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Short communication

Alpha and beta globin polymorphism in Italian islander sheep breeds

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ABSTRACT

Previous investigations have highlighted the elevated haemoglobin polymorphism of Apulian native sheep. Thanks to the use of highly resolving analytical procedures (PAGIF, AUT-PAGE and RP-HPLC), the alpha globin genetic system (HBA) has been shown to exhibit both qualitative and quantitative variations (HBA1L, HBA1A, HBA1D, HBA2L, HBA2H, HBA3L, HBA3H, and HBA4H), while three allelic variants have been detected at the beta globin locus (HBBA, HBBB, and HBBI). The effect of a possibly adaptive response to the Apulian environmental conditions was suggested to account for both for the unusual presence of alpha haplotypes characterized by extra-numerary genes and the high frequency of the HBBB and HBBI alleles. Unfortunately, the few data in the literature regarding the HBA system polymorphism and the presence of the HBBI gene do not allow a comparison between Apulian sheep and those from other geographical areas. This work reports the results of a screening of Italian islander sheep breeds with a view to expanding the existing data set. A total of 465 blood samples were taken from 156 Comisana (C), 159 Sardinian (S) and 150 Valle del Belice (VdB) sheep belonging to 13 different flocks located in Apulia. Haemolysates were prepared from EDTA samples and analyzed by PAGIF, AUT-PAGE and RP-HPLC. Based on our results, the islander breeds exhibited slight differences in the HBA2H gene frequency compared to the HBA system of the Apulian native sheep; the most interesting finding was that though no HBA1D genes were recorded, the frequencies of triplicated haplotypes were very close, which supports the hypothesis that extra-numerary genes in Mediterranean environments may be an adaptive response to endemic tick borne parasites. Regarding the HBB locus, the results indicated that both Sicilian breeds exhibit an HBBI allele frequency of about 0.2, which is more than double that of previously reported data; this finding provided confirmatory evidence that HBBI is a rather common allele in Southern Italian sheep breed and suggested that an appropriate next step should be to investigate whether it presents the same frequency latitudinal cline as HBBB gene.

1. Introduction

A key milestone in the study of sheep haemoglobin polymorphism was the high variability of the alpha and beta globin genes found in Apulian sheep breeds (Pieragostini et al., 1994, 2005).

In particular, the presence in the alpha globin cluster of extra-numeral alpha globin genes encoding for different products led to the detection of an expression gradient which reduces efficiency from the 5′ to the 3′-end in ααα and in the αααα (Vestri et al., 1991, 1994). The greater expression of the 5′ gene had been previously reported in other mammalian species (Schon et al., 1982; Clegg et al., 1984), but the presence of suitable markers detected in sheep, showed that: (i) the expression gradient reduces efficiency from the 5′ to the 3′-end following a 30:14:6 pattern in ααα and a 30:14:5:1 pattern in αααα (Vestri

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Table 1
Sampling scheme defining how data were obtained.

Breed	Comisana							Sardinian					Valle del Belice			
Farm	B_1	B_2	D	E	L_1	M	Total	A	F	G	I	Total	C	H	L_2	Total
Ewes	20	18	35	36	18	12	139	30	35	36	33	134	52	33	9	94
Rams	2	1	7	4	2	1	17	10	5	3	7	25	38	7	11	56
Sample size	21	19	42	40	20	13	155	40	40	39	40	159	90	40	20	150
Flock size	26	20	400	150	200	13	809	260	800	203	417	1680	800	320	300	1420

et al., 1991, 1994); (ii) additional α-globin genes produce extra α-globin chains and, consequently, the carriers of triplicated or quadruplicated haplotypes may exhibit an unbalanced α/β-globin ratio. The related haematological pattern mimics a thalassemia-like syndrome and seems to impart some protection against endemic TBD parasites, analogously to thalassemic red blood cells where malaria parasite growth is impaired. It was also suggested that in Apulia the relatively high frequency of individuals characterized by unusual extra α-globin genes might be taken as a telltale signature of a positive selection (Pieragostini et al., 2003).

Similarly, information regarding the HBB locus (Huisman and Kitchen, 1968; Pieragostini et al., 1994, 2006) has highlighted that HBBA allele is somehow associated with a selective disadvantage in dry and hot climates. Moreover, at the beginning of the 1990s, in Sardinian and Altamurana sheep (a rare Apulian native breed) a beta globin gene (HBBI) was found to be responsible for an electrophoretically silent haemoglobin band referred to as the HbI (Manca et al., 1993). The two breeds had the same variant due to the 13 Gly → Ser point mutation and practically the same frequency (Di Luccia et al., 1995). This warranted interesting questions on the origin and spread of the mutation since the occurrence of gene flow was rather improbable given the distance between the breeding sites. Subsequently, the only reports concerning this issue regarded sheep from Corsica (Serreri et al., 1998) and a recent detection in the Gentile di Puglia sheep, another Apulian native breed (Pieragostini et al., 2006). Understanding the dynamics and fate of a mutation is fundamental to explain how it contributes measurably to the standing variation of populations.

The point to be clarified here is whether the phenomena detected in the haemoglobin systems of Apulian native breeds are the result of an adaptive response to the Mediterranean environment. The finding of similar mechanisms in other Mediterranean native breeds would substantially contribute to support this hypothesis. To this purpose the data set available needs to be increased because a comparison of the results with other ovine breeds is thwarted by the paucity of existing reference data. The aim of this work was to gain further insights into this field by investigating the haemoglobin polymorphism in native islander sheep breeds.

2. Materials and methods

2.1. Sampling

Blood samples were taken from purebred Sardinian, Comisana and Valle del Belice sheep in different farms in Apulia (Table 1).

2.2. Breeds

The Comisana sheep originated from the Maltese and Sicilian breeds in the late 19th and early 20th century in the Southeast region of Sicily (Web site of Breeds of Livestock-Comisana: www.ansi.okstate.edu/breeds/sheep/comisana/index.htm). Thanks to its suitability for both extensive and intensive production systems, it spread from Sicily throughout Italy, mainly to Tuscany, Lazio, Abruzzo, Basilicata, Apulia and Calabria. Also known as "red head" because of its characteristic red face, the Comisana is a medium-large breed with all the morphological characteristics of a good milk producer, such as refined head and a good quality udder whose attachment and size are well suited for mechanical milking. The Comisana breed is fit for the semi-arid Mediterranean environment and represents an important resource for the marginal areas of Sicily and the new Central and Southern diffusion zones of the Italian peninsula (Web site of Progetto di Miglioramento Genetico Razza Comisana, www.comisana.it/ing/english.html).

The Sardinian breed is a native breed of Sardinia off the coast of Italy. It originated from the local lowland sheep which were large, polled, and had white wool. Merino and Barbary sheep were also used in developing the breed. The males are occasionally horned while the females are polled (hornless). They are primarily kept for milk production. Pecorino Sardo DOP is a cheese made exclusively with the milk of Sardinian sheep from Sardinia and is certified in Europe as a quality authentic product from a specific area of production with detailed manufacturing procedures (Web site of Breeds of Livestock-Sardinian: www.ansi.okstate.edu/breeds/sheep/sardinian/index.htm).

The Valle del Belice is a breed of dairy sheep resulting from a cross of Pinzirita, Sardinian, and Comisana breeds and subsequent selection by breeders (Cappio-Borlino et al., 1997).

2.3. Phenotyping

Haemolysates were obtained following the traditional method. The tetramers were analyzed by PAGIF and globin chains were separated by AUT-PAGE following the procedures described in Pieragostini et al. (2006). PAGIF detects HbA and HbB bands, with HbI comigrating with HbB while in AUT-PAGE beta globin I migrates close to beta globin A. RP-HPLC was performed using the analytical Vydac large-pore (300 Å) C_4 column (Hesperia, CA), as described by Pieragostini et al. (2003). Chromatogram data were stored and analyzed by the 32 Karat Software interfaced with Beckman Coulter hardware, Inc., System Gold™.

2.4. Statistics

Gene frequencies were calculated by means of gene and allele counting. The standard deviation estimates were computed by the following formula: S.D. = $[p(1-p)/2N_e]^{1/2}$ where $N_e = 4MF/N$ was obtained considering that the males in the sample were all the males in the flock (Table 1).

2.5. Nomenclature

The gene nomenclature used in this study followed the guidelines for ruminants (Andresen et al., 1995) and the nomenclature for haplotypes followed the model suggested by Pieragostini et al. (2003).

3. Results and discussion

3.1. Alpha globin system

Table 2 compares the allele frequency at the alpha globin loci in the sheep breeds examined and the data in the liter-