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MC1R gene: comparison between different farm animal species

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RIASSUNTO – Il gene MC1R: confronto fra diverse specie animali di interesse zootecnico. *Negli animali di interesse zootecnico, il polimorfismo del gene MC1R coinvolto nella pigmentazione è stato studiato a livello molecolare soltanto nell'ultimo decennio. Si sono confrontate descrizioni fenotipiche e polimorfismi del gene MC1R in alcune razze bovine (Rendena, Bruna, Cabannina, Ottonese-Varzese e Pezzata Rossa) e nel cavallo (62 animali). Tutti gli animali feomelanici sono portatori delle mutazioni recessive allo stato omozigote indipendentemente dalla tonalità del mantello. I cavalli neri sono portatori dell'allele selvatico del gene MC1R e della mutazione recessiva del gene ASIP allo stato omozigote. Nei cavalli bai e nelle altre razze bovine studiate è presente, almeno allo stato eterozigote, l'allele selvatico. Nella Bruna e nella Cabannina è presente anche l'allele E1. Nel gene MC1R, sequenziato in capre di razza Bionda dell'Adamello e Nera di Verzasca, si è evidenziata l'unica mutazione descritta in letteratura. I risultati sono stati confrontati con le informazioni disponibili nella specie murina e umana.*

KEY WORDS: MC1R; coat colour; farm animals.

INTRODUCTION – Coat colour genes in farm animals have only been studied at molecular level over the last ten years, starting with Klungland *et al.* in 1995. Among the genes involved in pigmentation, MC1R, previously known as Extension locus, revealed polymorphisms related to red and black coat colour in different farm animal species. More exactly, the MelanoCortin-1 Receptor (MC1R) gene, specifically expressed in melanocytes, encodes for the homonymous G-protein coupled receptor involved in the regulation of the type of melanin synthesized. Melanocytes produce in fact two different types of melanins: the black or brown eumelanin and the yellow or red pheomelanin. The hair distribution of eu- and pheomelanins depends on the activity of MC1R, which is normally modulated by response to the melanocortin hormone, antagonized by the action of Agouti protein encoded by the ASIP gene. We previously studied the MC1R polymorphisms in some cattle reared in Italy (Crepaldi *et al.*, 2003) and we now present the data on MC1R in other Italian cattle breeds, horses and goats. The aim of this work is to compare the pigmentary phenotypes and causative MC1R mutations in different farm animals and model species, such as mouse and human, in order to highlight differences and similarities between phenotypes and MC1R polymorphisms. The relationship between phenotypes and Agouti locus is also discussed.

MATERIAL AND METHODS – *Animals*: We studied the principal polymorphisms of MC1R gene in five Italian cattle breeds (Rendena n. 19, Italian Brown n. 23, Ottonese-Varzese n. 12, Italian Red Spotted (IRS) n. 13 and Cabannina n. 18), and 62 horses, classifying the phenotype of each animal. Rendena, Italian Brown and Italian Red Spotted animals were sampled in their genetic centre. We also sequenced the MC1R gene in 8 goats from two breeds, Nera di Verzasca and Bionda dell'Adamello.
DNA analyses: The genomic DNA was extracted from whole blood using a commercial kit (Macherey Nagel). The PCR-RFLP analysis was done as in Crepaldi *et al.* (2003) for bovine samples and as in Marklund *et al.* (1996) for horse samples. We designed the primers to amplify and sequence genomic DNA of goats (data not shown).

RESULTS AND DISCUSSION – We observed a high variation in coat pigmentation within the studied cattle breeds. In fact it was possible to classify the IRS animals into 3 phenotypic classes: dark red (n.4), light red (n.4) and yellow/blonde (n.6). In the Rendena breed we observed 11 animals with brown coat colour and 9 with a dark brown coat colour. The 23 Italian Brown bulls were classified into three groups: ordinary brown (n.8), dark brown (n.13) and light brown (n.2). Within Cabannina breed we observed: ordinary brown (n.9), dark brown (n.6), light brown (n.2) and one registered animal with white coat colour and dark "glasses". We did not observe coat colour variation in 12 light red Ottonese-Varzese animals coming from the same farm.
The 62 horses were 32 chestnut (8 light, 16 red and 8 dark), 10 black and 20 bay.
All the pheomelanic cattle (IRS and Ottonese-Varzese) and horses (32 chestnuts) had the recessive "e" allele at the homozygous state. This mutation is due to a G310 deletion in cattle and a C901T substitution in horses in MC1R gene. No E^d or E^+ allele was observed in these animals. In cattle the "e" allele is a frameshift mutation in the 2nd transmembrane domain generating an incomplete receptor. In horses it is due to a non-conservative substitution (ser-phe) which is also in the 2nd transmembrane domain (Marklund *et al.*, 1996). Both are loss of function mutations. Our results confirm that the recessive mutation at Extension locus is present in horses and cattle with all the shades of red-yellow pheomelanic coat colour. Also in mouse a recessive frameshift mutation is known, which is due to a deletion at codon 183 in the 4th transmembrane domain (e allele). Unlike cattle and horses, the "e" mutation in mouse is linked only to a completely yellow phenotype (Jackson, 1997). In human the MC1R coding region is highly polymorphic with over 35 segregation sites identified to date. Some of these, located in the 2nd transmembrane domain, are essentially associated with red hair and pale skin (Rees, 2004).
The black horses studied were homozygous or heterozygous for the E^+ allele and we observed as reported by Rieder *et al.* (2002) the homozygous presence of the "a" allele, generated by a 11 bp deletion, in ASIP gene. It is worth mentioning that, at present, in horses and also in man (Rees, 2004), no gain of function mutation or black dominant mutation is known at MC1R gene. A black dominant E^d allele is present in cattle, due to a T296C substitution, as previously observed in Holstein Italian and in other black cattle (Crepaldi *et al.*, 2003; Russo *et al.*, 2004). This gain of function mutation is located, as is the bovine "e" allele, in the 2nd transmembrane domain, like 2 out of 3 mutations responsible for black pigmentation in mice (Jackson, 1997). The E^d allele was not observed in the cattle breeds studied in the present work.
The bay horses studied had the wild type allele at least at the heterozygous state. Also the other analysed cattle breeds (Rendena, Italian Brown, Cabannina) had animals with the wild allele. Moreover in the latter two breeds we also observed the presence of the E^1 allele, due to a 12 bp insertion in the 3rd intracellular loop of the receptor, previously observed in breeds with different coat colours: Aubrac, Gasconne, Brown, Salers (acc. n. AJ297819) and Tarentaise (Russo *et al.*, 2004; Maudet and Taberlet, 2000). In Italian Brown and Cabannina breeds the E^+ allele had a frequency equal to 57 and 30%, whereas the E^1 allele showed a frequency respectively of 43 and 70%. All possible allelic combinations have been observed for the different shades of brown phenotypes studied.
The data show that, as the above mentioned E^1 allele, also the wild type allele is present in cattle breeds with different coat colours from brown (Italian Brown, Cabannina and Rendena) to "white" Chianina (Crepaldi *et al.*, 2003).
In the 8 sequenced goats of Nera di Verzasca (n. 4) and Bionda dell'Adamello (n.4) breeds, respectively characterized by black and red phenotype, we found the SNP described by Li (acc. n.AY292287, Econogene Consortium). We found this mutation at the heterozygous state in 1 blonde and 3 black goats. We also observed that this mutation falls in the 5th transmembrane domain of the receptor. At present the distribution of this polymorphism in different goat populations are under evaluation by the Econogene Consortium. It is worth mentioning that to date no other polymorphisms are known in goat MC1R gene.
In conclusion it is interesting to observe that in mouse the loss of function mutation of MC1R causes only yellow hair, whereas in cattle, horses and human the loss of function MC1R mutations are associated with different pheomelanic shades from yellow to dark red. To date no mutations at the Agouti locus, causing black or

yellow colour, have been observed in cattle, goat and human. In man some recent experimental data support the hypothesis that MC1R is relatively resistant to the effect of ASIP gene. It is probable that in human and analogously in cattle, where no mutation for the Agouti locus are known and a large variation from red–yellow is associated with the ee genotype, MC1R is expressed at a higher level. Unlike mouse, there are probably more efficient promoter or transcriptional factors involved in this coat colour determination (Rees, 2003).
More generally the data show the importance of the comparison of MC1R genetic polymorphisms with the related phenotypes in different farm animals and model species highlighting the known presence of homologous mutations in pigmentation genes in human, mouse and farm animals (Jackson, 1997; Andersson, 2003). We believe, therefore, that it is important to describe the phenotype more precisely, as with biochemical analyses of melanins, and to study gene expression in order to better understand variability of coat colour within and between different breeds of the major farm animal species. This can help in deeply understanding the genetic of pigmentation in order to identify causative mutations of different coat colours useful in breed differentiation and in traceability and appreciation of their local animal products.

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