GENETIC STRUCTURE OF TURCANA BREED, SIBIAN ECOTYPE AT OVINE PRN-P LOCUS

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Abstract

Scrapie in sheep and goats, is the longest known and most widely spread of transmissible encephalopathies (T.S.E s) or prion diseases, that also include Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy (B.S.E) in cattle. The Ppn-p locus is polymorphic with known variability at codon 136, 154, 171 in sheeps, which are associated with different sensibility in experimental and natural spongiform encephalopaties. General the possible combinations of the 5 amino acids encoded by the 3 different codons will determine the existence of 15 possible genotypes. To put in evidence those polymorphisms at the ovine Prn-p locus, several methods are developed but the most accurate assay is the direct sequencing of the gene and the primer extension technique. The purpose of this study was to determine the genetic structure at Prp locus in 322 male of Turcana breed, Sibian ecotype, using primer extension technique (ABI 3130xl Genetic Analyzer), and to establish the risk groups of the susceptibility at scrapie disease.

Key words: scrapie, codon, Prnp locus, primer extension,genetic structure

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are a group of fatal neurodegenerative diseases including sheep and goat scrapie, bovine spongiform encephalopathy (BSE), and Creutzfeld-Jakob disease (CJD) in humans. The pathology of such diseases is characterized by vacuolation, neuronal loss and glial cell activation and proliferation. The pathological hallmark of TSE is the accumulation of an abnormal protein, named Prp-Sc, which is formed from the normal isoform (Prp-C).Prions are transmissible particles that are devoid of nucleic acid and seem to be composed exclusively of a modified protein (Prp-Sc).The normal, cellular PrP (PrP-C) is converted into PrP-Sc throught a posttranslational process during which it acquires a high B-sheet content. The presence of Prp-Sc is considered a marker for TSEs. Polymorphisms in the prion protein (PrP) gene are associated with different phenotypic expression of transmissible spongiform encephalopathies in animals and humans. In sheep, at least 10 different mutually exclusive polymorphism are present in PrnP gene. The polymorphism at codon 136 154 and 171 are associated with different sensibility at natural and experimental spongiform encephalopathies. General the possible combination at the 5 amino acids encoded by the 3 different codons will determine the existence of the 15 possible genotypes at the PrP locus [1, 2].

- ARR/ARR-sheep very resistant at scrapie
- ARR/AHQ, ARR/ARH, ARR/ARQ-sheep genetic resistant to scrapie but they need a special attention for using in selection programs
- ARQ/ARH, ARQ/AHQ, AHQ/AHQ, ARH/ARH, AHQ/ARH, ARQ/ARQ-sheep with lower genetic to scrapie
- ARR/VRQ-sheep sensitive to scrapie
- AHQ/VRQ, ARH/VRQ, ARQ/VRQ, VRQ/VRQ-sheep very sensitive to scrapie

MATERIALS AND METHODS

Biologic material:

Blood samples were collected from 322 male sheep (Turcana breed, Sibian ecotype) belonging to herds from Sibiu, Brasov, Salaj,

Caransebes, Cluj regions.The selection of biologic material was performed using the register of official control of productions in the aim of including these animals in the national plan for selection against scrapie disease [6].

The selection of genetically resistant sheep populations represents the basis of the recent strategies against ovine TSE in the European Union. In order to accomplish this goal several methods are used, the most accuracy methods are based on sequencing, and in this study we use the primer extension technique [3, 4]. In order to perform the primer extension technique for genotypization at PrnP locus is necessary to follow some steps:

- DNA extraction from the blood samples
- determination of the DNA quality and quantity
- template amplification
-. purification of the PCR products
- Primer extension technique
- performing the capillarity electrophoresis to determine the polymorphism - the analysis of results for electrophoretic profiles with GeneMapper^{R} software

DNA preparation from blood samples:

Blood samples were collected in tubes containing K3- EDTA and stored at -20^{o}C. The DNA from blood was extracted using MagnaPure LC DNA Isolation Kit (Roche).The quality and quantity of DNA was detected spectrophotometricaly using NanoDrop ND 1000 instrument.

In order to perform this precision genetic molecular analysis, is necessary to have a very good DNA samples with a purity between 1,8-2 (DO260/280nm), while values over two indicate a proteic contamination and the values under 1,8 indicate a ARN contamination.

Template amplification :

PCR amplification was carried out in a 25 µl reaction volume containing 2 µl of DNA (40-100 ng/ µl) 0,3 µl Taq polymerase (Promega), 2,5 µl PCR buffer (Promega), 1 µl dNTPmix and 2,5 µl Primer preSnaPshot mix.

The PCR amplification was performed in a DNA thermocycler (Mastercycler Eppendorf) and the cycles were: 1 x 3 minutes at 92^{o}C; 35x 60 seconds at 95^{o}C, 30 seconds la 62 ^{o}C, 30 seconds la 72^{o}C, and a final final extension cycle at 72^{o}C.

The PCR product purification: High Pure purification kit (Roche):

This operation need to be performed for move away the primers, unincorporated dNTP and the inhibitors. The purification was made with the kit HighPure 96UFcleanup kit, on columns and consisted in repeated centrifugation and wash with buffer solution [5].

Primer extension technique:

The mix for the reaction is composed with SnaPshot Multiplex Ready Reaction mix (Applied Biosystem) - 5 µl, Primers SnaPshot Mix – 1 µl, H_2O – 1 µl, and 2µl of the previously purified PCR product [7]. The primer extension reaction cycles are: 25 x 10 seconds at 96^{o}C, 5 seconds at 50^{o}C, 30 seconds at 60^{o}C. This reaction consist of a single nucleotide extension of primers complementary to the target DNA and adjacent to the polymorphic sites of interest. During this step, the primers incorporate fluorescently labelled dideoxynucleotides presented in the SNaPshot chemistry. Primers are designed with tails of varying lengths at the 5` end and their identification by size differences is performed in a capillary electrophoresis.

The presence of unincorporated dNTPs into the primer extension reaction was avoided by dephosphorylating deoxyribonucleotides with calf intestinal alkaline phosphatase.

Capillarity electrophoresis – 3130 xl Genetic Analyzer (Applied Biosystem)

One microliter of the reaction mixture was incubated for 5 minutes at 95^{o}C with 11,5 µl formamide and 0,5 µl Gene-Scan Lize size standard (Applied Biosystem). After capillarity electrophoresis, the electropherograms were analyzed by GeneScan 3.1. Software (Applied Biosystem) [7,8]

The analysis of results and data interpretation

The polymorphism in codons 136,154 and 171, is detected in electrophoregrams (fig 1, fig 2):

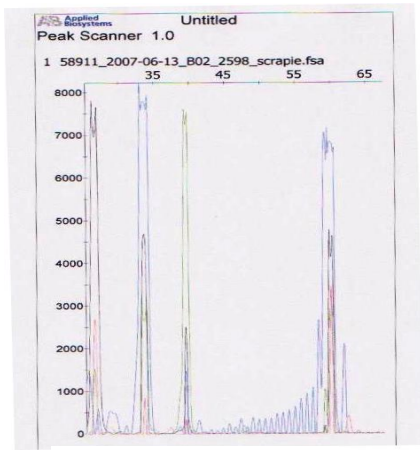

Fig.1 Genotype ARR/ARQ

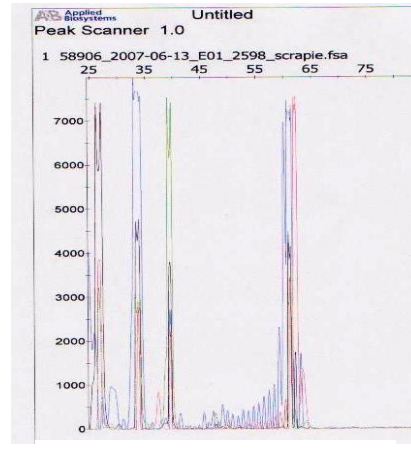

Fig.2 -. Genotype ARQ/VRQ

RESULTS AND DISCUSSION

From the data obtained in table 1, the frequency of the allele ARR in **Turcana breed-Sibian ecotype** is 35,4%, allele AHQ is 0,63%, ARH 2,95%, ARQ 54%, VRQ 6,06%, VHQ 0,97% and VRH 0,17%.

Table 1: Allele frequency at the PrP locus in Turcana breed, Sibian ecotype

Allele	ARR	AHQ	ARH	ARQ	VRQ	VHQ	VRH
Frequency	35,4%	0,63%	2,95%	54%	6,06%	0.79%	0.17%

After the genotyping at Prn-P locus of the 322 male sheeps of the Turcana breed – Sibian ecotype, we reach at the follow results (Table 2):

- 62 male (19,25%) had the ARR/ARR genotype, which is the genotype related to the highest resistance to scrapie and they represent 14, 63%
- 89 male (27,68%) had the genotypes ARR/AHQ, ARR/ARH, ARR/ARQ which are the genotype correlated with resistance to scrapie, but those require a particular attention to be use in selection programs
- 131 male (40,69%) had the genotypes ARQ/ARH, ARQ/AHQ, AHQ/AHQ, ARH/ARH, AHQ/ARH, ARQ/ARQ, which are the genotype correlated with lower resistance to scrapie

Table 2: The frequency of genotype in Turcana breed-Sibian ecotype

Risk intensity	RISK INTENSITY														
	I.Sheep genetically very resistant to scrapie	II. Sheep genetically resistant to scrapie but which require particular attention for use in selection programs			III. Sheep with low resistance to scrapie. Their use in selection scheme should be avoided						IV. Sheep sensitive to scrapie	V.Sheep very sensitive to scrapie which must be eliminated			
Genottype	ARR/ARR	ARR/AHQ	ARR/ARH	ARR/ARQ	ARQ/ARH	ARQ/AHQ	AHQ/AHQ	ARH/ARH	AHQ/ARH	ARQ/ARQ	ARR/VRQ	AHQ/VRQ	ARH/VRQ	ARQ/VRQ	VRQ/VRQ
Procent	19,25%	0	0,32%	27,36%	0,97%	6,51%	0,32%	0%	0%	32,89%	5,63%	0%	0%	6,84%	0

- 18 male had the ARR/VRQ, genotypes and those male are sensitive to scrapie and they represent 5,53%
- 22 male (6,84%) have been found with genotypes related with lower resistance to scrapie: AHQ/VRQ, ARH/VRQ, ARQ/VRQ, VRQ/VRQ, and is recommended to be sacrifice.

CONCLUSIONS

From comparison with the data obtained from literature we conclude that allele ARR frequency have medium values, while the allele VRQ frequency is superior and the rest of the alleles have comparative values.

By comparation in a study conducted in Germany on nine local and imported breeds the limits of the ARR allele frequency was found lying between 6.3% to breed **White Friesian** milk sheep and 69.1% **German black head**.

On breed **Coburg Fox** ARR allele frequency was found at a rate of 51.3%, the allele ARQ at a rate of 46%, AHQ 1.3% ARH 0.7%, VRQ 0.7%.At breed **Rhoen**, ARR allele frequency was found at a rate of 65.9%, the allele ARQ at a rate of 23.3%, AHQ 0.8%, ARH 8.2%, VRQ 1.8%.

On breed **Merinoland**, ARR allele frequency was found at a rate of 12.1%, the allele ARQ at a rate of 69.5%, AHQ 17% ARH 1.4%, VRQ. 0%

At **Shropshire** breed, ARR allele frequency was found at a rate of 23.9%, the allele ARQ at a rate of 39.8%, AHQ 36.3% ARH 0% VRQ 0%.

In the **Suffolk** breed, ARR allele frequency was found at a rate of 52.3%, the allele ARQ at a rate of 45.4%, AHQ 0%, ARH 1.7%, VRQ 0.6%.In the **Texe**l breed ARR allele frequency was found at a rate of 48.3%, the allele ARQ at a rate of 37.9%, AHQ 6%, ARH 5.2%, VRQ 2.6%

At **White Friesian** breed, the ARR allele frequency was found at a rate of 6.3%, the allele ARQ at a rate of 64.3%, AHQ 29.4% ARH 0% VRQ 0%.

In the **Texel** and **Rhoen** breeds the ARH allelic variant, meets with a frequency of 8.2% respectively 5.2%, while at the **Coburg Fox**, **Merinoland** and **Suffolk** is very rare, with a frequency below 2%.

From the analysis of these data we could conclude that it is necessary to apply the program of molecular marker-assisted selection, to increase the frequency of genotypes which gives natural resistance to scrapie in the breed Turcana-Sibian ecotype, which is the only efficient method of fighting against this disease, and to aling with the rest of U.E country where was applied such a national program.

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