Italian Journal of Animal Science

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

Preliminary study on MC1R polymorphism in some cattle breeds raised in Italy

P. Crepaldi, M. Marilli, C. Gorni, D. Meggiolaro, M. Cicogna & C. Renieri

To cite this article: P. Crepaldi, M. Marilli, C. Gorni, D. Meggiolaro, M. Cicogna & C. Renieri (2003) Preliminary study on MC1R polymorphism in some cattle breeds raised in Italy, Italian Journal of Animal Science, 2:sup1, 13-15

To link to this article: http://dx.doi.org/10.4081/ijas.2003.11675899

Preliminary study on MC1R polymorphism in some cattle breeds raised in Italy

P. Crepaldi[1], M. Marilli[1], C. Gorni[2], D. Meggiolaro[1], M. Cicogna[1], C. Renieri[3]

[1] Istituto di Zootecnia Generale – Università di Milano, Italy.
[2] Istituto di Zootecnica – Università del S. Cuore, sede di Piacenza, Italy.
[3] Dipartimento di Scienze Veterinarie – Università di Camerino, Italy.

RIASSUNTO – Ricerche preliminari sul polimorfismo del gene MC1R in alcune razze bovine allevate in Italia – *Il gene MC1R è stato analizzato in 193 soggetti appartenenti a 8 razze bovine, tramite PCR, per la presenza di due mutazioni ad effetto fenotipico noto sulla pigmentazione del mantello: la delezione G310 e la sostituzione T296C, associate rispettivamente al fenotipo feomelanico* (e) *ed eumelanico nero* (E^d). *Sessanta soggetti di razza Limousine e Pezzata Rossa Italiana presentano genotipo* e/e*; 27 soggetti di razza Frisona Italiana mostrano genotipo* E^d/E^d *mentre 2 genotipo* E^d/e. *Gli 84 soggetti appartenenti alle razze Cabannina, Chianina, Marchigiana e Piemontese non presentano tali mutazioni, analogamente a 18 soggetti di razza Romagnola, nella quale però si sono anche osservati 2 soggetti portatori dell'allele e allo stato eterozigote.*

KEY WORDS: cattle, coat colour, MC1R.

INTRODUCTION – Most of the Western European cattle breeds consist of standardised breeds with a definite coat colour (Renieri *et al.*, 1984). Thus coat colour could be useful to detect genetic markers for cattle breed identification. In cattle the pigmentation is determined by the distribution of two pigments: eu- and pheomelanin, producing brown or black and red to yellow pigmentation respectively. Tyrosinase, the rate-limiting enzyme involved in the synthesis of both melanins, is regulated by the melanocyte stimulating hormone (αMSH). This hormone and several other melanotropic peptides stimulate melanin formation in melanocytes by binding to the melanocortin-1-receptor (MC1R), a G-protein-coupled receptor encoded by the Extension gene (Robbins *et al.*, 1993). In addition, the amounts of eu- and pheomelanin in the melanocyte are controlled by the agouti gene encoding the Agouti Signal Protein (ASP), that acts as an antagonist of MSH signalling through the MC1R, even if its mechanism of action is controversial (Furumura *et al.*, 1998). At the MC1R *locus 3* principal alleles have been described (Klungland *et al.*, 1995): the wild type E^+ encodes the normal functional receptor for MSH, the dominant Ed due to a T/C substitution which changes the 99th amino acid to proline, leading to a MC1 receptor constitutively expressed, with high levels of eumelanin production. Finally the e allele contains a G-deletion which gives rise to a non-functional receptor and hence to low level of tyrosinase resulting in pheomelanin production. Recent studies on MC1R gene in cattle describe the presence of other 4 mutations (Graphodatskaya *et al.*, 2000; Rouzard *et al.*, 2000; Maudet and Taberlet, 2002). The aim of this research is to study the MC1R polymorphism in 8 cattle breeds raised in Italy. The study concerns only the two mutations E^d and e, resulting in a clear coat colour phenotype: the black eumelanic and the pheomelanic respectively.

MATERIAL AND METHODS – We analysed with two PCR-RFLP protocols the DNA of 193 animals belonging to Italian Holstein, Pezzata Rossa Italiana (PRI), Limousine, Cabannina, Chianina,

Marchigiana, Piemontese and Romagnola breeds, to detect the G310 deletion (e allele) and the T296C substitution (E^d allele), amplifying two regions of MC1R gene corresponding respectively to positions 193-593 and 192-329 (sequence accession number U39469). The primers used to amplify the first region of 401 bp were designed by means of Primer3 software, while the primers designed by Klungland *et al.* (1995) were used for the second region of 138 bp. Polymerase Chain Reactions (PCRs) of DNA samples, were accomplished in a i-Cycler (Biorad), by using the following primers and annealing temperatures:

M1 5' AAGAACCGCAACCTGCACT 3'
M2 5' GCTATGAAGAGGCCAACGAG 3', 62°C;
E5 5' CAAGAACCGCAACCTGCACT 3'
E6 5' GCCTGGGTGGCCAGGACA 3', 63°C.

The first 401 bp region amplified and purified was digested with MspI endonuclease. The deletion of guanine induces the removal of MspI restriction site leading to a 401 bp fragment; the absence of mutation produces two fragments, 116 and 285 bp long. The second region of 138 bp amplified and purified was digested with AciI endonuclease. The presence of the T296C substitution creates a new restriction site with the production of three short fragments of 8, 33 and 97 bp; the absence of mutation produces two fragments of 8 and 130 bp. The first MC1R region was sequenced by *M-Medical* in 9 animals of 4 breeds.

RESULTS AND CONCLUSIONS – The coat colour phenotypes of the considered breeds, according to Renieri *et al.* (1984), and the PCR-RFLP results for the e and E^d allele are showed in Table 1; the absence of these two mutations is indicated by the symbol E^+. The G310 deletion, corresponding to the e allele, has been observed at the homozygous state in all PRI and Limousine animals. Also 2 Italian Holstein and 2 Romagnola carried the e allele but at the heterozygous state. The T/C substitution at 296 nucleotide, corresponding to the E^d allele, has been observed only in the Italian Holstein breed. All the animals of this breed carry this allele: 27 out of 29 are homozigous for E^d. In some previous studies the recessive e allele has not been observed in a sample of 87 and 32 Holstein individuals (Rouzard *et al.*, 2000; Maudet and Taberlet, 2002). In 4 over the 5 other Italian breeds with the same pigmentation patterns, pheomelanic with eumelanic extremities, no one of these two mutations has been observed. Only in the Romagnola breed we found also the e allele at the heterozygous state in 2 animals. In small samples of Marchigiana, Chianina and Piemontese breed another T/C substitution in position 667 has been reported by Maudet and Taberlet (2002).

Table 1. Observed genotypes in 8 cattle breeds.

Breeds	Coat colour phenotypes	Animals (no.)	Genotypes		No. sample sequenced
PRI	blazed spotted pheom.	23	23	e/e	
Limousine	solid pheomelanic	37	37	e/e	
Italian	spotted eum. black	29	27	E^d/E^d	2
Holstein			2	E^d/e	2
Cabannina	pheom. with eum. extremities	20	20	E^+/E^+	
Chianina	pheom. with eum. extremities	20	20	E^+/E^+	1
Marchigiana	pheom. with eum. extremities	20	20	E^+/E^+	
Piemontese	pheom. with eum. extremities	24	24	E^+/E^+	3
Romagnola	pheom. with eum. extremities	20	18	E^+/E^+	
			2	E^+/e	1

eum.= eumelanic; pheom. = pheomelanic.

The results of this work suggest that the principal polymorphisms at MC1R locus could be useful for meat or milk traceability of Holstein, Limousine and PRI breeds. Chung *et al.* (2000) have already suggested the use of MC1R gene as breed-specific DNA marker to distinguish between the meat of a Korean pheomelanic breed (Hanwoo) and Holstein and Angus breeds, carrying the E^d allele. Also Maudet and Taberlet (2002) proposed a COP-PCR to detect Holstein's milk in French Registered Designation of Origin cheese, based on E^d allele detection. To find genetic markers for cattle breed characterisation of the other studied Italian breeds, other loci involved in coat colour should be analysed. It is worth noting the importance of the phenotypic coat colour description and classification of the sampled cattle to identify the involved loci and to better understand the results of molecular genetic analysis.

ACKNOWLEDGEMENTS – The authors are grateful to Breeder Associations for the provided blood samples. Research financied by FIRST 2001, Project Giovani Ricercatori 2000 (MURST) and Emilia Romagna Region: Project "Valorizzazione della razza bovina Romagnola attraverso la certificazione della carne per via molecolare".

REFERENCES – **Chung**, E.R., Kim, W.T., Kim, Y.S., Han, S.K., 2000. Korean J. An. Sci. 42:379-390. **Furumura**, M., Sakai, C., Potterf, S.B., Vieira, W.D., Barsh, G.S., Hearing, V.J., 1998. Proc. Nat. Acad. Sci. 95:7374-7378. **Graphodatskaya**, D., Joerg, H., Stranzinger, G., 2000. Veterinarni Medicina 45,10/11:290-295. **Klungland**, H., Vage, D.I., Gomez-Raya, L., Adalsteisson, S., Lien, S., 1995. Mammalian Genome 6:636-639. **Maudet**, C., Taberlet, P., 2002. J. Dairy Sci. 85:707-715. **Renieri**, C., Lauvergne, J.J., Valfrè, F., 1984. Riv. Zoot. Vet. 12, 5:310-317. **Robbins**, L.S., Nadeau, J.H., Johnson, K.R., Kelly, M.A., Roselli-Rehfuss, L., Baack, E., Mountjoy, K.G., Cone, R.D., 1993. Cell 72:827-834. **Rouzard**, F., Martin, J., Gallet, P.F., Delourme, D., Goulemot-Leger, V., Amigues, Y., Menissier, F., Leveziel, H., Julien, R., Oulmouden, A., 2000. Genet. Sel. Evol. 32:511-520.