High prevalence of prion protein genotype associated with resistance to chronic wasting disease in one Alberta woodland caribou population

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ABSTRACT. Chronic wasting disease (CWD) is a prion disease found in deer, elk and moose in North America and since recently, wild reindeer in Norway. Caribou are at-risk to encounter CWD in areas such as Alberta, Canada, where the disease spreads toward caribou habitats. CWD susceptibility is modulated by species-specific polymorphisms in the prion protein gene (Prnp). We sequenced Prnp of woodland caribou from 9 Albertan populations. In one population (Chinchaga) a significantly higher frequency of the 138N allele linked to reduced CWD susceptibility was observed. These data are relevant for developing CWD management strategies including conservation of threatened caribou populations.

KEYWORDS. caribou, chronic wasting disease, conservation, genetic resistance, prion protein, prion protein gene polymorphism

INTRODUCTION

Chronic wasting disease (CWD) is a prion disease which utterly occurs in wild and captive cervids, including mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus), Rocky Mountain elk (Cervus canadensis) and Shira’s moose (Alces alces shirasi).1,2 Just recently, the first CWD case in free-ranging reindeer (Rangifer tarandus tarandus) was diagnosed in southern Norway.3 CWD was first recognized in the 1960s in Colorado and has been burgeoning across North America, in at least 23 states in the US and 2 provinces of Canada.4 Prion diseases are a group of fatal neurodegenerative disorders that affect humans...
and animals and are caused by the conformational conversion of the cellular prion protein (PrPC) into the pathogenic isoform PrPSc. PrPSc is the sole component of prions and accumulates in the brains of infected individuals. In addition, environmental prions have been experimentally demonstrated to retain infectivity for years which contributes to horizontal transmission among cervids. Like in prion diseases of other species, species-specific amino acid polymorphisms in the cervid PrP are associated with susceptibility, incubation time, and pathology of CWD. In most cases, the occurrence of at least one non-wild type allele of Prnp is correlated with a reduced susceptibility to CWD infection and prolonged incubation times. The caribou prion protein primary structure is identical to the mule and white-tailed deer PrP, except for the positions of species-specific polymorphisms.

Caribou are threatened and declining in Alberta, whereas mule and white-tailed deer populations are stable or expanding. Considering the geographic spread of CWD and the proof of susceptibility of reindeer to CWD, it is likely that eventually caribou encounter CWD, which will be detrimental for the already declining populations.

Four polymorphisms were discovered in 3 Alaskan caribou (Rangifer tarandus granti) herds at amino acid positions 2 (valine [V] or methionine [M]), 129 (glycine [G] or serine [S]), 138 (serine [S] or asparagine [N]) and 169 (valine [V] to methionine [M]), respectively. Given the relevance of Prnp polymorphisms for CWD transmission and pathogenesis, it is important to determine the distribution of PrP polymorphisms among caribou populations that are likely to overlap with CWD positive herds in the future. Since CWD has been now well-established in Alberta, Canada, we analyzed the Prnp coding sequence of animals from 9 Albertan woodland caribou (Rangifer tarandus caribou) populations, including both the mountain and the boreal ecotypes. We did not identify novel polymorphic codons; however, we found a significantly increased frequency of the 138N allele in the Chinchaga population (boreal ecotype). These results allow predictions on the disease susceptibility of caribou populations on a genetic basis, and add important information regarding conservation management of this declining species.

RESULTS

Of the 5 polymorphic sites identified previously in Alaska caribou the single base change at base 438 is a synonymous substitution at codon 146 (N to N) and codon 2 locates within the N-terminal signal peptide (amino acid 1 to 22) which is cleaved upon post-translational modification. Therefore, we focused on variations in the sequence encoding mature PrP (without N- and C-terminal signal peptides), specifically at codon 129, 138 and 169.

There are 16 populations of woodland caribou (Rangifer tarandus caribou) currently existing in Alberta, Canada, which can be further classified into 2 ecotypes based upon geography and migration patterns: the boreal ecotype (with individuals typically sedentary) and the mountain ecotype (with some individuals sedentary and other migratory). We analyzed a total of 118 samples collected from 9 woodland caribou populations. No polymorphism was detected at codon 169 in any of the samples; thus, the PrP genotypes at codon 129 and codon 138 were categorized by individual populations (Table 1). A significant difference in allele frequencies at codon 138 between populations was detected by Fisher’s Exact test (p = 1.056e-08). Interestingly, a statistically significant difference was observed when frequencies of the 138N and 138S alleles, respectively, were compared using Fisher’s Exact test between the Chinchaga population and all other populations, grouped into the boreal or mountain ecotype (Fig. 1). If the caribou populations excluding Chinchaga population were classified into either mountain or boreal ecotypes, in populations of the mountain ecotype (Jasper, Narraway, Redrock Prairie Creek and A La Peche), the allele frequencies of 129G
and 138S are 1.0 and 0.821, respectively; in caribou populations of the boreal ecotype (Cold Lake, East-side Athabasca River, Slave Lake and Little Smoky), the allele frequencies of 129G and 138S are 0.989 and 0.913, respectively. The Chinchaga population is also classified as boreal ecotype. Whereas the 129G frequency is with 0.967 similar to the other populations, the frequency of the 138S allele is 0.417, which is significantly lower than in the other populations of either ecotype. Furthermore, we observed that as many as 36.7% of the animals of the Chinchaga population are homozygous for the 138N allele (Fig. 1). In summary, we have identified a woodland caribou population (Chinchaga) with an uncommonly high frequency of the 138N Prnp allele.

**DISCUSSION**

To date, 16 polymorphic sites in the Prnp gene have been discovered among the cervid family. Amino acid 138 (S/N) has been found to be polymorphic in the Prnp gene of caribou (Rangifer tarandus spp.), and fallow deer (Dama dama) but not in white-tailed or mule deer which only carry the allele encoding serine. In mule and white-tailed deer a Prnp pseudogene (Prnpψ) was identified which exclusively encoded N at position 138. However, Prnpψ was not found in old world deer such as reindeer, elk, fallow deer or caribou, arguing against the possibility that the higher frequency of the 138N allele found in caribou of the Chinchaga population is due to Prnpψ.

Asparagine at codon 138 has been associated with at least partial protection against CWD infection as in reindeer experimentally inoculated with CWD prions animals that harbored at least one 138N allele did not develop prion disease until termination of the experiment, suggesting genetic protection. Similarly, fallow deer (Dama dama) with the 138N PrP genotype did not develop CWD when they were exposed to infected mule deer and environmental CWD prions. Along this line, in vitro conversion of cervid PrP-138N into a protease-resistant form reminiscent of PrPSc was less efficient than the conversion of PrP-138S.

We demonstrate that caribou of the Chinchaga population exhibit significantly higher
frequencies of the 138SN and 138NN Prnp genotypes in comparison to the other 4 boreal populations, the mountain populations of woodland caribou (*Rangifer tarandus caribou*), and 3 herds of Alaskan caribou (*Rangifer tarandus grantii*). The Chinchaga is a large natural foothills region in north-western Alberta, with a large portion of this area disturbed by industrial development. Most likely due to human activities, the Chinchaga caribou population is not self-sustaining; the estimated population size is about 250 animals. It is not
clear why this high frequency of the 138N-encoding Prnp allele is only confined to the Chinchaga population but not to other boreal woodland caribou populations. Gene flow from other caribou populations can be one possible explanation, and an extensive microsatellite assignment test of 808 individuals revealed that the genetic characteristics of 3 diverse caribou subspecies, including Grant’s caribou (Rangifer tarandus granti), barren-ground caribou (Rangifer tarandus groenlandicus) and woodland caribou (Rangifer tarandus caribou) are equally mixed in the Chinchaga population. This suggests genetic admixture in this herd. This population is provincially classified as woodland caribou; however, from a genetic perspective it cannot be simply fitted in any subspecies.

The Chinchaga region has been included in the Alberta Woodland Caribou Recovery Plan for planning and implementing recovery actions. Our data suggest a reduced genetic susceptibility of Chinchaga caribou to CWD, therefore indicating lower risk of disease when compared with other populations that are also declining. Overall, our results also highlight the importance of investigating Prnp allele frequencies to inform authorities on potential CWD-resistant caribou herds. Currently there is no efficient approach available to eliminate environmental contamination and spread of CWD, however, the possibility of preserving CWD-protective gene lines needs to be considered.

**MATERIALS AND METHODS**

**Blood Samples**

We used a total number of 118 anticoagulated whole blood samples of 9 Albertan caribou populations which were collected at different time points during caribou capture conducted between 2002–2008 and were stored at -80°C. The protocol for sample collection was approved by the University of Calgary Life & Environmental Sciences Animal Care Committee (LESACC; Study Id: AC16–0195). Of these 9 populations, Chinchaga (n = 30), Cold Lake (n = 10), East-side Athabasca River (n = 15), Slave Lake (n = 10) and Little Smokey (n = 11) belong to the boreal ecotype; the other 4 populations: Japer (n = 10), Narraway (n = 13), Redrock Prairie Creek (n = 10) and A La Peche (n = 9) are classified as the mountain ecotype. Numbers of samples were chosen to represent about 10% of individuals of each population.

**Genomic DNA Extraction, PCR and Sequencing**

Genomic DNA extraction from blood was done by using a commercial kit (DNeasy Blood and Tissue Kit, Qiagen) according to the manufacturer’s recommendation. One hundred ng of genomic DNA were used as a template in PCR to amplify the sequence encoding the mature PrP in the third exon of the Prnp gene. The PCR primers were designed to complement sequences of the N- and C-terminal signal peptides (amino acid 1 to 24 and 25 to 256) of the caribou (Rangifer tarandus ssp.) prion protein gene. PCR cocktail components were as follows: 5 μl of 10X Pfu reaction buffer (Agilent), 2 μl of 5 mM dNTP, 2 μl of 10 μM forward primer (5'-CCT AGT TCT CTT TGT GGC CAT GTG-3'), 2 μl of 10 μM reverse primer (5'-TGA GGA AAG AGA TGA GGA GGA TCA C-3'), and 0.5 μl of Pfu polymerase. The PCR reactions were performed by following an initial denaturation at 94°C for 4 minutes, and 39 cycles of denaturation (94°C, 30 seconds), annealing (62°C, 30 seconds), extension (72°C, 1 minute) and a final extension at 72°C for 10 minutes. PCR products were analyzed on agarose gel, purified and sequenced using the forward and reverse primers used for PCR amplification (University of Calgary CoreDNA service or Eton Bioscience, San Diego). In addition, several PCR products which showed no polymorphisms at codon 129, 138, 146 and 169 were chosen for cloning (Zero Blunt™ TOPO™ PCR Cloning Kit; Invitrogen). Inserts of 10 single clones per PCR product were sequenced to confirm the absence of polymorphic residues.
DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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