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Identification of the causative mutation of the albinism determining the white coat colour of the Asinara donkey breed

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Asinara donkey (Asino dell'Asinara) breed is quite unique as it is characterized by a completely white coat colour that might be due to albinism. This breed is from the Asinara island (Sardinia, Italy) and is protected by the Asinara Island National Park in which about one hundred of these donkeys live. Apart from the coat colour, the other phenotypic features of this breed are similar to those of the Sardo donkey (Asino Sardo) population. In this study, we analysed the donkey tyrosinase (TYR) gene as a strong candidate for the albinism in the Asinara donkeys. TYR is a key melanocyte enzyme involved in the melanin production pathways. Disrupting mutations in the TYR gene cause different forms of albinism in many other mammals. A total of 40 donkeys (17 white Asinara donkey and 23 coloured donkeys of other breeds) were sampled and genomic DNA was extracted from hair roots. All five exons and parts of the intronic and flanking regions of the TYR gene were amplified from 10 donkeys (7 white Asinara and 3 coloured animals) using primers designed on the corresponding horse gene sequence retrieved from the EquCab2.0 genome version. Amplicons were sequenced using Sanger sequencing technique. A few missense mutations were identified in addition to several nucleotide differences between the two species, Equus asinus and Equus caballus. In particular, a missense mutation that occured in exon 1 was homozygous in all white donkeys whereas the alternative allele was always present in the coloured animals. The amino acid substitution involves a highly conserved amino acid position in all vertebrates that is in the binding site for the copper in the first catalytic domain (CuA) of the enzyme. A PCR-RFLP protocol was set up to genotype all donkeys sampled confirming the sequencing results: only white donkeys were homozygous for the identified missense mutation. These results confirm that the white coat colour of the Asinara donkeys is determined by a recesive albinism caused by a mutation in the TYR gene, opening new possibilities to establish conservation plans of the endangered Asinara donkey breed.

Acknowledgements
We thank Parco Nazionale dell'Asinara for the collaboration. This work was funded by University of Bologna RFO project.

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Effect of β-lactoglobulin (LGB) and k-casein (CSN3) alleles on some characteristics of milk produced by Cinisara cows

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It is well known that β-lactoglobulin (LGB) and k-casein (CSN3) alleles affect milk cheese making properties. The purpose of this study was to analyse the A and B allele distribution at the LGB and CSN3 loci in Cinisara breed and their influence on milk and cheese making production traits. The Cinisara is a Sicilian breed which, according to DAD-IS database (FAO), consists of about 5,000 individuals and is reared mainly for milk production which is usually transformed into Caciocavallo Palermitano, a typical stretched-curd cheese. We typed 314 lactating cows at the LGB and CSN3 loci by means of RFLP-PCR analyses. The frequencies of LGB A and B alleles were 0.22 and 0.78, respectively; the frequencies of CSN3 A and B alleles were 0.46 and 0.54, respectively. Individual milk samples were analysed for total nitrogen (TN) and non-casein nitrogen (NCN) according to FIL-IDF standard procedure. The coagulation properties were measured using the Formagraph. Data were analysed using ANOVA procedure in which the fixed effect of CSN3 genotype or the fixed effect of LGB genotype was evaluate. The results of this study show that also in Cinisara the LGB A and B alleles are associated with significantly different effects on NCN (lower for BB genotype, $P<0.001$), and casein index (higher value for BB genotype, $P<0.0001$). As a consequence, the LGB alleles are associated also with significantly different effects on coagulation properties such as coagulation time (r) and curd firming time (k20) (lower value for BB genotype, $P<0.011$, $P<0.050$, respectively); while they are not associated with different effects on curd firmness (a30 and a2r). CSN3 A and B alleles are not associated with different effects on NCN, and casein index. On the contrary, they are associated with significantly different effects on coagulation properties: BB genotype shows lower value for coagulation time (r) ($P<0.0001$) and curd firming time (k20) ($P<0.0001$); higher value for curd firmness (a30) ($P<0.0001$) and curd firmness (a2r) ($P<0.0001$) than AA genotype. Our results confirm again that LGB and CSN3 genotypes affect cheese making properties and show that Cinisara cows, due to higher frequencies of alleles with positive effect on cheese making properties, produce a high quality milk for cheese processing.

Acknowledgements
The research was funded by the Regione Siciliana, Italy.