Accurate Genomic Predictions for Chronic Wasting Disease in U.S. White-Tailed Deer

Christopher M. Seabury,*1 David L. Oldeschulte,* Eric K. Bhattarai,* Dhruti Legare,* Pamela J. Ferro,*
Richard P. Metz,* Charles D. Johnson,* Mitchell A. Lockwood,* and Tracy A. Nichols**

*Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas, †Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, Texas, ‡Genomics Core, Texas A&M AgriLife Research, College Station, Texas, §Texas Parks and Wildlife Department, Austin, Texas, and **USDA-APHIS-VS-Cervid Health Program, Fort Collins, CO

ABSTRACT The geographic expansion of chronic wasting disease (CWD) in U.S. white-tailed deer (Odocoileus virginianus) has been largely unabated by best management practices, diagnostic surveillance, and depopulation of positive herds. Using a custom Affymetrix Axiom single nucleotide polymorphism (SNP) array, we demonstrate that both differential susceptibility to CWD, and natural variation in disease progression, are moderately to highly heritable ($h^2 = 0.337 \pm 0.079 - 0.637 \pm 0.070$) among farmed U.S. white-tailed deer, and that loci other than PRNP are involved. Genome-wide association analyses using 123,987 quality filtered SNPs for a geographically diverse cohort of 807 farmed U.S. white-tailed deer ($n = 284$ CWD positive; $n = 523$ CWD non-detect) confirmed the prion gene (PRNP; G96S) as a large-effect risk locus ($P$-value < 6.3E-11), as evidenced by the estimated proportion of phenotypic variance explained (PVE $\geq 0.05$), but also demonstrated that more phenotypic variance was collectively explained by loci other than PRNP. Genomic best linear unbiased prediction (GBLUP; n = 123,987 SNPs) with k-fold cross validation ($k = 3$; $k = 5$) and random sampling ($n = 50$ iterations) for the same cohort of 807 farmed U.S. white-tailed deer produced mean genomic prediction accuracies $\geq 0.81$; thereby providing the necessary foundation for exploring a genomically-estimated CWD eradication program.

The fatal wasting syndrome known as chronic wasting disease (CWD) was first observed among captive mule deer (Odocoileus hemionus) and black-tailed deer (Odocoileus hemionus columbianus) within several Colorado wildlife facilities during the late 1960s, and histologically recognized as a prion disease by the late 1970s (Williams and Young 1980; Moreno and Telling 2018). Thereafter, CWD was described in free-ranging U.S. mule deer (Odocoileus hemionus columbianus; hereafter WTD) and moose (Alces alces shirasi), with subsequent diagnostic surveillance suggesting an irrevocable geographic expansion of the disease among farmed and free-ranging populations of these species (Moreno and Telling 2018; Gavin et al. 2019; Osterholm et al. 2019). At present, at least 26 U.S. states and multiple Canadian provinces are known to be affected by CWD (Moreno and Telling 2018; Gavin et al. 2019; Osterholm et al. 2019). Likewise, Norway, Finland, and the Republic of Korea have also reported CWD in free-ranging reindeer (Rangifer tarandus; Norway), moose (Norway, Finland), and imported elk (Korea) (Moreno and Telling 2018; Gavin et al. 2019; Osterholm et al. 2019). The implementation of modern best management practices, including containment and depopulation of positive herds, has not prevented the emergence of CWD in new geographic areas (Moreno and Telling 2018; Gavin et al. 2019; Osterholm et al. 2019). Therefore, a need currently exists to develop novel strategies to reduce the prevalence of CWD among farmed deer and elk.

MATERIALS AND METHODS

Study overview

Herein, we investigate differential susceptibility to CWD among farmed U.S. WTD by utilizing genomic DNA samples from CWD positive and CWD non-detect WTD to perform next-generation sequencing with variant prediction for the construction and validation of a medium density SNP array. Thereafter, we use the array in conjunction with PRNP genotypes to conduct genome-wide association
analyses (GWAA’s) and produce marker-based heritability estimates. Finally, we conclude our study by utilizing the genome-wide SNP data to deploy genomic prediction equations with k-fold cross validation to assess the potential for developing a genomically-estimated CWD eradication program.

Animal resources, CWD diagnostics, and DNA isolation

Frozen whole blood samples (n = 448) and rectal biopsies (n = 37) from farmed U.S. white-tailed deer (WTD; both sexes) were available within an existing USDA APHIS repository that was created via federal CWD surveillance activities; including depopulations of CWD positive herds (USDA APHIS, Fort Collins, CO). All herds included both CWD positive (n = 256) and CWD non-detect (n = 229) WTD positive herds (USDA APHIS, Fort Collins, CO). All herds included from farmed U.S. white-tailed deer (WTD; both sexes) were available to assess the potential for developing a genomically-estimated CWD eradication program.

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TAG ATC TCG GTG GTC GCC GTA TCA TT-3'; Hind_T, 5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACC XXX XXX XAC ACT CTG TCA CAC GAC GCC GCT CCA TTT CA-3'; Pst_T, 5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACC XXX XXX XAC ACT CTG TCA CAC GAC GCC GCT CCA TTT CA-3'; Hind_B, 5'-AGC TAG ATC GGA AGA TCG TGG TGT AGG GAA AGA GTG TXT XXX XXX GTG TAG ATC TCG GTG GTC GCC GTA TCA TT-3'; Pst_B, 5'-AGA TCG GAA GAG CTG GTG GTA GGG AAA GAG TGT XXX XXX XAG TGT AGA TCT CCG TGG TCG CCT CAT TAC T-3'; Adaptor-B_T, /5Phos/GAT CGG AAG AGC ACA GCT CTG AAC TCC AGT CAC-3'; Adaptor- B_B, 5'-GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TAC TAC-3'; P5_Select, /5Biotin/TEG/AAT GAT ACG GCG ACC ACC GAG ATC TAC AC-3'; Flp-F1: 5'-ATG GAT ACG GCG ACC ACC GAG ATC TAC AC-3'; Flp-R1: 5'-CAA GCA GAA GAC GGC ATA CGA GAT-3'; TDX, 5'-CAA GCA GAA GAC GGC ATA CGA GAT XXX XXT GAT TCG AGT AGT CTA-3'.

Sequence analysis and affymetrix axiom array design
All Illumina sequences were trimmed for quality and adapters using CLC Genomics Workbench 10.1.1 (Qiagen), as previously described (Halley et al. 2014; Halley et al. 2015; Sollars et al. 2017). All trimmed reads were mapped to the WTD genome assembly (GCF_002102435.1 Ovir.te_1.0; https://www.ncbi.nlm.nih.gov/assembly/GCF_002102435.1/) using the CLC Genomics Workbench 10.1.1 reference mapping algorithm (Seabury et al. 2011; Halley et al. 2014; Halley et al. 2015; Sollars et al. 2017). Variant prediction was performed using a probabilistic approach implemented within CLC Genomics Workbench 10.1.1 (Halley et al. 2014; Halley et al. 2015; Oldeschulte et al. 2017; Sollars et al. 2017). This algorithm estimates error probabilities from quality scores, and uses these probabilities to determine the most likely allele combination per nucleotide position, thus facilitating a user-specified minimum probability threshold (P ≥ 0.95) for variant prediction, and variant quality scores (Halley et al. 2014; Halley et al. 2015; Oldeschulte et al. 2017; Sollars et al. 2017). Additional variant prediction parameters and filters were similar to those recently described (Seabury et al. 2011; Oldeschulte et al. 2017), and the probabilistic approach produced evidence for 6,268,706 variants, which included 5,561,550 putative SNPs (P ≥ 0.95; Minor Allele Frequency ≥ 0.01). Variant prediction results were exported from CLC as a single variant call formatted file (VCF), which was used for SNP array design. Briefly, the VCF file was filtered according to the Affymetrix Axiom myDesign guidelines for SNP submission (http://www.affymetrix.com/support/technical/byproduct.affx/prodcept= axiom_custom_agrigenomics) using a custom python script as follows: Retain only bialleic SNPs with minimum depth = 10, maximum depth = 150, minimum minor allele frequency (MAF) ≥ 0.15, minimum SNP quality score = 30, identify probe overlaps for exclusion, and prioritize variants that maximize array density (i.e., A/T and C/G take up two spaces on the array). The python script as well as more detailed documentation are available in Additional File 30 (DRYAD). The targeted number of SNPs for submission to Affymetrix was ≥ 200,000; to facilitate internal Affymetrix scoring (VCF), which was used for SNP array design.

PRNP and affymetrix axiom array genotyping
PRNP genotyping for missense variants at codons 37, 95, 96, 116, and 226 was performed at GeneSeek Neogen (Lincoln, NE) as part of an existing commercial genotyping by sequencing (GBS) service. Briefly, the functional PRNP gene was PCR amplified using primers designed to be exclusionary to a processed pseudogene as previously described (O’Rourke et al. 2004), and the resulting amplicons were purified via AMPure XP beads as recommended by the manufacturer (Beckman Coulter); thus allowing for the creation of barcoded Illumina Nextera XT DNA libraries and amplicon sequencing on an Illumina MiSeq. PRNP genotypes were called from the aligned read pileups at GeneSeek Neogen, and delivered in text format. Affymetrix Axiom 200K genotyping was also performed at GeneSeek Neogen using the established Affymetrix best practices workflow; with genotypes delivered in text format. Affymetrix quality control thresholds were DQC ≥ 0.82, QC call rate ≥ 97%, passing samples in the project ≥ 95%, and average call rate for passing samples ≥ 97%. Collectively, 860 WTD samples with the desired metadata (i.e., sex, age, U.S. general region) passed all Affymetrix QC filters; each with 125,968 SNP array genotypes, and paired PRNP genotypes, thus yielding a combined set of 125,973 SNP genotypes for analysis. Fifty-three WTD did not have CWD diagnostic data at the time of study. SNPs which did not convert on the Affymetrix Axiom 200K WTD array were primarily due to call rates below the best practices threshold (n = 37,197), and failures to meet thresholds in two or more best practices criteria (n = 36,045). All SNP conversion types are comprehensively summarized in DRYAD (Additional File 32).

GWAS and genomic prediction with cross validation
Prior to all analyses, a comparative marker map was created by aligning the WTD PRNP sequence and all Affymetrix Axiom 200K probe sequences with ARS-UCD1.2 (GCA_002263795.2) via blastn, thus providing comparative evidence for the origin of the array SNPs (i.e., autosomal vs. non-autosomal); which was necessary because the draft de novo WTD genome assembly (GCF_002102435.1 Ovir.te_1.0) is unanchored (i.e., by maps or in situ hybridization). The comparative marker map was joined to the combined set of all genotypes (PRNP + Affymetrix Axiom array), and quality control analyses were performed in SVS v8.8.2 or v8.8.3 (Golden Helix), Initially, pairwise IBS distances were computed to identify twins and duplicate samples. Eight samples present in both repositories (USDA APHIS, TPWD) were purposely duplicated for use as process controls, and correctly identified by IBS/IBD estimates (Additional File 31 in DRYAD). Eight additional samples were also identified as either duplicates or potential twins. In all cases, only the sample with the highest call rate was retained for further analyses. Additional quality control analyses and filtering were as follows: sample call rate (< 97% excluded), and thereafter, SNP filtering by call rate (> 15% missing excluded), MAF (< 0.01 excluded), polymorphism (monomorphic SNPs excluded), and Hardy-Weinberg Equilibrium (excludes SNPs with HWE P-value < 1e-25), which yielded 123,987 SNPs for all analyses. PRNP SNPs which failed to endure quality control filtering included codons 95 (MAF < 0.01) and 116 (monomorphic), whereas codons 37, 96, and 226 remained. All GWAA’s and genomic predictions with k-fold cross validation were performed on 807 WTD with recorded metadata that included sex, age, U.S. general region of origin (i.e., Midwest, Northeast, South), and CWD diagnostics. CWD phenotypes used in all analyses were: CWD Scores (0 = non-detect, 1 = lymph node positive, 2 = lymph node and obex positive); CWD Binary (0 = non-detect, 1 = lymph node positive and/or obex positive). However, at the time of study, one WTD (sample ID: MQ6Q) only possessed diagnostic data for a
CWD positive tonsil biopsy, and thus was assigned a CWD Score and a CWD Binary phenotype of “1.” All WTD GWAA’s were performed using a mixed linear model with variance component estimates, as described and implemented in EMMAX, and executed in SVS v8.8.2 or v8.8.3 (Golden Helix), where all genotypes are also recoded as 0, 1, or 2, based on the incidence of the minor allele (Kang et al. 2010; Segura et al. 2012; Seabury et al. 2017; Smith et al. 2019). Briefly, the general mixed model can be specified as:

$$y = Xf \beta + Zu + \epsilon$$

where $y$ represents a $n \times 1$ vector of observed CWD phenotypes, $X$ is a $n \times m$ matrix of fixed effects, $\beta$ is a $f \times 1$ vector representing the coefficients of the fixed effects, $u$ represents the unknown random effect, and $Z$ is a $n \times t$ matrix relating the fixed effect to the CWD phenotypes of interest (Kang et al. 2010; Segura et al. 2012; Seabury et al. 2017; Smith et al. 2019). Herein, it is necessary to assume that $\text{Var}(u) = \sigma^2_u K$ and $\text{Var}(\epsilon) = \sigma^2_\epsilon I$, whereby $\text{Var}(y) = \sigma^2_u ZKZ^T + \sigma^2_\epsilon I$, but in this study $Z$ represents the identity matrix $I$, and $K$ represents a relationship matrix of all WTD samples. To solve the mixed model equation using a generalized least squares approach, we must first estimate the relevant variance components (i.e., $\sigma^2_u$ and $\sigma^2_\epsilon$) as previously described (Kang et al. 2010; Segura et al. 2012; Seabury et al. 2017; Smith et al. 2019). Moreover, because previous WTD studies indicate that the probability of CWD infection increases with age (Grear et al. 2006), and that CWD may disparately affect male and female WTD in different U.S. regions, including differences in clinical disease progression and mortality (Grear et al. 2006; Edmunds et al. 2016), the possibility for different CWD strains must be considered (Bistaffa et al. 2019). Therefore, the following fixed-effect covariates were specified for comparison for GWAA: sex, age, U.S. region of origin, and the total number as well as types (0 = none-detected; 1 = lymph node only; 2 = lymph node and obex) of CWD positive diagnostic tissues, with one exception (i.e., sample ID: MQ6Q3), as noted above.

For all genomic prediction analyses involving k-fold cross validation, we used GBLUP as previously described (Taylor 2014) and implemented in SVS v8.8.2 or v8.8.3 (Golden Helix), where the variance components were again estimated using the REML-based EMMAX technique (Kang et al. 2010) with a genomic relationship matrix ($G$) (VanRaden 2008; Taylor 2014). For WTD GBLUP analyses, consider the general mixed model equation:

$$y = Xf \beta + u + \epsilon,$$

across $n$ WTD samples where fixed effects specified as $B_f$ include the intercept and any additional covariates (i.e., U.S. region, sex, age); but also assume $\text{Var}(\epsilon) = \sigma^2_\epsilon I$, as above, and that the random effects $u$ are additive genetic merits (i.e., genomically estimated breeding values or GEBVs) for these WTD samples, which are produced from $m$ markers as $u = Ma$, where $M$ is a $n \times m$ matrix, and $\alpha$ is a vector where $\alpha_k$ is the allele substitution effect (ASE) for marker $k$. In this study, we used overall normalization for matrix $M$, as implemented in SVS v8.8.2 or v8.8.3 (Golden Helix), and explored solutions with and without gender corrections (i.e., full dosage compensation, no dosage compensation) (Taylor 2014), to produce GEBVs for all WTD samples as well as estimates of ASE for all SNPs. Moreover, considering that all training sets samples precede the validation set, we define $Z = [\emptyset]$, where the width and height of $I$ is given as $n_t$, the width of the zero matrix is given as $n_o$, and the height of the zero matrix is $n_t$. Thus we can partition $u, X_f$, and $y$ according to their origin (i.e., training vs. validation set) as $u = \begin{bmatrix} u_t & u_o \end{bmatrix}$,

$$X_f = \begin{bmatrix} X_{ft} \\ X_{fo} \end{bmatrix}, \ y = \begin{bmatrix} y_t \\ y_o \end{bmatrix},$$

and compute a genomic relationship matrix using all samples for use with the EMMA technique (Kang et al. 2010) to implement a mixed model for the training set as follows:

$$y_t = X_{ft} \beta + Zu + \epsilon_t,$$

where $\text{Var}(u) = \sigma^2_u G$ and $\text{Var}(\epsilon) = \sigma^2_\epsilon GZGZ^T$. Finally, we predict the validation set phenotypes as:

$$y_o = X_{fo} \beta + u_o,$$

from the intercept and any validation covariates $X_{fo}$ as well as the predicted values of $u_o$. Additional formulae and supporting documentation are available at https://doc.goldenhelix.com/SVS/latest/svsmanual/mixedModelMethods/overview.html#gb- blupproblemstmt. Notably, prior to this study, GEBVs were not estimated or utilized in WTD, and thus they cannot be expected to be intuitive or easily understood by U.S. WTD farmers or relevant regulatory agencies. However, the predicted CWD binary phenotypes are both intuitive and easily understood as estimates of enhanced or reduced susceptibility to CWD. Because GBLUP predicts CWD binary phenotypes across a range of values (i.e., 0 to 1), SVS v8.8.2 and v8.8.3 (Golden Helix) considers predicted values of 0.5 and higher as “1”, and < 0.5 as “0”, thus facilitating the calculation of important summary statistics which require binary classifications. Justification for rounding is evident within the histograms representing the frequency distributions of the predicted CWD binary phenotypes (Fig. S1), the relevant GEBVs (Fig. S2), and the relationship between the predicted CWD binary phenotypes and the relevant GEBVs (Fig. S3); with an obvious break that occurs at 0.50 (Fig. S1-S3). Binary summary statistics for GBLUP-based genomic predictions with k-fold ($k = 3; k = 5$) cross validations ($n = 50$ iterations) were computed in SVS v8.8.2 or v8.8.3 (Golden Helix) as follows: Area Under the Curve as $\text{AUC} = \frac{1}{m} \sum_{i=1}^{m} \text{ACC}_i$, with $\text{ACC}_i$ the frequency distributions of the predicted CWD binary phenotypes across a range of values ($0 \leq \text{ACC}_i \leq 1$), Mathews Correlation Coefficient as $\text{MCC} = \frac{TP \cdot TN - FP \cdot FN}{\sqrt{(TP+FN)(FP+TN)(TP+FP)(FN+TN)}}$, where $TP$ is the number of true CWD positives (from CWD diagnostics); $TN$ is the number of true CWD non-detects (from CWD diagnostics), $FP$ is the number of false positives, and $FN$ is the number of false non-detects among the predicted CWD binary phenotypes; Genomic Prediction Accuracy as $\text{ACC} = \frac{TP}{TP+FP}$; Sensitivity (true positive rate) as $\text{TPR} = \frac{TP}{TP+FN}$; Specificity (true negative rate) as $\text{SPC} = \frac{TN}{TN+FP}$; Root Mean Square Error as $\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (\hat{y}_i - y_i)^2}$. For the GBLUP predicted CWD Scores (0, 1, 2), we also produced and report relevant summary statistics from the k-fold ($k = 3; k = 5$) cross validations ($n = 50$ iterations) computed in SVS v8.8.2 or v8.8.3 (Golden Helix) as follows: Pearson’s Product-Moment Correlation Coefficient as $r_{y,y} = \frac{\sum_{i=1}^{n} \left(y_i - \bar{y} \right) \left(\hat{y}_i - \bar{\hat{y}} \right)}{(n-1) \sigma_n \sigma_{\hat{n}}}$ where $s_n$ and $s_{\hat{n}}$ are the standard deviations; Residual Sum of Squares as $RSS = \sum_{i=1}^{n} (\hat{y}_i - y_i)^2$; Total Sum of Squares $= \sum_{i=1}^{n} (y_i - \bar{y})^2$; R-Squared as $R^2 = 1 - \frac{RSS}{\text{TotalS}}$; Root Mean Square Error as $\text{RMSE} = \sqrt{\frac{RSS}{n}}$; Maximum Absolute Error as $\text{MAE} = \frac{1}{n} \sum_{i=1}^{n} |y_i - \hat{y}_i|$.

**Randomizing and blinding**
All GBLUP-based k-fold ($k = 3; k = 5$) cross validations (i.e., CWD binary; CWD-scores) were performed using automated random sampling to define the validation set (i.e., to predict on) and the training set, for the specified values of $k$. 1436 | C. M. Seabury et al.
Data availability
Accession codes are as follows: Illumina sequence data (SRA: SRR10313416-SRR10313421); genotype and phenotype data (DRYAD https://doi.org/10.5061/dryad.xd2547dcw).

RESULTS AND DISCUSSION
Using three reduced representation libraries (n = 374 farmed U.S. WTD) and Illumina paired-end sequencing for reference mapping and variant prediction, we successfully constructed a custom...
Affymetrix Axiom 200K SNP array for the WTD (See Methods). All probe sequences were aligned to the new PacBio long-read bovine genome assembly (ARS-UCD1.2; GCA_002263795.2), thus creating a comparative marker map. Thereafter, we genotyped a cohort of farmed WTD diagnostically classified (See Methods) as CWD positive (n = 284) and CWD non-detect (n = 523) from three U.S. geographic regions (Midwest, Northeast, South). Genome-wide association analyses were conducted using a mixed linear model with genomic relationship matrix and variance component analysis, thus yielding marker-based heritability estimates (GRM heritability), as implemented in EMMAX (Kang et al. 2010; Segura et al. 2012). All GWAA’s were carried out using 123,987 quality filtered SNPs for two dependent variables including a binary case-control variable (0 = non-detect; 1 = CWD positive) (Figure 1), and an interval variable (CWD-scores) which simultaneously reflects both the total number of CWD positive diagnostic tissues (i.e., 0, 1, 2) as well as the positive tissue types (i.e., 1 = lymph node only; 2 = lymph node and obex; Figure 2); with non-zero CWD-scores accurately modeling the natural progression of disease (Thomsen et al. 2012). Across all GWAA’s (Figure 1, Figure 2), GRM heritability estimates were moderate to high (i.e., $h^2 = 0.337 \pm 0.079 \div 0.637 \pm 0.070$); thus confirming that differential susceptibility to CWD in WTD is under genetic control, and that host genomic background also influences variation in disease progression. Herein we also confirm the PRNP gene as a major risk locus, and specifically, the codon 96 missense variant (G96S; binary case-control P-value $< 6.30E-11$; CWD-scores P-value $< 1.49E-15$) as well as one upstream promoter SNP (Affx-574071595; CWD-scores P-value $< 5.40E-06$) as being significantly associated with differential susceptibility to CWD, and with variation in natural disease progression among WTD (Figure 1, Figure 2, Table S1) (O’Rourke et al. 2004; Seabury et al. 2007). However, it should also be noted that 11 CWD-positive WTD possessed the codon 96S genotype, and the proportion of phenotypic variance explained (PVE) by even the largest-effect PRNP SNPs detected across all GWAA’s (G96S, Promoter Affx-574071595) was $< 0.11$ (Figure 1, Figure 2), indicating that loci other than PRNP influence differential susceptibility and disease progression. These results are compatible with prior analyses performed in mice; where incubation times for transmissible spongiform encephalopathies were largely influenced by a genetic architecture independent of PRNP.

Figure 2 EMMAX genome-wide association analyses (GWAA) for Chronic Wasting Disease (CWD) in farmed U.S. white-tailed deer (Odocoileus virginianus; hereafter WTD) using an interval variable (CWD-scores) to simultaneously reflect both the total number of CWD positive diagnostic tissues (i.e., 0, 1, 2) as well as the positive tissue types (i.e., 1 = lymph node only; 2 = lymph node and obex). All dual-panel manhattan plots depict -log10 P-values and the proportion of phenotypic variance explained (PVE) by white-tailed deer marker-effects on the y-axis, and the comparative position of all probe sequences on the x-axis, as inferred by blastn alignment with the bovine genome (ARS-UCD1.2). All analyses include diagnostically confirmed CWD positive (n = 284) and CWD non-detect (n = 523) WTD, and marker-based GRM heritability estimates ($h^2 = \sigma_e^2 / (\sigma_e^2 + \sigma_g^2)$) (Kang et al. 2010; Segura et al. 2012; Seabury et al. 2017; Smith et al. 2019). a, EMMAX GWAA for CWD-scores with no fixed-effect covariates, high GRM heritability estimates ($h^2 = 0.589 \pm 0.069$) and relevant positional candidate genes. b, EMMAX GWAA for CWD-scores with fixed-effect covariates (sex, age, U.S. region), high GRM heritability estimates ($h^2 = 0.515 \pm 0.075$) and relevant positional candidate genes.
bovine spongiform encephalopathy (BSE), various prion diseases including scrapie (i.e., M. domesticus), and Creutzfeldt-Jakob disease (i.e., B. taurus), among others. Interestingly, an investigation of all GWAA (n = 123,987 SNPs), only 61 SNPs met a nominal significance level for polygenic traits (P-value ≤ 5E-05) (Wellcome Trust Case Control Consortium 2007; Seabury et al. 2017), with 17 detected in more than one GWAA. This is relatively unsurprising since EMMAX is known to produce conservative P-values; particularly when large-effect regions exist (Zhou and Stephens 2012). Moreover, the architecture of both investigated CWD traits (Figure 1, Figure 2) is such that few large-effect regions exist (i.e., PVE ≥ 0.03); but together with many smaller-effect regions, a significant proportion of the phenotypic variance can be explained. Interestingly, an investigation of all GWAA’s revealed many of the same positional candidate genes (Figure 1, Figure 2; PVE ≥ 0.02); the majority of which have been implicated in the pathophysiology of various prion diseases including scrapie (i.e., ACSL4, CA3, KLF6), bovine spongiform encephalopathy (i.e., NPSA3, BACH2, EPHA7, ITGA4), spontaneous and familial Creutzfeldt-Jakob disease (i.e., HIST1H4D/OPCML, LAMA3, TTC7B), and various other neurodegenerative conditions including Alzheimer’s (i.e., DKG1, SRRP1, SLCA4A4) and Parkinson’s disease (BCL11B) (Scherrer et al. 2007; Tang et al. 2009; Tortosa et al. 2011; Silver et al. 2012; Cohen et al. 2013; Lambert et al. 2013; Filali et al. 2014; Lee et al. 2014; Kipkorir et al. 2015; Xerra et al. 2016; Esteve et al. 2019; Majer et al. 2019). Summary data for all GWAA’s and positional candidate genes (Figure 1, Figure 2) as well as the corresponding P–P plots are provided in the supplementary information (Table S1; Additional Files 1-5 in DRYAD).

To investigate the potential for developing a genomically-estimated CWD eradication program for farmed WTD, we used genomic best linear unbiased prediction (GLUP) in conjunction with k-fold cross validation and random sampling (Taylor 2014). Briefly, WTD data (i.e., genotypes, CWD diagnostic phenotypes, and other metadata) were randomly partitioned into k-subsamples (k = 3, k = 5), and one of these subsamples was then selected (i.e., within a discrete fold) to serve as the validation set for genomic prediction; thus the GLUP model was fit using the remaining (i.e., k - 1)-subsamples within that fold (i.e., the training data); until all subsamples were used for both training and prediction. All cross validations with random sampling were run for 50 iterations, with each iteration consisting of either three or five folds (k = 3, k = 5), and summary statistics were produced (See Table 1; Methods; Additional Files 6-29 in DRYAD). Binary GLUP models fit with no fixed-effect covariates (k = 3, k = 5) produced high mean genomic prediction accuracies (≥ 0.8167) and specificities (≥ 0.9101), with small standard deviations, but lower mean sensitivities (≤ 0.6496), indicating that false negatives pose the greatest challenge for reducing the prevalence of CWD via genomic prediction (Table 1; See Methods). Similar results were also obtained when binary GLUP models were fit using sex, age, and U.S. region of origin as fixed-effect covariates (k = 3, k = 5; Table 1). However, in addition to false negatives, some false positive genomic predictions were also observed, most likely due to underlying genomic susceptibility coupled with either very early stages of disease (i.e., CWD non-detect diagnostically) and/or differences in exposure (Tsairidou et al. 2014). All results were robust to the inclusion or exclusion of non-autosomal loci (i.e., X, MT; 123,987 SNPs vs. 120,808 SNPs, respectively), and to full dosage compensation vs. no dosage compensation when putative X-linked SNPs were included (Table 1; Additional Files 6-29 in DRYAD). GLUP models fit with the CWD-scores (0, 1, 2), thus reflecting the natural progression of disease, produced lower mean genomic prediction accuracies (i.e., ≤ 0.6007; See Methods; DRYAD), regardless of the inclusion or exclusion of fixed-effect covariates (sex, age, U.S. region), non-autosomal loci, or the implementation of full dosage compensation vs. no dosage compensation (k = 3, k = 5).

CONCLUSIONS

Herein, we demonstrate that differential susceptibility to CWD and variation in natural disease progression are both heritable, polygenic traits in farmed U.S. WTD, and that genome-wide SNP data can be used to produce accurate genomic predictions for risk (≥ 0.8167); thereby providing the first novel strategy for reducing the prevalence of CWD. Moreover, given the genomic architecture of these traits, we also demonstrate that PRNP genotyping alone cannot be expected to facilitate an eradication program, or to rapidly reduce the overall prevalence of CWD in farmed U.S. WTD.

AUTHOR CONTRIBUTIONS

C.M.S designed the research with input from T.A.N. M.A.L. and T.A.N. provided CWD diagnostic data, other relevant animal metadata, and biological samples for DNA isolation from existing agency repositories. C.M.S. and D. L. isolated and quantified DNA with assistance from F.P. E.K.B. prepared hair cards and quantified DNA. R.P.M. and C.D.J. prepared and sequenced reduced representation libraries. C.M.S. performed trimming, reference mapping, and variant prediction. C.M.S. designed the Affymetrix Axiom 200K SNP array with assistance from D.L.O. D.L.O. performed blastn searches to create comparative marker maps for the array. D.L.O. submitted reads to NCBI SRA. C.M.S. performed quality filtering of genotypes, genome wide association analyses with marker-based heritability estimates, alignment with positional candidate genes, and genomic predictions with k-fold cross validation and summary statistics. C.M.S. wrote the manuscript; incorporating input from D.L.O., E.K.B., D.L., P.F., R.P.M., C.D.J., M.A.L., and T.A.N. All authors edited and approved the manuscript.

ACKNOWLEDGMENTS

This material was made possible, in part, by a Cooperative Agreement from the United States Department of Agriculture’s Animal and Plant Health Inspection Service (APHIS). It may not necessarily express APHIS’s views. CMS acknowledges this support from USDA-MIS-Animal and Plant Health Inspection Service (grant AP17VSSPRS00C126).
as well as support from Texas Parks and Wildlife Department (grant 475613) and a charitable gift from the American Chronic Wasting Disease Foundation.

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Communicating editor: D. J. de Koning