2008

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JOURNAL OF VIROLOGY, Oct. 2008, p. 10318–10320 Vol. 82, No. 20
0022-538X/08/$08.00+0 doi:10.1128/JVI.00710-08

Scrapie Resistance in ARQ Sheep[▽]

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Received 31 March 2008/Accepted 8 July 2008

Variation in the ovine prion protein amino acid sequence influences scrapie progression, with sheep homozygous for $A^{136}R^{154}Q^{171}$ considered susceptible. This study examined the association of survival time of scrapie-exposed ARQ sheep with variation elsewhere in the ovine prion gene. Four single nucleotide polymorphism alleles were associated with prolonged survival. One nonsynonymous allele (T112) was associated with an additional 687 days of survival for scrapie-exposed sheep compared to M112 sheep (odds ratio, 42.5; $P = 0.00014$). The only two sheep homozygous for T112 (TARQ) did not develop scrapie, suggesting that the allelic effect may be additive. These results provide evidence that TARQ sheep are genetically resistant to development of classical scrapie.

The transmissible spongiform encephalopathies (TSEs) are a group of invariably fatal neurodegenerative diseases including Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy (BSE) of cattle, and chronic wasting disease of deer. A key feature of TSEs is conformational change occurring in a normal host protein, the prion protein (PrP), resulting in a protease-resistant isoform of the protein. Scrapie is the predominant TSE of sheep, occurs in both classical and atypical forms, and is a significant disease problem in flocks where it occurs. Susceptibility to classical scrapie is strongly associated with the amino acid sequence of PrP, particularly at positions 136, 154, and 171. The VRQ haplotype is considered the most susceptible, while ARR is considered resistant to classical scrapie (2). Susceptibility to atypical scrapie has been associated with L141F (2, 14, 17). The most common haplotype, ARQ, is considered susceptible, yet it is known that not all exposed ARQ/ARQ sheep develop scrapie. Moreover, at least nine distinct genetic subtypes of ARQ are known to exist in sheep (9). This raises the possibility that a resistant ARQ subtype may exist in some sheep populations.

Increasing the prevalence of 171R and decreasing the prevalence of 136V have constituted a goal of scrapie control programs in the European Union and United States. Additional studies have suggested that susceptibility to scrapie is influenced by variation at other PrP residues (20). The purpose of this study was to evaluate the influence of polymorphisms spanning the entire ovine prion gene (*PRNP*) on survival of scrapie-exposed sheep.

Material for this experiment was obtained from a previous study by Foote et al., in which a group of 103 Suffolk sheep was orally inoculated with sheep-derived scrapie infectious material (7). These animals were followed for up to 10 years, with scrapie diagnosis based on clinical and histopathological criteria. DNA and phenotypic information were obtained from these animals and used in the present study. Nucleotide sequences of these DNA samples were obtained from six PCR amplicons distributed across the ovine *PRNP* gene, as previously described (9, 10).

Cumulative survival of ARR/ARR, ARR/ARQ, and ARQ/ARQ sheep in this study was consistent with the dominant resistance conferred by ARR and indicated a biphasic response of ARQ sheep (Fig. 1) (16). A biphasic survival curve has also been reported in other studies, suggesting that this may be a more general effect and not simply an artifact of this study (1). For the present study, ARQ/ARQ sheep were grouped as short (survival time, <800 days) or prolonged (>800 days) survivors based on this biphasic survival curve. Data from animals that died from other causes without evidence of scrapie were excluded. Statistical analysis was performed using the SPSS 15.0 software program (SPSS Inc., Chicago, IL). Sequence was assembled and genotypes of individual sheep determined using the phred, phrap, consed, and polyphred software programs (5, 6, 8, 15). *PRNP* haplotypes were assigned using haplotype tagging single nucleotide polymorphism (SNP) loci (htSNPs [9]), and PHASE 2.11 software (18, 19).

Coding region sequence was obtained from the 98 available samples. Sheep with *PRNP* sequences encoding ARR ($n = 27$) or VRQ ($n = 1$) were excluded from further analysis. High-quality sequence for all amplicons was obtained from 53 of the remaining 72 available samples, and individual sheep genotypes were determined for each of the 12 htSNP loci described by Green et al. using sequence data (9). Haplotype phase was assigned using these htSNP genotypes and PHASE 2.11. Of the 12 htSNP *PRNP* haplotypes described by Green et al., only haplotypes 2, 3, and 4 were present at frequencies over 5% (43.4, 12.3, and 34.9%, respectively); the remaining haplotypes were grouped as "other" (9.4% total) (9). Association of htSNP haplotypes with prolonged survival of scrapie-exposed sheep was estimated using logistic regression assuming additivity (21). Using a reverse Wald procedure (in which haplotypes that do not significantly contribute to the regression model based on a Wald statistic of >0.10 are removed from the model in a stepwise manner), only haplotype 4 remained

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▽ Published ahead of print on 16 July 2007.

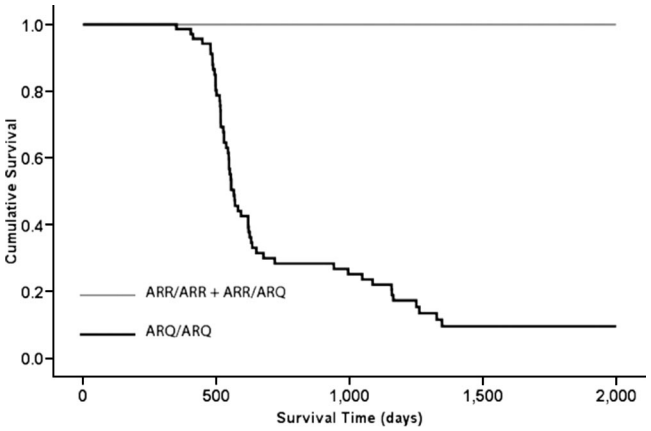

FIG. 1. Cumulative survival of sheep with ARQ/ARQ ($n = 71$) and ARR/ARR or ARR/ARQ ($n = 26$) haplotypes following oral exposure to scrapie.

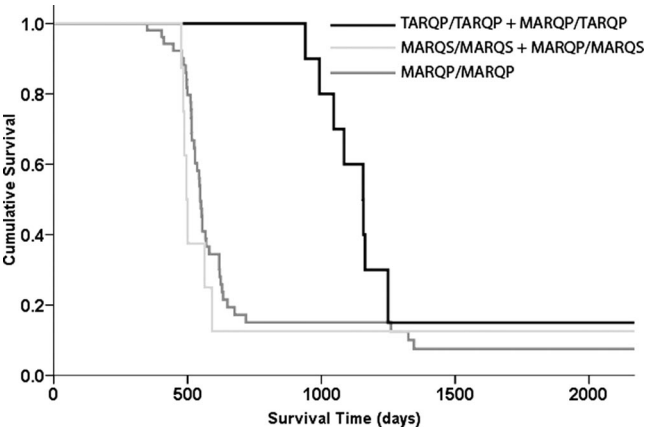

FIG. 2. Cumulative survival of sheep with MARQP, TARQP, and MARQS haplotypes following oral exposure to scrapie.

in the model (odds ratio, 5.3; 95% confidence interval, 1.79 to 15.57). Haplotype 4 alone was able to correctly classify 75.5% of individuals in the study as short or prolonged survivors, indicating a strong association of haplotype 4 with the outcome of scrapie exposure.

To further resolve the association of haplotype 4 with prolonged survival, genotypes were obtained for all polymorphic SNP loci with a minor allele frequency of >0.10 from sheep bearing haplotype 4. These were tested for association by multiple χ^2 analysis with Bonferroni's correction for multiple sampling. Four SNP loci were significantly associated with prolonged survival (Table 1). While all four SNP loci were in strong linkage disequilibrium (LD), two loci, 8252 and 22614, were in perfect LD in this sample and were the most strongly associated with prolonged survival. SNP 8252 is in intron 2, while SNP 22614 is in the PrP coding region and is nonsynonymous, resulting in a threonine at residue 112 instead of methionine (M112T).

The 136, 154, and 171 codon haplotypes were extended to include M112T and P241S (another polymorphic locus in this sample). Sheep were classified with the extended haplotypes (MARQP/MARQP [$n = 51$], TARQP/TARQP [$n = 2$] plus MARQP/TARQP [$n = 7$], and MARQS/MARQS [$n = 1$] plus MARQP/MARQS [$n = 9$]), and Cox regression was used to analyze the relationship between haplotype and survival, which indicated a nearly twofold increase in survival time per unit time for sheep bearing TARQP versus those bearing MARQP or MARQS (odds ratio = 1.91; 95% confidence interval, 1.47 to 2.49) (Fig. 2). Similarly, the median survival time of TARQP sheep (1,157 days) was slightly more than twice that of MARQP sheep (544 days; $P = 0.0002$). Survival times of MARQP and MARQS (499 days) sheep did not differ significantly, indicating that the P241S locus does not appreciably influence susceptibility to oral scrapie exposure. The T112 allele and the highly linked alleles at loci 8252, 8126, and 3264 are highly predictive of prolonged survival in scrapie-exposed sheep. It is notable that all seven heterozygous TARQP sheep developed scrapie, though with delayed onset, while the two homozygous TARQP sheep did not develop scrapie, suggesting both an additive effect and the possibility that TARQP homozygotes are highly resistant to scrapie.

The T112 allele is of particular interest because it results in a PrP which is relatively resistant to in vitro conversion to the protease-resistant PrP^{sc} isoform (3). In addition, there are epidemiologic and anecdotal reports of decreased representa-

TABLE 1. Association of SNP alleles polymorphic in haplotype 4 sheep

SNP locus[a]	Alleles	Region	MAF[b]	Odds ratio	Fisher's exact *P* value[c]
3264	gaaat-(A,T)-tttct[d]	Promoter	0.36	18.8	0.00448
5622	tcccc-(G,C)-ccccc	Promoter	0.04		
8026	aatac-(T,A)-atcat	Intron 1	NA[e]		
8126	agttt-(T,G)-Qaagg	Intron 1	0.07	18.8	0.00448
8183	caagc-(T,C)-gaagc	Exon 2	0.24		
8252	cctgc-(A,G)-gaatc	Intron 2	0.43	40.0	0.00035
8328	gatca-(C,G)-aaatc	Intron 2	0.17		
14330	ctaga-(T,C)-agcta	Intron 2	0.03		
22614	caaca-(T,C)-gaagc	Exon 3/CDS	0.05	40.0	0.00035
23856	tgatg-(C,T)-ttttR	Exon 3	0.17		
23961	ctcca-(G,A)-tactt	Exon 3	0.37		

[a] Number corresponds to nucleotide position in sequence under GenBank accession no. DQ077504.
[b] Minor allele frequency in sheep diversity panel (9).
[c] Bonferroni's corrected cutoff is 0.0045.
[d] Polymorphic loci are in capital letters.
[e] Not analyzed (not previously observed).

tion of TARQ in scrapie-positive samples, though these suffered from small sample sizes due to the low frequency of T112 in sheep populations (12, 13). Furthermore, it remains possible that one or more of the three other polymorphisms in strong LD with 22614 (M112T), or other unrecognized polymorphisms which could be in LD with this locus, might be responsible for the observed phenotype. It is also important to note an increasing body of literature that indicates genetic effects on susceptibility may be PrP strain specific, with ARR sheep at least somewhat susceptible and F141 sheep more susceptible to atypical scrapie (14, 17, 20). Sheep with ARR are also susceptible to BSE (4, 11). The effects of genotype on survival described in this work may not be evident in sheep challenged with atypical scrapie or BSE.

In conclusion, a set of ovine *PRNP* SNPs in strong LD were identified that associate with prolonged survival of scrapie-exposed ARQ sheep. One of these SNP loci is nonsynonymous, M112T, and PrP^{c} proteins bearing the T allele have been previously shown to be refractory to in vitro conversion to PrP^{sc}, suggesting a possible mechanism for prolonged survival. These results indicate that sheep with the ARQ haplotype are not uniformly susceptible to scrapie. These results also have implications for scrapie eradication programs, where ARQ sheep have previously been considered as a homogenous group, leading to losses of economically important sheep germplasm.

We thank Tammy Sorensen, Gennie Schuller-Chavez, Renee Godtel, Bob Lee, Steve Simcox, and Jacky Carnahan for technical assistance and sequencing support; Bucky Herman, Phil Anderson, Jim Wray, and Randy Bradley for database support; and Joan Rosch for administrative support.

This research was supported by the USDA National Research Initiative, competitive grant no. 2005-35212-15890, and the Agricultural Research Service. B. T. Green was partially supported by DARPA project 5438-32000-023-01R.

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