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PrP genotypes and experimental scrapie in orally inoculated Suffolk sheep in the United States

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One-hundred and three United States Suffolk sheep were inoculated orally with a scrapie agent preparation and monitored for clinical disease and histopathological lesions characteristic of scrapie. A retrospective study of the polymorphisms at codon 171 of the prion protein (PrP) gene was performed on these sheep. All 63 sheep that developed scrapie during the observation period were homozygous for the glutamine 171 (171-QQ) PrP allele. Twelve 171-QQ sheep failed to develop disease. All 5 sheep homozygous for arginine (171-RR) and all 23 heterozygous (171-QR) sheep remained free of scrapie.

Introduction

Scrapie is a naturally occurring neurodegenerative disease of sheep. The disease is experimentally transmissible to cattle, goats and laboratory animals via oral, parenteral and intracerebral routes using homogenates of brain or lymphoid tissues from infected animals (Pattison & Millson, 1961; Zlotnik & Rennie, 1963; Pattison, 1965; Kimberlin *et al.*, 1975; Clark *et al.*, 1995). The mode of transmission from ewe to lamb or between adults under field conditions is not known. However, oral exposure to foetal membranes or to pastures grazed by infected animals has been implicated as a possible route of vertical and horizontal transmission (Brotherston *et al.*, 1968; Pattison *et al.*, 1972; Dickinson *et al.*, 1974; Hadlow *et al.*, 1982; Onodera *et al.*, 1993).

The causative agent of scrapie does not appear to be a conventional micro-organism. Infectivity in tissues from experimentally infected animals is associated with a relatively protease-resistant isoform (PrP-Sc) (Bolton *et al.*, 1982; Prusiner, 1982; McKinley *et al.*, 1983; Diringer *et al.*, 1983; Merz *et al.*, 1984) of the cellular prion protein (PrP-C) (Oesch *et al.*, 1985; Basler *et al.*, 1986). The 'protein only' model for prion diseases proposes that disease is transmitted solely by PrP-Sc, which acts as a template for conversion of PrP-C to PrP-Sc by a nucleation or polymerization event (Gajdusek, 1993; Come & Lansbury, 1993).

Susceptibility to ovine scrapie is controlled by a combination of host genetics (Parry, 1979; Hunter *et al.*, 1989, 1991, 1992; Laplanche *et al.*, 1993; Westaway *et al.*, 1994; Belt *et al.*, 1995; Clouscard *et al.*, 1995) and the scrapie strain used to infect the host (Dickinson & Meikle, 1971; Goldmann *et al.*, 1994*a*). The ovine scrapie incubation period (*Sip*) gene (Dickinson *et al.*, 1968) is linked to, and probably synonymous with, the PrP gene (Carlson *et al.*, 1986; Goldmann *et al.*, 1990, 1991). The ovine PrP gene contains polymorphisms encoding amino acid changes at codons 112 (methionine or threonine), 136 (alanine or valine), 141 (leucine or phenylalanine), 154 (arginine or histidine) and 171 (arginine, glutamine or histidine) (Goldmann *et al.*, 1990; Laplanche *et al.*, 1993; Belt *et al.*, 1995; Hunter *et al.*, 1996). Polymorphisms at residues 136 and 171 are associated with susceptibility to both experimental and

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natural scrapie (Hunter *et al*., 1994; Westaway *et al*. 1994; Clouscard *et al*., 1995; Ikeda *et al*., 1995).

Two ovine scrapie strains have been defined by their action in Cheviot sheep of defined PrP genotypes (Goldmann *et al*., 1994*b*). Subcutaneous challenge with the isolate SSBP/1, the prototype for strain A, produces disease in Cheviot sheep that are homozygous or heterozygous for valine at codon 136 (Goldmann *et al*., 1994*a*; Maciulus *et al*., 1992); sheep homozygous for alanine at codon 136 survive subcutaneous challenge. Polymorphisms at codons 154 and 171 modulate the survival times in 136-AV sheep with natural scrapie (Hunter *et al*., 1996). Valine 136 is the predominant allele in naturally infected sheep of several breeds, including Swaledale, Romanov, Ile de France, Shetland, Scottish Halfbred and Bleu du Maine (Hunter *et al*., 1992; Laplanche *et al*., 1993; Hunter *et al*., 1993, 1994). Valine 136 is a rare allele in Suffolk sheep (Westaway *et al*., 1994) but has been reported at low frequency in Japan and the United States (Ikeda *et al*., 1995; O'Rourke *et al*., 1996).

Experimental challenge with isolate CH1641, the prototype strain C, results in disease in sheep homozygous for glutamine (171-QQ) (Goldmann *et al*., 1994*a*). Heterozygous (171-QR) or homozygous arginine (171-RR) sheep survive challenge by the intracerebral route. 171-QQ is the predominant genotype of naturally infected sheep of several breeds, notably Suffolk sheep in the United States and Japan (Westaway *et al*., 1994; Ikeda *et al*., 1995; O'Rourke *et al*., 1996). In this study, we examined the association of codon 171 genotype with susceptibility of Suffolk sheep to scrapie following oral exposure. We report that scrapie occurred only in sheep of the PrP genotype 171-QQ; all 24 171-QR sheep and all 5 171-RR sheep in the study remained scrapie free.

Methods

■ **Animal inoculation.** Animals and inoculation protocols were described earlier (Foote *et al*., 1993). Briefly, in 1980, Suffolk sheep were inoculated by the oral route with 30 ml of 10% (w/v) suspensions of pooled brain and spleen from Suffolk sheep infected with third- and fourth-passage Suffolk scrapie agent. Sheep were housed in two groups and observed for clinical disease. Histology was performed on all animals after death. Diagnosis was made on the basis of clinical signs and confirmed by histopathological examination of brain tissue by or under contract with the National Veterinary Services Laboratory, Ames, Iowa, USA.

■ **Genetic analysis.** DNA was extracted from blood or tissues by phenol–chloroform extraction (Maciulus *et al*., 1992). Codon 171 genotyping of Suffolk sheep samples was performed by oligonucleotide hybridization to a PrP PCR product using probes specific for alleles encoding glutamine, arginine and histidine (O'Rourke *et al*., 1996). Codon 136 determination was performed by *Bsp*HI digestion of PCR amplified products (Hunter *et al*., 1993; Maciulus *et al*., 1992).

■ **Statistical analysis.** Disease susceptibility of Suffolk sheep with the PrP 171 allele QQ was compared to susceptibility of sheep with PrP 171 alleles QR or RR by survival analysis using a lifetable procedure (SAS Institute, Inc., 1985), due to right censored data (i.e. sheep dying of non-scrapie causes during the range of observed scrapie incubation periods).

Results

PrP genotypes represented in this study

The inoculation trial was initiated before our current understanding of PrP genotypes. Thus, the distribution of genotypes in the study group represents the frequencies of those genotypes in the flocks from which the sheep were purchased in 1979. Genotype frequencies at codon 171 were 0·72 (QQ), 0·23 (QR) and 0·05 (RR), which are not significantly different ($P < 0·05$) from that of a large sample of United States Suffolk sheep reported earlier (O'Rourke *et al*., 1996). Alleles encoding 136-V and 171-H were not found in this group.

PrP genotypes and scrapie in Suffolk sheep

The oral inoculation trial was initiated with 141 Suffolk sheep (Foote *et al*., 1993). The earliest diagnosis of scrapie occurred in a sheep that survived for 349 days after inoculation. Therefore, only the 103 sheep surviving longer than 349 days are included in this study (Table 1).

Sixty-three of the 103 orally inoculated Suffolk sheep developed histopathological signs of scrapie. All 63 of the histopathologically positive sheep were of the genotype 136-AA, 171-QQ. Survival times in scrapie-affected sheep ranged from 349 days to 1346 days; mean survival time was 622 days ± 240 days. Fifty-four of the 63 sheep with histopathological signs of scrapie had clinical signs of ataxia, weight loss or wool loss for times ranging from 3 to 135 days (mean = 50 days, SD = 35) before euthanasia or death. Two sheep exhibited clinical signs for longer times (213 and 539 days). The last histopathologically positive sheep survived to 1346 days after inoculation.

Table 1. PrP genotypes and disease outcome in Suffolk sheep inoculated orally with Suffolk-passaged scrapie agent

PrP genotype		With scrapie*		Without scrapie†	
Codon 136	Codon 171	No. of sheep	Mean days survived (SD)	No. of sheep	Mean days observed (SD)
AA	QQ	63	622 (240)	12	1454 (1146)
AA	QR	0	NA	23	1803 (1100)
AA	RR	0	NA	5	2201 (697)
Totals…		**63**		**40**	

* Histopathological signs of scrapie were present.
† Histopathological signs of scrapie were not present.
NA, Not applicable.

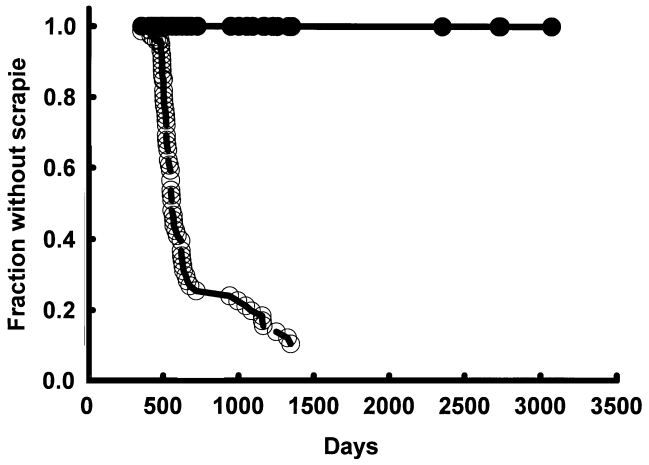

Fig. 1. Comparative survival of Suffolk sheep by genotype following oral exposure to scrapie agent. Symbols: ●, 171-QR or 171-RR; ○, 171-QQ.

Twelve sheep homozygous for glutamine (171-QQ), 23 sheep heterozygous for glutamine/arginine (171-QR) and 5 sheep homozygous for arginine (171-RR) failed to develop clinical or histological lesions of scrapie. Of these sheep, 20 171-QR and 4 171-RR sheep were observed for more than 1346 days, the longest incubation time of scrapie-positive sheep. Five of the sheep with the genotype 171-QR were observed for more than 3000 days.

Disease susceptibility of sheep with the 171-QQ genotype was compared with that of sheep with the 171-QR or 171-RR genotypes. The survival distribution function of the 171-QQ allele was significantly different from that of the 171-QR or RR group with a probability of $> 99\%$ ($P < 0{\cdot}0001$) (Fig. 1).

Discussion

The Suffolk sheep in this study were inoculated orally with a Suffolk-passaged scrapie homogenate. Our results demonstrate a very strong association of disease with the 171-QQ genotype using this scrapie isolate. This finding is consistent with a smaller study of British Cheviot sheep challenged subcutaneously or intracerebrally with the prototype strain C scrapie agent (Goldmann *et al.*, 1994*a*). The incubation times of scrapie-affected sheep in our study varied widely and 12 of 75 171-QQ sheep remained scrapie free. There are several possible explanations for the varying response to inoculation in these sheep. Additional polymorphisms within the PrP open reading frame or in the flanking regions may modulate incubation time or reduce susceptibility (Hunter *et al.*, 1996). Alternatively, uptake of the agent following oral inoculation of weaned lambs may vary among individuals, depending on rumen contents and maturity.

This study and earlier observations (Westaway *et al.*, 1994; Belt *et al.*, 1995; Clouscard *et al.*, 1995; Ikeda *et al.*, 1995; O'Rourke *et al.*, 1996) support the use of PrP genotyping in selection of Suffolk sheep with genotypes associated with lower susceptibility to clinical scrapie (171-RR and 171-QR) (Hosie & Dawson, 1996). However, no data have yet been reported regarding the possible accumulation of PrP-Sc or infectivity in extraneural tissues of inoculated or naturally exposed sheep with the 171-QR or 171-RR genotypes. Thus, although it appears likely that 136-AA, 171-QR (or 171-RR) genotypes are associated with resistance to clinical scrapie, it is premature to conclude that these Suffolk sheep represent no risk to offspring or susceptible flockmates until information on the presence or absence of a carrier state is available.

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References

Basler, K., Oesch, B., Scott, M., Westaway, D., Walchii, M., Groth, D. F., McKinley, M. P., Prusiner, S. B. & Weissmann, C. (1986). Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell* **46**, 417–428.

Belt, P. B. G. M., Muileman, I. H., Schreuder, B. E. C., Ruijter, J. B., Gielkens, A. L. J. & Smits, M. A. (1995). Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. *Journal of General Virology* **76**, 509–517.

Bolton, D. C., McKinley, M. P. & Prusiner, S. B. (1982). Identification of a protein that purifies with the scrapie prion. *Science* **218**, 1309–1311.

Brotherston, J. G., Renwick, C. C., Stamp, J. T., Zlotnik, I. & Pattison, I. H. (1968). Spread of scrapie by contact to goats and sheep. *Journal of Comparative Pathology* **78**, 9–17.

Carlson, G. A., Kingsbury, D. T., Goodman, P. A., Coleman, S., Marshall, S. T., DeArmond, S. J., Westaway, D. & Prusiner, S. B. (1986). Linkage of prion protein and scrapie incubation time genes. *Cell* **46**, 503–511.

Clark, W. W., Hourrigan, J. L. & Hadlow, W. J. (1995). Encephalopathy in cattle experimentally infected with the scrapie virus. *American Journal of Veterinary Research* **56**, 606–612.

Clouscard, C., Beaudry, P., Elsen, J. M., Milan, D., Dussaucy, M., Bounneau, C., Schelcher, F., Chatelain, J., Launay, J. M. & Laplanche, J. L. (1995). Different allelic effects of the codons 136 and 171 of the prion protein gene in sheep with natural scrapie. *Journal of General Virology* **76**, 2097–2101.

Come, J. H. & Lansbury, P. T., Jr (1994). Predisposition of prion protein homozygotes to Creutzfeldt–Jakob disease can be explained by a nucleation-dependent polymerization mechanism. *Journal of the American Chemical Society* **116**, 4109–4110.

Dickinson, A. G. & Meikle, V. M. (1971). Host-genotype and agent effects in scrapie incubation: change in allelic interaction with different strains of agent. *Molecular and General Genetics* **112**, 73–79.

Dickinson, A. G., Stamp, J. T., Renwick, C. C. & Rennie, J. C. (1968). Some factors controlling the incidence of scrapie in Cheviot sheep injected with a Cheviot-passaged scrapie agent. *Journal of Comparative Pathology* **78**, 313–321.

Dickinson, A. G., Stamp, J. T. & Renwick, C. C. (1974). Maternal and lateral transmission of scrapie in sheep. *Journal of Comparative Pathology* **84**, 19–25.

Diringer, H., Gelderblom, H., Hilmert, H., Ozel, M., Edelbluth, C. & Kimberlin, R. H. (1983). Scrapie infectivity, fibrils and low molecular weight protein. *Nature* **306**, 476–478.

Foote, W. C., Clark, W., Maciulis, A., Call, J. W., Hourrigan, J., Evans, R. C., Marshall, M. R. & Decamp, M. (1993). Prevention of scrapie transmission in sheep, using embryo transfer. *American Journal of Veterinary Research* **54**, 1863–1868.

Gajdusek, D. C. (1993). Genetic control of nucleation and polymerization of host precursors to infectious amyloids in the transmissible amyloidoses of brain. *British Medical Bulletin* **49**, 913–931.

Goldmann, W., Hunter, N., Foster, J. D., Salbaum, J. M., Beyreuther, K. & Hope, J. (1990). Two alleles of a neural protein gene linked to scrapie in sheep. *Proceedings of the National Academy of Sciences, USA* **87**, 2476–2480.

Goldmann, W., Hunter, N., Benson, G., Foster, J. D. & Hope, J. (1991). Different scrapie-associated fibril proteins (PrP) are encoded by lines of sheep selected for different alleles of the Sip gene. *Journal of General Virology* **72**, 2411–2417.

Goldmann, W., Hunter, N., Smith, G., Foster, J. & Hope, J. (1994*a*). PrP genotype and agent effects in scrapie; change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *Journal of General Virology* **75**, 989–995.

Goldmann, W., Hunter, N., Smith, G., Foster, J. & Hope, J. (1994*b*). PrP genotypes and the *Sip* gene in Cheviot sheep form the basis for scrapie strain typing in sheep. *Annals of the New York Academy of Sciences* **724**, 296–299.

Hadlow, W. J., Kennedy, R. C. & Race, R. E. (1982). Natural infection of Suffolk sheep with scrapie virus. *Journal of Infectious Diseases* **146**, 657–664.

Hosie, B. D. & Dawson, M. (1996). Scrapie genotyping for Suffolk sheep. *Veterinary Record* **138**, 215–216.

Hunter, N., Foster, J. D., Dickinson, A. G. & Hope, J. (1989). Linkage of the gene for the scrapie-associated fibril protein (PrP) to the Sip gene in Cheviot sheep. *Veterinary Record* **124**, 364–366.

Hunter, N., Foster, J. D., Benson, G. & Hope, J. (1991). Restriction fragment length polymorphisms of the scrapie-associated fibril protein (PrP) gene and their association with susceptibility to natural scrapie in British sheep. *Journal of General Virology* **72**, 1287–1292.

Hunter, N., Foster, J. D. & Hope, J. (1992). Natural scrapie in British sheep: breeds, ages and PrP gene polymorphisms. *Veterinary Record* **130**, 389–392.

Hunter, N., Goldmann, W., Benson, G., Foster, J. D. & Hope, J. (1993). Swaledale sheep affected by natural scrapie differ significantly in PrP genotype frequencies from healthy sheep and those selected for reduced incidence of scrapie. *Journal of General Virology* **74**, 1025–1031.

Hunter, N., Goldmann, W., Smith, G. & Hope, J. (1994). The association of a codon 136 PrP gene variant with the occurrence of natural scrapie. *Archives of Virology* **137**, 171–177.

Hunter, N., Foster, J. D., Goldmann, W., Stear, M. J., Hope, J. & Bostock, C. (1996). Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. *Archives of Virology* **141**, 809–824.

Ikeda, T., Horiuchi, M., Ishiguro, N., Muramatsu, Y., Kai-Uwe, G. D. & Shinagawa, M. (1995). Amino acid polymorphisms of PrP with reference to onset of scrapie in Suffolk and Corriedale sheep in Japan. *Journal of General Virology* **76**, 2577–2581.

Kimberlin, R. H., Walker, C. A. & Millson, G. C. (1975). Interspecies transmission of scrapie-like diseases. (Letter.) *Lancet* ii, 1309–1310.

Laplanche, J. L., Chatelain, J., Westaway, D., Thomas, S., Dussaucy, M., Brugere-Picoux, J. & Launay, J. M. (1993). PrP polymorphisms associated with natural scrapie discovered by denaturing gradient gel electrophoresis. *Genomics* **15**, 30–37.

McKinley, M. P., Bolton, D. C. & Prusiner, S. B. (1983). A protease-resistant protein is a structural component of the scrapie prion. *Cell* **35**, 57–62.

Maciulis, A., Hunter, N., Wang, S., Goldmann, W., Hope, J. & Foote, W. C. (1992). Polymorphisms of a scrapie-associated fibril protein (PrP) gene and their association with susceptibility to experimentally induced scrapie in Cheviot sheep in the United States. *American Journal of Veterinary Research* **53**, 1957–1960.

Merz, P. A., Rohwer, R. G., Kascsak, R. J., Wisniewski, H. M., Somerville, R. A., Gibbs, C. J. & Gajdusek, D. C. (1984). Infection specific particle from the unconventional slow virus diseases. *Science* **224**, 437–440.

Oesch, B., Westaway, D., Walchi, M., McKinley, M. P., Kent, S. B. H., Aebersold, R., Barry, R. A., Tempst, P., Teplow, D. B., Hood, L. E. and others (1985). A cellular gene encodes scrapie PrP27-30 protein. *Cell* **40**, 735–746.

Onodera, T., Ikeda, T., Muramatsu, Y. & Shinagawa, M. (1993). Isolation of scrapie agent from the placenta of sheep with natural scrapie in Japan. *Microbiology and Immunology* **37**, 311–316.

O'Rourke, K. I., Melco, R. P. & Mickelson, J. R. (1996). Allelic frequencies of an ovine scrapie susceptibility gene. *Animal Biotechnology* **7**, 155–162.

Parry, H. B. (1979). Elimination of natural scrapie in sheep by sire genotype selection. *Nature* **277**, 127–129.

Pattison, I. H. (1965). Scrapie in the Welsh mountain breed of sheep and its experimental transmission to goats. *Veterinary Record* **77**, 1388–1390.

Pattison, I. H. & Millson, G. C. (1961). Experimental transmission of scrapie to goats and sheep by the oral route. *Journal of Comparative Pathology* **71**, 171–176.

Pattison, I. H., Hoare, M. N., Jebbett, J. N. & Watson, W. A. (1972). Spread of scrapie to sheep and goats by oral dosing with foetal membranes from scrapie-affected sheep. *Veterinary Record* **90**, 465–468.

SAS Institute, Inc. (1985). *SAS User's Guide: Statistics*. Version 5, 527.

Prusiner, S. B. (1982). Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136–144.

Westaway, D., Zuliani, V., Cooper, C. M., Da Costa, M., Neuman, S., Jenny, A. L., Detwiler, L. & Prusiner, S. B. (1994). Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie. *Genes & Development* **8**, 959–969.

Zlotnik, I. & Rennie, J. C. (1963). Further observations on the experimental transmission of scrapie from sheep and goats to laboratory mice. *Journal of Comparative Pathology* **73**, 150–162.

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