Characterizing the spatial patterns of chronic wasting disease susceptibility in white-tailed deer

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Executive Summary

White-tailed deer occupy a diverse array of landscapes and have been expanding their range in Ontario; this popular big-game species harbors an array of zoonotic and potentially zoonotic diseases. One notable disease is the transmissible spongiform encephalopathy known as chronic wasting disease (CWD). Two important developments have made monitoring the CWD prion of importance to Ontario: i) the detection of CWD in nearly all bordering jurisdictions; and ii) the recent transmission of CWD to nonhuman primates, suggesting human populations could be susceptible. There is a genetic basis (i.e. the PRNP gene) for the susceptibility and expression of symptoms of CWD in white-tailed deer, the spatial patterns of which could influence spread of the disease; however, the prevalence and spatial patterning of variants of the PRNP gene have not been characterized in Ontario. We sequenced the PRNP gene and quantified variation in white-tailed deer in Ontario. We spatially characterized this variation (main deliverable) and characterized variation relative to other regions with and without CWD. Variation in four known positions (referred to as SNPs) with links to reduced expression of CWD symptoms did not vary across the province and are consistent with populations that have no or recent detections of CWD. Our bioinformatic pipeline that streamlines analysis will be made publicly available, facilitating future assessments. We discuss how these data and information can be integrated into the OMNRF CWD surveillance program; specifically, we recommend continued sampling of tissue for CWD detection and continued genotyping in high-risk areas (i.e. southeastern Ontario). Further, we discuss how these findings can be used to better plan for the potential for future detections of CWD in Ontario.

Overview

Warming winters have permitted the steady northward expansion of wild animals and the zoonoses they harbour. White-tailed deer occupy a diverse array of landscapes and have expanded their range in Ontario over the last several decades; this popular big-game species harbours an array of zoonotic and potentially zoonotic diseases. One notable disease is the transmissible spongiform encephalopathy known as chronic wasting disease (CWD; Sigurdson 2008). Two important developments have made monitoring the CWD prion of utmost importance to Ontario: i) the detection of CWD in all but one bordering jurisdiction; and ii) the recent transmission of CWD to non-human primates, suggesting human populations could be susceptible. Moreover, CWD is 100% fatal in deer and in some states has resulted in die-offs of deer that could cause population declines. Considering that white-tailed deer are a commonly hunted species in Ontario, and a farmed game animal in the province, the transmission of CWD to deer in Ontario has the potential to cause dramatic economic impacts, with uncertain human health implications. A genetic basis for the susceptibility and expression of symptoms of CWD in white-tailed deer is known to exist (O'Rourke et al. 2004; Wilson et al. 2009). Specifically, variation at the PRNP gene has shown to cause variation in how quickly deer display symptoms of CWD, and how long they shed prions to the environment (Table 1). This variation, in turn, could influence the rate and nature of spread of CWD. The aim of this project was to characterize the prevalence and spatial patterning of genetic variation in the prion protein (PRNP) gene in order to assess the potential utility for informing surveillance for and future monitoring of CWD.

Spatial characterization of PRNP genetic variation across Ontario

Samples were taken from the OMNRF repository containing tissues samples from yearly CWD surveillance that rotates throughout the province using a risk-based model. No cervid farm with white-tailed deer volunteered to provide samples for this analysis. Samples from all the three regions encompassing the WMUs in which white-tailed deer are harvested most commonly were sampled systematically such that we had equal representation of each sex per WMU, and spatial representation of the entire province with sizeable white-tailed deer populations (Fig. 1). DNA was extracted from all samples and the PRNP gene was sequenced using a standardized assay made available by the University of Alberta. All sequencing was conducted at the University of Guelph. We developed a bioinformatics pipeline to rapidly assess, filter, and identify variants in the PRNP gene. This protocol meets the standards of human genomic studies and has been made publicly available (https://gitlab.com/WiDGeT_TrentU). A total of 631 Ontario samples passed quality control and were analyzed (Fig. 2; Table 2). No major differences were detected between the three regions in the prevalence of different variants of the PRNP gene (Fig. 2; Table 3). Novel variants were detected (Fig. 2), but we stress that their influence on CWD (e.g. Table 1) is currently unknown. The frequency of mutations known to influence CWD relative to other regions is shown in Table 2 and largely matches past work in areas with no or recent detections of CWD.

Implications for Ontario deer management and CWD surveillance

There have been no confirmed cases of CWD in white-tailed deer in Ontario. OMNRF conducts annual surveillance for the disease, that rotate across the province using a risk-based model. It has been suggested that CWD containment efforts could be informed through genetic testing and redirecting management resources accordingly (Brandt et al. 2016). Given the similarity in allele frequencies across the Ontario (Table 3), there is currently no information from this project that points to PRNP frequency

being used in the decision process as it pertains to CWD screening due to the lack of spatial variation. However, allele frequencies are known to vary in CWD positive vs CWD negative areas (Wilson et al. 2009; Brandt et al. 2016). This suggests that continued genetic screening might be warranted in high-risk areas as it could indicate (undetected) CWD on the landscape.

The findings of the research form the basis for next steps in better informing decision making about the control of any potential future CWD outbreaks. The lack of spatial variation in PRNP variants indicates that spread will follow natural movement pathways for white-tailed deer. The genetic information obtained from this work can be used to assess the natural gene flow of white-tailed deer in Ontario allows for the simulation of the most likely patterns of CWD spread throughout the province. This work constitutes next steps in this project that would be impossible without the baseline information collected so far.

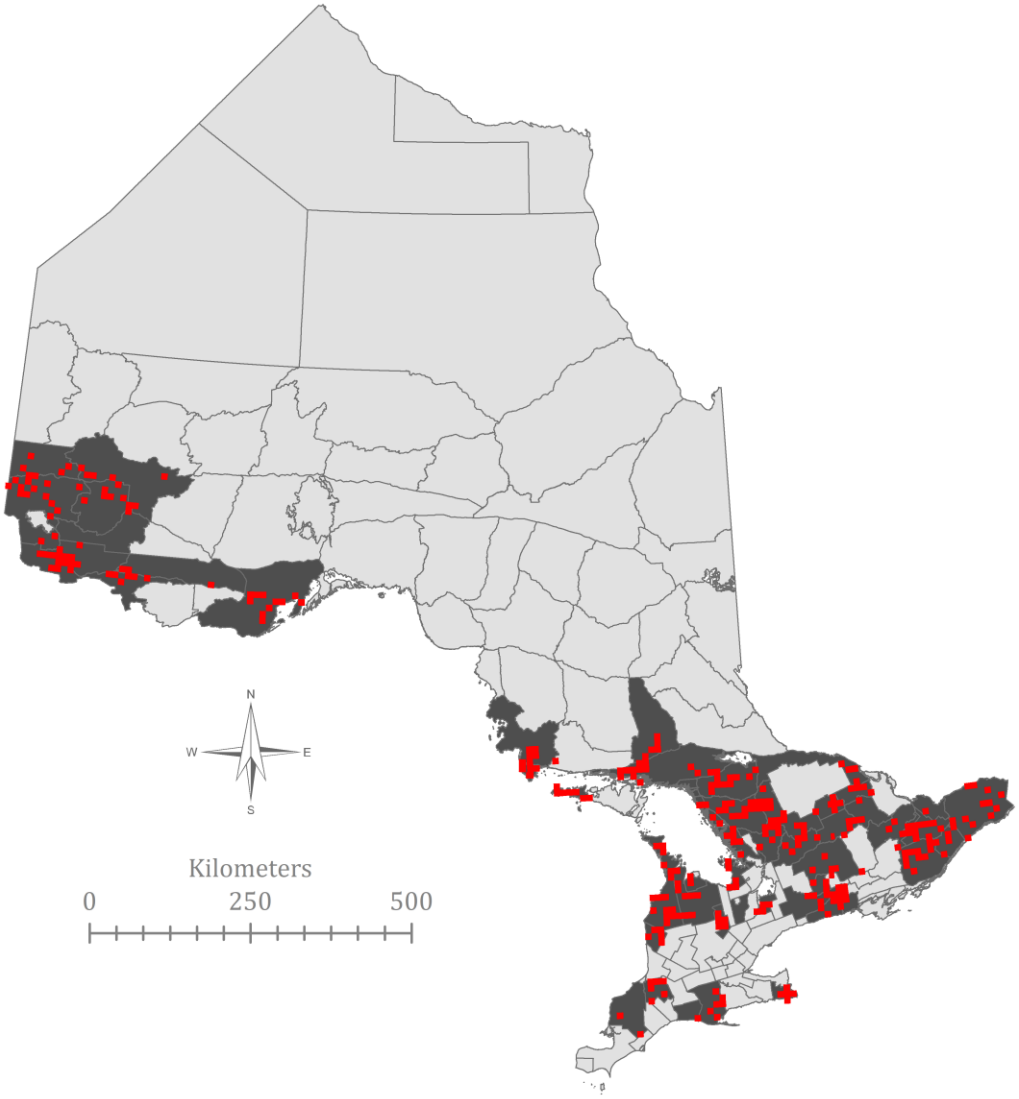

Figure 1. Sample sites (red) of white-tailed deer analyzed in this report (*n* = 631). Dark grey denotes WMUs with deer samples used in this study.

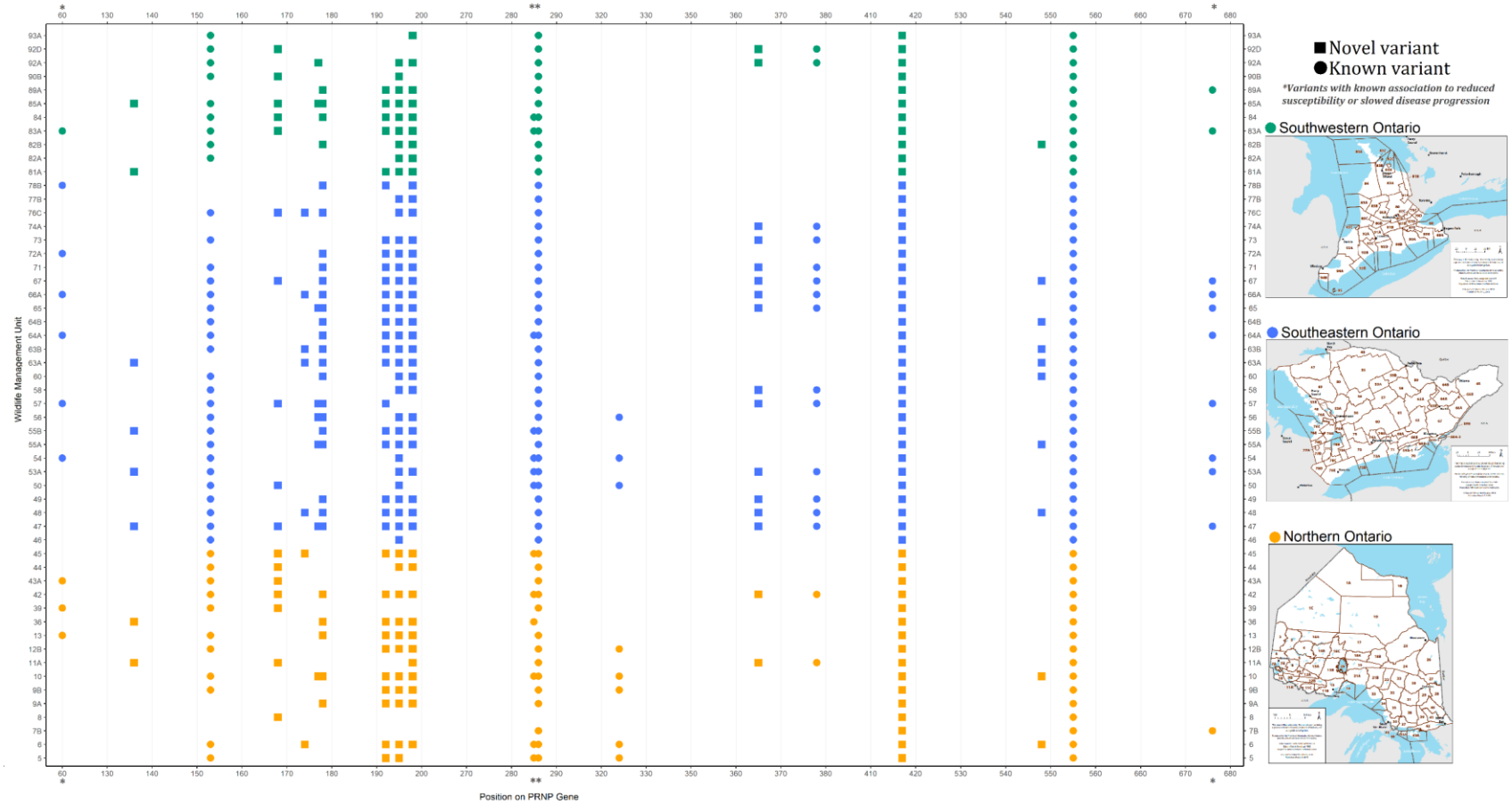

Figure 2. Overview of the PRNP genes and variants detected in 631 wild white-tailed deer samples from across Ontario

Table 1. Known alleles (genetic variants) on the PRNP gene that influence chronic wasting disease. The reference and alternate alleles and amino acids are provided.

Position	Ref	Alt	Codon	Ref	Alt	Influence
60	C	T	20	D	-	Reduced susceptibility. Displays linkage disequilibrium with nt285
285	A	C	95	Q	H	Reduced susceptibility
286	G	A	96	G	S	Reduced susceptibility & slower clinical progression of disease
676	C	A	226	Q	K	Involved with pathogenic process, protective against CWD

Table 2. Frequency of genetic variants (alleles, shown by nucleotide (nt) position) known to influence chronic wasting disease (CWD). For reference, two studies that assayed the same alleles in deer populations with CWD are shown. The reference allele frequency is shown on the left side of the hash (Ref/Variant); see Table 1.

Location	**Sample size**	**Collection year**	**Years with CWD at time of study**	**nt60**	**nt285**	**nt286**	**nt676**	**Citation**
ON, CA	631	2002-2017	0	0.979/0.021	0.970/0.030	0.661/0.340	0.962/0.038	-
AB & SK, CA	227	Not stated	AB=4, SK=13	0.94/0.06	0.99/0.01	0.74/0.26	0.98/0.02	Wilson et al., 2009
Illinois, USA	196	2002-2006	6	0.92.0.08	0.94/0.06	0.99/<0.01	0.99/0.01	Kelly et al., 2008

Table 3. Frequency of genetic variants (alleles, shown by nucleotide (nt) position) known to influence chronic wasting disease (CWD) in three Ontario regions. The reference allele frequency is shown on the left side of the hash (Ref/Variant); see Table 1.

Location	Sample size	nt60	nt285	nt286	nt676
Northern ON	189	0.984/0.016	0.963/0.037	0.698/0.302	0.989/0.011
Southeastern ON	314	0.975/0.025	0.981/0.019	0.624/0.376	0.955/0.045
Southwestern ON	128	0.984/0.016	0.953/0.047	0.695/0.305	0.938/0.063

References

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