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Genetic risk assessment for atypical scrapie in Turkish native sheep breeds

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ABSTRACT

Scrapie, a fatal transmissible spongiform encephalopathy (TSE), occurs in two phenotypes; classical and atypical. The aim of this study was to assess the genetic risk and identify the PRNP polymorphisms for atypical scrapie in a total of 1110 healthy sheep from 18 Turkish native sheep breeds. There were 10 alleles and 23 genotypes observed based on codons 136, 141, 154 and 171 of PRNP gene. The ALRQ allele was predominant for all breeds. The AFRQ allele, associated with the susceptibility to atypical scrapie, was detected in only İvesi. The other susceptible allele, ALHQ, was found at low frequencies in Akkaraman, Kangal Akkaraman, Güneykaraman, Kıvırcık, Sakız, Dağlıç and Gökçeada breeds. Generally, the ALRQ/ALRQ genotype, which is resistant to atypical scrapie, was predominant in all breeds. Among the most susceptible genotypes to atypical scrapie, only ALHQ/ALHQ was found in this study. In addition, the ARR/ARR genotype, which has been reported in lots of atypical scrapie positive sheep from various countries, was detected in almost all Turkish native sheep breeds. According to our results, it is propounded that the susceptibility to atypical scrapie increased from eastern to western part of Turkey. Although it seems that Turkish native sheep breeds are safe from atypical scrapie, the occurrence of susceptible genotypes should be taken into consideration.

1. Introduction

Scrapie is a fatal nervous disease that affects small ruminants and is the prototype of transmissible spongiform encephalopathies (TSEs) or prion diseases. It is characterized by the accumulation of an abnormal, protease-resistant isoform (PrP^{Sc}) of the cellular prion protein (PrP^{C}) in some tissues of infected animals (Prusiner, 1998). In sheep, susceptibility to classical scrapie has been largely proven to be controlled by polymorphisms of the PRNP gene at codons 136, 154 and 171. Sheep with VRQ were highly susceptible to classical scrapie and had a short survival period after challenge with scrapie; whereas sheep with ARR were resistant to classical scrapie under field and laboratory conditions (Hunter, 1997; Goldmann, 2008).

In 1998, a new type of scrapie called scrapie Nor98 was detected in Norway and has become known as Nor98, Nor98-like or atypical scrapie (Benestad et al., 2003). After this time, atypical scrapie cases have been diagnosed not only in several European countries including Germany, France, Belgium, Sweden, Ireland, Portugal, Great Britain, Switzerland, Poland and Italy (Buschmann et al., 2004; De Bosschere et al., 2004; Gavier-Widén et al., 2004; Onnasch et al., 2004; Orge et al., 2004; Everest et al., 2006; Saunders et al., 2006; Lühken et al., 2007; Mazza et al., 2010), but also in USA (Loiacono et al., 2009), the Falkland Islands (Epstein et al., 2005), Canada (Mitchell et al., 2010) and New Zealand (Kittelberger et al., 2010). Atypical scrapie cases differ from classical scrapie in several features, including the neuroanatomical distribution of the histopathological lesions and of PrP^{Sc} in the brain, and the pattern of PrP^{Sc} deposits (Benestad et al., 2003). In 2005, the European

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Food Safety Authority (EFSA) defined diagnostic criteria for classical scrapie and atypical scrapie, based on the results of Western blot pattern of the pathogenic prion protein, histopathology, immunohistochemistry, age and epidemiology (EFSA, 2005).

As a contagious disease, classical scrapie is often clustered within flocks and regions. Infected animals usually die at the end of the clinical course of the disease when they are between two and four years of age. In contrast to classical scrapie, atypical scrapie is usually detected in older (5 to >10 years of age) animals (Benestad et al., 2008; Fediaevsky et al., 2010a). While sheep carrying PrP genotypes with VRQ and/or ARQ alleles are considered most susceptible to classical scrapie (Hunter, 2007), PrP genotypes that include alleles AHQ and/or AFRQ are more susceptible to atypical scrapie (Benestad et al., 2003; Moum et al., 2005; Arsac et al., 2007; Moreno et al., 2007; Fediaevsky et al., 2010b).

One of the first full descriptions of the PrP genetics for atypical scrapie was provided by Moum et al. (2005). Performing four codon genotyping (codons 136, 141, 154, and 171) on 38 cases of atypical scrapie, they came to three major conclusions. Firstly, all animals were of AA_{136} PrP genotype. The VRQ allele conferring susceptibility to classical scrapie was completely absent from Nor98 cases. Secondly, there was an over-representation of animals carrying the AHQ allele, in HH_{154} homozygous and HR_{154} heterozygous genotypes. Thirdly, the $AF_{141}RQ$ allele appeared to confer higher susceptibility to atypical scrapie than the $AL_{141}RQ$ allele. Indeed, the $AF_{141}RQ$ allele conferred a higher risk than the AHQ allele.

A case–control study was designed to study risk factors of atypical scrapie in France (Fediaevsky et al., 2009). According to this study, PrP genotypes were linearly classified by levels of genetic risk for atypical scrapie as five different groups. Group 1 was thought to be most resistant to atypical scrapie whereas group 5 was thought to be most susceptible. Fediaevsky et al. (2009) revealed that they did not find any risk factor associated with an infectious origin of scrapie and atypical scrapie could be a spontaneous disease influenced by genetic and metabolic factors.

The results of another case–control study in France demonstrated that there were no atypical scrapie cases among the ALRR/VLRQ, ALRQ/ALRH, ALRQ/VLRQ, and VLRQ/VLRQ genotypes. The ALHQ/ALHQ, AFRQ/ALHQ and AFRQ/AFRQ genotypes were associated with the highest risks of atypical scrapie compared to ALRQ/ALRQ. Within classical scrapie cases, the VLRQ/VLRQ animals presented the highest risk compared to ALRQ/ALRQ. In addition, the authors detected a significant risk of atypical scrapie for sheep carrying the ALRR/ALRR genotype (Fediaevsky et al., 2010b).

The earliest evidence of sheep domestication was found in certain parts of the Near East, with Turkey as an area of major importance. Archeological data suggest two different areas with independent sheep domestication events in Turkey: the upper Euphrates valley in eastern Turkey (particularly, the Çatal Höyük and Aşıklı Höyük sites). The Zagros Mountains on the border of Turkey and Iran is also recognized as a primary center of sheep domestication (Bruford et al., 2003; Zeder, 2008). Thus, it is likely that the Turkish native sheep breeds of today are one of the oldest living descendants of their first domesticated ancestors and Anatolian (Turkish) native breeds may be special in maintaining very valuable genetic diversity. Therefore they must be explored with regard to genetic markers. In Turkey, there have been no official reports about the cases of classical and atypical scrapie. Although there were a few studies on PrP genotyping for classical scrapie (Ün et al., 2008; Alvarez et al., 2011; Meydan et al., 2012), we could not come across any studies about the atypical scrapie in Turkish native sheep breeds. The aim of this study was to genotype all eighteen Turkish native sheep breeds in order to determine polymorphisms of the PRNP gene and evaluate the genetic susceptibility to atypical scrapie.

2. Materials and methods

2.1. Sampling and DNA extraction

In this study, a total of 1110 unrelated healthy sheep were randomly (regardless of age and sex) sampled from 18 Turkish native breeds, İvesi (Awassi, $n = 100$), Akkaraman ($n = 100$), Kangal Akkaraman ($n = 100$), Morkaraman ($n = 100$), Güneykaraman ($n = 30$), Kıvırcık ($n = 140$), Norduz ($n = 35$), Karakaş ($n = 35$), Sakız (Chios, $n = 50$), Herik ($n = 45$), Hemşin ($n = 55$), Dağlıç ($n = 40$), Karayaka ($n = 45$), Tuj (Tushin, $n = 45$), Çine Çaparı ($n = 40$), Gökçeada (Imroz, $n = 50$), Karagül (Karakul, $n = 50$) and Zom ($n = 50$) breeds.

Blood samples were collected from the jugular vein into EDTA containing tubes, transported to the laboratory and stored at −20 °C until genomic DNA extraction, which was carried out using a salting-out method (Miller et al., 1988).

2.2. PCR assay and DNA sequencing

The fragment of 771 bp in length, which covered open reading frame of PRNP gene and codons 136, 141, 154 and 171, was amplified by PCR with forward (5′-ATG GTG AAA AGC CAC ATA GGC AGT-3′) and reverse (5′-CTA TCC TAC TAT GAG AAA AAT GAG-3′) primers suggested by Sipos et al. (2002). PCR products resolved by electrophoresis on 2% agarose gels. After gel electrophoresis, the amplicons were purified using a Qiamp Mini Kit (QIAGEN, Valencia, CA, USA). The purified samples were sequenced by a Big Dye Terminator Chemistry on an ABI 3100 Avant Automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The DNA sequences were analyzed using the Sequencing Analysis Software Version 3.3 (Applied Biosystems, Foster City, CA, USA).

2.3. Statistical analysis

Genotype (X_{ij}) and gene ($\hat{x}_i$) frequencies were estimated as following formulas (Nei, 1987);

$$X_{ij} = \frac{n_{ij}}{n} \quad \text{and} \quad \hat{x}_i = \frac{2n_{ii} + \sum n_{ij}}{2n}$$

where X_{ij} is genotypic frequency of A_iA_j; n_{ij} and n_{ii} are the number of individuals for heterozygous (A_iA_j) and homozygous (A_iA_i) genotypes, respectively; $\hat{x}_i$ is the gene frequency of A_i and n is the total number of individuals sampled from the population.

3. Results

The alleles and genotypes observed based on codons 136, 141, 154 and 171 of PRNP gene and their frequencies are summarized in Table 1. The most frequent allele in each of the eighteen breeds was ALRQ with the frequencies ranging from 0.445 to 0.757. The AFRQ allele, associated with the susceptibility to atypical scrapie, was detected in only İvesi with the frequency of 0.006. The other susceptible allele to atypical scrapie, ALHQ, was detected in Akkaraman (0.005), Kangal Akkaraman (0.010), Güneykaraman