Gene Reports 4 (2016) 213–217

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Genetic variation of the prion protein gene (PRNP) in alpaca (*Vicugna pacos*)

M.S. Vermette [a], J.A. Schleining [b], J.J. Greenlee [c], J.D. Smith [a,*]

[a] Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, 1600 S 16th St., Ames, IA, USA
[b] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, 1600 S 16th St., Ames, IA, USA
[c] Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, 1920 Dayton Ave., Ames, IA, USA

ARTICLE INFO

Article history:
Received 30 December 2015
Received in revised form 5 June 2016
Accepted 7 June 2016
Available online 8 June 2016

Keywords:
Camelid
TSE
Spongiform
Encephalopathy

ABSTRACT

Transmissible spongiform encephalopathies (TSE) are caused by accumulation of a misfolded form of the prion protein (PrP). The normal cellular isoform of PrP is produced by the prion gene (*PRNP*) and is highly expressed in the central nervous system. Currently, there is an absence of information regarding the genetic sequence of alpaca *PRNP* and the potential susceptibility of this species to TSE. The objective of this study was to sequence the open reading frame of the alpaca prion gene and analyze this sequence for variation within the alpaca population and for homology to TSE-susceptible species. We sequenced the open reading frame of the prion gene of 40 alpacas of Huacaya or Suri descent. Length polymorphisms were identified within the sampled population. A subset (15%) of animals contained an additional 24 base pairs within the putative octapeptide repeat region. This polymorphism was independent of breed and sex. The majority (52.5%) of animals were heterozygous, possessing both longer and shorter alleles. Comparison with proven TSE-susceptible species (sheep, cattle, deer) revealed the following amino acid sequence variations: I6M, A16V, M17T, G92del, Q95_G96insG, N111S, R167K, N/T177S, I206V, S225Y, Y228S, Q230G, and L237del. Sequence alignment showed high homology compared to camel (>95%), sheep (>88%), cattle (>87%) and deer (>88%) *PRNP* sequence. This study demonstrates intraspecies variability within the *PRNP* open reading frame in alpacas and overall high sequence homology to TSE-susceptible species, providing foundational data for further research on the potential susceptibility of alpacas to TSE.

1. Introduction

Transmissible spongiform encephalopathies (TSE), also known as prion diseases, are a group of fatal neurodegenerative diseases that affect both animals and humans. These diseases include scrapie in sheep and goats, Creutzfeldt-Jakob disease in humans, chronic wasting disease (CWD) in deer and elk, and bovine spongiform encephalopathy (mad cow disease) in cattle. The prion protein (PrP^{C}), encoded by the prion gene (*PRNP*), is detergent-soluble and sensitive to proteinase K digestion. This protein is normally expressed in humans and animals, with greatest levels of expression in the central nervous system. It is generally recognized that prions, composed of PrP^{Sc} (the misfolded, disease-associated form of the prion protein), are formed from PrP^{C} by a posttranslational process that results in a profound change in conformation (Prusiner, 2013). The transformation and accumulation of PrP^{Sc}, a detergent insoluble form that is relatively resistant to proteases, is considered the central event in TSE pathogenesis (Aguzzi et al., 2001; Xu et al., 2012). Sequence analysis of *PRNP* in a number of animal species has demonstrated a high degree of conservation across mammals, typically with >50% sequence identity relative to humans (Schatzl et al., 1995; Wopfner et al., 1999).

Prion transmission within the same species is an efficient process, whereas transmission between species is usually less efficient or not possible ('species-barrier' phenomenon) (Hagiwara et al., 2013). However, the barrier sometimes can be overcome or negligible depending on the combination of donor and recipient animal PrP, or adaptation of prion strains to new hosts. For example, the CWD agent of mule deer has been experimentally transmitted by intracranial (IC) inoculation to cattle by first and second passage. On first passage, only 5 of 13 inoculated animals (38%) showed evidence of PrP^{Sc} amplification within 2 to 5 years post-inoculation (Hamir et al., 2005). Second passage resulted in 100% of inoculated cattle succumbing to infection within 16.5 months post-inoculation (Hamir et al., 2006a), demonstrating interspecies transmission and adaptation of the agent to the bovine host. Experimental interspecies transmission of the scrapie agent to cervids has also been achieved (Greenlee et al., 2011), demonstrating interspecies transmission potential for this TSE agent as well. These findings could be of concern to the alpaca industry given the potential

* Corresponding author at: Veterinary Pathology, 2714 Vet Med Bldg, 1600 S 16th St., Ames, IA 50011-1250, United States.
E-mail address: jdismith@iastate.edu (J.D. Smith).

http://dx.doi.org/10.1016/j.genrep.2016.06.004
2452-0144/

for physical interaction between alpacas and CWD-infected free-ranging cervids and/or scrapie-infected domestic sheep.

The alpaca is a part of the family Camelidae, along with the dromedary camel, Bactrian camel, llama, guanaco, and vicugna. There are two breeds of alpaca, Suri and Huacaya, both of which are found in the United States. These animals are used for fiber production and are considered the most color-diverse fiber-producing animals in the world. To date, there have been no reported cases of TSE in the alpaca. Whether this is a function of resistance of this species to TSE agents in general, underdiagnosis of alpacas with neurologic disease, or the relatively small US alpaca population and, therefore, exposure of a small number of animals to TSE agents, such as CWD and scrapie, remains to be determined. There is an absence of information about *PRNP* in the alpaca (*Vicugna pacos*). Within the Camelidae family, *PRNP* sequence has only been reported for Bactrian and dromedary camel (Kaluz et al., 1997; Xu et al., 2012). The aim of the current study was to elucidate the alpaca *PRNP* sequence and compare it to the *PRNP* sequences of other domesticated animals with proven susceptibility to TSE agents.

2. Materials and methods

Blood was collected from 40 alpacas ranging in age from 2 to 13 years and representing both Suri ($n = 20$) and Huacaya ($n = 20$) breed types (Table 1). Blood was obtained from the jugular vein via standard venipuncture procedure, placed into EDTA, and stored at 4 °C or −20 °C until processing. Genomic DNA was extracted from whole blood samples using the Roche HighPure PCR Template Preparation Kit (Roche Diagnostics Corporation, Indianapolis, IN, USA) and amplified by PCR using primer pairs (forward primer (7064F) 5′-TAGGACGC TGACACCCTCTT-3′ and reverse primer (R1) 5′-CCCACTATGAGGAAAA TGAG-3′; Integrated DNA Technologies, Coralville, IA) designed based on a combination of published camelid *PRNP* sequence data (Xu et al., 2012) and initial data analysis using an alternate forward primer (5′ ATCCTGGTTCTCTTTGTGGT-3′). Thermal cycling parameters comprised an initial denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 20 s, annealing at 58 °C for 20 s, and extension at 72 °C for 1 min, and ended by a final 10 min extension at 72 °C on an Applied Biosystems thermal cycler (Life Technologies, Carlsbad, CA) under standard buffer conditions (Herculase II Fusion DNA Polymerase, Agilent, Santa Clara, CA, USA). Each reaction contained 5 μL 5× Herculase II Reaction Buffer, 0.5 μL deoxyribonucleotide triphosphates (dNTPs), 0.63 μL of 7064F and R1 primers, 1.25 uL Dimethylsulfoxide (DMSO), 14.74 μL distilled water, 0.25 μL Herculase II fusion DNA polymerase, and 2 μL of extracted DNA. PCR products were confirmed by electrophoresis on 1% agarose gel and imaged using a gel imaging system (G:BOX; Syngene, Frederick, MD, USA) with GelRed DNA stain (GelRed, Phoenix Research, Candler, NC, USA). Samples resulting in more than one band on gel electrophoresis were separated using stab extraction with a 20 μL pipette tip. The sample was then pipetted into 100 μL of UltraPure DNase/RNase-free distilled water (Life Technologies, Carlsbad, CA, USA) and frozen at −20 °C until use. The samples were amplified using PCR parameters as described above. PCR products were then diluted at 1:10 with UltraPure DNase/RNase-free distilled water and analyzed using a 1% agarose gel to confirm a clean extraction. PCR products were then purified using an Amicon Ultra-0.5 mL centrifugal filter (EMD Millipore, Billerica, MA, USA) to remove unincorporated dNTPs and primers, then sequenced using Applied Biosystems 3100 genetic analyzer (Life Technologies, Carlsbad, CA, USA) with Big Dye Terminator chemistry (PE-Applied Biosystems, Carlsbad, CA, USA) using primers 7064F and R1. Initial sequence analysis revealed the necessity of additional primers to ensure accurate sequence identity; therefore, internal primers were created using the dromedary camel *PRNP* sequence (*Camelus dromedarius*, GenBank accession number Y09760) and Geneious version R6 (Biomatters, Auckland, New Zealand; Kearse et al., 2012). These primers were named based on location within the camel *PRNP* and included: 306F (5′-GAACAAGCCCAGTA AGCCGA-3′), 620F (5′-AGATGATGGAGCGCGTAGTG-3′), 327R (5′TTTC GGCTTACTGGGCTTGT-3′), 453R (5′-ACGGTCCTCATAGTCGTTGC-3′), 493R (5′-ACACTTGGTTGGGGTAACGG-3′), and 606R (5′-GGTGAAGTTC TCCCCCTTGG-3′). These primers provided overlapping sequence data (≥3× coverage) using the same sequence techniques described above. All sequence data was then analyzed and aligned against currently available GenBank consensus sequences of *PRNP* for cattle (*Bos taurus*, GenBank accession number FJ907304), sheep (*Ovis aries*, GenBank accession number HM803994), dromedary camel (*Camelus dromedarius*, GenBank accession number Y09760), Bactrian camel (*Camelus bactrianus*, GenBank accession number HQ204566.1), red deer (*Cervus elaphus*, GenBank accession number Y09761.1), and white-tailed deer (*Odocoileus virginianus*, GenBank accession number AF156185.1) to determine percent identity (pairwise alignment) using Geneious version R6.

Table 1
Animal information.

Bands by gel electrophoresis	Sample #	Age (yrs)	Sex	Breed
Double	11	6	F	Huacaya
	12	6	F	Huacaya
	14	2	F	Huacaya
	15	9	F	Huacaya
	16	6	F	Huacaya
	18	5	F	Huacaya
	22	6	F	Huacaya*
	24	7	F	Huacaya*
	26	10	F	Huacaya*
	29	5	F	Huacaya*
	30	5	F	Huacaya*
	23	13	M	Huacaya*
	27	7	M	Huacaya*
	32	Unk**	C/M	Suri
	36	Unk**	C/M	Suri
	37	Unk**	C/M	Suri
	39	Unk**	C/M	Suri
	1	8	F	Suri
	2	9	F	Suri
	6	7	F	Suri
	10	Unk**	F	Suri
High MW/long	13	5	F	Huacaya
	17	10	F	Huacaya
	21	7	M	Huacaya*
	25	11	M	Huacaya*
	31	Unk**	C/M	Suri
	33	Unk**	C/M	Suri
Low MW/short	19	5	F	Huacaya
	20	7	F	Huacaya
	28	7	M	Huacaya*
	34	Unk**	C/M	Suri
	35	Unk**	C/M	Suri
	38	Unk**	C/M	Suri
	40	Unk**	C/M	Suri
	3	11	F	Suri
	4	9	F	Suri
	5	10	F	Suri
	7	8	F	Suri
	8	7	F	Suri
	9	6	F	Suri

Unk = unknown, F = female, M = male, C/M = castrated male.
* Accoyo line.
** ≥6 years of age.

3. Results

Complete alpaca open reading frame *PRNP* nucleotide sequence and deduced PrP amino acid sequence were determined (Fig. 1). Sequence data was submitted to NCBI's GenBank (accession numbers KT692714 and KT692715). On gel electrophoresis, variability was discovered among PCR products (Fig. 2). Three distinct banding patterns were observed: DNA fragments from 6 alpacas (15%) had a higher molecular weight (approximately 800 bp), DNA fragments from 13 alpacas