Short Paper

Genotyping of PRNP coding region for scrapie in Indian sheep

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Summary

Prions are unprecedented infectious pathogens that cause a group of invariably fatal neurodegenerative disease by an entirely novel mechanism. The conformational change in prion proteins results in a change from a predominantly α-helical protein to a β-sheet form, which causes scrapie in sheep and goat. The present study was carried out to identify polymorphisms of the prion protein gene (*PrP*) at the codons (136, 154 and 171) responsible for the susceptibility and resistance of the scrapie disease in the sheep. The ARQ is the most frequent allele which is less susceptible, but may get scrapie. The highly sensitive VRQ and resistant ARR alleles were not present in the Mandya sheep. Genotype ARQ/ARQ, ARQ/AHQ, and AHQ/AHQ were found in the analyzed population with 40.00, 40.00 and 20.00% respectively, showing little resistance to scrapie and require careful selection when used for breeding. Six groups (variants) were found in SSCP (single-strand conformation polymorphism) i.e., out of each group one sample was sequenced. Sequencing (accession No. KF207876-79) of samples allowed the identification of 5 other new polymorphisms on *PrP* gene at codon positions 98(S/R), 147(D/E), 175(Q/R), 184(N/H) and 189(Q/L). Absence of ARR allele in the Mandya sheep should be taken into considerationfor the implementation of a preventive selection programme to avoid erosion of the genetic stock.

Key words: Scrapie, Allele, *PrP* gene, Single nucleotide polymorphism, Sheep

Introduction

Prion diseases or transmissible spongiform encephalopathies (TSE) is a neurodegenerative disease that can inflict on humans and animals, characterized by dementia and movement disorder. The gene responsible for this disease is commonly called as PRNP (prion protein gene). It is a fatal neurodegenerative prion disease which is both genetic and infectious. It is characterized by the accumulation of an abnormal isoform of the prion protein (PrP^{Sc}) in the central nervous system. (Babar *et al*., 2009; Choudhary and Gupta, 2013c) Histopathological changes of the brain are comprised of a fine vacuolation, also termed spongiosis, reactive changes of astrocytes (gliosis) and variable loss of neurons. A significant relationship with susceptibility and resistance of sheep to scrapie was observed for polymorphisms at codons 136(A/V), 154(R/H) and 171(Q/R/H/K) of *PrP* gene (Baylis and Goldman, 2004; Choudhary *et al*., 2012, 2013a, b). Polymorphisms at other codons are rare and have not yet been associated with classical scrapie susceptibility (Piestrzyñska-Kajtoch and Rejduch, 2006; Wiœniewska *et al*., 2006).

Materials and Methods

Sample collection and DNA extraction

Blood samples were collected aseptically by jugular vein puncture of 50 animals (Mandya sheep) in a sterile vacutainer. Genomic DNA was isolated using standard procedure (Sambrook *et al*., 1989).

PCR and SSCP analysis

PCR was carried out on about 50 ng genomic DNA. Amplifications were performed in a MJ PTC 100 thermal cycler (MJ Research Inc., USA). The PCR products were denatured at 85°C, followed by rapid cooling (Kutzer *et al*., 2002; Mead *et al*., 2003). The samples were loaded on 12% polyacrylamide gels containing 0.2% glycerol. Electrophoresis was performed using Protean-II xi cells (Bio-Rad Laboratories). The gels were stained in silver nitrate using a standard protocol (Bassam *et al*., 1991) and were dried on Gel Dryer (Bio-Rad Laboratories).

PCR-SSCP profile

PCR product amplicons developed using *PrP* forward primers (5'-caa ggt ggt agc cac agt ca-3') and reverse primers (5'-cca cca ctc gct cca tta tc-3') of approximately 358 bp were generated for *PrP* gene exon-3 partial CD's covering 352 to 700 nucleotide positions. The SSCP (single-strand conformation polymorphism) amplimers were resolved on non-denaturing gel. Polyacrylamide gels of Mandya sheep breed are shown in Fig. 1. A total of 6 variants were designated as A, B, C, D, E, and F (Table 1). The classification of SSCP variants was given based on conformational changes in the band patterns.

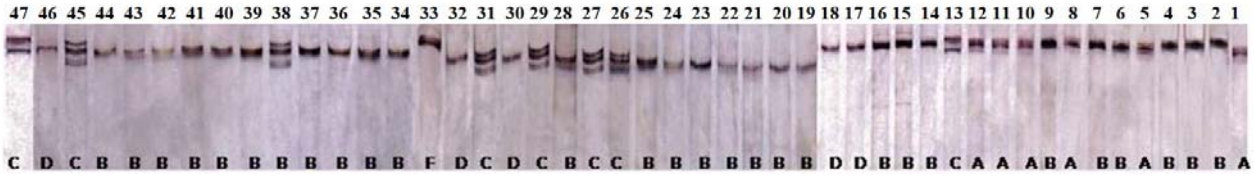

Fig. 1: SSCP gels showing different band pattern (variants)

Table 1: PCR-SSCP variants and their frequency (%) in Mandya sheep breed

Sr. No.	Variant	Variant name	Frequency (%)
1	A	Seq 1	12.76
2	B	Seq 2	57.45
3	C	Seq 3	14.90
4	D	Seq 4	10.63
5	E	Seq 5	2.13
6	F	Seq 6	2.13

PCR cleanup and sequencing

The PCR-SSCP amplicons were identified for sequencing based on different haplotypes observed on gels for genomic DNA products. PCR products were treated with shrimp alkaline phosphatase and exonuclease III in 96 well plates, incubated at 37°C for 30 min followed by 80°C for 10 min. Out of this, 1-5 µL PCR product was used in sequencing. PCR products were loaded in to a 96 well format and analyzed on ABI 370 DNA analyzer (Applied Biosystem, USA). All sequences were analyzed and single nucleotide polymorphism (SNPs) were identified. The SSCP alleles were identified using the nomenclature followed by Zhou *et al*. (2005) and compared with amino acid of the codon.

Sequencing results of PCR amplicons

The sequencing chromatograms were viewed and edited by ClustalW and BioEdit software and submitted to GenBank at accession No. KF207876-79 which showed identities with GenBank accession No. AJ000734-39.

Results

In order to determine the incidences of scrapie, the farmers and flock owners of Mandya sheep were interviewed. All the farmers under study stated that they had never observed any symptoms explained to them for the incidence of the scrapie. Further, the veterinary staff of the respective areas also confirmed that neither of the sheep in the sampled flocks showed any symptom of scrapie nor have they received any case of clinical scrapie alive or carcass for postmortem showing any prevalence of the disease in sheep of these areas.

Protein translation of *PrP* gene sequences

The deduced amino acid sequence based on Mandya sheep *PrP* gene exon-3 (partial CD's) is presented in Fig. 2.

Single nucleotide polymorphism in *PrP* gene

Polymorphism was observed at codon 98, 147, 154, 175, 184, and 189. No polymorphism was found on codon 136(AA) and 171(QQ).

Discussion

Scrapie belongs to the most intriguing group of diseases, the prion diseases, comprising slowly developing fatal neurodegenerative conditions in sheep and other animal species, as well as humans. Scrapie in sheep is the oldest of these diseases, being known in Europe for more than 250 years, and is often characterized as the prototype of prion disease. The association between scrapie susceptibility and sheep encoding the prion protein (*PrP*) 136(A/V), 154(R/H) and 171(Q/R/H/K) allele is very strong.

The ARQ allele was the most frequent allele in Mandya sheep. The highly risky allele VRQ and highly resistant allele ARR were not found in any of the sampled population. The ARQ/ARQ genotype was most frequent, and was experimentally challenged with scrapie (Vaccari *et al*., 2007). ARQ/ARQ is considered as slightly resistant to scrapie (DEFRA, 2007). The second genotype observed was AHQ/AHQ, which is considered as a slightly susceptible genotype for the scrapie (Arsac *et al*., 2007). The third genotype was ARQ/AHQ which is found to be partially resistant in Greece sheep (Billinis

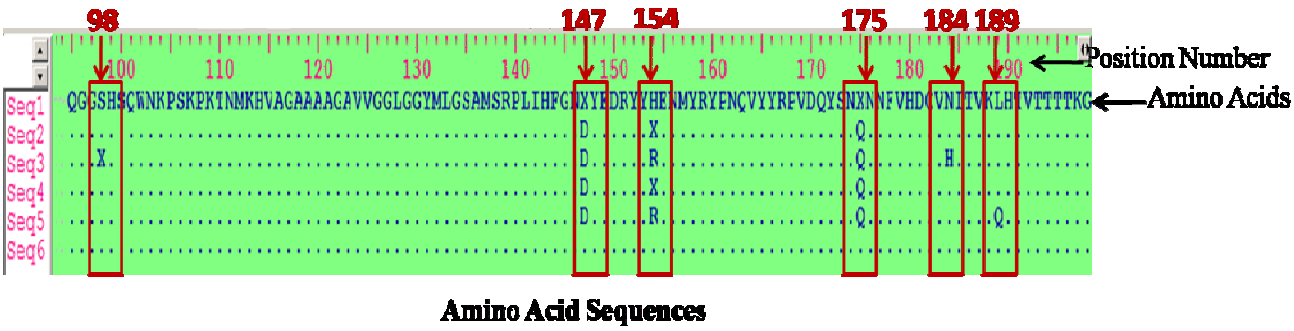

Fig. 2: Comparison of amino acid sequence in *PrP* gene

et al., 2004).

AA allele was present at codon 136. The prevalence of this allele in indigenous sheep is indicative of the susceptibility of indigenous sheep to scrapie; however, no report has so far been published where any animal carrying this allele in India contracted the disease. This could have been either due to small sample size or due to the other management practices that could be important in prevention of onset of disease conditions. At codon 154 three types of alleles RR, RH and HH were present. Polymorphism at codon 154 (R/H) has also been perceived to play a role in susceptibility to scrapie. A recent study in Greece suggested that presence of H154 is likely to confer higher susceptibility to scrapie in Chios-breed sheep (Ekateriniadou *et al*., 2007), which lack V136. 'Q' allele at 171 loci alone has been reported to be positively correlated with naturally occurring incidence of scrapie in sheep (Hunter *et al*., 1994; De Silva *et al*., 2003). In the present study, all the genotypes at locus 171 were of QQ type, in homozygous condition, but none of the animals have shown any clinical symptoms of scrapie so far.

Other than principal SNPs, polymorphisms were noted for 5 new positions at codon 98(S/R), 147(D/E), 175(Q/R), 184(N/H) and 189(Q/L). These are new SNPs which were observed in this indigenous breed as no earlier studies reported these polymorphisms but as yet numbers are still small to conclude whether these polymorphisms result in resistance/susceptible to all classical scrapie strains. In total 6 SNPs have been observed in this study including the principal SNPs at 136, 154 and 171 codons. The significance of the polymorphism at other codons in prevention of scrapie disease susceptibility in indigenous breed cannot be ruled out because sheep carrying known susceptible alleles at 136 AA, at 154 RR, RH, HH and at 171 QQ did not show any symptoms of naturally occurring scrapie. Further, these results carry specific significance in confirming the earlier concept of Hunter *et al*. (1994) where it has been suggested that despite the positions of susceptible alleles in animal, the symptoms of naturally occurring scrapie may not appear as additional factors might be involved in control of scrapie including genotype x environment interactions.

In one study documented by Zlotnik and Katiyar (1961), four animals died because of scrapie infection in Himalayan region of India. However, their genotypes were not studied using molecular markers. Though the ARR genotype is absent and observed genotypes also show susceptibility to scrapie in Mandya sheep, no animal was found positive for this disease. These results suggest that either some mutation or polymorphism at other codons or loci is protecting the animals from becoming infected with prion protein or it may be that the management practices of sheep reared solely on range grazing without any concentrate supplements probably prevents the onset of scrapie disease symptoms. These results have important bearing on prevention of scrapie disease in sheep globally through traditional management of sheep on forages. It has been very well documented even in case of BSE outbreaks in cattle population of the United Kingdom that the animals fed on animal protein only showed the symptoms of mad cow syndrome.

In this case, our results are of special significance, however, before this concept is applied globally, more detailed genetic survey in larger population is warranted by molecular markers using high throughput screening protocols.

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