The shortest known prion protein gene allele occurs in goats, has only three octapeptide repeats and is non-pathogenic

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The prion protein (PrP) gene modulates the incidence and incubation periods of transmissible spongiform encephalopathies of sheep, goats, mice and man. Here, a new caprine PrP allele encoding the shortest naturally occurring PrP protein so far described is reported. This variant contains only three instead of the usual five copies of a short peptide repeat [Pro-Gln/His-Gly-Gly-(Gly)-Trp-Gly-Gln] characteristic of PrP, with an additional Trp to Gly substitution in codon 102. Fifteen out of 111 genotyped goats carried the novel PrP allele and 14 survived without signs of disease for at least 4 years. One goat heterozygous for the polymorphism was challenged experimentally with SSBP/1-scrapie and succumbed after an unusually long incubation period.

The 33–35 kDa glycoprotein prion protein (PrP) is essential for the development of transmissible spongiform encephalopathies (TSEs or prion diseases) such as scrapie, bovine spongiform encephalopathy (BSE) or Creutzfeldt–Jakob disease (CJD). Many single amino acid substitutions have been described for this protein and several have been associated with the incidence of natural disease and modulation of incubation period of iatrogenic, or experimental, disease (e.g. Brown, 1994; Hunter, 1997). A different kind of protein variation is found in a series of glycine-rich octa- or nonapeptide sequences in the N-terminal region of the protein. A PrP allele found in cattle, sheep and goat encodes five of these sequences in the following arrangement: nonapeptide P1 (1×), octapeptide P2 (3×) and nonapeptide P3 (1×) (Fig. 1a) (Goldmann et al., 1990, 1991, 1996). Human PrP has a similar structure except that a deletion of glycine converts P3 into a fourth P2 octapeptide (1×P1, 4×P2) (Kretzschmar et al., 1986). In cattle the most common PrP allele has six repeats (Hunter et al., 1994). A reduction to four repeats has been described for rare human PrP alleles and for some other primates (Diedrich et al., 1992; Schätzl et al., 1995). Several rare PrP alleles with a total repeat number larger than five have been described in man (Goldfarb et al., 1991; Brown, 1994). In all of these allelic variants only full octapeptide repeats are inserted or lost. Identical octapeptides can sometimes be distinguished at the DNA level; for example, P2 can be encoded by DNA repeat sequences R2 or R3 (Fig. 1a).

Human PrP variants with more than nine repeated octapeptides are associated with the incidence of CJD (Goldfarb et al., 1991). However, there is little evidence in

(a) Normal allele (codons 54–102)

... Pro Gln Gly Gly Gly Gly Trp Gly Gln P1
CCT CAG GGA GGG GTG GTG CAG R1

... Pro His Gly --- Gly Trp Gly Gln P2
CCC CAT GGA GTG GTG GTG GGC CAA R2

... Pro His Gly --- Gly Trp Gly Gln P2
CCT CAG GGA GTG GTG GTG GGC CAA R3

... Pro His Gly --- Gly Gly Trp Gly Gln P2
CCC CAT GTG GTG GTG GGA CAG R4

... Pro His Gly Gly Gly Gly Trp Gly Gln P3
CCA CAT GTG GGA GTG GTG CAG R5

Gly Gly Ser His Ser Gln Trp...
GTG GTG AGC AAC ATG CAG TGG

(b) Mutated allele (codons 63–78 deleted)

... Pro Gln Gly Gly Gly Gly Trp Gly Gln P1
CCT CAG GGA GGG GTG GTG CAG R1

... Pro His Gly --- Gly Gly Trp Gly Gln P2
CCC CAT GTG --- GTG GTG GGA CAG R4

... Pro His Gly Gly Gly Gly Trp Gly Gln P3
CCA CAT GTG GGA GTG GTG CAG R5

Gly Gly Ser His Ser Gln Gly
GTG GTG AGC AAC ATG CAG GGG

Fig. 1. Protein and DNA sequence of codons 54–102 in normal (five-repeat) and codons 63–78 in mutated (three-repeat) goat PrP alleles. P1–P5, octa- and nonapeptides; R1–R5, DNA repeats. Amino acids are shown in the three-letter code.
humans for an association of a repeat number between six and nine with disease, and the association of four-repeat PrP variants with CJD remains unresolved (Palmer et al., 1993; Cervenakova et al., 1994). Laplanche et al. (1995) reported an association between an unclassified case of dementia and a six-repeat PrP allele; whereas Kenney et al. (1995) found a rare seven-repeat polymorphism with no disease association. In cattle there is no association of the six-repeat PrP variant with BSE incidence (Hunter et al., 1994).

The role of this PrP octapeptide region is not understood, although binding of metal ions to synthetic peptides of these sequences has been reported (Hornshaw et al., 1995). Equilibrium dialysis studies using recombinant hamster PrP revealed selective binding of approximately two Cu^{2+} ions per protein molecule (Stöckel et al., 1998). Each metal ion may be chelated by one histidine and a glycinyl carbonyl group in a structure formed by two octapeptide repeats (Stöckel et al., 1998) and PrP may exist and function as a Cu-metalloprotein in vivo (Brown et al., 1997). All five-repeat PrP alleles have four histidine residues in the region of codons 54–95 (Fig. 1a) and an increase or decrease by two repeat sequences would double or halve, respectively, the Cu^{2+}:PrP stoichiometry. During the course of a study on disease linkage to the PrP gene in goats (Goldmann et al., 1996), we discovered in healthy goats a PrP allele with a novel 48 bp deletion (equivalent to two octapeptide repeats) in the open reading frame.

Goat PrP coding DNA was purified from peripheral leukocytes and the PrP coding region was amplified by PCR as described in Goldmann et al. (1991, 1996). Direct sequencing with Sequenase (Amersham) of amplified DNA fragments confirmed the presence of a three-repeat PrP allele (Fig. 1b) encoding the shortest naturally occurring PrP protein so far described. The 48 bp size difference between the PCR fragments of normal and mutant PrP was used to separate allele-specific DNA fragments and to sequence the entire open reading frame. This revealed an additional T to G transversion (nucleotide 323 of sequence GenEMBL X91999) leading to a codon 102 amino acid substitution from tryptophan (Goldmann et al., 1996) to glycine in the three-repeat allele (Fig. 1b). Tryptophan is encoded in the equivalent codon position (e.g. 99 for human PrP) in all other species. Tryptophan fluorescence spectroscopy following Cu^{2+} binding to PrP has been interpreted to show interaction of the indole ring of an octapeptide tryptophan with the Cu^{2+} centre (Stöckel et al., 1998). However, it is conceivable that the Trp_{102} (and His_{99}) can also influence Cu^{2+} binding and the mutation of the Trp_{102} residue to Gly_{102} modulates a Cu^{2+}-binding function of the prion protein. Caprine PrP alleles with single amino acid dimorphisms in codons 142 (Ile or Met), 143 (His or Arg) and 240 (Ser or Pro) have been described before (Goldmann et al., 1996), the three-repeat allele encoded Ile_{142}, His_{143} and Ser_{240} and no further variation was found. The three-repeat/Gly_{102} allele was found in three Siberian goats, 11 of their offspring and a single British dairy goat, whereas 96 other goats of various breeds genotyped in parallel had a (5:5) homozygous PrP genotype. Fourteen out of 15 (5:3) animals remained healthy to the age of 4.5 to 5.5 years, when they were culled for herd management reasons. The usual age of onset for natural scrapie is 2–4 years in sheep (Hunter et al., 1996) and goats (Wood et al., 1992) and our data therefore suggest that the three-repeat/Gly_{102} allele is non-pathogenic in a heterozygous genotype. As all 15 goats encoding the three-repeat/Gly_{102} allele were heterozygous (5:3), a breeding programme for (3:3) homozygous goats has been initiated.

In a retrospective analysis of experimental scrapie, the genotypes of five goats inoculated at the same time intracerebrally with scrapie isolate SSBP/1 (Goldmann et al., 1996) were compared. Four of these goats, all with homozygous (5:5) genotype, succumbed to scrapie after an incubation period of 620 ± 23 SEM days as expected from the mean incubation period of 603 ± 38 SEM days established for SSBP/1 scrapie in goats (Goldmann et al., 1996). The fifth animal, with a (5:3) genotype, succumbed to scrapie 968 days post-inoculation. Experimental challenge experiments are planned to confirm this observation. In human TSEs, distinct PrP pathologies of the molecular layer of the cerebellum have been described in patients with different numbers of octapeptide repeats (Vital et al., 1998). The levels of cerebellar vacuolization in all five goats was sparse or nil with no difference between the genotypes. In contrast, other brain areas, e.g. the thalamus, showed high levels of vacuolization in all five goats also without genotype association (J. Foster, W. Goldmann & N. Hunter, unpublished observations).

Our data suggest that a deletion of two repeats and/or a non-conservative amino acid substitution in codon 102 of the PrP gene does not lead to increased scrapie incidence and might even be beneficial to a scrapie-infected goat. Linkage of alleles with reduced peptide repeat number to disease in humans may therefore be less likely and could support the argument that the human four-repeat PrP polymorphism is not associated with CJD. Fischer et al. (1996) recently described transgenic mouse lines overexpressing an artificial three-repeat PrP protein variant in a PrP null background. These transgenic mice, when challenged with scrapie, developed scrapie with incubation periods dependent on PrP levels and infectivity titres comparable to normal mice, but lower PrP^{Sc} levels in the final stages of disease than the levels found in normal mice.

Prolonged incubation periods of experimental TSEs have been described recently in association with a goat PrP allele carrying a methionine substitution at codon 142 (Goldmann et al., 1996). Significantly extended incubation periods of experimental scrapie were also found in hemizygous (PrP^{+/−})
five-repeat allele, the bottom band (3-rep) represents the mutated, three-homozygous goat (lane 5). The top band (5-rep) represents the normal, amplified from a (5:3) heterozygous goat (lanes 1–4) and a (5:5) homozygous goat (lane 5). The top band (5-rep) represents the normal, amplified from a (5:3) heterozygous goat (lanes 1–4) and a (5:5) homozygous goat (lane 5). The top band (5-rep) represents the normal, amplified from a (5:3) heterozygous goat (lanes 1–4) and a (5:5) homozygous goat (lane 5). The top band (5-rep) represents the normal, amplified from a (5:3) heterozygous goat (lanes 1–4) and a (5:5) homozygous goat (lane 5). The top band (5-rep) represents the normal, amplified from a (5:3) heterozygous goat (lanes 1–4) and a (5:5) homozygous goat (lane 5).

Fig. 2. (a) Ethidium bromide-stained PrP cDNA-specific fragments amplified from a (5:3) heterozygous goat (lanes 1–4) and a (5:5) homozygous goat (lane 5). The top band (5-rep) represents the normal, five-repeat allele, the bottom band (3-rep) represents the mutated, three-repeat allele. (b) Protein PrP<sup>Sc</sup> purified from brain and visualized by immunodetection (anti-PrP antibody 1B4) for (5:5) homozygote (lanes 6, 7) and (5:3) heterozygote (lanes 8, 9) without PNGase F (lanes 6, 8) or with PNGase F (lanes 7, 9). Band marked with asterisk is PNGase F. (c) Disease-specific protein PrP<sup>Sc</sup> purified from goat brain and visualized by immunodetection (anti-PrP antibody 1B4) for (5:5) homozygote (lanes 6, 8) or with proteinase K (lanes 11, 13) without proteinase K (lanes 10, 12).

mice when compared with PrP<sup>+/+</sup> wild-type mice, suggesting a relationship between the PrP<sup>C</sup> expression level and incubation period (Manson et al., 1994). We therefore investigated whether the 968-day incubation period observed in this one goat might be due to nil or low expression of the three-repeat/Gly<sub>102</sub> allele. Total RNA from frozen tissues was extracted with guanidinium thiocyanate followed by centrifugation in cesium chloride solution (Sambrook et al., 1989). Total RNA was used for gene-specific cDNA synthesis with oligonucleotide 316 (GCTCCACACTCAGCTCCATTACTTG) and subsequent standard PCR amplification of PrP cDNA specific fragments with oligonucleotides A023 (CTGACA-GCCGCAAGCGTGGAG) and 961 (GGTGAAGTTCTCC-CCTTGGTGGT). As shown in Fig. 2(a), wild-type and mutated allele from the affected heterozygous (5:3) goat (lanes 1–4) are both transcribed, but it appears that, on average, slightly less DNA fragment (70–90%) was produced from the mutated allele than from the wild-type. Whether a structural change in the three-repeat PrP mRNA has led, for example, to a change in mRNA stability remains to be established: our results suggest a reduced level of three-repeat PrP mRNA.

We also analysed the level of normal PrP (PrP<sup>C</sup>) in the affected (5:3) heterozygote and a wild-type (5:5) homozygous goat by immunoblotting of PrP<sup>Sc</sup> separated by SDS–PAGE. PrP<sup>C</sup> was deglycosylated with N-glycosidase F (PNGase F) to enhance the size separation of the two PrP variants (Fig. 2b, lanes 7 and 9) (Chen et al., 1995). The three-repeat/Gly<sub>102</sub> PrP<sup>C</sup> was expressed at a similar level (±20%) to wild-type PrP<sup>C</sup> in this affected goat; its expression was also not significantly different from a (5:5) homozygote (Fig. 2b). Preparations of PrP<sup>Sc</sup> (Hope et al., 1986; Farquhar et al., 1989) from the affected (5:3) goat brain were positive (Fig. 2c, lane 13); there was not, however, sufficient PrP<sup>Sc</sup> to distinguish between that produced from the wild-type and mutant alleles. Altogether, our data imply that the observed long incubation period in the affected heterozygous (5:3) goat is not due to the complete absence of expression of the mutated allele.

The cellular function of the PrP protein is not known, but it has been speculated that the Cu<sup>2+</sup>-binding capacity of the octapeptide region provides evidence for PrP being a Cu<sup>2+</sup>-transport protein potentially important in Cu<sup>2+</sup> homeostasis. Cells from goats with a (5:3) or (3:3) PrP genotype could therefore be used as models for the investigation into PrP as a metalloprotein. Further scrapie challenges will also clarify the association of this allele and the octapeptide region of PrP with incubation period and pathogenesis of TSEs.

References


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