Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes

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Summary. Natural scrapie in a closed flock of South Country Cheviot sheep has resulted in 45 deaths between 1986 and 1995. Of these cases, 35 sheep have been analysed for disease-linked PrP gene polymorphisms and all encode valine at codon 136 on at least one allele with 77% homozygous (VV₁₃₆) and 23% valine/alanine heterozygotes (VA₁₃₆). Mean survival time was 907 and 1482 days for VV₁₃₆ and VA₁₃₆ scrapie affected animals respectively. VV₁₃₆ animals were all at great risk of disease if allowed to live long enough. However scrapie occurred only in a specific subgroup of VA₁₃₆ sheep, survival advantage depending on VA₁₃₆ animals being heterozygous for other polymorphisms at codons 154 or 171. The flock history has been recorded in great detail since its foundation in 1960 however there was no strong evidence for simple maternal or paternal transmission of disease other than inheritance of PrP genotype.

Introduction

A South Country Cheviot flock, founded in 1960, has been selected into two lines differing in their response to experimental challenge with scrapie [9]. Response to SSBP/1 (scrapie sheep brain pool/1) is under the control of a single gene, Sip, with two alleles, sA and pA [8], with sA being dominant [12]. Different sources of scrapie infected inocula have different transmission characteristics: SSBP/1 (termed an A group isolate) has shorter incubation periods in Sip²⁻ carriers (positive line) than in Sip²⁺ (negative line) sheep, but CH1641 (a C group scrapie isolate) and BSE have shorter incubation periods in some negative line sheep than in positive line animals [10, 14, 15]. The flock has been completely closed (genetically isolated) since 1962 and is known as the Neuropathogenesis Unit (NPU)Cheviot flock to differentiate the sheep from others of the Cheviot breed.

Polymorphisms of the sheep PrP gene are so closely associated with incubation time differences (and the alleles of Sip) in NPU Cheviots [18, 21] that the
PrP gene and Sip are believed to be synonymous. In NPU Cheviot sheep, valine at codon 136 (V\textsubscript{136}) is linked to Sip\textsuperscript{SA} whereas alanine at codon 136 (A\textsubscript{136}) is linked to Sip\textsuperscript{PA}. Positive line animals with short incubation periods (167 days +/- 5) following subcutaneous (sc) inoculation with SSBP/1 are VV\textsubscript{136} (Sip\textsuperscript{SAsA}), whereas those with longer incubation periods (322 days +/- 16) are VA\textsubscript{136} (Sip\textsuperscript{PApA}). Negative line animals are all AA\textsubscript{136} (Sip\textsuperscript{PApA}) and these resist sc challenge with SSBP/1. Inevitably, when crosses involve heterozygotes (Sip\textsuperscript{SApA}) in the positive line, a small number of Sip\textsuperscript{PApA} (AA\textsubscript{136}) animals are produced and these also resist sc SSBP/1 inoculation. Challenge with CH1641 or BSE causes disease in only a proportion of both positive and negative lines and does not associate primarily with codon 136 variation. Instead, animals encoding glutamine at codon 171 (Q\textsubscript{171}) on both PrP alleles (QQ\textsubscript{171}) succumb to intracerebral (ic) inoculation whereas those having one allele with arginine (R\textsubscript{171}) have much longer incubation period (RQ\textsubscript{171}) and those with two (RR\textsubscript{171}) are resistant [19, 20].

Although SSBP/1 targets animals carrying V\textsubscript{136}, a minor influence of codon 171 is seen since VA\textsubscript{136} animals which are also RQ\textsubscript{171} have a longer incubation period (364 days +/- 17) than those which are QQ\textsubscript{171} (260 days +/- 15). Similarly with CH1641 and BSE, although the major effect on incubation period depends on the codon 171 genotype, animals encoding V\textsubscript{136} (VV\textsubscript{136} or VA\textsubscript{136}) have longer incubation periods than AA\textsubscript{136} sheep [20]. Incidence of natural scrapie in other breeds has also been associated with variation at codons 136 and 171 [2, 4, 23, 26, 32].

PrP genotypes are now used to predict accurately the response of NPU Cheviot sheep to experimental challenge with SSBP/1, CH1641 and BSE [19] and the flock is thus a valuable resource for transmission and pathogenesis studies [13, 15]. However a low incidence of natural scrapie has been apparent for some time in the positive line (Sip\textsuperscript{SA} carriers) [14]. Accumulation of genotype information has now revealed that natural scrapie has occurred only in animals of specific PrP genotypes. This paper describes the natural scrapie outbreak within the NPU Cheviot flock and discusses hypothetical links with maternal or paternal transmission, PrP genotype and ovine lymphocyte antigens.

### Materials and methods

**Sheep and flock breeding strategy**

All sheep in this study were from the NPU Cheviot flock. Detailed records held for every animal (> 5,000) since the foundation of the flock provided the pedigree data. The flock was formed in 1960 with 15 rams (born in 1960 and bought at auction) and 300 ewes (born 1957–1959 on three different farms believed to be scrapie-free). From 1962, when nine ewes (born 1957–1959) were introduced, the flock has been completely closed. All foundation animals were injected (usually sc) with SSBP/1 scrapie after mating (males) and after lamb weaning (females). The policy of challenging every animal was continued for many years to consolidate the selection lines and to study the genetics of control of susceptibility differences. Animals were mated up to three times prior to challenge but never following challenge, thus avoiding any possibility of maternal transmission of experimentally induced disease to