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Original investigation

MHC class II DRB1 and DQA2 gene polymorphisms in four indigenous breeds of sheep (*Ovis aries*)

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ABSTRACT

Major histocompatibility complex (MHC) contains highly polymorphic genes, coding receptors that participate in the immune responses. Information of MHC polymorphism from domestic animals could be used in genetic improvement programs and could contribute to the understanding of the impact of domestication or human influence. Here, we studied the highly polymorphic genes *DRB1* and *DQA2* in four Mediterranean breeds of domestic sheep (*Ovis aries*), using SSCP method in combination with sequencing. In total 48 *Ovar-DRB1* and 46 *Ovar-DQA2* alleles were detected. For both genes high levels of polymorphism were recorded; *DRB1* had higher functional polymorphism in the peptide binding region (PBR) whereas *DQA2* had a remarkable amino acid polymorphism at full-length exon. This polymorphism detected in *O. aries* is consistent with heterozygote and rare allele advantage, the main mechanisms of balancing selection.

Introduction

Livestock breeds are disappearing at an annual rate of 5%, or 6 breeds per month. In Europe, half of all breeds of domestic animals that existed at the turn of the 20th century have become extinct, and 43% of the remaining breeds are endangered. Many communities of South Europe are extraordinarily rich in animal breeds genetic diversity (FAO, 2015). However, many of these indigenous breeds and diversity are endangered as the Green Revolution approach (high-input, high-tech, and high-yielding livestock breeds) has very nearly extinguished the rich reservoirs of animal genetic diversity that they have selected and improved for generations (FAO, 2015). For many decades the dominant model for agricultural production has been based on imported genetic stock and technology. But this approach of importing industrial animal breeds to boost productivity of livestock is now being rethought, in recognition of the fact that native breeds are far more likely to be productive under low-input conditions (FAO, 2015). Therefore, is imperative to record and analyse the genetic biodiversity of indigenous livestock breeds.

MHC genes code for proteins with central role in immune responses, are essential for infectious disease resistance in vertebrates (Hedrick and Kim, 2000) and are highly polymorphic. Because of their role in the adaptive immune response of vertebrates, they can be useful for investigating the role of natural selection on genetic diversity in animal populations (Bernatchez and Landry, 2003). Major histocompatibility complex (MHC) genes were used in genetic and evolutionary population studies for quantifying adaptive genetic variation in wild animal populations of conservation concern (Sommer, 2005; Ujvari and Belov, 2011) and for the investigation of potentially effective mechanisms driving genetic polymorphisms (Seifertová et al., 2015).

The ovine MHC (ovine leukocyte antigen, OLA) is among those that are well characterized. The OLA-MHC is located on the long arm of ovine chromosome 20 (OAR 20q15–20q23) and has similar structure and organization to that of other mammals (Mahdy et al., 1989). However there are significant differences between OLA-MHC and to homologous regions of human MHC like the replace of DP region with the DY (Wright et al., 1994; Deverson et al., 1991) and a variation in the number of DQA loci, varying even among sheep breeds (Snibson et al., 1998; Hickford et al., 2004). The construction of a continuous BAC clone contig that fully covers the entire ovine MHC region revealed that ovine MHC is interrupted by a large piece of autosome insertion via a hypothetical chromosome inversion that constitute ~25% of ovine chromosome 20 with unknown evolutionary consequence (Li et al., 2012). All of the above suggest that the highly dynamic of OLA-MHC makes it worthwhile to test its function and to compare MHC evolution in ruminants.

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Within the MHC class II region of ruminants, two subregions exhibit polymorphism, DR and DQ (Amills et al., 1998), but, in contrast to other mammals, extensive variation is also observed in the DQ subregion that usually is less polymorphic (Escayg et al., 1996). Extensive polymorphism of the DQ subregion, along with the absence of the DP subregion (Scott et al., 1987; Deverson et al., 1991), suggests that ovine DQ has a more important role in the induction of adaptive immunity than in other species. In sheep, two genes termed as *DQA1* and *DQA2* have been identified (Scott et al., 1991) and are both being polymorphic. However, in a minority of haplotypes the *DQA1* locus appears to be absent (*DQA1* null; Fabb et al., 1993) and is replaced by a second locus termed, *DQA2-like* as it is more related to *DQA2* (Hickford et al., 2004). These *DQA2-like* sequences (found also in goat and cattle), appear functional and they have probably arisen from an ancient interlocus recombination between *DQA1* and *DQA2* loci predating Bovidae speciation (Ballingall et al., 2015).

In this study, the objectives were to assess the nature and extent of polymorphism of the DRB1 and DQA2 genes in four sheep breeds (Friesarta, Comisana, Butsiko with white head and red head) simultaneously. These heritable differences between several breeds can create opportunities to breed animals for enhanced resistance to the disease. Such studies being part of an integrated control strategy can contribute to large-scale data collection (both within and between breeds) and help breeders select individuals for enhanced resistance to a variety of diseases.

Butsiko breed (Orino of Epirus, orino = mountainous) descends from the Greek breeds Vlahiko, Sarakatsniko and Grammoustiano and appeared first at the end of 18th century. Their population is decreasing and several breeders try to maintain pureblood flocks. They are resistant to the bad weather conditions of the mountainous areas and are well adapted to climate with high humidity, steep slopes and low feed requirements. Friesarta breed is named after Arta (Greek region) as they come from the crossing of Friesian and Arta flocks in the years of 1960's. They locate mainly in Western Greece and produce large amounts of milk used for cheese production. Animals of this breed are susceptible to pneumonic diseases and are adapted to wet and cold weather conditions. Comisana breed is indigenous to central and northern Sicily, Italy and its name derives from the comune of Comiso, in the province of Ragusa. It originated from the Maltese and Sicilian breeds in the late 19th and early 20th century. It is raised principally in the provinces of Caltanissetta, Enna and Palermo, but is found in many other Italian provinces and has also been exported to other Mediterranean countries. However, the breed from Comiso (RG) is in any case an endangered native breed and for this reason alone it should be protected.

DRB1 exon 2 was chosen as it encodes functionally important peptide-binding sites for presentation to T cells, and usually is highly polymorphic (Hughes and Nei, 1990; Hughes and Yeager, 1998) and DQA2 was chosen because it is the most polymorphic DQ gene. Moreover, both DRB1 and DQA are well studied genes in non-model organisms permitting data comparison of genetic diversity among species (Sommer, 2005).

Material and methods

Samples and DNA isolation

Blood samples from 102 sheeps were analysed. 10 ml of each blood sample was stored with anticoagulant (EDTA, 0.5M pH 8.0) at −20 °C. Fifty three samples belong to Butsiko race (provided by a breeding station in Trikala area, Central Greece), 19 samples belong to Friesarta (provided by a breeding station in Arta area, West Greece) and 30 samples belong to Comisana race (provided by an Italian breeding station). DNA isolation from blood was performed using PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA 92008, USA) according to the manufacturer's instructions.

PCR, SSCP, and sequencing

MHC class II *DRB1* and *DQA2* exon2 sequences (258 bp and 242 bp, respectively) were obtained by polymerase chain reaction (PCR) reaction using locus-specific pairs of primers. The pair of primers that we used for DRB1 exon 2 amplification was Fw 5′ TATCCCGTCTCTGCAGCACATTTC 3′ and Rv 5′ TCGCCGCTGCACACTGAAACTCTC 3′ (Amills et al., 1995). DRB1 Fw primer attaches to the first intron second exon boundary while DRB1 Rv primer is complementary to the 3′ end of the second exon. The amplification reactions were carried out on 200–300 ng of genomic DNA in a 50 μl final volume containing 49 μl compound of PCR buffer (final concentration 1 × buffer, 2 mM MgCl2, 10 mM each dNTP, 25 pmol/μl each primer and 5 U Taq polymerase). After 4 min of initial denaturation (95 °C), 35 cycles were run on a thermal cycler each comprising of 40-s denaturation (95 °C), 50-s annealing (57 °C), and 40-s extension (72 °C) followed by a final extension for 10 min. The primers for the *DQA2* gene were constructed according to Hickford et al. (2004), DQA2 Fw 5′ ACTACCAATCTCATGGTCCCTCT 3′ and DQA2 Rv 5′ GGAGTAGAATGGTGGACACTTACC 3′. The amplification reactions and cycling parameters were the same ones as described for DRB1.

All samples were screened for polymorphisms in exon 2 of the *DRB1* and *DQA2* loci by single-strand conformation polymorphism (SSCP) analysis. Five microliters of the PCR sample was mixed with 10 μl of the denaturating buffer (95% formamide, 0.05% bromophenol blue, 10 mM NaOH, 0.05% xylene cyanol, 10% glycerol) before being heated for 2 min at 95 °C, 2 min at 97 °C, and 5 min at 99 °C. The samples after denaturation were immediately cooled on ice. After that, for *DRB1*, the samples were loaded into a 1.5-mm thick, 10% acrylamide gel and were electrophoresed in 0.5 × TBE at 210 V overnight (18 h) at room temperature. For *DQA2*, the samples were loaded in a 1.5-mm thick 12% acrylamide gel and were electrophoresed in 0.5× TBE at 230 V overnight (18 h) at room temperature. The resulting bands were visualized by silver staining. PCR products that showed the same SSCP pattern were grouped,and representative samples from each profile were purified using QIAquick PCR purification kit (Qiagen cat. no. 28106). Homozygous samples were sequenced directly and bidirectionally by Macrogen Inc., with the Sanger method whereas PCR products from heterozygous samples were ligated first into the pGEM-T Easy vector (Promega), and transformed into Escherichia coli DH5a-competent cells to separate the two alleles. After the blue/white selection, a number of positive clones were picked and grown on a small scale. Plasmid DNA was isolated (Eppendorf FastPlasmid purification kit), subjected to PCR–SSCP analysis, and seven clones for each SSCP profile were sequenced by Macrogen Inc. in both directions.

Data analysis

Nucleotide and amino acid sequences were aligned using ClustalW (Larkin et al., 2007). The allelic frequency distribution, expected (He) and observed (Ho) heterozygosities (Table 3) were estimated with GENETIX software (Belkhir, 1999). The calculation of the relative rate of non-synonymous and synonymous substitutions has been performed according to the modified method of Nei and Gojobori (1986), applying the correction of Jukes and Cantor for multiple hits using MEGA version 5 (Tamura et al., 2011). MEGA5 was also used to calculate pairwise nucleotide and amino acid differences and to construct the neighbour-joining tree using the genetic distances of Jukes and Cantor (1969) with 10,000 boot-