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Abstract

Transmissible Spongiform Encephalopathies (TSE's) are neurodegenerative disorders characterized by a long generation time, spongy degeneration in the cerebral gray matter, neuronal loss and proliferation and hypertrophy of glial cells. An abnormal form of the prion protein (PrP) plays a major part in TSE pathogenesis and has been hypothesized to be the only component of the infectious agent. Some animals exposed to scrapie, the TSE affecting sheep and goats, seem to be resistant to development of the disease. Alleles encoding amino acid substitutions at codons 136 (A/V) and 171 (Q/R/H) have been associated with scrapie resistance. Other amino acid substitutions at codons 112 (M/T), 137 (M/T), 141 (L/F), 154 (R/H), and 211 (R/Q) have been reported but not associated with scrapie resistance. It may be possible to reduce the incidence of ovine scrapie by increasing the frequency of resistant genotypes (AA-136, RR-171, or QR-171). Thus, an important consideration is the frequency of these genotypes in different breeds of sheep. In this study, the genetic sequence for codons 104-175 was determined for at least ten animals of ten sheep breeds (n=207). Genotypes at codons 112, 136, 154, and 171 were determined. For codon 136, the frequency of the susceptible allele (V) was less than 0.20 in all breeds. In contrast, the frequency of the susceptible allele (Q) at codon 171 ranged from 0.27 (St. Croix) to 0.96 (Hampshire). In addition, a previously unreported substitution was found at codon 143 (H/R), with frequencies as high as 0.40.

Introduction

Scrapie, a neurodegenerative disorder affecting sheep and goats, is one of a group of diseases known as transmissible spongiform encephalopathies (TSEs). Up until the 1960's, it was believed that scrapie and other TSEs were caused by a slow acting virus. Alper *et al.* (1967)

demonstrated that the scrapie agent was resistant to levels of UV radiation that would destroy most known pathogens, including viruses. Prusiner *et al.* (1981) found a protein that was in scrapie infected tissues but not in normal tissues. It was then shown (Prusiner, 1982) that the protein was present in two isoforms. Based on these findings, Prusiner (1982) formulated the "protein-only" hypothesis for TSE transmission.

The prion protein (PrP) is expressed in nervous tissue and in the lymphoreticular system. PrP^{c} is the normal form and PrP^{sc} is the abnormal, disease causing form (Figure 1). It is thought that the abnormal form serves as a template for a posttranslational misfolding of the normal cellular protein. Accumulation of the abnormal, protease resistant PrP^{sc} is associated with disease. Another protein, protein X, is thought to take part in this refolding process and may be one of the reasons for species specificity of prion diseases (Prusiner 1996).

The ovine PrP gene sequence contains three exons and covers over 20 kb of DNA (Westaway *et al.* 1994). The PrP open reading frame (ORF) is contained in exon three. There have been seven polymorphisms reported in the ovine ORF (Bossers *et al.* 1996); two of these (codons 136 and 171) have been associated with scrapie susceptibility. Perhaps amino acid substitutions at these sites increase the chances of PrP^{sc} propagation when an animal is exposed to scrapie. Animals differ in PrP amino acid sequences and therefore differ in susceptibility to the scrapie agent. Furthermore, alleles encoding these amino acid changes vary between breeds as do the polymorphisms that are most important in scrapie susceptibility. Therefore, it would be interesting to know allele frequencies at the various polymorphic sites in breeds of sheep. The purpose of this project was to determine allele frequencies at PrP polymorphisms in ten different breeds of sheep.

Materials and Methods

Animals. Genomic DNA was obtained from blood of 207 sheep from ten different breeds: Columbia (n=15), Dorper (n=10), Dorset (n=10), Hampshire (n=12), Polypay (n=25), Rambouilet (n=15), Southdown (n=74), Saint Croix (n=15), Suffolk (n=16), and Targhee (n=15). Each animal was selected from a distinct bloodline in order to get a representative, non-related sampling of the breed.

PrP Genotyping. Sequence information for the PrP gene between codons 104 and 175 was obtained using cycle sequencing. Primers used in the study included:

PrP352: 5'- GTCAAGGTGGTAGCCACAGT - 3'

PrP751: 5'- GCCTGGGATTCTCTCTGGTA - 3'

Primer 5a: 5'- AAGGTGGTAGCCACAGTCAGTGGA - 3'

Genomic DNA was amplified using primers PrP352 and PrP751. The PCR contained 100 ng genomic DNA, 0.2 mM of dGTP, dCTP, dATP and dTTP, 10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 10 ρmol each primer, and 2.5 units of *AmpliTaq* DNA polymerase in a 100 uL volume. PCR conditions were as follows: 1 cycle of 5 min at 95 ^{0}C, 2 min at 60 ^{0}C, 2 min at 72 ^{0}C, and then 34 cycles of 1 minute at 94 ^{0}C, 1 min at 60 ^{0}C, and 1 min at 72 ^{0}C.

Amplified product (25 μL) was then electrophoresed on a small 6% nondenaturing polyacrylamide gel to check the size (771 bp) and quality of the product. An aliquot of the reaction (25 μL) was precipitated using ammonium acetate and isopropyl alcohol, dried, and then re-suspended in 15-20 uL of TE. The samples were then sequenced.

The ThermoSequenase radiolabeled terminator cycle sequencing kit (Amersham Life Science, Arlington Heights, IL) was used for sequencing. Purified PCR product (1 uL) as

prepared above was added to the sequencing reaction which contained 7.92 ng primer 5a (O'Rourke *et al.* 1996), 2 uL sequencing buffer, 2 uL ThermoSequenase, 2 uL termination master mix and 0.5 uL of appropriate [α-^{33}P] ddNTP. Samples were overlaid with one drop mineral oil and cycled as follows: 1 cycle of 5 min at 95 ^{0}C, and then 30 cycles of 30 sec at 95 ^{0}C, 30 sec at 60 ^{0}C, and 1 min at 72 ^{0}C. Samples were stopped with 4 uL formamide stop buffer and then loaded onto a 6% Long Ranger Gel (FMC Bioproducts, Rockland ME). After appropriate running times, gels were wrapped in saran wrap and exposed to x-ray film for 48-72 hrs at -80 ^{0}C (Figure 2). Results obtained from the sequence data are summarized in tables 1-3.

Results

The genetic sequence for PrP codons 104 to 177 was determined for 207 sheep of ten different breeds. Five polymorphic sites within this region were found. Allele frequencies at PrP171, previously associated with scrapie susceptibility, were strikingly different. The frequencies ranged from 0.27 (St. Croix) to 0.96 (Hampshire) for Q-171, the "susceptible" amino acid (Table 1). At PrP136, the A/A genotype is associated with resistance. Three breeds (Polypay, Targhees and Suffolks) were homozygous for A-136; however, there were animals with the genotype A/V in the other seven breeds, and frequencies ranged from 0.04 to 0.40 for this susceptible genotype (Table 2). In this study, variation of the PrP amino acid sequence was found at three other sites, PrP112, PrP143 and PrP154. Substitutions at PrP112 and 154 have been reported but have not yet been linked to scrapie susceptibility. Variation at PrP112 occurred in three breeds (Dorset, Hampshire, and Southdown). Frequencies of the threonine at this site ranged from 0.1-0.25. Variation at PrP154 occurred in Columbia, Rambouilet and Targhee breeds with histidine frequencies ranging from 0.03-0.17. The substitution at PrP143

has not been previously reported. Variation at this site occurred in five breeds (Columbia, Dorset, Hampshire, Polypay and Southdown). The arginine substitution was present at frequences as high as 0.40 (Table 3).

In conclusion, sheep breeds differ in frequency of PrP susceptibility/resistant genotypes. It may be possible to reduce the incidence of scrapie by selecting for resistant genotypes within a breed.

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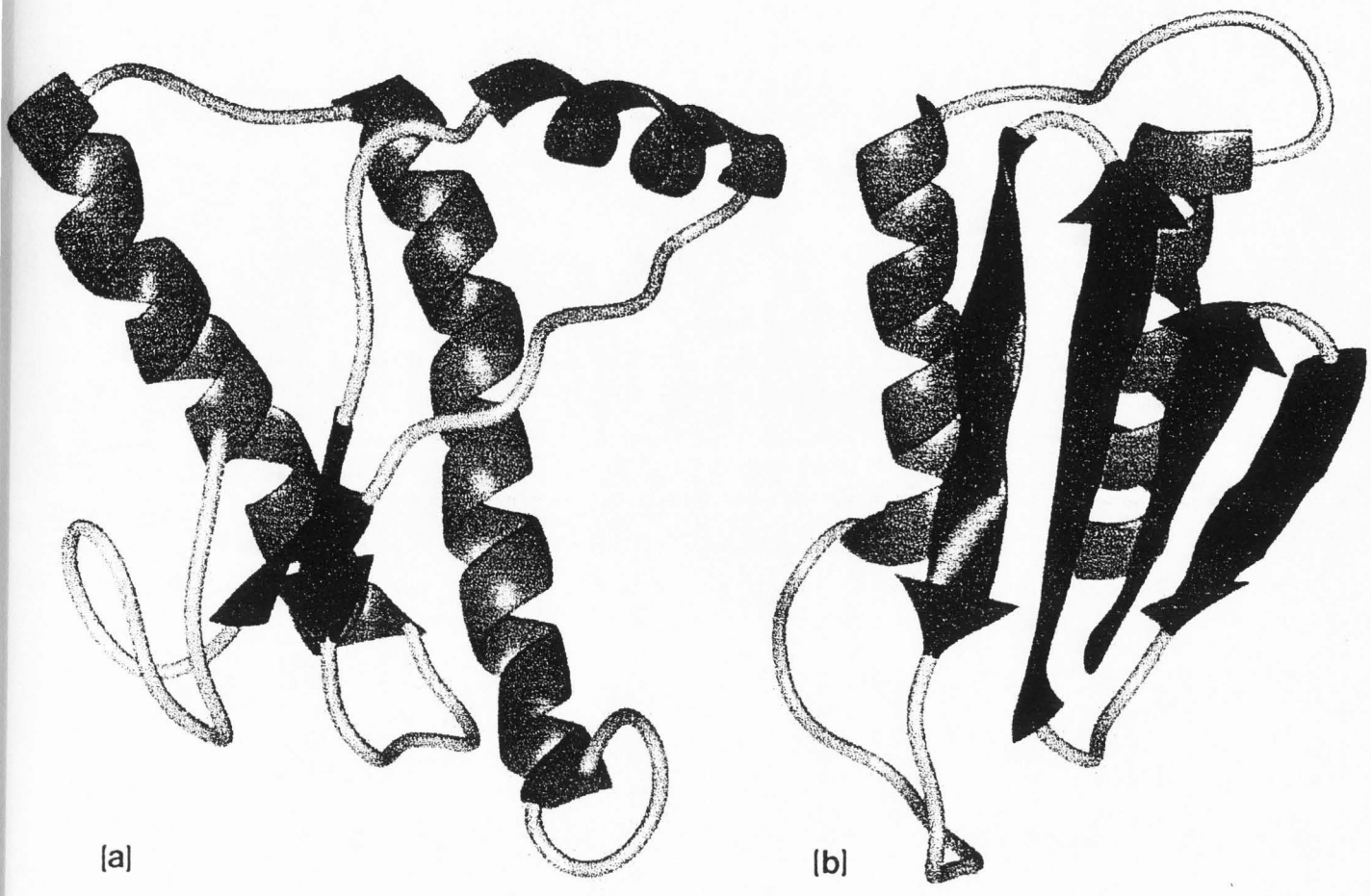

Figure 1. Computer generated images (Fred Cohen): a) NMR structure PrP^{c} residues 121-231 and b) predicted structure of PrP^{sc}. Note the increased levels of β-sheet in the PrP^{sc} structure.

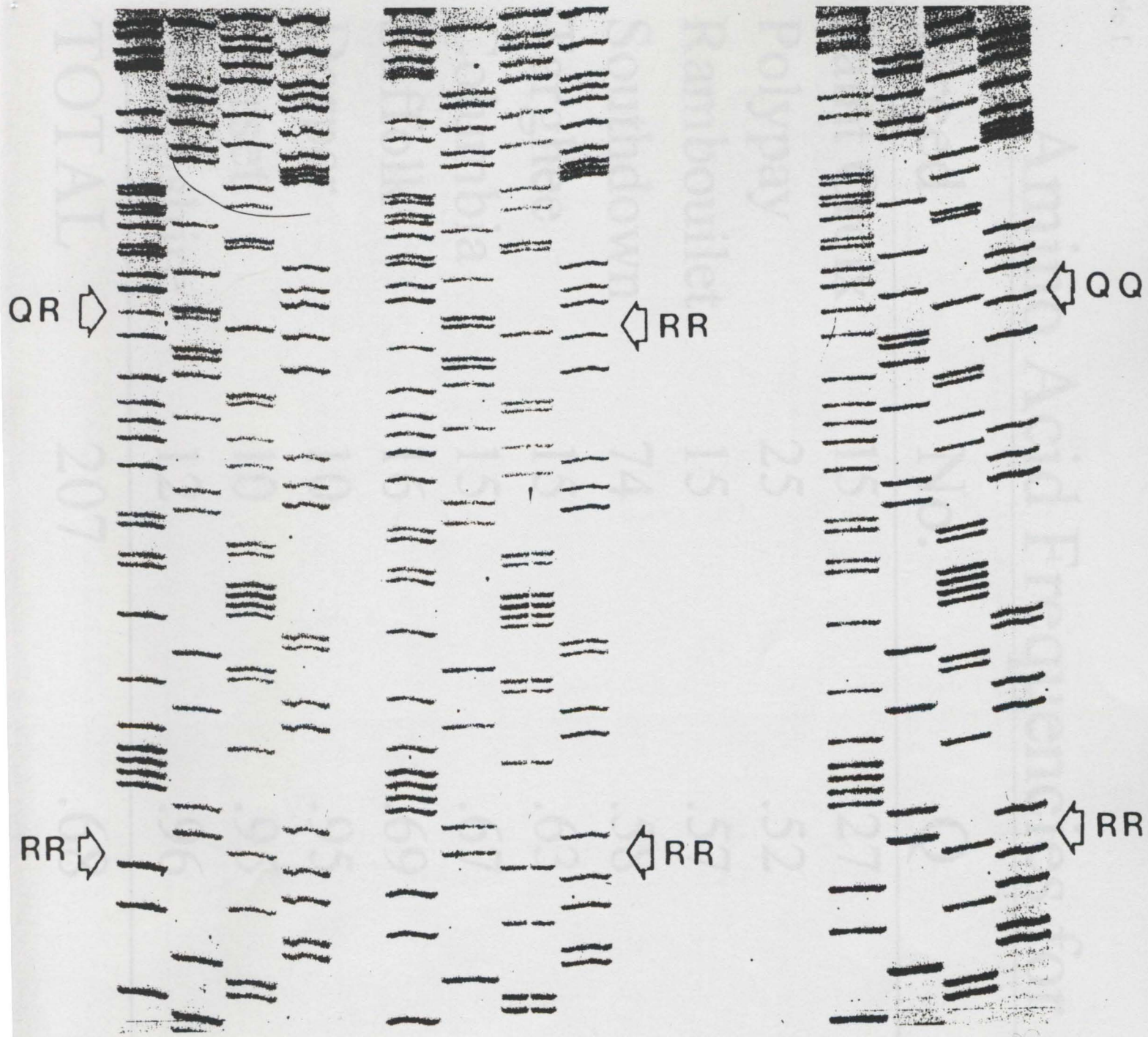

Figure 2. Autoradiograph showing nucleotide polymorphisms at codon 171. Note the heterozygous animal (Q-CAG and R-CGG). The sequence for codon 154 (R-CGT) is also shown.

Table 1.

Amino Acid Frequencies for PrP171

Breed	No.	Q	R
Saint Croix	15	.27	.73
Polypay	25	.52	.48
Rambouilet	15	.57	.43
Southdown	74	.58	.42
Targhee	15	.63	.37
Columbia	15	.67	.33
Suffolk	16	.69	.31
Dorper	10	.95	.05
Dorset	10	.95	.05
Hampshire	12	.96	.04
TOTAL	207	.68	.32

Table 2.

Genotype Frequencies at PrP136

Breed	No.	A/V	A/A
Columbia	15	.13	.87
Dorper	10	.40	.60
Dorset	10	.40	.60
Hampshire	12	.33	.67
Rambouilet	15	.20	.80
Southdown	74	.04	.96
Saint Croix	30	.20	.80

All other breeds were homozygous A/A at PrP136

Table 3.

Amino Acid Frequencies at other PrP Codons

Breed	PrP112 (M/T)	PrP143 (H/R)	PrP154 (R/H)
Columbia	all M	.80/.20	.83/.17
Dorper	all M	all H	all R
Dorset	.90/.10	.60/.40	all R
Hampshire	.75/.25	all H	all R
Polypay	.98/.02	.94/.06	all R
Rambouilet	all M	.97/.03	.97/.03
Southdown	.94/.06	.96/.04	all R
Saint Croix	all M	all H	all R
Suffolk	all M	all H	all R
Targhee	all M	all H	.93/.07