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Prion protein gene sequence and chronic wasting disease susceptibility in white-tailed deer (Odocoileus virginianus)

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1 Prion protein gene sequence and chronic wasting disease susceptibility in white-tailed deer
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31
32
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34 Natural Resources; MCMC, Markov Chain Monte Carlo; *PRNP*, prion protein gene; PrP, Prion
35 protein; TSE, transmissible spongiform encephalopathy.
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45 Running title: Prion gene sequence of deer in the Illinois and Wisconsin CWD outbreak
46

47 **Abstract**

48 The sequence of the prion protein gene (*PRNP*) affects susceptibility to spongiform
49 encephalopathies, or prion diseases in many species. In white-tailed deer, both coding and non-
50 coding single nucleotide polymorphisms have been identified in this gene that correlate to
51 chronic wasting disease (CWD) susceptibility. Previous studies examined individual nucleotide
52 or amino acid mutations; here we examine all nucleotide polymorphisms and their combined
53 effects on CWD. A 626 bp region of *PRNP* was examined from 703 free-ranging white-tailed
54 deer. Deer were sampled between 2002 and 2010 by hunter harvest or government culling in
55 Illinois and Wisconsin. Fourteen variable nucleotide positions were identified (4 new and 10
56 previously reported). We identified 68 diplotypes comprised of 24 predicted haplotypes, with the
57 most common diplotype occurring in 123 individuals. Diplotypes that were found exclusively
58 among positive or negative animals were rare, each occurring in less than 1% of the deer studied.
59 Only one haplotype (C, odds ratio 0.240) and two diplotypes (AC and BC, odds ratios of 0.161
60 and 0.108 respectively) has significant associations with CWD resistance. Each contains
61 mutations (one synonymous nucleotide 555C/T and one nonsynonymous nucleotide 286G/A) at
62 positions reported to be significantly associated with reduced CWD susceptibility. Results
63 suggest that deer populations with higher frequencies of haplotype C or diplotypes AC and BC
64 might have a reduced risk for CWD infection – while populations with lower frequencies may
65 have higher risk for infection. Understanding the genetic basis of CWD has improved our ability
66 to assess herd susceptibility and direct management efforts within CWD infected areas.
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68 **Introduction**

69 Transmissible spongiform encephalopathies (TSEs) or prion diseases are fatal

70 neurological disorders caused by the misfolding of a common protein (PrP^{C}) into an infectious

71 conformation (PrP^{SC}). [1] Chronic wasting disease (CWD) is a TSE that occurs in free ranging

72 cervids, including white-tailed deer, mule deer, elk, and moose. [2, 3] Originally CWD was

73 described in the 1970s in northern Colorado and southern Wyoming[4, 5] and has since spread to a

74 number of other US states and Canadian provinces. [6, 7] The first case of CWD detected east of

75 the Mississippi river was in Wisconsin in 2002 [8] and then Illinois later that year. [9-12]

76 CWD is transmitted horizontally (and possibly vertically [13]) within white-tailed deer by

77 pathogenic prions shed from the infected host in blood, saliva, urine and feces. [14, 15] Furthermore,

78 prions have been shown to persist in the environment potentially remaining infectious and

79 causing CWD infection long after affected deer have dispersed. [16-18] Cross-species infection of

80 TSEs are rare but fatal, most notably being variant Creutzfeldt-Jakob disease which is the result

81 of bovine spongiform encephalopathy transmission to humans. [19] Chronic wasting disease is not

82 known to affect humans; though, there is no consensus among researchers about the possibility

83 of human infection. Studies using mouse [20] and primate [21] models suggest a strong barrier to

84 human disease transmission. However, under certain experimental conditions cervid PrP^{SC} is

85 capable of converting human PrP^{C} to produce PrP^{SC}. [22, 23]

86 The *PRNP* gene was shown to affect prion disease susceptibility and progression in

87 several species. [10, 24-29] Because CWD is influenced by the expressed protein [1], many studies

88 have focused on the inferred amino acid sequence of *PRNP*. In white-tailed deer two

89 polymorphisms at amino acid positions (aa) Q95H and G96S have been detected that are

90 associated with reduced disease susceptibility. [26, 27, 30] In a captive deer herd in Nebraska,

91 individuals with at least one copy of serine (S) at aa96 were less likely to test positive for CWD.
92 [30] Similarly, free ranging deer in Wisconsin were found to have reduced susceptibility to CWD
93 among individuals with a histidine (H) at aa95 or one copy of aa96S; however, complete genetic
94 resistance was not detected and further analysis linked aa96S to slowed disease progression. [26, 27]
95 Analyses of the *PRNP* nucleotide sequences corroborated the significance of aa95 and aa96, but
96 also revealed that synonymous mutations were associated with CWD susceptibility. [10, 28]
97 Furthermore, the cumulative number of nucleotide deviations (both synonymous and non-
98 synonymous) from the database derived consensus sequence for *PRNP* was found to have a
99 negative correlation with the probability of CWD infection among white-tailed deer. [10]
100 A majority of the studies to this point have focused on single locus polymorphisms and
101 CWD susceptibility, with few considering naturally occurring diplotype sequences. One study
102 examined four amino acid genotypes consisting of the Q95H and G96S loci (QQ/GG, QQ/GS,
103 QQ/SS, and QH/GG) among free ranging white-tailed deer in Wisconsin, finding only genotypes
104 with at least one copy of S less likely to be CWD positive. [29] It is important to note that only two
105 amino acid loci were examined and synonymous changes were not addressed. Here we examine
106 the nucleotide sequence of the *PRNP* gene comparing all observed combinations of synonymous
107 and non-synonymous mutations in white-tailed deer. In this study we explore the relationship
108 between *PRNP* diplotypes and CWD disease status with the goal of better understanding disease
109 susceptibility.
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114 **Results**

115 *PRNP* sequences were determined for 703 deer by PCR and Sanger sequencing: 579
116 tested for CWD (105 testing positive, 474 for which CWD was not detected) and 124 that were
117 not tested. Analyses of disease risk were performed using a reduced dataset (N= 240) consisting
118 of deer originating in counties with more than five cases of CWD confirmed by government
119 monitoring between 2002-2010 (henceforth referred to as the "CWD infection area"; Figure 1).
120 Additional tested and untested samples were obtained from areas with low risk of CWD
121 (counties with fewer than five or no confirmed cases at the time of sampling) to better detect
122 sequence variations, haplotypes, and diplotypes. Statistical significance was determined by
123 logistic regression using the most frequent haplotype, diplotype, genotype, or nucleotide as the
124 reference level. Within the analyzed 626 bp region of the *PRNP* gene 14 variable positions were
125 identified, 10 previously reported [10, 28] and four novel sites (299G/A, 308A/T, 367G/A, and
126 372G/A). Of the 14 variable sites six are non-synonymous (three novel and three previously
127 reported) and result in a change to the amino acid sequence (Table 1). It is important to note that
128 mutations at nt299, nt308, and nt367 (aa100S/N, aa103N/I and aa123A/T respectively) are of
129 interest as the human equivalents (aa97, aa100, and aa120 respectively) are in close proximity to
130 polymorphisms associated with prion disease susceptibility in humans. [31-33]

131 Haplotypes were generated from unphased sequences using PHASE v2.1. [34, 35] Twenty-
132 four haplotypes were predicted from 703 deer (N=1406 possible haplotype copies), with
133 haplotype A occurring most frequently (Table 1). Nine haplotypes are found exclusively among
134 negative (haplotypes L, Q, S, T, and W), positive (haplotype R) or untested deer (haplotypes U,
135 V, and X); however, each of these haplotypes is rare with a frequency of occurrence less than 1%
136 (Table 1). Seventeen haplotypes occurred within the CWD infection area and only haplotype C is

137 significantly less likely to be found among deer infected with CWD (P < 0.001, OR = 0.240 and
138 95% CI = 0.104-0.503) (Table 2).

139 A total of 68 unique diplotypes were identified among all sampled deer (including both
140 tested and untested individuals from all sampled areas, N=703). Diplotype AB is the most
141 frequently detected, occurring in 123 deer (positive, negative, and untested deer; Table 3). One
142 diplotype (BF) is found exclusively among negative deer; nine individuals (1%) carried this
143 diplotype, of which two are found within the CWD infection area (Table 3 and 4). Fifty
144 diplotypes are considered rare having a frequency of occurrence less than 1% and 38 of these are
145 found exclusively among positive, negative or untested deer (3, 26, and 9 diplotypes
146 respectively). Diplotypes AC and BC are significantly less likely to be found among deer
147 infected with CWD (P < 0.05). Odd ratios for these diplotypes are 0.161 and 0.108 (95% CI =
148 0.024-0.654 and 0.006-0.623) respectively (Table 4).

149 Previous studies determined that polymorphisms aa95H and aa96S (nt285C and nt286A
150 respectively) were significantly associated with reduced CWD susceptibility [10, 26-29]. We
151 reexamined these positions within our data (including only deer from the CWD infection area,
152 N=240), confirming aa96S as having a significant effect in reducing infection. We observed no
153 difference in infection between deer with aa95H or aa95Q (nt285A or nt258C; N = 12 and 468
154 respectively; P = 0.076). However, CWD is less common among aa96S deer than aa96G deer
155 (nt286A and nt286G respectively; N = 58 and 422 respectively; P < 0.001, OR= 0.295, 95% CI =
156 0.146-0.556) (Table 5). Furthermore, deer aa96 heterozygous are significantly less likely to be
157 CWD positive compared to deer aa96G homozygous (P = 0.001, OR = 0.247, 95% CI = 0.104-
158 0.552) (Table 5). Among the observed two locus genotypes (aa 95/96) only QQ/GS is significant
159 (P < 0.001), having a reduced odds ratio (OR =0.247, 95% CI = 0.101-0.542)(Table 5). It is

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160 important to note that while we did not observe a significant difference in infection among deer
161 with the aa95H allele or aa96SS genotype, susceptibility might only be evident with a larger
162 sampling providing greater statistical power.
163
164
165 **Discussion**

166 In this study, we find reduced susceptibility to CWD infection among white-tailed deer
167 with haplotype C (Table 2). We still observed individual deer positive for CWD with this
168 haplotype, demonstrating a reduced susceptibility rather than a complete genetic resistance as is
169 seen with other TSEs (e.g. scrapie [36, 37]). This haplotype had two different polymorphisms, 1
170 synonymous and 1 non-synonymous, both reported to be associated with decreased infection;
171 nt286A (aa96S) and nt555T [10, 26, 29, 30]. Other haplotypes have similar mutations at nt286 and
172 nt555 (eg. haplotypes I, Q, and S); though, within the CWD infection area these haplotypes are
173 not found at all (haplotype Q), occur infrequently ($f < 0.01$, haplotypes I and S), or are found
174 exclusively among positive deer (haplotype I). A number of other haplotypes have the same
175 mutations at either nt286 or nt555; again most are absent (haplotypes H, V, W and X), infrequent
176 ($f < 0.01$, haplotypes N and P), or are found abundantly among positive deer (haplotype B) in the
177 CWD infection area (Table 2). Rarity of these haplotypes prevents any meaningful association
178 with changes in susceptibility (Table 2). The effects of mutations at nt286 and nt555 alone or in
179 concert are unclear as other haplotypes with these polymorphisms occur infrequently and with
180 varied susceptibility. An even larger sampling may be necessary to resolve this interaction.

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181 Neither haplotypes with aa95H (nt285C) had a significantly reduced susceptibility to
182 CWD (Table 2). Some previous studies reported the occurrence of this mutation among CWD
183 negative deer only, which was interpreted as CWD resistance. [26, 29] In this study and in the study
184 by Kelly *et al.* [10] the aa95H mutation was found among deer positive for CWD; however, we
185 find in a larger sampling (N=240) the frequency of aa95H to be lower than that found by Kelly
186 *et al.* [10] and not significantly associated with resistance. We cannot preclude the importance of
187 this mutation given that a significant difference in disease susceptibility may be possible with an
188 even larger sample size providing greater statistical power (data not shown).

189 The presence of aa96S has been associated with slowed disease progression, longer life
190 span among captive deer, [26, 27] and does not appear to affect the rate at which prions are shed
191 from infected individuals. [38] Additionally, CWD infected mule deer have been found to excrete
192 pathogenic prions while asymptomatic. [39] This contributes to concerns that wild deer with aa96S
193 may be shedding infectious prions into the environment for longer periods of time than deer
194 lacking the mutation, but are not symptomatic or detectable by immunohistochemical
195 procedures. On the other hand, studies using epidemiological modeling suggest that deer with
196 aa96S under certain conditions may have a selective advantage for CWD resistance over those
197 without. [40] With our data, we are unable to make accurate conclusions about detection, longevity,
198 or increased risks of exposure to infectious prions. Nonetheless, our results do corroborate the
199 importance of the polymorphism at G96S in reduced CWD susceptibility (Table 5). [26, 30]

200 Kelly *et al.* [10] found a negative correlation between the number of nucleotide deviations
201 from the *PRNP* consensus sequence and CWD infection. The database derived consensus
202 sequence reported is the same as the most common haplotype (haplotype A) in this study (Table
203 1). Haplotype C has two deviations from haplotype A; other haplotypes were found containing

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204 more deviations but were exceedingly rare (Table 1). These haplotypes (namely haplotypes I, N,
205 Q, S, and X) were largely absent among CWD positive deer (only two positive deer were found
206 each with a single copy of haplotype I) and their combined frequency was less than 1%. An
207 increased number of polymorphisms may improve resistance to CWD, but the large sample size
208 of this study (N=703) suggests that haplotypes with more than two nucleotide deviations are rare
209 and would not be likely to have an appreciable effect on resistance or susceptibility within the
210 population.

211 Examination of *PRNP* diplotypes revealed that individuals with at least one copy of
212 haplotype C (specifically AC and BC) were less likely to test positive for CWD (Table 4). Other
213 diplotypes containing at least one copy of haplotype C (mutations at aa96S and nt555T) had a
214 low frequency of occurrence (< 1%); therefore, individually these less frequent diplotypes may
215 not be significant for CWD resistance but they could play a vital role in decreasing population-
216 level susceptibility by increasing the frequency of the C haplotype over time through inheritance
217 (i.e. herd immunity). Under ideal circumstances, determining genetic association with disease
218 status is examined under controlled experimental conditions to account for all confounding
219 factors; [41, 42] though, this is not always possible when studying free ranging animals. To address
220 this, other studies have attempted to use matched-case or paired-case control design to increase
221 the likelihood that samples have a similar genetic background. [29] For this study, perfectly paired
222 samples were not obtainable due to the nature of sampling through management and hunter
223 harvest. Nonetheless, negative deer were selected from available samples to match with positive
224 deer on the basis of age, sex, and geographic origin to minimize any potential bias. Additional
225 samples were randomly selected outside of the CWD infection area. To avoid spurious results,
226 statistical analyses were restricted to deer originating in the infected area as these animals are

227 more likely to have been exposed to the disease than deer from counties without identified cases
228 of CWD. The relationship between *PRNP* sequence and CWD status was found in multiple
229 geographic locations at distances greater than the average home range of Illinois white-tailed
230 deer [43-45] (i.e. deer with haplotype C were not restricted to one county and were found throughout
231 the study area), suggesting that relatedness and family groups were not a confounding factor and
232 that these results are a strong indication of low genetic susceptibility.

233 The *PRNP* gene is variable within all species with some mutations affecting susceptibility
234 to TSEs. [46-48] Scrapie infection in sheep is the classic example of genetic resistance to a prion
235 disease, where individuals with two copies of amino acid sequence V136, R154, Q171 are
236 susceptible to scrapie, and those with two copies of the sequence A136, R154, R171 are
237 resistant. [36, 37] Changes in the protein coding sequence have been shown to affect the ability of
238 pathogenic prions to convert normal prion proteins [31]; accordingly, many studies have heavily
239 examined the amino acid variations associated with CWD. Synonymous or silent mutations are
240 often overlooked, but may have a greater effect on protein expression and conformation than
241 expected. [49-51] [52, 53] Other studies have found significant associations between individual
242 synonymous mutations and CWD susceptibility. [10, 28] The specific mechanisms involved between
243 nucleotide variation (specifically synonymous mutations) and CWD are not known, but the rate
244 at which PrP^{C} conformations that are more favorable to PrP^{SC} conversion are produced may be
245 slowed by the presence of certain synonymous mutations. [51] Due to the low frequency of
246 haplotypes with similar mutations as haplotype C, we cannot accurately conclude whether or not
247 the specific combination of mutations or any one mutation alone is responsible for reduced CWD
248 susceptibility. Nevertheless, haplotype and diplotype analyses provide more insight in gene-

249 disease association than those restricted to alleles and genotypes [54] which are unable to detect
250 additive effects.

251 A solid understanding of the genetics of CWD in white-tailed deer is vital to improve
252 management of CWD on the landscape. Most TSEs are found in domestic or captive animals
253 where management of infected individuals is feasible. For example, scrapie infected flocks can
254 be handled through a process generally involving genetic testing, removal and destruction of
255 infected or suspect animals, followed by decontamination of facilities and equipment. [55]
256 Containment of free ranging deer in wild populations potentially infected with CWD and
257 decontamination of the environment is not reasonably possible. The long term effects of CWD
258 are not yet known but it is conceivable that an unmanaged infected population would be
259 gradually extirpated as the disease progresses [56, 57] or at least reduced to low densities with high
260 disease prevalence. [58, 59] Either outcome would have severe ecological effects (e.g. deer play a
261 major role in affecting plant communities [60] and as a prey source [61, 62]) as well as negative
262 economic impacts to hunting. Overall disease prevalence has remained at relatively low levels in
263 Illinois compared to Wisconsin.[11] It is important to note that at the time of sampling, CWD had
264 been found in six Illinois counties and has since been detected in 14.[9] Complete eradication of
265 CWD among free ranging white-tailed deer may not be possible; however, an active containment
266 effort in Illinois appears to have prevented significant increases in prevalence.[9,11,12] Further
267 examination of *PRNP* haplotype and diplotype frequencies across northern Illinois and southern
268 Wisconsin in conjunction with population structure and movement [45, 63, 64] will be useful in
269 identifying localities with greater or reduced susceptibility risk. Effectiveness of CWD
270 containment efforts can be aided through genetic testing and redirecting management resources.
271

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272 **Materials and Methods**

273 *Deer Sampling and CWD Testing*

274 Seven hundred three samples were collected between 2002 and 2010 from wild free-
275 ranging white-tailed deer in Illinois and southern Wisconsin from both public hunting and
276 government culling. For Illinois samples, obex and retropharyngeal lymph nodes were tested
277 using USDA approved immunohistochemical (IHC) procedures to detect protease-resistant prion
278 protein (PrP^{SC}) at the Illinois Department of Agriculture Diagnostic Laboratories in Galesburg or
279 Centralia and most positives were confirmed at the National Veterinary Services Laboratory.
280 Untested samples originated from areas where CWD had not been detected or where there was a
281 low risk at the time of sampling; these were included to determine the extent of *PRNP*
282 variability. Tissues samples (skeletal muscle, mainly tongue) were archived for both CWD
283 positive and negative deer. Wisconsin samples were tested for CWD by the Wisconsin
284 Veterinary Diagnostic Laboratory by IHC or ELISA based procedures with all positives
285 confirmed by IHC. At the time of sampling, detailed information including location (1.6 x 1.6
286 km area), sex, and age was recorded. Deer for this study were selected from a larger sampling;
287 those originating outside of the CWD infected area were chosen randomly. Within the infected
288 area to minimize bias, CWD negative deer were selected to match with positive deer on the basis
289 of age, sex, and geographic origin.

290

291 *PRNP Amplification and Sequencing*

292 Genomic DNA was isolated from skeletal muscle using the Wizard Genomic DNA
293 purification kit (Promega, Madison, WI) following the manufacturer's recommended protocol. A
294 626 bp region of the PRNP gene was amplified by polymerase chain reaction using previously

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295 published primers CWD-13 and CWD-LA [65] or primers 223 and 224. [30] Amplification was
296 performed in 40 ul reaction volumes following previously published protocols. [10, 65]
297 PCR amplicons were purified using the Wizard SV Gel and PCR Clean-Up System
298 (Promega, Madison, WI). Products were then sequenced using the BigDye Terminator system
299 (ABI), purified, and resolved on an ABI 3730XL DNA Sequencer at the University of Illinois
300 Keck Center for Functional and Comparative Genomics. The software *Sequencher* (Gene Codes
301 Corporation, Ann Arbor, MI) was used to edit and concatenate sequences. The identities of DNA
302 sequences were confirmed using NCBI BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi)
303 and variable positions were identified by comparison to published DNA sequence. Open reading
304 frames were confirmed and sequences translated in MEGA v6.0. [66] Sequences were checked for
305 the absence of the aa138 mutation to ensure that all sequences were *PRNP* and not the processed
306 pseudogene [26]; asparagine (N) at aa138 (nt413A) would indicate amplification of the
307 pseudogene. If aa138N was detected with primers CWD-13 and CWD-LA, then the sequence
308 was verified with primers 223 and 224 which were specifically designed to only amplify the
309 functional gene.[30] Though it is possible that this mutation could also occur in the functional gene,
310 we did not observe aa138N in any deer when both primer sets were used.
311
312 *Analysis*
313 Haplotypes were generated from unphased sequences using PHASE v2.1. [34, 35] Markov
314 chain Monte Carlo (MCMC) samples were taken from a minimum of 100,000 steps, with a
315 discarded burn-in of 10,000; samples were drawn every 100 MCMC steps. Five repetitions were
316 performed and haplotype frequencies compared to verify consistent assignment. Logistic
317 regression was calculated for haplotype, diplotype, genotype, or nucleotide, with each variable

318 treated as categorical data and the most frequent for each as the reference level. Disease status
319 was binary, with infected deer as one and uninfected deer as zero. Odds ratios were calculated
320 for significant variables (alpha 0.05); ratios less than one were considered to have reduced CWD
321 susceptibility. All calculations were performed in R version 3.0.0 [67] with R Studio v0.98.1083. [68]
322
323
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332
333
334 **Conflict of interest:**

335 The authors have declared that there is no conflict of interest.
336
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338

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522 **Figures and Tables**

523

524 **Figure 1.** Map of Illinois (orange) and Wisconsin (pink), showing the study area for samples

525 collected between 2002 and 2010. Samples were collected from all counties in grey by hunter

526 harvest or government culling. Counties within the CWD infection area (at least five confirmed

527 cases of CWD during the sample period) are darkly shaded; statistical analyses of CWD

528 susceptibility were restricted to individuals originating from these locations thus increasing the

529 probability of disease exposure. Number of samples from each county is indicated below the

530 county name.

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Table 1. Variable nucleotide positions for reconstructed haplotypes within the 629bp region of the *PRNP* gene.

Haplotype	60	153	243	285	286	299*	308*	324	367*	372*	378	438	555	676	N_m	f	(+)	(-)	NT
A	C	C	T	A	G	G	A	A	G	G	G	C	C	C	-	0.30	76	282	70
B	C	C	T	A	G	G	A	A	G	G	G	C	T	C	1	0.25	75	219	55
C	C	C	T	A	A	G	A	A	G	G	G	C	T	C	2	0.16	9	173	47
D	C	T	T	A	G	G	A	A	G	G	G	C	C	C	1	0.12	30	110	28
E	C	C	T	A	G	G	A	A	G	G	G	T	C	C	1	0.05	6	45	15
F	T	C	T	C	G	G	A	A	G	G	G	C	C	C	2	0.03	2	38	9
G	T	C	T	A	G	G	A	A	G	G	G	C	C	C	1	0.03	5	33	3
H	C	C	T	A	G	G	A	A	G	A	G	C	T	C	2	0.01	0	13	6
I	C	C	A	A	A	G	A	A	G	G	G	C	T	C	3	0.01	2	3	9
J	C	C	T	A	G	G	A	G	G	G	G	C	C	C	1	0.01	1	8	0
K	T	C	T	A	G	G	A	A	G	G	G	C	C	A	2	< 0.01	0	4	1
L	C	C	T	A	G	G	A	A	A	G	G	C	C	C	1	< 0.01	0	4	0
M	C	C	T	A	G	A	A	A	G	G	G	C	C	C	1	< 0.01	0	3	1
N	T	C	T	C	A	G	A	A	G	G	G	C	C	C	3	< 0.01	0	3	1
O	T	T	T	A	G	G	A	A	G	G	G	C	C	C	2	< 0.01	1	3	0
P	C	C	T	A	A	G	A	A	G	G	G	C	C	C	1	< 0.01	1	2	0
Q	C	C	T	A	A	G	A	A	A	G	G	C	T	C	3	< 0.01	0	2	0
R	C	T	T	A	G	G	A	A	G	G	G	C	T	C	2	< 0.01	2	0	0
S	C	C	T	A	A	A	A	A	G	G	G	C	T	C	3	< 0.01	0	1	0
T	C	T	T	A	G	G	A	A	G	G	A	C	C	C	2	< 0.01	0	1	0
U	C	T	T	A	G	G	T	A	G	G	G	C	C	C	2	< 0.01	0	0	1
V	C	T	T	A	A	G	A	A	G	G	G	C	C	C	2	< 0.01	0	0	1
W	T	C	T	A	A	G	A	A	G	G	G	C	C	C	2	< 0.01	0	1	0
X	T	C	A	A	A	G	A	A	G	G	G	C	C	C	3	< 0.01	0	0	1

Haplotypes were generated from unphased sequences in PHASE v2.1. Nucleotide positions are based on Kelly et al. 2008. Non-synonyous mutations are shaded, and the four novel mutations are indicated by asterisks. N_m is the number of nucleotide deviations from haplotype A, which is the most abundant haplotype among all sampled deer. f is the frequency of haplotypes among all sampled deer. The number of haplotype copies (N=1406) are shown among (+) CWD positive deer, (-) CWD negative deer, and (NT) deer that were not tested for CWD.

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538 **Table 2.** Disease association for unique *PRNP* haplotypes
539 among deer within the CWD infection area.

Haplotype	*f*	(+)	(-)	P-val	Odds Ratio
A	0.34	76	87	-	-
B	0.31	75	74	0.513	-
C	0.11	9	43	< 0.001	0.240 (0.104-0.503)
D	0.11	30	22	0.166	-
E	0.05	6	17	0.070	-
F	0.02	2	9	0.086	-
G	0.02	5	5	0.836	-
I	< 0.01	2	0	0.992	-
J	< 0.01	1	3	0.408	-
K	< 0.01	0	2	0.992	-
M	< 0.01	0	2	0.992	-
N	< 0.01	0	1	0.995	-
O	< 0.01	1	2	0.651	-
P	< 0.01	1	1	0.924	-
R	< 0.01	2	0	0.992	-
S	< 0.01	0	1	0.995	-
T	< 0.01	0	1	0.995	-

540 Haplotypes were generated from unphased sequences in PHASE v2.1. To
541 avoid spurious results, this analysis includes only deer from the CWD
542 infection area (counties with at least five confirmed cases of CWD). Only
543 haplotypes that occurred in the CWD infection area are shown. *f* is the
544 frequency of each haplotype. The number of haplotype copies (N=480) are
545 shown among (+) CWD positive deer and (-) CWD negative deer. Odds
546 ratios and 95% confidence intervals (parentheses) are shown for significant
547 parameters ($P < 0.05$) determined by logistic regression against haplotype
548 A, as it occurs most frequently among the sampled deer.
549

550 **Table 3.** Frequency of *PRNP*
551 diplotypes among all sampled
552 white-tailed deer.

Diplotype	*f*	(+)	(-)	NT
AB	0.17	32	73	18
AA	0.10	13	49	7
AC	0.08	2	40	14
BC	0.08	1	38	14
AD	0.07	8	32	10
BB	0.06	12	26	5
BD	0.06	12	22	6
CC	0.04	1	25	4
CD	0.03	1	18	3
AE	0.03	3	10	6
AF	0.02	2	9	4
DD	0.02	4	10	1
AG	0.02	1	10	1
BE	0.02	1	10	0
EC	0.02	1	8	2
BG	0.01	3	7	0
BF	0.01	0	9	0
DG	0.01	1	7	1
Rare (N=50)	< 0.01	7	71	28

553 Diplotypes were determined from unique
554 *PRNP* sequences. *f* is the frequency of
555 diplotypes among all sampled deer. The
556 number of deer (N=703) with each
557 diplotype are shown for (+) CWD positive
558 deer, (-) CWD negative deer, and (NT)
559 deer that were not tested for CWD. Fifty
560 diplotypes were considered rare, each
561 occurring in less than 1% of the total
562 sampled deer and are summarized
563 collectively.
564

565 **Table 4.** Disease association with *PRNP* diplotypes among white-tailed
566 deer within the CWD infection area.

Diplotype	*f*	(+)	(-)	P-val	Odds Ratio
AB	0.26	32	31	-	-
AA	0.12	13	15	0.701	-
BB	0.08	12	8	0.474	-
BD	0.08	12	7	0.346	-
AC	0.06	2	12	0.023	0.161 (0.024-0.654)
AD	0.06	8	6	0.668	-
BC	0.04	1	9	0.040	0.108 (0.006-0.623)
AE	0.03	3	4	0.691	-
CD	0.03	1	5	0.144	-
BE	0.02	1	4	0.216	-
BG	0.02	3	2	0.693	-
CC	0.02	1	4	0.216	-
DD	0.02	4	1	0.237	-
AF	0.02	2	2	0.975	-
EC	0.02	1	3	0.339	-
AI	0.01	2	0	0.995	-
BR	0.01	2	0	0.995	-
BF	0.01	0	2	0.995	-
BO	0.01	0	2	0.995	-
EJ	0.01	0	2	0.995	-
EF	0.01	0	2	0.995	-
PC	0.01	1	1	0.982	-
DG	0.01	1	1	0.982	-
Rare (N=15)	< 0.01	3	12	> 0.050	-

567 Diplotypes were determined from unique *PRNP* sequences. To avoid spurious results,
568 this analysis includes only deer from the CWD infection area (counties with at least
569 five confirmed cases of CWD). Only diplotypes that occurred in the CWD infection
570 area are shown. *f* is the frequency of each diplotype. The number of deer (N=240)
571 with each diplotype are shown for (+) CWD positive deer and (-) CWD negative deer.
572 Odds ratios and 95% confidence intervals (parentheses) are shown for significant
573 parameters ($P < 0.050$) determined by logistic regression against diplotype AB, as it
574 occurs most frequently among the sampled deer. Rare diplotypes (N=15) each
575 occurred in less than 1% of deer in this reduced dataset and are summarized
576 collectively.
577

578 **Table 5.** Confirmation of *PRNP* nucleotide positions 285 and 286 (amino acid
579 positions 95 and 96) previously reported as significant for reduced CWD
580 susceptibility.

Locus	Nt	AA	*f*	(+)	(-)	P-val	Odds Ratio
Allele							
285	A	Q	0.975	208	260	-	-
	C	H	0.025	2	10	0.076	-
286	G	G	0.879	198	224	-	-
	A	S	0.121	12	46	< 0.001	0.295 (0.146-0.556)
Single Position Genotype							
285	AA	QQ	0.954	103	126	-	-
	AC	QH	0.041	2	8	0.139	-
	CC	HH	< 0.01	0	1	0.987	-
286	GG	GG	0.796	95	96	-	-
	GA	GS	0.167	8	32	0.001	0.253 (0.104-0.552)
	AA	SS	0.038	2	7	0.127	-
Two Position Genotype							
285/286	AA/GG	QQ/GG	0.758	93	89	-	-
	AA/GA	QQ/GS	0.163	8	31	< 0.001	0.247 (0.101-0.542)
	AA/AA	QQ/SS	0.033	2	6	0.169	-
	AC/GG	QH/GG	0.033	2	6	0.169	-
	AC/GA	QH/GS	0.004	0	1	0.991	-
	AC/AA	QH/SS	0.004	0	1	0.991	-
	CC/GG	HH/GG	0.004	0	1	0.991	-

581 To avoid spurious results, this analysis includes only deer from the core CWD infection area
582 (counties with at least five confirmed cases of CWD). Nt is the nucleotide at each position,
583 and AA is the resulting amino acid for each nucleotide mutation. *f* is the frequency of each
584 variable. The number of alleles (N=480) or genotypes (N=240) is shown for (+)CWD positive
585 deer and (-)CWD negative deer. Odds ratios and 95% confidence intervals (parentheses) are
586 shown for significant parameters ($P < 0.05$) determined by logistic regression.
587
588
589