negli introni (sei nell'introne 1, uno nell'introne 5 e quattro nell'introne 8) e cinque negli esoni (uno nell'esone 1; due nell'esone 6 e due nell'esone 7). Tre SNP posizionati negli esoni 1, 6 e 7 sono non-sinonimi e determinano rispettivamente le seguenti variazioni Pro19Leu, Ala238Val, and Met341Ile. Tutti gli SNPs identificati sono polimorfici tra Bos taurus, Bos indicus e Sanga, nessuno discrimina le tre razze (Chianina, Mucubal e Goudali) caratterizzate dall'avere la cute pigmentata scura, rispetto alle altre.

Parole chiave: *SNPs, Pigmentazione,* SLC24A5*, Bovini.*

Introduction

In mammals, pigmentation results primarily from the synthesis of melanin pigments: black or brown eumelanin and red or yellow pheomelanin. Melanins are synthesized in specialised organelles, melanosomes, located within melanocyte cells. The colour of hair and skin is mainly due to form, content, transfer and accumulation of melanosomes in the neighbouring keratinocytes. In dark skin, melanosomes are larger and more abundant and contain primarily eumelanin, whereas pheomelanin prevails in melanosomes of fair skin and red or yellow hair. The genetic of pigmentation is complex with more than one hundred loci in which gene mutations have been associated to hair and skin coat colour changes in model organisms, particularly in mice. Comparative mapping of about sixty of these loci permitted the identification of the corresponding orthologous genes in several mammalian species (Bennet and Lamoreux, 2003). Mutations within these orthologous genes have often been associated with analogous phenotypes or genetic diseases in both humans and mice, the most studied species (Sturm, 2006). For example, the *MC1R* (Melanocortin 1 Receptor) gene shows polymorphisms in several mammalian species associated with similar phenotypes: black hair, red hair and fair skin in man, mice, cattle and other species (Jackson, 1997). Likewise, the *SLC24A5* gene has recently been indicated as responsible for the golden phenotype (hypopigmentation of skin melanophores) in zebrafish and for the lighter skin pigmentation in humans (Lamason *et al.*, 2005).

In cattle, the role of *MC1R* in the eumelanin/pheomelanin switch has been well characterized and different alleles at this locus have been identified (Klungland *et al.,* 1995). Others coat colour genes such as *TYRP1* (Tyrosine related protein 1) and *SILV* (silver homolog), for which there is a known association with pigment dilution in some breeds (Berryere *et al.,* 2003, 2007; Gutiérrez-Gil *et al.*, 2007) and *ASIP* (coding for Agouti Signaling Protein), for which no polymorphisms in the coding sequence have been found (Royo *et al.*, 2005), have been investigated.

Among the genes affecting coat colour, the *golden* gene, *slc24a5*, a potassium dependent sodium/calcium exchanger, was identified only recently (Lamason *et al.*, 2005). The human *SLC24A5* gene plays an

important role in human skin pigmentation promoting melanin deposition through maturation of the melanosome (Lamason *et al.*, 2005). Human *SLC24A5* mRNA rescued melanin pigmentation when injected into golden zebrafish embryos, demonstrating functional conservation of mammalian and fish polypeptides across vertebrate evolution (Lamason *et al.*, 2005).

Up to now, the only coding SNP identified in human *SLC24A5* coding sequence is a G to A transition determining an alanine to threonine amino acid change. Such SNP is located at codon 111 in the third exon of *SLC24A5* (International HapMap - release 16c.1). Lamason *et al.* (2005) reported that the frequency of G to A transition (rs1426654) ranges from 98.7 to 100% in several European-American populations. This indicates that the threonine variant is almost ubiquitous in populations with lighter skin pigmentation but rare or absent in dark skinned African, Indigenous American and East Asian populations in which the ancestral alanine allele reach a frequency of 93 to 100%. A deficit of heterozigosity at loci flanking *SLC24A5* in the European-American population has also been observed suggesting the effect of directional selection in this or on a tightly linked gene (Müller and Kelsh, 2006).

The bovine *SLC24A5* gene map on chromosome 10 (Btau_3.1, XM_001249737 and NC_007308, LOC781411), is composed of nine exons and spans 22,931 bp. At the moment no SNPs are reported in dbSNP (NCBI).

The aim of this work was to compare genomic sequences of the bovine *SLC24A5* gene of individual cattle with dark pigmented and light pigmented skin to identify nucleotide variations and determine their potential association with the above-mentioned phenotype. We targeted eight Italian and three European *Bos taurus* breeds, two African *Bos indicus* breeds and two African sanga breeds (originated from crosses between *Bos taurus* and *Bos indicus*).

Material and methods

A total of 31 animals belonging to eight Italian and three European cattle breeds (4 Italian Red Spotted, 3 Italian Brown, 2 Italian Holstein, 2 Chianina, 2 Limousin, 2 Romagnola, 2 Grey Alpine, 2 Hereford, 2 Angus and 2 Hungarian Grey), two African sanga breeds (2 Mucubal and 2 Humbe from Angola) and two African zebu breeds (2 Azawak from Niger and Bourkina Faso, 2 Goudali from Niger) were sampled.

Among the analysed breeds the Italian Chianina, African Goudali and African Mucubal breeds have dark pigmented skin. The others have light pigmented skin.

DNA from all the samples was isolated from whole blood using GenElute™ Mammalian Genomic DNA kit (Sigma-Aldrich Corp, St. Louis, MO, USA) following the manufacturer's instruction. The sequence of the bovine *SLC24A5* gene was retrieved from NCBI databases (XM_001249737 and NC_007308) and six primer pairs were designed to amplify the nine exons of the gene (Table 1).

PCR amplifications were carried out using Platinum® Taq DNA Polymerase High Fidelity Kit (Invitrogen) following the manufacture's instruction.

All 31 samples were amplified with the set of primers N° 2, while the other sets were used on a subset of 16 animals: 2 Italian Red Spotted, 2 Italian Holstein, 2 Chianina, 2 Italian Brown, 2 Azawak, 2 Goudali, 2 Humbe and 2 Mucubal.

The PCR products obtained were purified using enzymatic reaction (with Shrimp Alkaline Phosphatase - Roche and Exonuclease I - New England BioLabs) and sequenced in out-sourcing (DNA sequenc-

Table 1. Primers used for bovine *SLC24A5* amplification and sequencing and size of the PCR product. The primers were designed based on NC_007308 and XM_001249737 sequences.

	No. of set	Primer forward	Primer reverse	bp
exon1-intron1-exon2	1	5′-TCTCCCTTCCCAAGGCTGCAC-3′	5′-GGCATCACTATTCTGGGAAGT-3′	1261
exon3-intron3-exon4	2	5′-CCTTAGGACTGTCCCAGGATG-3′	5′-CACATTAGATAGTAAGCCACA-3′	278
exon5-intron5	3	5′-ACTTGAAGAAGTAACAGTGTA-3′	5′-GTACTCTCTGTCATTGTTTTA-3′	197
exon6	4	5′-GTATGAAGGAACTTTACTGCT-3′	5′-AGTTTTGCTAGTAACAGACTTA-3′	331
exon7	5	5′-AATATAGCTTCTTAAATGGAC-3′	5′-GTTCTTATATACAACTGATTA-3′	342
exon8-intron8-exon9	6	5′-TAGTGAATCTATAAGATTTGT-3′	5′-GCCCTTCCCCATCATTCACAT-3′	987

ing service, CRIBI, University of Padova, (http://bmr.cribi.unipd.it/). Sequences were aligned and compared for SNPs identification using the program BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit/html).

Nucleotide and amino acidic identity and similarity indexes between human *SLC24A5* (NM_205850) and bovine *SLC24A5* genes were calculated by similarity matrix BLOSUM62 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Results and discussion

The coding sequence nucleotide similarity between the entire human and cattle *SLC24A5* gene resulted 0.89. Amino acid similarity resulted 0.94 with 0.90 identity with the alanin human variant. In particular, in the two species considered, the third exon is 84 nucleotides long, with nucleotide identity value of 0.89 and amino acidic identity and similarity equal to 1.

The primer pairs designed allowed the amplification of 3204 nucleotides that cover the entire coding region and four out of the eight introns (introns 1, 3 and 8 and part of intron 5). A total of sixteen SNPs were identified: eleven in introns (six in intron 1, one in intron 5 and four in intron 8) and five in exons (one in exon 1, two in exon 6 and two in exon 7). Three SNPs located in exons 1, 6 and 7 were non synonymous determining Pro19Leu, Ala238Val, and Met341Ile amino acid changes, respectively, as shown in Table 2 and 3. None of the SNPs discriminate the three breeds (Chianina, Mucubal and Goudali) characterized by dark pigmented skin from the others. No SNP has been detected in the exon 3 even when an extra set of fifteen animals from seven breeds was sequenced. The further investigation of exon 3 was motivated by the fact that in humans a G to A transition associated with different skin pigmentation has been described (Lamason *et al*., 2005).

No SNP was found within the taurine breeds even when characterized by different skin pigmentation. Since a striking reduction of polymorphisms near the *SLC24A5* gene in the European human populations has been observed, the absence of polymorphism in the taurine breeds needs more in-depth investigation. Conversely, a rather high number of polymorphic sites was identified comparing taurine, zebu and sanga

Table 2. SNPs detected by alignment of the *SLC24A5* sequences and genotypes found in the analysed breeds.

Location[1]	Position[2]	SNPs	aa change	Taurine genotype		Zebu genotype		Sanga genotype	
				dark skin	light skin	dark skin	light skin	dark skin	light skin
Exon 1	56	c.56T>C	p.Leu19Pro	TT	TT	TT	TT	TT/TC	TT
Exon 1	84	c.84G>A	Synonymous	GG	GG	GA	AA	AA	AA
Intron 1	213	c.121+92C>T		CC	CC	CT	TT	TT	TT
Intron 1	354	c.121+233C>T		CC	CC	CT	TT	CC/TT	CT/TT
Intron 1	445	c.121+324G>A		GG	GG	AG	AA	AA	AA
Intron 1	521	c.121+400C>T		CC	CC	CC	CC	CT/TT	CC/CT
Intron 1	522	c.121+401A>G		AA	AA	GG	GG	GG	GG
Intron 1	712	c.991-279G>C>A		GG	GG	CC	CC	CC/CA	CC/CA
Intron 5	1828	c.590+7G>T		TT	TT	TT	TT	TG	TG
Exon 6	2073	c.713C>T	p.Ala238Val	CC	CC	CC	CC/CT	CC/CT	CC/CT
Exon 7	2444	c.900C>T	Synonymous	TT	TT	TT	TT/CC	CT/CC	CT/CC
Exon 7	2567	c.1023G>C	p.Met341Ile	GG	GG	GG	GG	GG/GC	GG/GC
Intron 8	2912	c.1183+24G>A		GG	GG	GG	GG	GG	GA
Intron 8	2950	c.1183+62delA		AA	AA	AA	AA/A-	AA/A-	AA/A-
Intron 8	2967	c.1183+79A>T		AA	AA	AA	AA	AA	AT
Intron 8	3053	c.1183+165T>C		TT	TT	TT/CT	CC	CT	CC

[1] Exon and intron numbers are followed by their designations according to sequences NC_007308 and XM_001249737.

[2] Nucleotide position are numbered according to the first base of sequence as it appears in GeneBank (EF362803).

Table 3. SNPs detected in the analyzed breeds (no.=number of animals analysed).

	Breeds				
SNPs	Taurine breeds no.=8	Azawak no.=2	Goudali no.=2	Humbe no.=2	Mucubal no.=2
c.56 T>C	TT	TT	TT	TT	CT/TT
c.84 G>A	GG	AA	AG	AA	AA
c.121+92 C>T	CC	TT	TC	TT	TT
c.121+233 C>T	CC	TT	CT	CT/TT	CC/TT
c121+324 G>A	GG	AA	AG	AA	AA
c.121+400 C>T	CC	CC	CC	CT/CC	TT/CT
c121+401 A>G	AA	GG	GG	GG	GG
c991-279 G>C>A	GG	CC	CC	CA/CC	CA/CC
c.590+7 G>T	TT	TT	TT	TG	TG
c.713 C>T	CC	CT/CC	CC	CT/CC	CT/CC
c.900 C>T	TT	CC/TT	TT	TC/TT	TC/TT
c.1023 G>C	GG	GG	GG	GG/GC	GG/GC
c.1183+24 G>A	GG	GG	GG	GA	GG
c.1183+62 delA>-	AA	A-/AA	AA	A-/AA	A-/AA
c.1183+79 A/T	AA	AA	AA	AT	AA
c.1183+165 T>C	TT	CC	TT/CT	CC	CT

breeds and within zebu breeds.

In humans, although skin colour is considered a selective trait significant for evolution, only few major loci involved in its control have been found. Lamason and colleagues (2005) showed that *SLC24A5* is a major locus affecting skin colour in humans and that the paler skin in European-American population results directly from the threonine variant. Moreover, the presence of this variant seems to allow other related loci, such as *MC1R,* to mutate and express

phenotypically the mutated protein variants. In fact, the *MC1R* wild type resulted fixed in African human populations where the ancestral alanine allele of *SLC24A5* reaches a frequency of 93% to 100%. A constitutively functional *MC1R* is probably essential for dark skin in mice (Barsh, 2003), although gain-of-function mutations have not yet been reported in humans (Harding *et al.*, 2000).

Conclusions

Our preliminary results suggest that non synonymous SNPs at the *SLC24A5* gene exist in bovines, as in humans. However, in cattle the *SLC24A5* coding sequence does not seem to play a central role in skin pigmentation. In fact none of the SNPs identified discriminate the three breeds with dark pigmented skin from the others with lighter skin. In any case, the absence of any polymorphism in taurine breeds and the higher level of variation in sanga and zebu breeds seem quite interesting. Further studies may be extended to the promoter region of the *SLC24A5* gene and to other key genes involved in skin and coat colour variation such as *MC1R*, still poorly investigated in zebu breeds.

We gratefully wish to acknowledge Prof. Riccardo Bozzi (Dipartimento di Scienze Animali, Università di Firenze, Italy) for providing zebu and sanga samples, and Prof. Paolo Ajmone-Marsan (Istituto di Zootecnica, Università Cattolica del S. Cuore, Piacenza, Italy) for helpful comments on the manuscript.

This work was supported by grants from Cariplo, 2003.0721/11.8094.

REFERENCES

Barsh, G.S., 2003. What controls variation in human skin color?. Plos Biol. 1:19-22.

Bennet, D.C., Lamoreux, M.L., 2003. The color loci of mice - a genetic century. Pigment Cell. Res. 16:333-344.

Berryere, T.G., Oulmouden, A., Schmutz, S.M., 2007. Genetic studies of coat colors and hair length in Highland cattle. P547 in Proc. 15th Int. Conf. on Plant and Animal Genomes, San Diego, CA, USA.

Berryere, T.G., Schmutz, S.M., Schimpf, R.J., Cowan, C.M., Potter, J., 2003. *TYRP1* is associated with dun coat colour in Dexter cattle or how now brown cow? Anim. Genet. 34:169-175.

Felius, M., 1995. Cattle breeds: an encyclopedia. Ed. Misset, Doetinchem, The Netherlands.

Gutiérrez-Gil, B., Wiener, P., Williams, J.L., 2007. Genetic effects on coat colour in cattle: dilution of eumelanin and phaeomelanin pigments in an F2-Backcross Charolais × Holstein population. BMC Genet. 8:56-68.

Harding, R.M., Healy, E., Ray, A.J., Ellis, N.S., Flanagan, N., Todd, C., Dixon, C., Sajantila, A., Jackson, I.J., Birch-Machin, M.A., Rees, J.L., 2000. Evidence for Variable Selective Pressures at MC1R. Am. J. Hum. Genet. 66:1351-1361.

International HapMap Consortium, 2003. The international HapMap project. Nature 426:789-796.

Jackson, I.J., 1997. Homologous pigmentation mutations in human, mouse and other model organisms. Hum.Mol. Gen. 6:1613-1624.

Klungland, H., Våge, D.I., Gomez-Raya, L., Adalsteinsson, S., Lien, S., 1995. The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. Mamm. Genome 6:636-639.

Lamason, R.L., Mohideen, M.A., Mest, J.R., Wong, A.C., Norton, H.L., Aros, M.C., Jurynec, M.J., Mao, X., Humphreville, V.R., Humbert, J.E., Sinha, S., Moore, J.L., Jagadeeswaran, P., Zhao, W., Ning, G., Makalo-

wska, I., McKeigue, P.M., O'donnell, D., Kittles, R., Parra, E.J., Mangini, N.J., Grunwald, D.J., Shriver, M.D., Canfield, V.A., Cheng, K.C., 2005. SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. Science 310:1782-1786.

Müller, J., Kelsh, R.N., 2006. A *golden* clue to human skin colour variation. BioEssays 28:578-582.

Royo, L.J., Alvarez, I., Fernandez, I., Arranz, J.J., Gomez, E., Goyache, F, 2005. The coding sequence of the ASIP gene is identical in nine wild-type coloured cattle breeds. J. Anim. Breed. Genet. 122:357-360.

Sturm, R.A., 2006. A *golden* age of human pigmentation genetics. Trends Genet. 22:464-468.