ROLE OF MELANOCORTIN 1 RECEPTOR (MC1R) GENE POLYMORPHISMS IN THE TRACEABILITY OF HUNGARIAN GREY CATTLE'S MEAT PRODUCTS

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Introduction

Traceability is defined as a system able to maintain the identification of animals or animal products, all along the production chain: from the farm to the retailer (McKean, 2001). In the last few years traceability has become more important due to the consumers' increasing attention to food quality matters. The consumers' lack of confidence is due to several reasons including both food safety (e.g.: BSE, dioxin crisis, avian influenza) and socio-economic changes (e.g.: new habits, demand for organic products) (Dalvit et al, 2007). European food legislation is very strict and traceability systems based on tags or labels have become mandatory in all EU countries. Traceability of products of traditional and protected animal species is especially important, as these products are of higher value and the higher price may greatly contribute to the preservation of these breeds.

Dalvit et al. (2007) distinguish three main types of traceability systems: conventional, geographical and genetic traceability. In our research work we applied the g*enetic traceability* system, which is based on the identification of an animal or its product through the study of DNA. *Species identification* of meat products is important because of religious, health, social and economic reasons. DNA analyses are used mainly for the differentiation of fish, avian species and for distinguishing wild boar and domestic swine. *Breed genetic traceability* allows the assignment or the exclusion of the breed of origin to a product (Dalvit et al, 2007). Such system is mainly applied for the valorisation of PDO or PGI products. Coat colour is usually considered as an aid for cattle breed identification. Therefore, the analyses of mutations in some genes affecting coat colour may serve as diagnostic tools for assigning an animal to or excluding from a given cattle breed (Maudet – Taberlet, 2002).

Pigmentation in cattle (and in general in all mammals) is determined by the presence or absence of melanins in the hair (Searle, 1968). The relative amounts of eumelanin (black/brown pigments) and feomelanin (yellow/red pigment) produced in melanocytes are controlled by two loci, extension and agouti. The extension locus codes for the melanocortin 1 receptor (MC1R) gene that is expressed in melanocytes (Robbins et al, 1993). MC1R controls pigmentation through the regulation of tyrosinase activity. The role of agouti locus in forming coat colour varieties of domestic animals has not been fully clarified, yet. Several research groups (Royo et al, 2005; Girardot et al, 2005; Graphodatskaya et al, 2006) did not find any polymorphisms in the coding region of the ASIP gene of cattle breeds with different coat colours. Therefore, other genes (TYRP1,

DCT, KIT) besides the MC1R gene are suggested to be responsible for forming different coat colours of cattle breeds. In cattle, the first studies identified three alleles of the melanocortin 1 receptor gene: a) the dominant allele, $\mathbf{E^D}$ which gives black colour, b) the recessive allele, **e** which produces red/yellow coat colour in homozygote animals and c) the wild-type allele, $\mathbf{E^+}$ which may produce a variety of colours.

In order to identify breed specific markers which can be used for the traceability of beef products, particularly for products made from Hungarian Grey Cattle meat, the presence and distribution of the three MC1R alleles were studied in some cattle breeds kept in Hungary.

Material and methods

Analyses were performed by using DNA extracted from blood and hair samples and the genomic DNA provided by the Laboratory of Central Agricultural Office (MgSzH) *(Table 1)*. For the analysis of product traceability, different dry-products made from Hungarian Grey meat and raw cattle meat were used.

Table 1:

Number of animals, type and origin of samples

Breed	Number of animals (n)	Sample	Origin of samples
Hungarian Grey	90	blood, hair	Hortobágy
	14	genomic DNA	MgSzH Laboratory
Hungarian Simmental	20	blood, hair	Derecske
	14	genomic DNA	MgSzH Laboratory
Limousine	10	hair	Hajdúszoboszló
Charolais	17	hair	Léh
Holstein	30	blood	Hód-Mezőgazda Zrt., Pély-Tiszatáj Agrár Rt.,Nagykun Mg.Rt.,NarivoÁllattenyésztő és Növénytermesztő Kft.
	19	genomic DNA	MgSzH Laboratory

The analysed samples of processed meat products were as follows: raw Hungarian Grey cattle meat, raw cattle meat (breed is unknown), Hungarian Grey cattle salami, Hungarian Grey cattle salami with paprika, sausage, stew made of Hungarian Grey cattle meat with red-wine and biosalami with paprika.

Genomic DNA was purified from blood samples by using the method of Zsolnai – Orbán (1999), and from hair samples by the method of FAO/IAEA (2004). For the analyses of listed meat products and raw meat DNA was purified by using E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek., USA) according to manufacturer's protocol.

PCR primers (Crepaldi et al, 2003) were used to amplify MC1R gene fragments. PCR was performed using a GeneAmp PCR Sytem 9700 (Applied Biosystems) thermal cycler in a total volume of 20µl containing the DNA template (50-100 ng/µl); 0,2 mM dNTP mix (Pharmacia Biotech, USA); GoTaq Flexi DNA Ploymerase (5u/µl) (Promega, Medison, USA); 5x buffer (Promega, Medison, USA); 25 mM $MgCl_2$ (Promega, Medison, USA) and the forward and reverse primers (10 pmol/µl) (Invitrogen Corporation, California, USA).
PCR profile for the analyses was as follows: 2 min. at 95ºC, 35 amplification cycles of 30 sec. at 95ºC, 30 sec. at the annealing temperature of the primers (61ºC), 30 sec. at 72ºC, then 5 min. at 72ºC.

PCR-amplified product size is 401bp containing the recognition sequence of restriction enzymes Msp1 and MspA1I. RFLP was performed by using a mix in a final volume of 10µl containing 0,5µl Msp1 or MspA1I restriction enzymes (10 u/µl) (Promega, Medison, USA); 1 µl of buffer; 1,4 µl of dH_2O; 0,1µl of BSA and 7 µl of PCR product. This enzyme digestion mixture was incubated for 3 hours at 37 ºC. Restriction analysed fragments were separated by gel electrophoresis on agarose gel and stained with ethidium-bromide.

Results and discussion

Using PCR-RFLP we separated three alleles of the MC1R gene: a) the dominant allele, $\mathbf{E^D}$ which gives black colour, b) the recessive allele, **e** which produces red/yellow coat colour in homozygote animals and c) the wild-type allele, $\mathbf{E^+}$ which may produce a variety of colours. Allele and genotype frequencies of the analysed breeds are shown in *Table 2.*

Table 2:

Allele and genotype frequencies of the analysed breeds

Breed	Coat colour	Number of animals (n)	Allele frequencies			Genotype frequencies					
			E^D	E^+	e	E^D/E^D	E^D/E^+	E^D/e	E^+/E^+	E^+/e	e/e
Hungarian Grey	grey	104	-	0.997	0.003	-	-	-	0.993	0.007	-
Hungarian Simmental	red and white	34	-	-	1.00	-	-	-	-	-	1.00
Limousine	red	10	-	-	1.00	-	-	-	-	-	1.00
Charolais	cream-coloured	17	-	-	1.00	-	-	-	-	-	1.00
Holstein	black and white	49	0.949	0.017	0.034	0.897	0.034	0.069	-	-	-

E^D (dominant allele): gives black colour
e (recessive allele): gives red/yellow coat colour in homozygote animals
E^+ (wild-type allele): produces a variety of colours

Nowadays, 40% of the beef sold in shops originates from culled Holstein cows, therefore the most important task is to be able to distinguish products made of Holstein's and Hungarian Grey cattle's meat. All analysed Holstein animals were black-and-white-coloured, therefore they had at least one copy of E^D allele. On the other hand, this dominant allele was not detectable in any of the analysed Hungarian Grey animals. Based on these results, we suggest that the analysis of MC1R polymorphisms enables the separation of the two breeds *(Table 3)*. This statement was supported by the results of analysing raw meat samples (one sample originated from Hungarian Grey cattle, while the breed of the other sample was unknown. The genotype of the control sample proved to be E^D/E^D, therefore we suggest it was taken from a Holstein animal).

Table 3:

Possibilities of separating breeds based on their MC1R genotypes

	Hungarian Grey	**Hungarian Simmental**	**Limousine**	**Charolais**	**Holstein**
Hungarian Grey					
Hungarian Simmental	+				
Limousine	+	-			
Charolais	+	-	-		
Holstein	+	+	+	+	

+: distinguishable from each other, -: not distinguishable from each other

Limousine and Charolais, having red and a diluted version of red coat colour, were found to be fixed for the recessive allele *e*. Although the wild-type allele was detectable in Simmental breeds of other countries (Russo et al, 2007), the Hungarian Simmental was found to be fixed for allele *e*. The fact that our samples originated from only two farms has to be considered when evaluating these results. The wild-type allele may be present in other populations. Identification of genetic markers responsible for forming spotting may increase the reliability of the separation of Simmental and Hungarian Grey cattle breeds.
The above statements are true only in the case of pure-bred animals.

Analyses of MC1R genotypes from processed meat products made of Hungarian Grey cattle meat

The above described PCR-RFLP method was used to analyse whether products made from Hungarian Grey cattle meat contain meat of other cattle breeds. Based on the results presented in *Table 4*, we concluded that all analysed processed products were probably made only from meat of Hungarian Grey animals, as the genotype E^+/E^+, characteristic to only the Hungarian Grey breed was detectable.

Table 4:

MC1R genotypes isolated from meat products

	Product				
	Salami	**Salami with paprika**	**Sausage**	**Stew with red-wine**	**Biosalami with paprika**
Genotype	E^+/E^+	E^+/E^+	E^+/E^+	E^+/E^+	E^+/E^+

In our opinion, therefore, the analysis of MC1R gene polymorphisms is a good tool for distinguishing products of the black and white Holstein and the Hungarian Grey cattle and for preventing any possible frauds.

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