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Large-scale prion protein genotyping in Canadian caribou populations and potential impact on chronic wasting disease susceptibility

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Abstract

Polymorphisms within the prion protein gene (*Prnp*) are an intrinsic factor that can modulate chronic wasting disease (CWD) pathogenesis in cervids. Although wild European reindeer (*Rangifer tarandus tarandus*) were infected with CWD, as yet there have been no reports of the disease in North American caribou (*R. tarandus* spp.). Previous *Prnp* genotyping studies on approximately 200 caribou revealed single nucleotide polymorphisms (SNPs) at codons 2 (V/M), 129 (G/S), 138 (S/N), 146 (N/n) and 169 (V/M). The impact of these polymorphisms on CWD transmission is mostly unknown, except for codon 138. Reindeer carrying at least one allele encoding for asparagine (138NN or 138SN) are less susceptible to clinical CWD upon infection by natural routes, with the majority of prions limited to extraneural tissues. We sequenced the *Prnp* coding region of two caribou subspecies ($n = 986$) from British Columbia, Saskatchewan, Yukon, Nunavut and the Northwest Territories, to identify SNPs and their frequencies. Genotype frequencies at codon 138 differed significantly between barren-ground (*R. t. groenlandicus*) and woodland (*R. t. caribou*) caribou when we excluded the Chinchaga herd ($p < .05$). We also found new variants at codons 153 (Y/F) and 242 (P/L). Our findings show that the 138N allele is rare among caribou in areas with higher risk of contact with CWD-infected species. As both subspecies are classified as Threatened and play significant roles in North American Indigenous culture, history, food security and the economy, determining frequencies of *Prnp* genotypes associated with susceptibility to CWD is important for future wildlife management measures.

KEYWORDS

caribou, caribou conservation, chronic wasting disease, genotyping, prion protein

1 | INTRODUCTION

Chronic wasting disease (CWD) was first discovered in 1967 in captive mule deer in Colorado (Williams & Young, 1980). Since then, the disease has spread to wild and farmed cervids in 26 US states and three Canadian provinces (https://www.usgs.gov/media/images/distribution-chronic-wasting-disease-north-america-0; Hannaoui, Schatzl, & Gilch, 2017). Recently, CWD has also been reported in wild cervids in Europe (Benestad, Mitchell, Simmons, Ytrehus, & Vikøren, 2016). CWD is a prion disease, also known as a transmissible spongiform encephalopathy (TSE), of cervid species. Prion diseases are caused by conversion of the endogenous cellular prion protein (PrP^{C}) into its infectious isoform, PrP^{Sc}, which is the major component of prions (Prusiner, 1998). This conformational transition is catalysed by PrP^{Sc} coming into contact with PrP^{C} (Prusiner, 1998), and PrP^{C} is most abundantly expressed in the central nervous system (CNS) (Linden et al., 2008). The CNS is a major site of PrP^{Sc} conversion and accumulation, leading to neurodegeneration and ultimately death (Prusiner, 1998). In naturally occurring CWD, prions are acquired through peripheral routes, such as oral uptake from environmental reservoirs (Gilch et al., 2011). Prions are highly resistant to many major forms of inactivation (Giles, Woerman, Berry, & Prusiner, 2017). It is exceptionally challenging and not possible to contain and eradicate CWD to date, as prions are shed in excrement and bodily fluids of infected free-ranging cervids (Cheng, Hannaoui, et al., 2017; Haley et al., 2011; Henderson et al., 2015; Safar et al., 2008). This results in contamination of the environment, particularly soil, with prions that remain infectious for years (Bartelt-Hunt & Bartz, 2013; Kuznetsova, McKenzie, Cullingham, & Aiken, 2020; Pritzkow et al., 2018; Somerville et al., 2019). So far, wild and farmed cervid species naturally infected with CWD include white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*), elk (*Cervus canadensis*), red deer (*C. elaphus*) and moose (*Alces alces* sp.) in North America (Baeten, Powers, Jewell, Spraker, & Miller, 2007; Hannaoui, Amidian, et al., 2017; Kurt & Sigurdson, 2016; Pirisinu et al., 2018; Williams & Young, 1980, 1982, 1993), as well as reindeer (*Rangifer tarandus tarandus*), red deer (*C. elaphus*) and moose (*A. a. alces*) in Europe (Benestad et al., 2016; Pirisinu et al., 2018; Vikøren et al., 2019).

There are currently four caribou subspecies residing in North America: the Peary (*R. t. pearyi*), Grant's (*R. t. granti*), barren-ground (*R. t. groenlandicus*), and woodland (*R. t. caribou*) caribou (National Museum of Canada, 1962). Peary caribou populations are located in the Canadian Arctic, on islands north of the Northwest Territories (NT) and Nunavut (Mallory & Boyce, 2019). Barren-ground caribou herds migrate throughout vast areas of NT, Nunavut and Yukon, with historical ranges in Northern Alberta and Saskatchewan (COSEWIC, 2016). Grant's caribou, consisting mainly of the Porcupine herd, are found in Yukon and Alaska, and are also often classified as barren-ground caribou (Festa-Bianchet, Ray, Boutin, Côté, & Gunn, 2011). Woodland caribou are divided into two major ecotypes comprising boreal and mountain populations (Festa-Bianchet et al., 2011). Boreal woodland caribou populations span from British Columbia (BC) to Québec, while mountain woodland caribou mainly reside in BC, Yukon and NT (Festa-Bianchet et al., 2011). Many caribou populations in Canada are listed as either Threatened or Endangered (COSEWIC, 2016; Thomas & Gray, 2002). Recent observations of cervid populations in Saskatchewan show an overlap in habitat between white-tailed deer, including CWD-infected deer, and boreal caribou herds (Hebblewhite & Fortin, 2017; Hervieux et al., 2013; McLoughlin et al., 2019; Saskatchewan Ministry of Environment, 2013). Because CWD is becoming increasingly prevalent in free-ranging cervids in Saskatchewan (CWD map of positive tests, 2020; Kahn, Dubé, Bates, & Balachandran, 2004), this brings into light the possibility of CWD invading the *R. tarandus* populations in this province. All cervid species tested so far have been shown to be susceptible to CWD infection, albeit some only in experimental settings (Balachandran et al., 2010; Hamir et al., 2011; Mitchell et al., 2012; Moore et al., 2016; Nalls et al., 2013; Sigurdson et al., 1999). Furthermore, wild reindeer in Europe acquired CWD (Benestad et al., 2016). While as yet wild caribou in Canada are reportedly free from CWD, transmission is likely to happen naturally under current conditions.

The prion protein gene (*Prnp*) sequence is largely identical among cervid species, with only a small number of single nucleotide polymorphisms (SNPs) present in the gene pool (Robinson, Samuel, O'Rourke, & Johnson, 2012). Nevertheless, *Prnp* polymorphisms can modulate susceptibility to CWD (Angers et al., 2014; Green et al., 2008; Hannaoui, Amidian, et al., 2017; Hannaoui, Schatzl, et al., 2017; Johnson et al., 2011). Previous studies reported a *Prnp* polymorphism resulting in a substitution from serine to asparagine at codon 138 (S138N) unique to fallow deer (*Dama dama*) and reindeer/caribou (Hamir et al., 2011; Mitchell et al., 2012; Moore et al., 2016; Rhyan et al., 2011; Robinson et al., 2019). This polymorphism has been associated with lower susceptibility to CWD in both species under experimental conditions, depending on the route of prion infection (Mitchell et al., 2012; Moore et al., 2016; Rhyan et al., 2011). Fallow deer, naturally homozygous for asparagine at codon 138 (138NN), did not develop clinical disease nor accumulate detectable amounts of PrP^{Sc} in their CNS or lymphatic organs, after 6 years of exposure to CWD-infected mule deer (Rhyan et al., 2011). Nevertheless, fallow deer were susceptible to intracerebral CWD infection, where PrP^{Sc} and spongiform degeneration was detected in the CNS after a prolonged incubation period of 51 months post-infection, whereas deer usually succumb to the disease between 12–34 months (Hamir et al., 2011; Kahn et al., 2004). In two other studies, no PrP^{Sc} was found in the CNS of reindeer homozygous or heterozygous for asparagine at codon 138 (138SN or 138NN) infected peripherally with prions, with the exception of one animal (Mitchell et al., 2012; Moore et al., 2016). A prolonged CWD incubation period with the absence of typical clinical CWD symptoms was also seen in a 138SN reindeer infected orally (Mitchell et al., 2012). The S138N polymorphism is present in caribou herds in Alaska and Alberta, but not reported in wild reindeer in Norway so far (Cheng, Musiani, Cavedon, & Gilch, 2017; Güere et al., 2020; Happ, Huson, Beckmen, & Kennedy, 2007). Other *Prnp* polymorphisms in the

Rangifer species include codons V2M, G129S, N146n (synonymous substitution), V169M, N176D, S225Y, and a 24 base pair deletion in the octapeptide repeat region (Cheng, et al., 2017; Robinson et al., 2012; Wik et al., 2012). Recent studies reported that the prevalence of the 225S allele and a combination of the 2M-129S-169M variant was higher in CWD-positive wild reindeer from the Nordfjella region in Norway (Güere et al., 2020).

Here, we report the prevalence and distribution of *R. tarandus* prion protein polymorphisms obtained from 986 caribou samples from BC, Yukon, NT, Nunavut and Saskatchewan. We identified two novel prion protein polymorphisms, one in barren-ground (Y153F) and one in woodland (P242L) caribou. The frequencies of previously reported polymorphisms found in this study were not significantly different across herds and subspecies. At the herd level, the 138N allele was exceptionally prevalent in the Chinchaga boreal woodland caribou population in BC, in agreement with previous studies in Alberta (Cheng, Musiani, et al., 2017). When we excluded this woodland herd from the analyses, we found that codon 138 was significantly different between the two subspecies, with 138NN and 138SN genotypes being more frequent in barren-ground than woodland caribou. Notably, spatial differences were found among different herds, with the lowest prevalence of the 138N allele in woodland caribou of the mountain ecotype in BC, and the boreal woodland caribou herds in SK.

2 | MATERIALS AND METHODS

2.1 | Samples

We obtained a total of 986 frozen caribou blood samples or tissue from herds in BC (n = 397), NT/Nunavut (n = 462), Saskatchewan (n = 101) and Yukon (n = 26). These samples were collected between 2004–2018 and represent woodland and barren-ground caribou populations in western Canada (Figure 1). The NT/Nunavut samples consist of the barren-ground herds Bathurst (n = 87), Beverly and Ahiak (n = 107), Bluenose East (n = 90), Bluenose West (n = 50), Cape Bathurst (n = 27), Tuktoyaktuk Peninsula (n = 15) and woodland boreal herds Hay and Cameron (n = 54) and those from the North Slave region (n = 19). Yukon samples comprise of the Porcupine

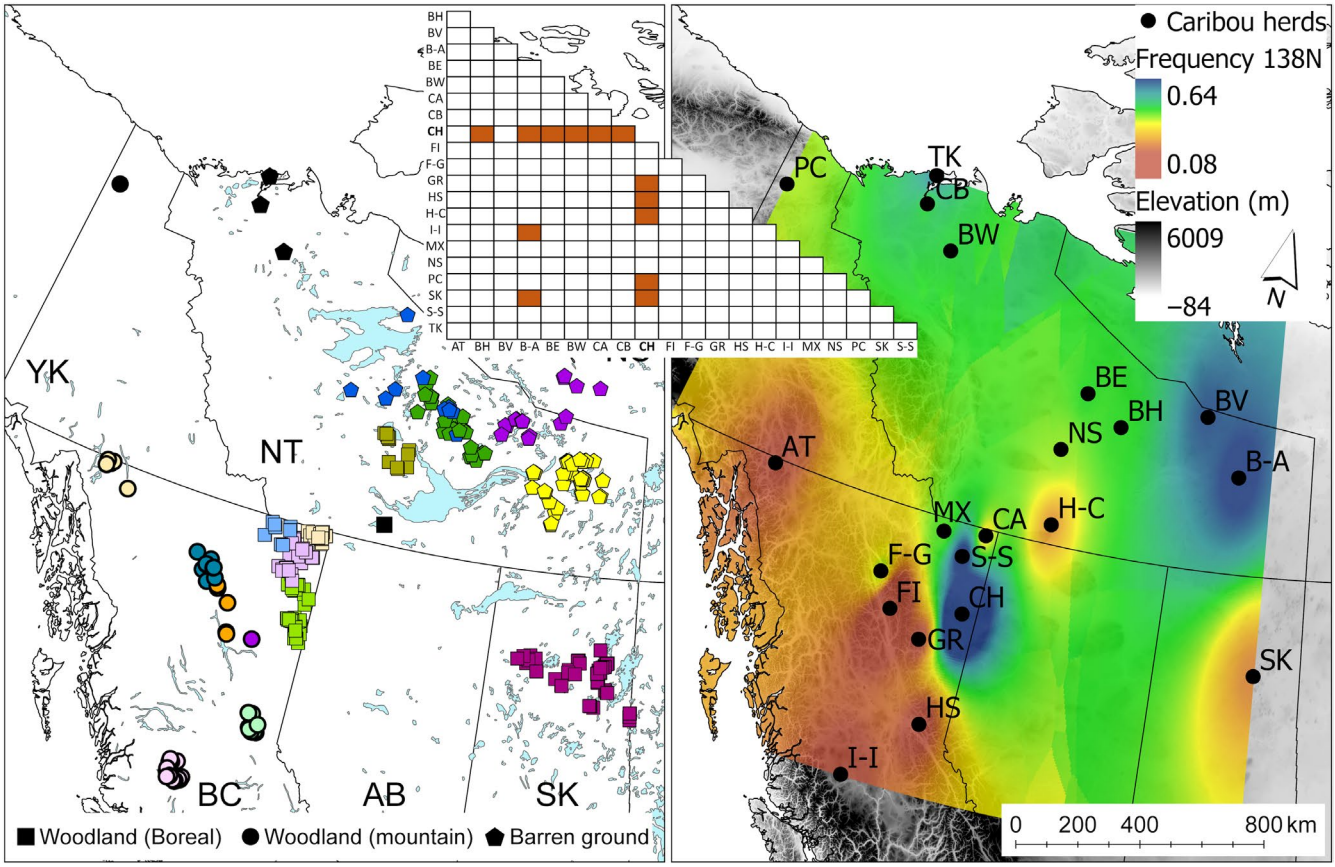

FIGURE 1 Left panel shows the distribution of caribou samples used in this study with colours representing the herds, and symbol shapes representing the subspecies/ecotype. Black symbols represent herds without individual coordinates. Top panel indicates significant pairwise genotypic comparisons among herds following p-value correction for multiple tests. Right panel is an interpolation of the 138N allele frequency across herds, individual coordinates were averaged for centroids. Herds are labelled using the following abbreviations: AT, Atlin; B-A, Beverly/Ahiak; BE, Bluenose east; BH, Bathurst; BV, Beverly; BW, Bluenose west; CA, Calendar; CB, Cape Bathurst; CH, Chinchaga; F-G, Frog/Gataga; FI, Finlay; GR, Graham; H-C, Hay/Cameron; HS, Hart south; I-I, Itcha-Ilgachuz; MX, Maxhamish; NS, North Slave; PC, Porcupine; SK, SK1; S-S, Snake-Sahtaneh; TK, Tuktoyaktuk

herd (*R. t. granti*) (n = 19), which will be included as barren-ground from here onwards, and woodland caribou of the mountain ecotype including Horseranch (n = 2), Little Rancheria (n = 2) and Carcross (n = 2) herds. BC woodland caribou herds included in this study were Calendar (n = 35), Chinchaga (n = 59), Parker (n = 3), Prophet (n = 9), Maxhamish (n = 37), Snake-Sahtaneh (n = 70), and Fort Nelson (n = 4) of the boreal ecotype and Muskwa (n = 6), Frog (n = 6), Itcha-Ilgachuz (n = 59), Wolverine (n = 10), Atlin (n = 26), Finlay (n = 8), Gataga (n = 8), Graham (n = 11), Pink Mountain (n = 17), and Hart South (n = 29) of the mountain ecotype. Out of the 986 samples, 14 animals did not have herd information.

2.2 | DNA extraction, polymerase chain reaction (PCR) and sequencing

We extracted DNA from frozen clotted blood using the MagMAX-96 DNA Multi-Sample Kit (ThermoFisher Scientific, cat #4413022) in a 96-well format following the manufacturer's protocol. The *Prnp* open reading frame in exon 3 was amplified using a protocol from Cheng, Musiani, et al. (2017). PCR reaction conditions were as follows: 10 μl of genomic DNA (equivalent to 100 ng of genomic DNA), 2 μl of 10 μM forward primer (5′- CCT AGT TCT CTT TGT GGC CAT GTG -3′ or 5′- GGG CAT ATG ATG CTG ACA CCC TCT TT -3′), 2 μl of 10 μM reverse primer (5′- TGA GGA AAG AGA TGA GGA GGA TCA C 3′ or 5′- GAG AAA AAT GAG GAA AGA GAT GAG GAG G -3′), 10 μl of 10x Pfu buffer (Agilent), 1 μl of 10 mM dNTP (Invitrogen), 0.5 μl of Pfu polymerase (Agilent) and nuclease-free water in a total reaction volume of 50 μl. Conditions for the PCR were 4 min of initial denaturation at 94°C, followed by 39 cycles of denaturation at 94°C for 30 s, annealing at 63°C for 30 s and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. The runs were performed in 96-well plates using the GeneAmp PCR System 9700 (Applied Biosystems) or the T100 (Bio-Rad). No-template controls were randomly included in every run to ensure no cross-contamination of samples. Amplified samples were run on a 2% agarose gel at 95V for 1 hr. Bands were visualized under UV light, excised using a clean blade, and amplified DNA was retrieved and purified using a commercial gel extraction kit (Qiagen, cat #28706). Sequencing of products were performed at Eton Bioscience (San Diego) with the first primer set used in the PCR reaction above.

2.3 | TOPO cloning

Gel-extracted PCR products from samples with novel SNPs detected in the chromatograms were cloned using the TOPO Zero Blunt cloning kit (ThermoFisher Scientific, cat #450245) and transformed into TOP10 cells (ThermoFisher Scientific, cat #C404010) according to the manufacturer's protocol. Ten colonies from each sample were screened for the *Prnp* insert using colony PCR with Taq DNA Polymerase (GenScript, cat #E00043) and the first primer set mentioned above. Plasmid DNA was extracted from positive colonies using a commercial kit (Omega Bio-tek, cat #D6945-2) and sent for sequencing at the University of Calgary core sequencing facility or Eton Bioscience (San Diego) using the common M13F and M13R primers present in the vector sequence.

2.4 | SNPs and statistical analyses

To find SNPs, both known and novel, caribou *Prnp* sequences were analysed using the sangeranalyseR package (Aneichyk et al., 2018; https://github.com/roblanf/sangeranalyseR) in R Studio and Geneious v.10.2.6 (Kearse et al., 2012; http://www.geneious.com). Statistical significance of genotype and allele frequencies between herds and subspecies were determined using using an exact G-test, chi-squared or Fisher's exact test in Genepop and/or the Genetics package (https://CRAN.R-project.org/package=genetics) in R Studio. *p*-values were adjusted using the *fdr* correction with the *p.adjust* function in R Studio. *Prnp* genotypes for each SNP were also analysed for deviations from Hardy-Weinberg Equilibrium, and for linkage disequilibrium using the Genetics package. To visualize the spatial variation in the frequency of 138N we generated a heat-map using the interpolation tool within ArcGIS Pro (ESRI Software). Centroid locations were estimated for herds without geographic coordinates, and we did not include herds with fewer than six individuals. Frog and Gataga herds were merged as they had small sample sizes, were spatially adjacent, and their genotype frequencies did not differ.

3 | RESULTS

Seven *Prnp* polymorphisms and a deletion from nucleotide (nt) 249 to 272 have been reported in caribou/reindeer (*R. tarandus* spp.). The polymorphisms are V→M at codon 2, G→S at codon 129, S→N at codon 138, N→n (synonymous substitution) at codon 146, V→M at codon 169, N→D at codon 176 and S→Y at codon 225 and (41,48,49,51). The 24 base pair (bp) deletion, the 176 N→D and the 225 S→Y polymorphisms have only been reported in European reindeer. All sequences from our North American caribou samples were homozygous for serine at codon 225 and none had the 24 bp deletion. The polymorphism at codon 2 is located in the signal peptide sequence of PrP^C, which is cotranslationally cleaved off, thus we excluded this codon from our analyses. Out of 986 samples, 708 successful reads were obtained for *Prnp* codon 129 (71.81%), 726 for codon 138 (73.63%), 697 for codon 146 (70.69%), 724 for codon 169 (73.43%) and 708 for codon 225 (71.81%). Only individuals with genotype data at codons 129, 138, 146 and/or 169 and herd data, and only herds with greater than five individuals, were included in the herd level analyses (n = 756). Statistical tests show that all alleles in the *Prnp* codons analysed here were in Hardy-Weinberg equilibrium (chi-squared and Fisher's exact tests), suggesting that none of these *Prnp* polymorphisms were under selection pressure. Pairwise linkage disequilibrium analyses also showed us that the majority of

SNP haplotype pairs, with the exception of codon 146, were genetically linked ($D' = 0.86–0.99$; $p < .05$). This is expected as these SNPs are located physically close to each other within the *Prnp* open reading frame (ORF) on exon 3.

We found two barren-ground caribou that were heterozygous for N and D at codon 176 (Figure S1). European reindeer are known to carry the D variant at codon 176, but this is the first report of its presence in caribou in North America. Furthermore, we found two novel *Prnp* SNPs that have not been previously reported in any species; a Y→F substitution at codon 153 in three NT barren-ground caribou (all heterozygous Y/F) and a P→L substitution at codon 242 in 12 BC woodland caribou (nine heterozygous P/L and three homozygous L/L). We performed TOPO cloning on all samples with possible SNPs to exclude any sequencing artifacts.

We assessed the genotypic differentiation between all caribou herds ($n = 21$ herds, with individuals >5 per herd, Frog-Gataga combined) at codons 129, 138, 146 and 169 in Genepop. Only codon 138 had significantly different pairwise comparisons following *p*-value correction; the majority of the significant comparisons were between Chinchaga and the other herds (Figure 1 and Table 1). We also performed Grubbs' test for outliers, and based on the N allele frequency at codon 138, the Chinchaga herd was determined an outlier amongst all caribou herds ($Z = 2.27$, $p < .05$). Mean allele frequency of 138N was 0.32 across all herds, while the frequency in the Chinchaga herd was 0.64. There was no significant difference between the barren-ground and woodland caribou subspecies at all codons (Table S1), but when we omitted the Chinchaga herd, the genotype frequencies at codon 138 between barren-ground and woodland populations differed significantly ($p < .01$) (Figure 2). In addition, codon 138 also significantly differed between barren-ground and woodland caribou of the mountain ecotype ($p < .05$) (Figure 2). These differences are highlighted in the heatmap for the 138N allele (Figure 1). We also show that individual animals can carry more than one polymorphic allele (Table 2). We report that the *Prnp* codon associated with reduced CWD susceptibility is found in higher frequencies in the Chinchaga woodland population, and higher in migratory barren-ground than woodland caribou populations when this herd is excluded from analyses.

4 | DISCUSSION

Currently, there have been no reports of CWD in wild caribou in North America. However, CWD has been identified in their wild relatives in Europe (reindeer) (Benestad et al., 2016). They are members of the same species, *R. tarandus*; hence, there is concern that CWD will expand, or has already transmitted, to caribou in the wild. The S138N *Prnp* polymorphism has been described in caribou and

TABLE 1 Herd allele frequencies at *Prnp* codon 138

Herd	Subspecies	Ecotype	Abbrev.	Sample size	Codon 138	
					N-freq	S-freq
Atlin	Woodland	Mountain	AT	9	0.222	0.778
Bathurst	Barren-ground	NA	BH	79	0.342	0.658
Beverly	Barren-ground	NA	BV	18	0.417	0.583
Beverly/Ahiak	Barren-ground	NA	B-A	77	0.435	0.565
Bluenose E	Barren-ground	NA	BE	83	0.343	0.657
Bluenose W	Barren-ground	NA	BW	46	0.348	0.652
Calendar	Woodland	Boreal	CA	34	0.294	0.706
Cape Bathurst	Barren-ground	NA	CB	26	0.346	0.654
Chinchaga	Woodland	Boreal	CH	51	0.637[a]	0.363
Finlay	Woodland	Mountain	FI	6	0.083	0.917
Frog/Gataga	Woodland	Mountain	F-G	11	0.318	0.682
Graham	Woodland	Mountain	GR	7	0.214	0.786
Hart South	Woodland	Mountain	HS	17	0.206	0.794
Hay/Cameron	Woodland	Boreal	H-C	45	0.256	0.744
Itcha-Ilgachuz	Woodland	Mountain	I-I	30	0.200	0.800
Maxhamish	Woodland	Boreal	MX	12	0.333	0.667
North Slave	Woodland	Boreal	NS	14	0.321	0.679
Porcupine	Barren-ground	NA	PC	19	0.316	0.684
SK woodland	Woodland	Boreal	SK	82	0.250	0.750
Snake-Sahtaneh	Woodland	Boreal	S-S	33	0.439	0.561
Tuktoyaktuk	Barren-ground	NA	TK	15	0.400	0.600

[a]Significantly different from most other herds.

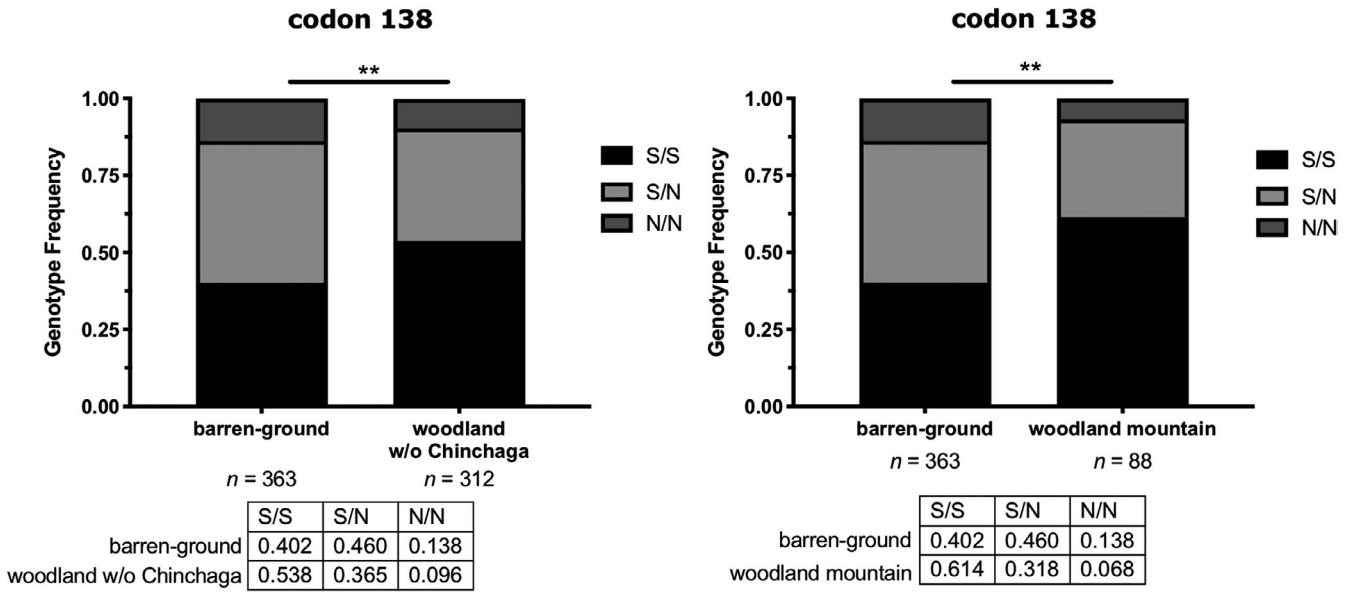

FIGURE 2 When the boreal woodland Chinchaga herd was omitted from statistical analyses, the difference in codon 138 between barren-ground and woodland caribou (left) and between barren-ground and woodland caribou of the mountatin ecotype (right) was statistically significant at a 5% significance level ($p = .002$ and .001, respectively). Tests were performed using a chi-squared test in the Genetics package in R Studio and graphs were generated in Graphpad Prism 8

TABLE 2 Number of individuals per haplotype group present in caribou herds in western Canada (codons 129/138/169; 146 is excluded as it is a synonymous substitution)

Caribou subspecies	GSV	GNV	GSM	GNM	SSV	SNV	SSM	SNM
Barren-ground (*R. t. groenlandicus*)	112	178	1	0	19	9	6	4
Woodland (*R. t. caribou*)	132	152	0	0	12	7	1	0

fallow deer (Cheng, Musiani, et al., 2017; Hamir et al., 2011; Happ et al., 2007; Wik et al., 2012) and the pseudogene of mule deer and white-tailed deer (Brayton, O'Rourke, Lyda, Miller, & Knowles, 2004; O'Rourke, 2004). Here, we report the prevalence of the S138N polymorphism in *Prnp* sequences of 726 caribou samples from two provinces (BC, Saskatchewan) and three territories (Yukon, NT, and Nunvaut) in western Canada. Our results give an overall picture of the 138N allele frequency being higher in the northern migratory barren-ground herds when compared to both boreal and mountain woodland caribou populations, with the exception of the boreal woodland Chinchaga herd. We also report the presence of the N176D polymorphism in caribou for the first time as well as two novel *Prnp* polymorphisms, Y153F and P242L. As the genotype frequencies in the other three polymorphisms (G129S, N146n and V169M) do not differ significantly between subspecies and herds, we will not discuss them further.

This S138N polymorphism is linked with strongly reduced susceptibility to clinical CWD upon natural routes of prion infection (Hamir et al., 2011; Mitchell et al., 2012; Moore et al., 2016; Rhyan et al., 2011). In fallow deer, where both alleles at codon 138 encode asparagine (138NN), peripheral exposure to CWD-infected deer did not result in disease (Rhyan et al., 2011). However, they are susceptible to intracerebral infection with white-tailed deer and elk CWD prions, with prolonged survival times ranging between 24 to 59 months post-infection (Hamir et al., 2011), indicating that 138N PrP^C per se can be converted into PrP^{Sc} and does not confer absolute resistance to prion infection. In reindeer, the 138N allele has been associated with reduced susceptibility to natural CWD infection routes in experimental settings. Only one animal had detectable prions in the obex, while in the rest prions were limited to peripheral organs (Mitchell et al., 2012; Moore et al., 2016). All reindeer that tested positive for CWD in the Nordfjella region in Norway are of the 138SS genotype, and the 138N allele has not been reported in that population of reindeer (Güere et al., 2020). In addition, the substitution from serine to asparagine at position 138 impacted in vitro prion conversion in a previous study (Raymond et al., 2000). Together, these data suggest this may be an important site for disease modulation. Structurally, serine is a small polar (hydrophilic) noncharged residue. An exchange to asparagine, which is a larger polar noncharged residue, can alter packing of side chains as serine may fit into pockets of bigger residues.

While the newly identified polymorphisms have no known exposure to CWD, we provide some insight into their potential role in disease susceptibility based on their locations and amino acid changes. The polymorphism at codon 242 is located in the C-terminal of the PrP^C sequence that is cotranslationally cleaved off when the GPI-anchor is added. Thus, it is unlikely that the P242L polymorphism plays a role in determining PrP^C properties that influence

the pathogenesis of CWD. The Y153F polymorphism is located within the first alpha-helix of PrP^C (Wopfner et al., 1999). Tyrosine (Y) is identical to phenylalanine (F) and only differ in a hydroxyl group present in tyrosine. A previous study reported that the YYR (Tyrosine-Tyrosine-Arginine) motif at position 149–151 in human PrP, corresponding to 152–154 in cervids, is exposed in the PrP^{Sc} conformation and constitutes a PrP^{Sc}-specific epitope (Paramithiotis et al., 2003). The Y153F substitution might impact recognition by PrP^{Sc}-specific antibodies developed based on the YYR motif, but whether it actually plays a role in PrP^C to PrP^{Sc} conversion is not known. The N→D polymorphism at codon 176, previously reported only in the Eurasian reindeer, was found in two NT barren-ground animals in this study. *R. tarandus* spp. is divided into two lineages based on microsatellite loci and mitochondrial DNA sequences (Yannic et al., 2014). The barren-ground populations belong to the Euro-Beringia clade that inhabit western Canada and Eurasia (Yannic et al., 2014). This shows the possibility that the *Prnp* gene variants are preserved within the lineage. Another explanation is the introduction of Eurasian reindeer into Alaska which were later herded into the Inuvik region in the 1930s, highlighting possibilities of introgression between caribou and reindeer in NT (Klein, 1980). Polymorphisms and their locations with respect to PrP^C are visualized in Figure 3.

Landscape features, such as major valleys and separation by rivers, have shown to be more accurate in describing caribou genetic structure than classification by subspecies and ecotype (Serrouya et al., 2012). We therefore examined genotypic differentiation at the herd level, finding Chinchaga was significantly different from most of the other herds at the 138 locus. Boreal woodland populations in the Chinchaga range, both in BC and Alberta, inhabit only the peatlands where lichen is abundant (Leech, Whittaker, & the Doig River First Nation, 2016; O'Leary, Saxena, & DeCoursey, 2002). Furthermore, the overlay with landscape motifs in Figure 1 indicates a possibility that the Chinchaga population in BC is located in a secluded habitat surrounded by higher elevation ground, which may result in reduced gene flow. These provide possible reasons for the high 138N allele prevalence in the Chinchaga caribou herd, where geographic isolation limits genetic flow to and from other adjacent herds. A similarly high 138N prevalence has also been previously reported in the Chinchaga caribou population in Alberta (Cheng, Musiani, et al., 2017). Although there are likely many factors impacting free-ranging caribou populations, we propose that the high 138N allele prevalence is advantageous should CWD expand to the Chinchaga population, as caribou in this area are at high risk of extinction (Leech et al., 2016; O'Leary et al., 2002).

Woodland boreal caribou in Saskatchewan are divided into two major populations, the northern SK1 range that inhabits the Boreal Shield and the southern SK2 range, further categorized into west, central and east, populating the Saskatchewan Boreal Plains (Saskatchewan Ministry of Environment, 2013). The SK2 range habitat overlaps with white-tailed deer, which are natural hosts of CWD (McLoughlin et al., 2019). Genetic information on these populations show that caribou in the SK2 range often migrate to the SK1 range but not vice versa, excepting high gene flow in the central range (Figure S2) (Priadka et al., 2018). Our results show that the majority of the SK1 range caribou harbor the wild-type 138SS *Prnp* genotype (97.5%), which is associated with susceptibility to natural CWD infection routes (Güere et al., 2020; Mitchell et al., 2012; Moore et al., 2016). Should these herds contract CWD, they will increase transmission probability to woodland and barren-ground caribou populations in NT. In this study, barren-ground caribou have significantly higher 138N *Prnp* allele frequencies (36.8%) than woodland caribou (27.9%), in particular of the mountain ecotype (22.7%), when the outlier Chinchaga herd was removed from the analyses. As barren-ground caribou migrate across vast areas of land (COSEWIC, 2016), they will pose a risk of widespread prion contamination if

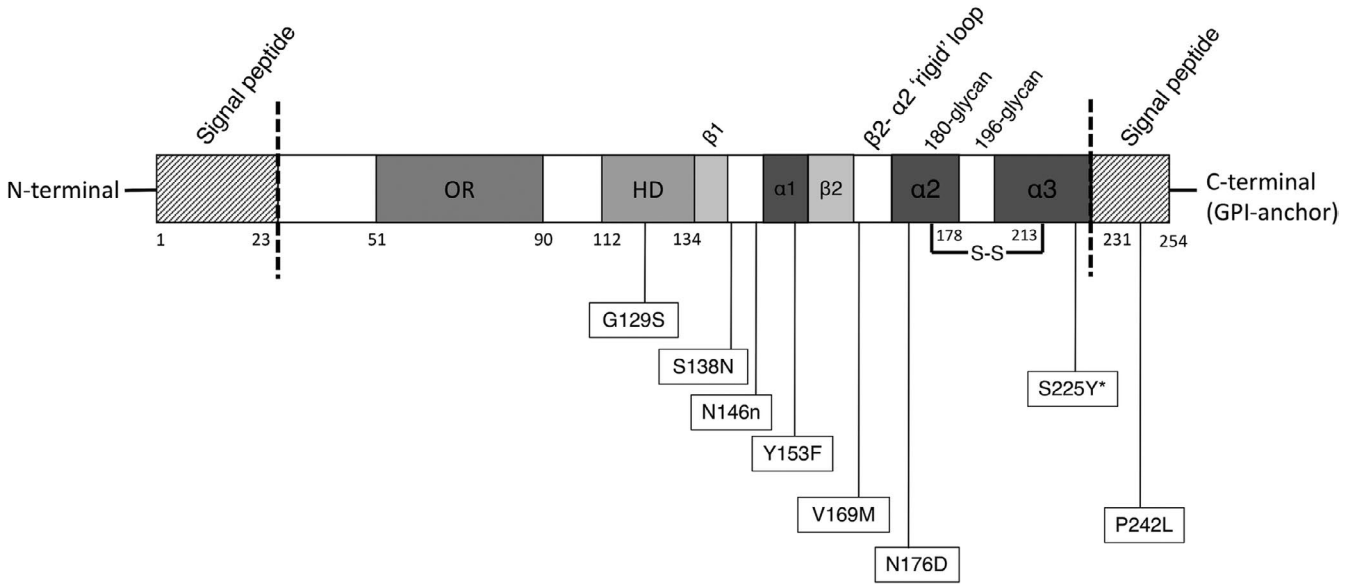

FIGURE 3 Positions of caribou polymorphisms reported in this study within the prion protein sequence. GPI, glycosylphosphatidylinositol; HD, hydrophobic domain; OR, octapeptide repeat. *All animals were homozygous serine (S) at codon 225

infected, eventually reaching the east and west coasts, and even Alaska.

Climate change and industrial development have reduced the size of remaining caribou habitat leading to range retraction and increased contact between caribou and other cervids (especially white-tailed deer) and predator species, either directly or indirectly (Bauduin, McIntire, St-Laurent, & Cumming, 2018; Dawe & Boutin, 2016; Hebblewhite & Fortin, 2017; Hervieux et al., 2013; Leech et al., 2016; Tennant et al., 2020). Predators have been shown to pass prions in their faeces after consuming CWD-infected cervids (Krumm, Conner, Hobbs, Hunter, & Miller, 2010; Nichols, Fischer, Spraker, Kong, & VerCauteren, 2015), and mountain lions preferentially prey on CWD-infected deer (Krumm et al., 2010), which further increases the probability of prion dissemination. Should wild caribou come into contact with these prions, an additional risk factor is imposed to their already Threatened status, as the disease has been shown to drive the decline of white-tailed and mule deer populations (DeVivo et al., 2017; Edmunds et al., 2016). Although CWD has not yet been detected in free-ranging caribou in Canada, transmission to these animals from infected captive or free-ranging cervids remains a concern for wildlife scientists and managers. Thus, CWD control measures and strategies for caribou are already part of current wildlife disease management guidelines (CWD map of positive tests, 2020; Gagnier, Laurion, & DeNicola, 2020; Williams, Miller, Kreeger, Kahn, & Thorne, 2002; Zimmer, Boxall, & Adamowicz, 2011). Our findings highlight herds with the highest susceptibility to CWD, assuming the 138N allele reduces susceptibility. Of the herds we examined, mountain caribou herds and those in the SK1 range in Saskatchewan are at the highest risk (Figure 1). We hope these findings help in mitigating CWD transmission to caribou by contributing to future decisions and planning of CWD surveillance and preventive measures in Canada.

In conclusion, the 138N *Prnp* allele, associated with less susceptibility to CWD, is found in caribou populations in Alaska (Happ et al., 2007), Alberta (Cheng, Musiani, et al., 2017), British Columbia, the Northwest Territories, Nunavut, Saskatchewan, and Yukon. It is more prevalent in barren-ground than woodland caribou populations, with statistical significance when the woodland Chinchaga herd was excluded from the analyses. This high 138N allele frequency in the Chinchaga range is probably due to landscape and geographic isolation. In the SK1 range in Saskatchewan, which overlaps with barren-ground caribou range, the 138N *Prnp* allele is present in much lower frequencies, thus increasing caribou susceptibility to contracting clinical CWD. This caribou population can serve as a gateway to possible CWD transmission from infected cervids, thus bringing into light the risk of CWD expansion into woodland and barren-ground caribou in western North America.

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AUTHOR CONTRIBUTIONS

M.I.A. wrote the paper. S.G. and M.I.A. designed the research. M.I.A., A.S., S.Y.S., and Y.H. performed the research. H.F., and P.D.M. provided samples. M.I.A., A.S., C.C., G.M., and S.G. analysed data. A.S., S.Y.S., H.F., P.D.M., C.C., G.M., and S.G. provided comments and edits to the manuscript.

DATA ACCESSIBILITY

Caribou ID, genotype, herd and capture coordinates are listed in Table S2. Novel *Prnp* polymorphisms are submitted to Genbank with accession numbers MT361766 and MT361767.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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