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Identification of a novel ovine PrP polymorphism and scrapie-resistant genotypes for St. Croix White and a related composite breed

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Abstract. Susceptibility to scrapie is primarily controlled by polymorphisms in the ovine prion protein gene (PRNP). Here, we report a novel ovine exon three PRNP polymorphism (SNP G346C; P_{116}), its association with the ovine ARQ allele ($P_{116}A_{136}R_{154}Q_{171}$), and two new genotypes (PARQ/ARR; PARQ/ARQ) for the St. Croix White (SCW) breed and a related composite (CMP) breed developed for meat production. The (P_{116}) polymorphism occurs between the N-terminal cleavage site and the hydrophobic region of the ovine prion protein, a region which exhibits extreme conservation across mammalian taxa. The relatively high frequency (0.75) of resistant ARR alleles and the absence of ARQ alleles for the SCW ewes used as breeding stock for CMP resulted in significant genic differentiation ($P = 0.0123$; S.E. = 0.00113). Additionally, the majority of the SCW (66.7%) and CMP (65.4%) sampled possessed genotypes considered resistant or nearly resistant to scrapie and experimental BSE (bovine spongiform encephalopathy.

Scrapie is an inevitably fatal transmissible spongiform encephalopathy (TSE) affecting sheep and goats. Polymorphisms within exon three of the ovine host-encoded prion protein gene (PRNP) at codons 136 (Alanine or Valine; A,V), 154 (Histidine or Arginine; H,R), and 171 (Glutamine, Arginine, or Histidine; Q, R, or H) are associated with variation in the phenotypic expression of scrapie including incubation period, clinical signs, and pathology (Bossers et al., 1996, 2000; reviewed by Hunter, 1997). Of the twelve possible alleles derivable from these polymorphisms, only five are commonly seen: $A_{136}R_{154}R_{171}$ (hereafter ARR), ARQ, VRQ, AHQ, and ARH (Belt et al., 1995). It should also be noted that seven additional ovine PRNP polymorphisms, exhibiting little or no association with the phenotypic expression of scrapie, have been described at codons 112, 127, 137, 138, 141, 151, and 211 (as referenced in Bossers et al., 2000). High susceptibility to scrapie is associated with the ovine VRQ allele, while the ARR allele is associated with resistance (Westaway et al., 1994; Belt et al., 1995; Hunter et al., 1996; Sabuncu et al., 2003). The AHQ allele may be associated with resistance in some ovine breeds, but not others, while the ARH allele is likely to be neutral (Dawson et al., 1998; Baylis et al., 2002a).

In the U.S. sheep population scrapie has only been confirmed in sheep homozygous for the PRNP allele encoding glutamine at codon 171 (Q/Q), regardless of breed (Westaway et al., 1994; O'Rourke et al., 1996, 1997, 2002). Moreover, the ovine PRNP genotype ARR/ARR is known to confer global resistance to scrapie and experimental BSE (for review of genotypes see Belt et al., 1995; Hunter, 1997; Baylis et al., 2002a, 2002b). The ARR/AHQ and ARR/ARQ genotypes are associated with nearly complete resistance to scrapie worldwide as well as incubation periods of >5 years following intracerebral

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challenge (IC) with BSE (Foster et al., 2001; Jeffrey et al., 2001; Baylis et al. 2002a, 2002b). The ARQ/ARQ genotype is generally associated with increased risk of scrapie worldwide although some breeds (e.g. Cheviot Sheep, UK) are relatively resistant (Baylis et al., 2002b). Sheep possessing the ARQ/VRQ genotype are at high risk of scrapie and experimental BSE (Baylis et al., 2002b). The ARR/VRQ genotype, somewhat variable by breed, is generally associated with rare to slightly elevated risk of scrapie as well as incubation periods of >5 years following IC with BSE (Belt et. al., 1995; Foster et al., 2001; Jeffrey et al., 2001; Baylis et al., 2002a, 2002b).

The St. Croix White (SCW) and White Dorper (WD) breeds are members of a larger group of sheep commonly referred to as hair sheep or hair breeds. Collectively, hair breeds make up a relatively small portion of the overall world sheep population and as a result have often escaped studies pertaining to scrapie or the PRNP locus in general, yet they are the predominant breed type found throughout the Caribbean and other tropical regions (Shelton, 1991; Godfrey and Collins, 1999). Additionally, hair breeds are commonly utilized in tropical regions worldwide for meat production and are valued for their resistance to Trichostrongyle (Mazzola, 1990; Godfrey and Collins 1999). In this study we investigated exon three PRNP genotypes and allelic variants for SCW as well as a related composite breed (CMP) developed for commercial meat production.

Materials and methods

Study animals

A total sampling of 33 sheep from Dorpcroix Sheep Farm (Hermleigh, TX USA) consisted of the following: six unrelated adult SCW (ewes) previously utilized as breeding stock for CMP, one full-blooded adult WD (ram) utilized as breeding stock for CMP, and 26 CMP (20 adult ewes, three ewe-lambs, and three adult rams). Composite animals (26 of n = 500 total farm) were developed for commercial meat production in 1998 and represent a synthetic breed resulting from an initial cross (SCW ewes × WD rams) followed by selection and crossing of animals exhibiting economically important traits such as overall hardiness and robust body stature. The WD and SCW sampled do not represent the sole founding stock for CMP. Study animals had no previous history or symptoms of scrapie at the time of publication.

DNA isolation and PRNP amplification

Genomic DNA was isolated from whole blood samples either by spotting whole blood on Whatman Bioscience FTA® Classic Cards, and following the preparation protocol provided by the manufacturer (Whatman Inc., Clifton, NJ), or through utilization of the SUPER QUICK-GENE DNA Isolation kit (Analytical Genetic Testing Center, Denver, CO).

The entire coding region for exon three of the ovine PRNP gene was amplified via PCR with the flanking synthetic oligonucleotides SAF1 and SAF2 (Prusiner et al., 1993). Thermal cycling parameters, as optimized in our laboratory, were as follows: 2 min at 96 °C; 4 cycles × 30 s at 96 °C, 30 s at 58 °C (–1 °C/cycle), 90 s at 65 °C; 31 cycles × 30 s at 96 °C, 30 s at 54 °C, 90 s at 65 °C; 15 min at 65 °C. Each 25-µl reaction included a 1.2-mm FTA punch or 100 ng genomic DNA, 400 µM dNTPs, 2.0 mM $MgCl_2$, 0.28 µM each primer, 1× reaction buffer, 1× MasterAmp™ PCR Enhancer (Epicentre, Madison, WI) and 1.0 unit *Taq* polymerase (Promega). PCR products were examined through agarose gel electrophoresis and purified using a Qiagen QIAquick PCR Purification Kit (Qiagen, Valencia, CA).

Sequencing

Purified PCR products were directly sequenced using a Big Dye™ Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA), the aforementioned PCR primers, and the following thermal parameters: 2 min at 96 °C; 35 cycles × 30 s at 96 °C, 20 s at 54 °C, 4 min at 60 °C; 5 min at 60 °C. Each 10-µl sequencing reaction included: 60 ng purified PCR product, 2 µl Big Dye™, 0.8 µM primer and 0.5× MasterAmp™ PCR Enhancer. Reactions were purified with G-50 sephadex columns (Biomax, Odenton, MD). Sequence fragments were separated and analyzed using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA), and are available through GenBank accession no. (AY350241–AY350275).

Validation techniques

Most samples were directly sequenced more than once. Representative alleles from each genotypic class with more than one single nucleotide polymorphism (SNP) were validated through cloning using a TOPO TA Cloning kit according to the manufacturer's recommendations (Invitrogen, Carlsbad CA). Plasmid DNA was isolated and purified using a Qiagen Plasmid Mini Kit as directed by the manufacturer (Qiagen Inc., Valencia CA). Insert sequencing for 12 clones was carried out via the sequencing method previously described with the following exceptions: 400 ng/reaction plasmid DNA, 50 °C anneal temperature, and (6.2 pmol/reaction) M13 forward and reverse primers.

Computer software and analysis

Ovine PRNP exon three genotypes and allelic variants were visualized using ABI PRISM SeqScape SNP Discovery and Validation Software version 1.01 (Applied Biosystems, Foster City, CA). Allele frequencies and tests of genic differentiation were calculated in GENEPOP (Raymond and Rousset, 1995).

Table 1. PRNP allele frequencies and observed genotype frequencies

Breed[a]		PRNP[b] allele	Total	Frequency	Genotype (obs.)	Total	Frequency %
WD		ARQ	2	1.0000	ARQ/ARQ	1	100
WD	Sum		2	1.0000		1	100
SCW		ARR	9	0.7500	ARR/ARR	3	50.0
		ARQ	0	0.0000	P_{116}ARQ/ARR	1	16.7
		VRQ	1	0.0833	ARR/AHQ	1	16.7
		AHQ	1	0.0833	ARR/VRQ	1	16.7
		ARH	0	0.0000			
		P_{116}ARQ[c]	1	0.0833			
SCW	Sum		12	1.0000		6	100
CMP		ARR	22	0.4231	ARR/ARR	4	15.4
		ARQ	23	0.4423	ARR/ARQ	12	46.2
		VRQ	2	0.0385	P_{116}ARQ/ARQ	4	15.4
		AHQ	1	0.0192	ARQ/ARQ	3	11.5
		ARH	0	0.0000	ARR/AHQ	1	3.8
		P_{116}ARQ[c]	4	0.0769	ARR/VRQ	1	3.8
					ARQ/VRQ	1	3.8
CMP	Sum		52	1.0000		26	100

[a] WD, White Dorper; SCW, St. Croix White; CMP, composite breed.
[b] Ovine PRNP exon 3.
[c] Alanine is the wild-type amino acid at ovine position 116.

Results and discussion

The frequencies of the five most common ovine PRNP exon three alleles (ARR, ARQ, VRQ, AHQ, and ARH), as verified via cloning, as well as a new allele ($P_{116}A_{136}R_{154}Q_{171}$; hereafter PARQ) for SCW and CMP are presented in Table 1. The novel (P_{116}) polymorphism associated with the PARQ allele is the result of an SNP (G→C) at ovine nucleotide position 346. The origin of the PARQ allele is likely SCW since the allele is present in SCW and CMP, but not in the WD sampled (Ta-

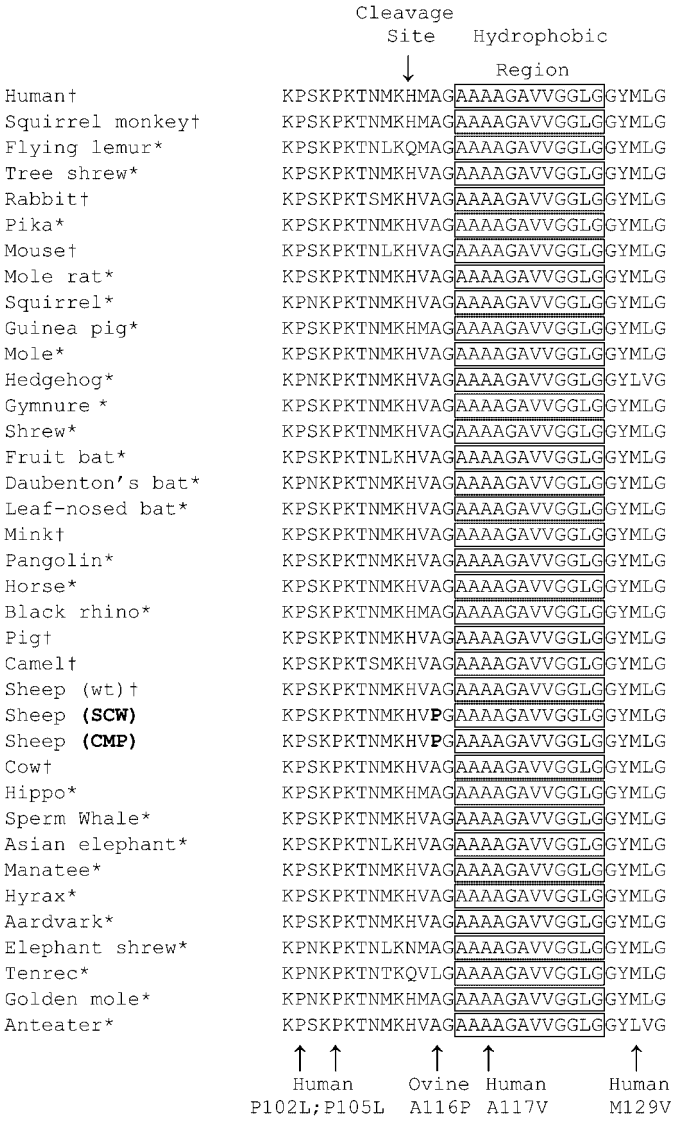

Fig. 1. Extreme conservation associated with the mammalian prion protein regions flanking the hydrophobic region, the hydrophobic region itself, AA position 116, and the N-terminal cleavage site. Proximally relevant human mutations also depicted (GSS mutations: P102L, P105L, and A117V; M129V associated with vCJD). Asterisk (*) indicates sequences generated by van Rheede et al. (2003). Sequences obtained from GenBank, as referenced and utilized in van Rheede et al. (2003), are indicated by a cross (†).

ble 1). We did not detect the ARH allele in our SCW, WD, and CMP samples (Table 1). Absence of the ARH allele has previously been reported in Scottish Blackface, Welsh Mountain, Swaledale, and Beulah breeds in the UK (Arnold et al., 2002). Of the 15 possible genotypes derivable from the five most common ovine PRNP exon three alleles we determined our samples for WD, SCW, and CMP to possess only six, collectively (Table 1). However, two new PRNP genotypes (PARQ/ARR and PARQ/ARQ) were detected for the SCW and CMP samples, thereby increasing the total number of distinct PRNP genotypes detected in this study to eight (Table 1). The distribution of PRNP exon three genotypes within the SCW, CMP, and WD sampled are depicted in Table 1. Absence of the ARQ allele combined with the relatively high frequency of the ARR allele in the SCW sampled results in significant (P = 0.0123; S.E. = 0.00113) genic differentiation between SCW and CMP.

The relationship between scrapie susceptibility or resistance, the novel PARQ allele, and/or the associated genotypes (PARQ/ARR; PARQ/ARQ), is presently unknown. However, the proline polymorphism noted at ovine amino acid position 116 occurs between the N-terminal cleavage site (between Lys_{112} and His_{113}; human numbering) and the hydrophobic region of the prion protein, a region exhibiting extreme conservation across mammalian groups (Fig. 1). The functional ability of the normal cellular prion protein (PrP^{C}) as a potential cell-surface receptor is most likely modulated by the proteolytic cleavage and removal of the N-terminal region of the protein (Harris et al., 1993; van Rheede et al., 2003). Furthermore, the amino acid residues immediately flanking the ovine (P_{116}) polymorphism are considered to play a major role in the interface between (PrP^{C}) and the pathogenic isoform (PrP^{Sc}) (Cohen and Prusiner, 1998). Currently, three pathogenic human mutations causing GSS (Gerstmann-Strausler-Scheinker syndrome; P102L; P105L; and A117V) and one human mutation strongly associated with the phenotypic expression of vCJD (variant Creutzfeld-Jakob disease, M129V) have been described within the regions of the prion protein immediately flanking the ovine (P_{116}) polymorphism (Fig. 1; for review see Collinge, 2001 and van Rheede et al., 2003).

In conclusion, we have demonstrated that resistant or nearly resistant genotypes exist for the majority of the CMP (65.4%) and SCW (66.7%) sampled, while the WD ram was determined to possess a susceptible genotype (ARQ/ARQ) (Table 1). Additionally, the identification of a novel ovine PrP polymorphism provides an opportunity for future challenge experiments to investigate the potential effect(s) of the PARQ allele as well as the PARQ/ARR and PARQ/ARQ genotypes.

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