Determining Which Codons Within the Prion Protein Gene (PRNP) Predict Susceptibility to Chronic Wasting Disease (CWD) of Cervid Populations in the United States.

Emma K. McDonald[1], Emily A. Wright[1], Emma K. Roberts[2], Asha E. Worsham[3], Warren C. Conway[4], Daniel M. Hardy[3], and Robert Bradley[1,5]

Department of Biological Sciences[1], Climate Sciences Center[2], Department of Cell Biology and Biochemistry, TTUHSC Graduate School of Biomedical Sciences[3], Department of Natural Resources Management[4], Natural Science Research Laboratory[5]

Introduction

- Chronic Wasting Disease (CWD) is a transmissible spongiform encephalopathy (TSE) that is highly contagious in deer.
- Previous studies have investigated amino acid and nucleotide substitutions and compared them to known susceptible codons which could help predict the degree of susceptibility.
- Known codons that identify reduced susceptibility have been found in White-Tailed deer (*Odocoileus virginianus and O. v. clavium*) include G96S and Q95H.
- New data suggests that a heterozygous allele (Y = C/T) at nucleotide 60 in White-Tailed deer (WTD) may heighten the degree of susceptibility.
- Previous studies suggest that a heterozygous state at codon 20 for mule deer (MD) greatly heightens the susceptibility to CWD.

Materials and Methods

Sampling

1. Obtain *PRNP* sequences from GenBank
2. Use deer samples from previous studies
3. Collect deer samples from unsampled localities

Lab Work

1. DNA extractions using buffy coat and the DNeasy kit
2. PCR and PCR cleanup using Exosap-IT
3. Cycle sequencing
4. Sephadex

Data analyses

1. Align DNA sequences using Mega XI
2. Check base calls using Sequencher
3. Use Baynesian & likelihood methods for phylogenetic analyses
4. Inspect nucleotide and amino acid sequences for substitutions and compare them to known susceptible codons

Management Implications

- The further spread of CWD in wild populations needs to be prevented. If the spread of CWD can be tracked using predicted susceptible codons, it will be easer to control.
- If susceptible codons can be identified within populations, there may be a potential solution by “breeding out” the known susceptible codons in deer, especially in TX.

Insight into the degree of susceptibility to CWD in deer will give a great advantage into knowing which populations are the most vulnerable. This can aid wildlife conservations and spread awareness to breeders about known susceptible codons. Additionally, warnings about the usage of deer in certain populations that have been predicted to have a high degree of susceptibility to CWD can be issued to hunters.

Objectives

- Examine the *PRNP* gene in populations of WTD and MD across the United States to find the appearance of nucleotide and amino acid substitutions (including both non-synonymous and synonymous).
- Further investigate the synonymous mutation of D at codon 20 in WTD for the prediction of susceptibility.
- Compare wild populations to breeder populations of deer.
- Predict the degree of susceptibility of populations and compare to standardized and novel CWD detection tests.

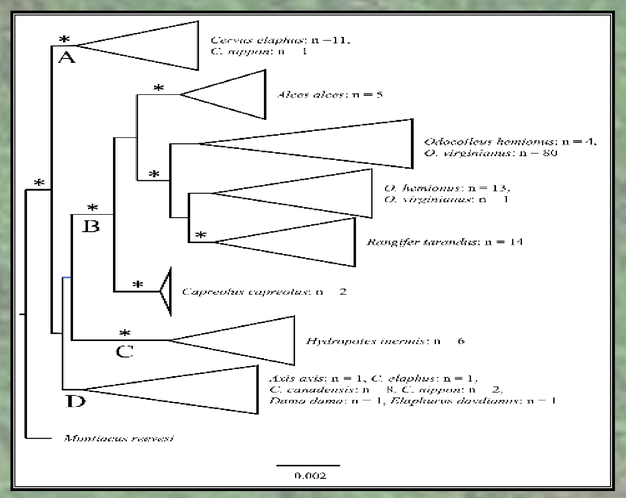

Figure 1. Phylogenetic tree generated from *PRNP* sequences and Bayesian inference analyses and the GTR+I+G model of evolution. Posterior probability values ≥ 0.95 are indicated by an asterisk and depict nodal support.

Preliminary Results

It appears that we have identified a synonymous substitution (nucleotide 60/codon 20) that predicts susceptibility to CWD. This needs to be confirmed by examining “known positive” samples as determined by the TVMDL testing method.

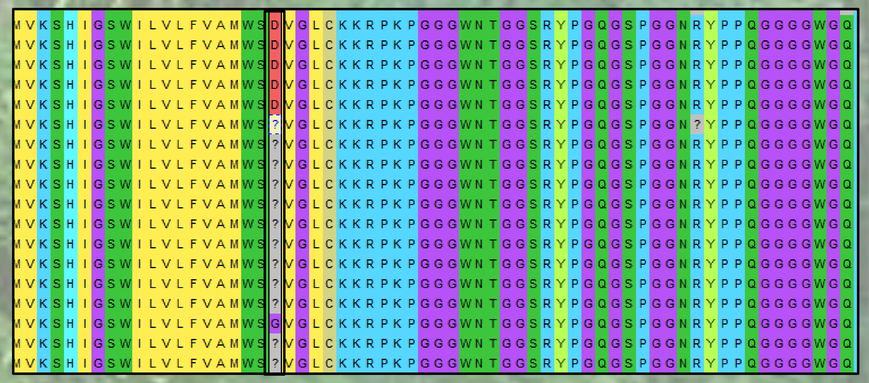

Figure 2. Question marks indicate individuals that are heterozygous at nucleotide 60 and demonstrate D, with either T or C.

Table 1. Individuals to test CWD-detected with biomarker test and the corresponding genotype.

	Nucleotide position	Codon	Major homozygote variants observed	Heterozygote variants observed	Minor homozygote variants observed
PRNP	60	20	CC	CT	TT
Biomarker test - CWD –			3	0	0
Biomarker test - CWD +			0	8	0
			Less likely to test positive	More likely to test positive	Most likely to test positive

Future Directions

- Use sampled localities to map changes to determine if geography influences the degree of susceptibility.
- Investigate 30 tissues samples (20 CWD + 10 CWD-) and compare to other findings by TVMDL and TTU biomarker test.
- Determine if the existence of synonymous mutations in humans, such as a single nucleotide polymorphism in exon 26 of the Multi-Drug Resistance 1 gene, and synonymous mutations in exon 3 of the PRNP gene in deer have similar deleterious consequences.

Acknowledgments

Thanks to M. Ashton who have assisted in lab work. Thanks to Julianna McDonald for help with editing. Thanks to the Natural Science Research Laboratory at TTU who provided samples for testing. Thanks to several state agencies (AK Department of Fish and Game; CA Department of Fish and Wildlife; GA Department of Natural Resources; ME Department of Inland Fisheries and Wildlife; MO Department of Conservation; MT Fish, Wildlife, and Parks; ND Game and Fish Department; SC Department of Natural Resources; TX Parks and Wildlife Department; WA Department of Fish and Wildlife; WI Department of Natural Resources) for assistance with tissue collection. Thanks to the Natural History Museum of Utah for a tissue loan. Special thanks to all hunters who knowingly or inadvertently contributed deer used in this study. Funding provided by the Bobby Baker Memorial Scholarship for Excellence in Scientific and Genomics Research.