Title

Prion gene (PRNP) haplotype variation in United States goat breeds (Open Access publication).

Journal

Genetics, selection, evolution : GSE, 40(5)

ISSN

0999-193X

Authors

White, Stephen
Herrmann-Hoesing, Lynn
O'rourke, Katherine
et al.

Publication Date

2008-09-01

DOI

10.1186/1297-9686-40-5-553

Peer reviewed

Genet. Sel. Evol. 40 (2008) 553–561
© INRA, EDP Sciences, 2008
DOI: 10.1051/gse:2008021

Available online at:
www.gse-journal.org

Original article

Prion gene (*PRNP*) haplotype variation in United States goat breeds *(Open Access publication)*

Stephen White[1,2*], Lynn Herrmann-Hoesing[1,2], Katherine O'Rourke[1,2], Daniel Waldron[3], Joan Rowe[4], Janet Alverson[1,2**]

[1]USDA, ARS, Animal Disease Research Unit, Pullman, WA 99164, USA
[2]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164, USA
[3]Texas Agricultural Experiment Station, San Angelo, TX 76901, USA
[4]School of Veterinary Medicine, University of California, Davis, CA 95616, USA

(Received 31 October 2007; accepted 13 May 2008)

Abstract – Scrapie eradication efforts cost 18 million dollars annually in the United States and rely heavily upon *PRNP* genotyping of sheep. Genetic resistance might reduce goat scrapie and limit the risk of goats serving as a scrapie reservoir, so *PRNP* coding sequences were examined from 446 goats of 10 breeds, 8 of which had not been previously examined at *PRNP*. The 10 observed alleles were all related to one of two central haplotypes by a single amino acid substitution. At least five of these alleles (M142, R143, S146, H154, and K222) have been associated with increased incubation time or decreased odds of scrapie. To the best of our knowledge, neither S146 nor K222 has been found in any goats with scrapie, though further evaluation will be required to demonstrate true resistance. S146 was more common, present in several breeds at widely varying frequencies, while K222 was observed only in two dairy breeds at low frequency. Overall, this study provides frequency data on *PRNP* alleles in US goats, shows the pattern of relationships between haplotypes, and demonstrates segregation of multiple scrapie-associated alleles in several breeds not examined before at *PRNP*.

scrapie / goat / polymorphism / resistance / prion

1. INTRODUCTION

Scrapie is a transmissible spongiform encephalopathy (TSE) that affects sheep and goats. Sequence changes in the *PRNP* gene that encodes prion protein have been associated with differential resistance and susceptibility to scrapie.

*Corresponding author: swhite@vetmed.wsu.edu
**Present address: USDA, APHIS, Helena, MT 59601, USA.

In particular, sheep have substitutions in amino acids 136, 154, and 171 with well-established allelic and genotypic effects on resistance to common scrapie strains [11]. This is consistent with genetic relationships to a wide variety of TSEs, including kuru and sporadic Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle, and chronic wasting disease in elk and deer [3,12–14,18,19]. Scrapie eradication programs worldwide involve breeding sheep based upon the most resistant alleles, and scrapie prevalence has been falling using such measures [16].

Goats may provide a reservoir for scrapie even if current efforts are completely successful at eradication in sheep, and goat surveillance will become a part of the overall scrapie eradication program in the US. Although there is some evidence that goat genetics plays an important role in goat scrapie, no related management measure has been implemented. Several *PRNP* alleles have been documented in goats including those with amino acid substitutions in codons 21, 24, 37, 49, 102, 110, 127, 133, 137, 142, 143, 146, 151, 154, 168, 211, 218, 220, 222, and 240 [1,2,5,8–10,15,20,26,27]. Silent changes have also been observed in codons 42, 107, 138, 179, 181, 202, 207, 219, and 231 [1,5,8,20,27]. Several alleles have been associated with increased incubation time or decreased odds of scrapie, including those with amino acid substitutions M142, R143, S146, D146, H154, K222, and a 3-octapeptide repeat [1,5,8,9,20,24]. This suggests that a genetic approach to scrapie reduction in goats may have merit.

This study examined sequence variants in *PRNP* present in 10 breeds of US goats; 8 of these breeds had never been examined at *PRNP*. In addition, the present study used allele discovery and frequency information to assess the potential for selective breeding of goats to augment scrapie eradication efforts.

2. MATERIALS AND METHODS

2.1. Animals

Blood was collected from 10 breeds of goats commonly found in the United States: Alpine, Angora, Boer, LaMancha, Myotonic (Tennessee Fainting Goat), Nubian, Oberhasli, Pygmy, Saanen, and Toggenburg. Approximately 30 private producers and university herds were solicited either directly or at goat shows for samples of US animals. When more than one animal was included from the same herd, producers and/or pedigree records assured the animals were unrelated.

2.2. Genotyping

DNA was isolated from 600 μL aliquots of blood using the PureGene Kit (Gentra Systems, Minneapolis, MN), following the manufacturer's protocols.

Approximately 100 ng of DNA was used as a template for amplification of the open reading frame of *PRNP* using the following primer pairs: GGCATTTGATGCTGACACC and CTGTAAGCGCCAAGGGTAT, corresponding to the nucleotide positions 22 234–22 252 and 23 171–23 189 of GenBank Accession No. U67922. Amplification was performed under a temperature regime of: 95 °C for 5 min; 95 °C for 30 s, 62–63 °C for 30 s, and 68–72 °C for 59–90 s over 30–34 cycles; and 68–72 °C for 7–10 min. Amplified products were sequenced by standard dideoxynucleotide analysis using BigDye v3.1 Chemistry (Applied Biosystems, Norwalk, CT) and primers: GGCATTTGATGCTGACACC and CTGTAAGCGCCAAGGGTAT, corresponding to the nucleotide positions 22 234–22 252 and 23 171–23 189 of GenBank Accession No. U67922, and CTGGGGTCAAGGTGGTAGCC and GGTGGTGACTGTGTGTTGCTTGA, corresponding to the nucleotide positions 276–295 and 560–582 of GenBank Accession No. EU253454. Two technicians extracted genotypes from sequence data independently under double-blind conditions and the results were confirmed by a third party. Polymerase chain reaction (PCR) clones were produced using primers (CAGAGCTTCTAGGGTCCTCAC and GACAGCAATAAAGAAATGCACA, corresponding to positions 21 857–21 877 and 24 334–24 355, respectively, in GenBank Accession No. U67922) and TOPO-TA Cloning Kit (Invitrogen, Carlsbad, CA) to verify haplotype phase. Sequencing primers for the clones were CTGTAAGCGCCAAGGGTAT and CAGAGCTTCTAGGGTCCTCAC, which correspond to positions 23 171–23 189 and 21 857–21 877 of GenBank Accession No. U67922.

2.3. Statistical analysis

Haplotypes were analyzed using PHASE v2 [22,23], with a 95% confidence threshold for haplotype predictions for individual animals. If a haplotype had not been previously reported, a PCR product was cloned to verify the predicted haplotype. Linkage disequilibrium was assessed using Haploview [4]. Simultaneous confidence intervals for allele frequencies were calculated using a published macro program [17] in SAS® v9.1 (SAS® Institute, Cary, NC).

3. RESULTS

Ten polymorphisms were observed in caprine *PRNP*, including eight that encode amino acid substitutions and two silent polymorphisms. The amino acid substitutions in codons 127, 142, 143, 146, 154, 211, 222, and 240 and their haplotypic configurations are shown in Table I. The silent polymorphisms were

Table I. *PRNP* haplotypes at the amino acid level.

Haplotype	Amino acid position							
	127	142	143	146	154	211	222	240
1	G	I	H	N	R	R	Q	P
2	–	–	–	–	–	–	–	S
3	S	–	–	–	–	–	–	–
4	–	M	–	–	–	–	–	–
5	–	M	–	–	–	–	–	S
6	–	–	R	–	–	–	–	–
7	–	–	–	S	–	–	–	–
8	–	–	–	–	H	–	–	S
9	–	–	–	–	–	Q	–	S
10	–	–	–	–	–	–	K	S

a G-A substitution in the third nucleotide positions of codon 42 and a T-C substitution in the third nucleotide position of codon 138 as previously described [8], and they were in tight linkage disequilibrium with the amino acid substitution in codon 240 (D' 0.988 and 0.983, respectively). All polymorphisms with at least 5% minor allele frequency within breed were found to be in agreement with Hardy-Weinberg proportions within breed.

The haplotype distributions are shown by breed with 95% confidence intervals in Table II (online). More than 75% of animals had haplotype confidence scores of 1, indicating known haplotypes with no prediction necessary. All the remaining animals had confidence scores of at least 95% for haplotype assignment computed by PHASE from population genotype frequency data using established methods [22,23]. Haplotype 5 was verified by cloning and sequencing since it had not been observed in previous reports, and the clones verified the haplotype predictions from PHASE. Haplotype 1 was the only haplotype observed in every breed examined. Haplotype 2 was common in all breeds except the Toggenburg and Myotonic breeds. Haplotypes 3, 4, 5, 8, 9, and 10 were observed only within dairy breeds.

4. DISCUSSION

Natural scrapie is found in both goats and sheep, and since goats and sheep often cohabitate, goats may serve as a scrapie reservoir for sheep. This is especially concerning since goats can have more subtle scrapie symptoms than sheep [6,21,25]. Sheep *PRNP* genetic testing has greatly aided sheep scrapie

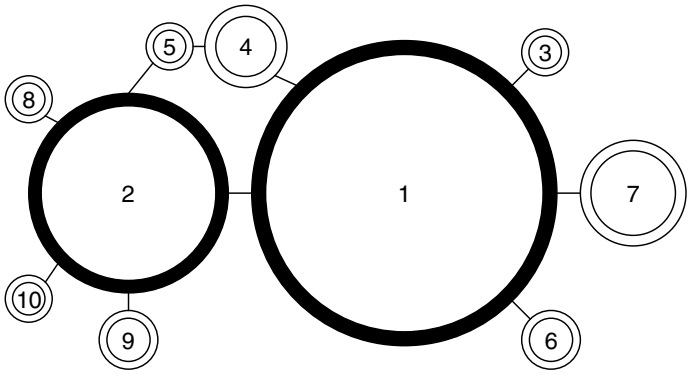

Figure 1. Haplotype relationships indicate two central alleles. Each numbered circle represents a haplotype at the amino acid level. The frequency of each haplotype is depicted by circle size. Lines connect haplotypes related by a single amino acid substitution, and loops formed by haplotype connections may indicate historical recombination events. The two haplotypes represented by black circles are related to all the remaining haplotypes by single amino acid substitutions.

eradication efforts in the US. Goat monitoring efforts will be phased into the US scrapie eradication program, and this study addressed the question of whether goat genetics can be used as a tool in such efforts. As a first step, this study identified 10 different *PRNP* haplotypes and their frequencies in 10 goat breeds. At least two variants have never been found in goats with scrapie. Three other variants have been associated with scrapie impact but not complete resistance, and breed differences were observed for all five scrapie-associated alleles.

Ten haplotypic alleles were observed in this sample of US goats with relationships suggesting a simple evolutionary history. Figure 1 shows two central haplotypes, and all other alleles related to one of these two by only a single amino acid substitution. The high frequency of these two haplotypes and their central position in the haplotype diagram suggest that the other alleles arose by mutation on the background of these two central haplotypes. The one exception is haplotype 5, which has been experimentally verified by cloning. Haplotype 5 probably arose by recombination to combine the M142 and S240 variants on one haplotype (see Fig. 1), and to the best of our knowledge this is the first report of a recombinant *PRNP* haplotype in goats. Several haplotypes were only observed in dairy breeds, indicating low levels of historical gene flow from the dairy breeds into other breed types.

The S146 and K222 alleles (found on haplotypes 7 and 10, respectively) are especially promising since relatively large numbers of animals with each allele

have been present in scrapie-exposed herds but no scrapie positive animal has been reported with even a single copy of either allele [1,20,24]. The S146 allele was a relatively common minor allele at 12.3% frequency overall, and it was found in 7 of 10 breeds examined including breeds used primarily for dairy, meat, and other purposes. This study added Alpine, LaMancha, Nubian, Myotonic, and Pygmy goats to the list of breeds in which S146 has been observed. In these US goats, S146 was always the minor allele, but its within breed frequency ranged from 35.2% (Boer) to 1.4% (Alpine). In contrast, the K222 allele was only observed in two breeds at low frequencies (5.4% in Toggenburg and 1.0% in LaMancha). This study adds these two breeds to the list of known carriers of K222.

Three other *PRNP* variants have been associated with incomplete scrapie resistance and were observed in US goats. The M142 variant has been associated with extended scrapie incubation time [8] and had previously been found in Saanen and mixed-breed dairy goats [1,7,8,15]. The M142 variant (observed on haplotypes 4 and 5) was present at 6.7% frequency overall and was found only in dairy animals. These results added Alpine, Oberhasli, Toggenburg, and LaMancha as known breeds in which this variant has been observed, with frequencies ranging from 43.2% in Toggenburgs to 4.0% in LaMancha goats. Also, both R143 and H154 were observed in US goats (on haplotypes 6 and 8, respectively) and have been associated with reduced scrapie [2,5,24], but not with complete resistance. This study demonstrates for the first time segregation of R143 in Nubian, Pygmy, and Angora goats, and confirms a previous finding of segregation in Boer goats [27]. This study also adds Saanen goats as a breed in which H154 is known to segregate.

In summary, this study documents the presence of 10 *PRNP* alleles in US goats and shows the relationships between segregating haplotypes. Eight of the ten breeds examined had not previously been examined for *PRNP* allelic variation, and several scrapie-associated alleles were found to segregate in these breeds. However, additional data are necessary to demonstrate full genetic scrapie resistance before genetic approaches to goat scrapie can serve as a useful adjunct to current efforts in scrapie eradication.

ONLINE MATERIAL

The supplementary file (Tab. II) supplied by the authors is available at: http://www.gse-journal.org.

Table II. *PRNP* haplotype frequencies with 95% confidence intervals for alleles by breed.

ACKNOWLEDGEMENTS

We thank Heather Garcia, Codie Hanke, Will Harwood, David Herndon, and Jim Reynolds for excellent technical assistance; Bruce Mackey for statistical support; and Jan Carlson (UC Davis), Michelle Fonda, Eileen Farrel, Laura Chenel (Chenel's Dairy), Barbara van Zee (van Zee Ranch), Fauna Smith (Wingwood Farm), Jennifer Bice (Redwood Hill Farm), Lisa Shepard, Phil Sponenberg, Desiree Nelson, Eileen Kuhlman, Cindy Wolf, Helen Snyder, Marcia St. John (Okanogan Oberhaslis), Fern and Laurie Acton, Emma Karel, and Lori Fuller (Washington State University goat herd), and many other private producers for submission of blood samples. This research was supported by a grant from the USDA Agricultural Research Service (5348-32000-026-00D).

REFERENCES

[1] Acutis P.L., Bossers A., Priem J., Riina M.V., Peletto S., Mazza M., Casalone C., Forloni G., Ru G., Caramelli M., Identification of prion protein gene polymorphisms in goats from Italian scrapie outbreaks, J. Gen. Virol. 87 (2006) 1029–1033.

[2] Agrimi U., Conte M., Morelli L., Di Bari M.A., Di Guardo G., Ligios C., Antonucci G., Aufiero G.M., Pozzato N., Mutinelli F., Nonno R., Vaccari G., Animal transmissible spongiform encephalopathies and genetics, Vet. Res. Commun. 27 (Suppl. 1) (2003) 31–38.

[3] Aguzzi A., Prion diseases of humans and farm animals: epidemiology, genetics, and pathogenesis, J. Neurochem. 97 (2006) 1726–1739.

[4] Barrett J.C., Fry B., Maller J., Daly M.J., Haploview: analysis and visualization of LD and haplotype maps, Bioinformatics 21 (2005) 263–265.

[5] Billinis C., Panagiotidis C.H., Psychas V., Argyroudis S., Nicolaou A., Leontides S., Papadopoulos O., Sklaviadis T., Prion protein gene polymorphisms in natural goat scrapie, J. Gen. Virol. 83 (2002) 713–721.

[6] Capucchio M.T., Guarda F., Pozzato N., Coppolino S., Caracappa S., Di Marco V., Clinical signs and diagnosis of scrapie in Italy: a comparative study in sheep and goats, J. Vet. Med. A Physiol. Pathol. Clin. Med. 48 (2001) 23–31.

[7] Foster J.D., Parnham D., Chong A., Goldmann W., Hunter N., Clinical signs, histopathology and genetics of experimental transmission of BSE and natural scrapie to sheep and goats, Vet. Rec. 148 (2001) 165–171.

[8] Goldmann W., Martin T., Foster J., Hughes S., Smith G., Hughes K., Dawson M., Hunter N., Novel polymorphisms in the caprine *PrP* gene: a codon 142 mutation associated with scrapie incubation period, J. Gen. Virol. 77 (1996) 2885–2891.

[9] Goldmann W., Chong A., Foster J., Hope J., Hunter N., The shortest known prion protein gene allele occurs in goats, has only three octapeptide repeats and is non-pathogenic, J. Gen. Virol. 79 (1998) 3173–3176.

[10] Goldmann W., Perucchini M., Smith A., Hunter N., Genetic variability of the *PrP* gene in a goat herd in the UK, Vet. Rec. 155 (2004) 177–178.
[11] Hunter N., Scrapie: uncertainties, biology and molecular approaches, Biochim. Biophys. Acta 1772 (2007) 619–628.
[12] Jewell J.E., Conner M.M., Wolfe L.L., Miller M.W., Williams E.S., Low frequency of PrP genotype 225SF among free-ranging mule deer (*Odocoileus hemionus*) with chronic wasting disease, J. Gen. Virol. 86 (2005) 2127–2134.
[13] Juling K., Schwarzenbacher H., Williams J.L., Fries R., A major genetic component of BSE susceptibility, BMC Biol. 4 (2006) 33.
[14] Kashkevich K., Humeny A., Ziegler U., Groschup M.H., Nicken P., Leeb T., Fischer C., Becker C.M., Schiebel K., Functional relevance of DNA polymorphisms within the promoter region of the prion protein gene and their association to BSE infection, FASEB J. 21 (2007) 1547–1555.
[15] Kurosaki Y., Ishiguro N., Horiuchi M., Shinagawa M., Polymorphisms of caprine *PrP* gene detected in Japan, J. Vet. Med. Sci. 67 (2005) 321–323.
[16] Lynn T., Grannis J., Williams M., Marshall K., Miller R., Bush E., Bruntz S., An evaluation of scrapie surveillance in the United States, Prev. Vet. Med. 81 (2007) 70–79.
[17] May W.L., Johnson W.D., A SAS macro for constructing simultaneous confidence intervals for multinomial proportions, Comput. Methods Programs Biomed. 53 (1997) 153–162.
[18] O'Rourke K.I., Besser T.E., Miller M.W., Cline T.F., Spraker T.R., Jenny A.L., Wild M.A., Zebarth G.L., Williams E.S., *PrP* genotypes of captive and free-ranging rocky mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease, J. Gen. Virol. 80 (1999) 2765–2769.
[19] O'Rourke K.I., Spraker T.R., Hamburg L.K., Besser T.E., Brayton K.A., Knowles D.P., Polymorphisms in the prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer, J. Gen. Virol. 85 (2004) 1339–1346.
[20] Papasavva-Stylianou P., Kleanthous M., Toumazos P., Mavrikiou P., Loucaides P., Novel polymorphisms at codons 146 and 151 in the prion protein gene of Cyprus goats, and their association with natural scrapie, Vet. J. 173 (2007) 459–462.
[21] Sofianidis G., Psychas V., Billinis C., Spyrou V., Argyroudis S., Papaioannou N., Vlemmas I., Histopathological and immunohistochemical features of natural goat scrapie, J. Comp. Pathol. 135 (2006) 116–129.
[22] Stephens M., Donnelly P., A comparison of Bayesian methods for haplotype reconstruction from population genotype data, Am. J. Hum. Genet. 73 (2003) 1162–1169.
[23] Stephens M., Smith N.J., Donnelly P., A new statistical method for haplotype reconstruction from population data, Am. J. Hum. Genet. 68 (2001) 978–989.
[24] Vaccari G., Di Bari M.A., Morelli L., Nonno R., Chiappini B., Antonucci G., Marcon S., Esposito E., Fazzi P., Palazzini N., Troiano P., Petrella A., Di Guardo G.,

Agrimi U., Identification of an allelic variant of the goat *PrP* gene associated with resistance to scrapie, J. Gen. Virol. 87 (2006) 1395–1402.
[25] Wood J.N., Done S.H., Pritchard G.C., Wooldridge M.J., Natural scrapie in goats: case histories and clinical signs, Vet. Rec. 131 (1992) 66–68.
[26] Wopfner F., Weidenhofer G., Schneider R., von Brunn A., Gilch S., Schwarz T.F., Werner T., Schatzl H.M., Analysis of 27 mammalian and 9 avian *PrP*s reveals high conservation of flexible regions of the prion protein, J. Mol. Biol. 289 (1999) 1163–1178.
[27] Zhang L., Li N., Fan B., Fang M., Xu W., *PRNP* polymorphisms in Chinese ovine, caprine and bovine breeds, Anim. Genet. 35 (2004) 457–461.