

Genotype by environment interactions for chronic wasting disease in farmed US white-tailed deer

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Abstract

Despite implementation of enhanced management practices, chronic wasting disease in US white-tailed deer (*Odocoileus virginianus*) continues to expand geographically. Herein, we perform the largest genome-wide association analysis to date for chronic wasting disease ($n = 412$ chronic wasting disease-positive; $n = 758$ chronic wasting disease-nondetect) using a custom Affymetrix Axiom single-nucleotide polymorphism array ($n = 121,010$ single-nucleotide polymorphisms), and confirm that differential susceptibility to chronic wasting disease is a highly heritable ($h^2 = 0.611 \pm 0.056$) polygenic trait in farmed US white-tailed deer, but with greater trait complexity than previously appreciated. We also confirm *PRNP* codon 96 (G96S) as having the largest-effects on risk ($P \leq 3.19E-08$; phenotypic variance explained ≥ 0.025) across 3 US regions (Northeast, Midwest, South). However, 20 chronic wasting disease-positive white-tailed deer possessing codon 96SS genotypes were also observed, including one that was lymph node and obex positive. Beyond *PRNP*, we also detected 23 significant single-nucleotide polymorphisms (P -value $\leq 5E-05$) implicating ≥ 24 positional candidate genes; many of which have been directly implicated in Parkinson's, Alzheimer's and prion diseases. Genotype-by-environment interaction genome-wide association analysis revealed a single-nucleotide polymorphism in the lysosomal enzyme gene *ARSB* as having the most significant regional heterogeneity of effects on chronic wasting disease ($P \leq 3.20E-06$); with increasing copy number of the minor allele increasing susceptibility to chronic wasting disease in the Northeast and Midwest; but with opposite effects in the South. In addition to *ARSB*, 38 significant genotype-by-environment single-nucleotide polymorphisms (P -value $\leq 5E-05$) were also detected, thereby implicating ≥ 36 positional candidate genes; the majority of which have also been associated with aspects of Parkinson's, Alzheimer's, and prion diseases.

Keywords: white-tailed deer; chronic wasting disease; GxE interaction; GWAA; *PRNP*

Introduction

Chronic wasting disease (CWD) was initially recognized as a fatal wasting syndrome in captive mule deer (*Odocoileus hemionus*) and black-tailed deer (*Odocoileus hemionus columbianus*) housed within several Colorado wildlife research facilities during the late 1960s, with subsequent histological characterization as a prion disease by the late 1970s (Williams and Young 1980; Moreno and Telling 2018). Following initial characterization, CWD has subsequently been detected in free-ranging US elk (*Cervus elaphus nelsoni*), mule deer, white-tailed deer (*Odocoileus virginianus*; hereafter WTD), and moose (*Alces alces shirasi*), with further geographic expansion of the disease noted among farmed and free-ranging populations of these species (Moreno and Telling 2018; Gavin et al. 2019; Osterholm et al. 2019). Relevant to the geographic expansion of CWD, development and implementation of modern management practices, including the establishment of surveillance or containment zones, as well as depopulation of positive herds, have not prevented CWD from emerging in new geographic areas; with multiple Canadian provinces and at least 26 US states currently affected by CWD (Moreno and Telling 2018; Gavin et al. 2019; Osterholm et al. 2019). Moreover, Norway, Finland, and the

Republic of Korea have also reported CWD in free-ranging reindeer (*Rangifer tarandus*; Norway), moose (*Alces alces*; Norway, Finland), and imported elk (Korea) (Moreno and Telling 2018; Gavin et al. 2019; Osterholm et al. 2019); thereby emphasizing the global expansion of CWD among several susceptible species within the family Cervidae. However, recent studies hypothesize that some forms of transmissible CWD may have emerged from sporadic CWD in Norwegian reindeer, moose, and perhaps red deer (*Cervus elaphus*) (Mysterud et al. 2020; Güere et al. 2021).

A recent genome-wide association study demonstrated that differential susceptibility to CWD, and natural variation in disease progression, are both moderately to highly heritable polygenic traits among farmed US WTD, and that loci other than *PRNP* are involved (Seabury et al. 2020). Moreover, genomic prediction accuracy related to differential susceptibility to CWD, as estimated by cross validation, was also shown to be high; thereby underscoring the potential for reducing susceptibility in farmed US WTD via genomic prediction (Seabury et al. 2020). However, no information currently exists regarding the potential for genotype-by-environment (GxE) interactions with respect to differential susceptibility to CWD. Herein, we employ genome-wide

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association analyses (GWAA) to further investigate the genomic basis for differential susceptibility to CWD in farmed US WTD using a larger and more geographically diverse sample than previously reported (Seabury et al. 2020), and subsequently confirm the high heritability of differential susceptibility to CWD. Additionally, we use two GWAA approaches to evaluate the potential for significant GxE interactions with respect to differential susceptibility to CWD among farmed US WTD. The results of this study provide the first genome-wide report on GxE interactions related to CWD, and are expected to positively augment genomic prediction programs aimed at reducing susceptibility among farmed US WTD.

Materials and methods

Study overview

In the present study, we utilize mixed linear models with genomic relationship matrices (GRM) to further investigate the genomic basis of differential susceptibility to CWD in farmed US WTD; including the potential for significant GxE interactions. Initially, we conduct standard GWAA with GRM heritability estimated using EMMAX (Kang et al. 2010; Segura et al. 2012), but also produce heritability estimates on the liability scale using GCTA (Lee et al. 2011; Yang et al. 2011) for 1,170 farmed US WTD. Thereafter, we use an implementation of EMMAX (Kang et al. 2010; Vilhjalmsón 2012; Smith et al. 2019) where interaction-term covariates may be specified; with the environmental variable expressing the US region of origin for each individual WTD (i.e. Northeast, Midwest, South) specified as the interaction term for a GxE GWAA. Finally, we also perform region-specific (i.e. Northeast, Midwest, South) GWAA for differential susceptibility to CWD using EMMAX (Kang et al. 2010; Segura et al. 2012), and thereafter, utilize a meta-based approach employing Cochran's Q-test for heterogeneity (Cochran 1954; Willer et al. 2010) to further confirm WTD single-nucleotide polymorphisms (SNPs) displaying evidence for significant GxE interactions with respect to CWD.

Animal resources, CWD diagnostics, and DNA isolation

Herein, we utilized animal repository resources, including CWD immunohistochemistry (IHC) diagnostic data ($n=523$ CWD non-detect, $n=284$ CWD positive), PRNP genotypes, and Affymetrix Axiom SNP array genotypes for 807 farmed US WTD from a previous study (Seabury et al. 2020). These data included regional representation from the US Northeast ($n=35$), Midwest ($n=291$), and South ($n=481$), as previously described (Seabury et al. 2020). In the present study, we expand upon our prior dataset ($n=807$) to collectively include 1,170 farmed US WTD from the Northeast ($n=286$), the Midwest ($n=322$), and the South ($n=562$) by obtaining 363 additional farmed US WTD samples (both sexes) from an existing USDA APHIS repository that was created via federal CWD surveillance activities; including depopulations of CWD positive herds (USDA APHIS, Fort Collins, CO). Thus, the current dataset includes both CWD positive ($n=412$) and CWD nondetect ($n=758$) WTD (Thomsen et al. 2012) from 22 US farms with CWD prevalence ranging from 0.01 to 1.00 (Supplementary Table 1). For new WTD enrolled in the present study ($n=363$), all CWD diagnostic classifications were based upon IHC (i.e. IHC of lymph node, obex, rectal, tonsil biopsy) that was performed at the USDA National Veterinary Services Laboratory (NVSL) in Ames Iowa (Thomsen et al. 2012). Genomic DNA for 363 additional farmed US WTD was isolated from ear fibroblast biopsies using the LGC

sbeadex tissue purification kit (LGC) with automation at GeneSeek Neogen (Lincoln, NE) (Seabury et al. 2020). Thereafter, WTD genomic DNAs were quantified and assessed for purity (260/280 ratio) using a Nanodrop (ThermoFisher).

PRNP and Affymetrix Axiom array genotyping

All PRNP genotyping ($n=363$ farmed US WTD) for missense variants at codons 37, 95, 96, 116, and 226 was performed at GeneSeek Neogen (Lincoln, NE) via commercial genotyping by sequencing service (Seabury et al. 2020). Briefly, to prevent chimeric PRNP amplicons that obscure genotype-phenotype relationships, the functional PRNP gene was PCR amplified using primers exclusionary to a processed pseudogene (O'Rourke et al. 2004), with all amplicons purified via AMPure XP beads as recommended by the manufacturer (Beckman Coulter); thereby facilitating the generation of barcoded Illumina Nextera XT DNA libraries and amplicon sequencing on an Illumina MiSeq. All WTD PRNP genotypes for codons 37, 95, 96, 116, and 226 were called from the reference-aligned read pileups at GeneSeek Neogen, and delivered in text format (Seabury et al. 2020). Genotyping on the custom Affymetrix Axiom 200K SNP array for 363 farmed US WTD was also performed at GeneSeek Neogen using the Affymetrix best practices workflow; with genotypes delivered in text format (Seabury et al. 2020). Specifically, Affymetrix quality control thresholds implemented for the present study were $DQC \geq 0.82$, QC call rate $\geq 95\%$, passing samples in the project $\geq 95\%$, and average call rate for passing samples $\geq 97\%$, as previously described and utilized (Seabury et al. 2020). For the present study, a total of 1,170 WTD samples passed all Affymetrix QC filters; each with 125,585 SNP array genotypes, and paired PRNP codon genotypes, thus yielding a combined set of 125,590 SNP genotypes for analysis. However, only 1,151 WTD samples contained all possible metadata (i.e. sex, age, US region of origin, farm code, CWD diagnostic outcome), and could be used for all analytical approaches explored in the present study.

GWAA and GxE interactions

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), utilization of a comparative marker map (ARS-UCD1.2; GCA_002263795.2) is necessary to provide comparative evidence for the origin of the array and PRNP SNPs (i.e. autosomal vs. nonautosomal), as previously described (Seabury et al. 2020). After joining the comparative marker map to the combined set of all WTD genotypes (PRNP + Affymetrix Axiom array), quality control analyses were performed in SVS v8.9.0 (Golden Helix), including verification of sample call-rate ($\geq 95\%$), and pairwise IBS distances to identify twins and duplicate samples among 1,170 US farmed WTD. No duplicate samples were detected. Further quality control analyses and filtering were as follows: 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 $< 1e-25$), thereby yielding 121,010 SNPs for all EMMAX GWAA involving 1,170 US farmed WTD. PRNP SNPs which failed to endure quality control filtering included only codon 116 (monomorphic), whereas codons 37, 95, 96, and 226 remained. To further investigate the genomic basis for differential susceptibility to CWD in farmed US WTD using a larger and more geographically diverse sample than previously reported, we performed GWAA on 1,170 WTD using a mixed linear model with variance component estimates, as implemented in EMMAX, and executed in SVS v8.9.0, with all genotypes recoded as 0, 1, or 2, based on the incidence of

the minor allele (Kang et al. 2010; Segura et al. 2012; Neibergs et al. 2014; Seabury et al. 2017, 2020; Smith et al. 2019). However, within an additive model, hemizygous males may only possess 0 or 1 copy of an X-linked minor allele (excluding the pseudo-autosomal region), whereas females may possess 0, 1, or 2 copies of the minor allele. A gender correction reflecting these ploidy differences was utilized to recode putative X-linked genotypes prior to EMMAX-GRM analyses (Neibergs et al. 2014; Taylor 2014; Seabury et al. 2017, 2020). The disease phenotype used for all WTD analyses was CWD Binary (0 = nondetect, 1 = CWD positive for one or more diagnostic tissues including lymph node, obex, rectal, and/or tonsil). The mixed model utilized in the present study can be generally specified as: $y = X\beta + Zu + \epsilon$, where y represents a $n \times 1$ vector of CWD diagnostic phenotypes, X is a $n \times f$ matrix of fixed effects, β is a $f \times 1$ vector representing the coefficients of the fixed effects, u is the unknown random effect, and Z is a $n \times t$ matrix relating the random effect to the CWD diagnostic phenotypes (Kang et al. 2010; Segura et al. 2012; Seabury et al. 2017, 2020; Smith et al. 2019). Herein, we must assume that $\text{Var}(u) = \sigma_g^2 K$ and $\text{Var}(\epsilon) = \sigma_e^2 I$, such that $\text{Var}(y) = \sigma_g^2 ZKZ' + \sigma_e^2 I$, but in the present WTD 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, the variance components (i.e. σ_g^2 and σ_e^2) were estimated using the REML-based (restricted maximum likelihood) EMMA approach (Kang et al. 2010), with stratification accounted for and controlled using a GRM (G) (VanRaden 2008), as computed from the filtered WTD genotypes (PRNP + Affymetrix Axiom array) (Seabury et al. 2020). GRM heritability estimates ($h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$) for differential susceptibility to CWD were produced as previously described (Kang et al. 2010; Segura et al. 2012; Seabury et al. 2017, 2020; Smith et al. 2019). Likewise, because the proportion of CWD cases included herein (i.e. 35% of 1,170 farmed WTD) is larger than the weighted mean CWD prevalence (i.e. 26%) across all farms included in the present study, we also estimated the heritability on a liability scale (Lee et al. 2011) using GCTA v1.93 (Yang et al. 2011) across a range of values for prevalence (i.e. prevalence = 0.01, 0.05, 0.10, 0.15, 0.20, 0.25, 0.26 as the weighted mean in the present study, 0.30, and 0.35). To conduct a GxE GWAA, we used the same filtered WTD data and disease phenotype (CWD Binary) in conjunction with an implementation of EMMAX (Kang et al. 2010; Vilhjalmsjon 2012; Smith et al. 2019) whereby interaction-term covariates may be specified; with the environmental variable expressing the US geographic region of origin for each WTD (Northeast, Midwest, South) specified as the interaction term. The basis of this approach is rooted in full vs. reduced model regression (Neibergs et al. 2014; Smith et al. 2019), where interaction-term covariates are included in the model as follows: Each specified interaction-term covariate serves as one reduced-model covariate; Each specified interaction-term covariate is also multiplied, element by element, with each SNP predictor (i.e. SNP \times geographic origin) to create an interaction term to be included in the full model. Specifically, given n observations of a WTD disease phenotype (CWD Binary) that is influenced by m fixed effects and n instances of one random effect, with one or more GxE effects (e) whereby the interaction is potentially with one predictor variable, we model this using a full and a reduced model. The full model can be specified as $y = X_c\beta_{kc} + X_i\beta_{ki} + X_k\beta_{kp} + X_{ip}\beta_{ip} + u_{full} + \epsilon_{full}$, and the reduced model as $y = X_c\beta_{krc} + X_i\beta_{kri} + X_k\beta_{krp} + u_{reduced} + \epsilon_{reduced}$, where y is an n -vector of observed WTD CWD phenotypes, X_c is an $n \times m$ matrix of m fixed-effect covariates, X_i is an $n \times e$ matrix of e fixed terms being tested for GxE interactions, X_k is an n -vector

containing the covariate or predictor variable that may be interacting, and X_{ip} is an $n \times e$ matrix containing the e interaction terms created by multiplying the columns of X_i element-by-element with X_k . Herein, all β terms correspond to the X terms as written above, and to the full or the reduced model, as specified, with u and ϵ representing the random effect and error terms, respectively (Smith et al. 2019). Similar to the EMMAX method without interactions (Kang et al. 2010; Segura et al. 2012), we approximate this by finding the variance components once, utilizing the parts of the above equations that are independent of X_k as follows: $y = X_c\beta_{cvc} + X_i\beta_{ivc} + u_{vc} + \epsilon_{vc}$, where vc indicates the variance components. To estimate the variance components, we must again assume that $\text{Var}(u_{vc}) = \sigma_g^2 K$ and $\text{Var}(\epsilon_{vc}) = \sigma_e^2 I$, whereby $\text{Var}(y) = \sigma_g^2 K + \sigma_e^2 I$ (Kang et al. 2010; Vilhjalmsjon 2012; Smith et al. 2019). The REML-based EMMA technique can then be used to estimate the variance components σ_g^2 and σ_e^2 as well as a matrix B (and its inverse) whereby $BB' = H = \frac{\text{Var}(y)}{\sigma_e^2} = K + \frac{\sigma_g^2}{\sigma_e^2} I$, as previously described and utilized in a large-sample analysis (Smith et al. 2019). Thereafter, for every WTD SNP marker (k), we can compute (via EMMAX-type approximation) the full and reduced models as: $B^{-1}y = B^{-1}X_c\beta_{kc} + B^{-1}X_i\beta_{ki} + B^{-1}X_k\beta_{kp} + B^{-1}X_{ip}\beta_{ip} + B^{-1}(u_{full} + \epsilon_{full})$ for the full model, where $B^{-1}(u_{full} + \epsilon_{full})$ is assumed to be an error term proportional to the identity matrix, and as $B^{-1}X_c\beta_{krc} + B^{-1}X_i\beta_{kri} + B^{-1}X_k\beta_{krp} + B^{-1}(u_{reduced} + \epsilon_{reduced})$ for the reduced model, where $B^{-1}(u_{reduced} + \epsilon_{reduced})$ is assumed to be an error term proportional to the identity matrix (Smith et al. 2019). To estimate the significance of the full vs. reduced model using the EMMAX GxE approach, an F -test was performed (Kang et al. 2010; Vilhjalmsjon 2012; Smith et al. 2019); with all analyses executed and evaluated by constructing Manhattan plots within SVS v8.9.0 (Golden Helix, Bozeman, MT). Finally, although SVS computes the full model described above and outputs all β values, it only performs an optimization of the reduced model computation; to determine the residual sum of squares of the reduced-model equation, and thus estimate the full vs. reduced model P -value via F -test (Kang et al. 2010; Vilhjalmsjon 2012). This general approach is highly efficient for large-sample analyses (Smith et al. 2019); with the reduced model optimization used to solve: $MB^{-1}y = MB^{-1}X_k\beta_{krp} + \epsilon_{MB}$, where $M = (I - QQ')$, and Q is derived from performing the QR algorithm, as $QR = B^{-1}[X_c|X_i]$. Additional formulae and documentation are available at <https://doc.goldenhelix.com/SVS/latest/svsmanual/mixedModelMethods/overview.html#gblupproblemstmt>. Notably, because the probability of CWD infection is likely to increase with age (Grear et al. 2006), and may also disparately affect male and female WTD in different US regions, including differences in clinical disease progression and mortality (Grear et al. 2006; Edmunds et al. 2016), we explored several model fits for comparison as follows: GWAA with no fixed effect covariates; GWAA with sex, age, and US region of origin as fixed effect covariates; GWAA with sex, age, and US farm of origin (i.e. for farms with ≥ 10 deer available for analysis) as fixed effect covariates; GxE GWAA with no fixed effect covariates; GxE GWAA with sex and age as fixed effect covariates. A farm variable was not used as a fixed effect covariate for EMMAX GxE GWAA because farm and US region of origin are collinear. For all EMMAX analyses, genomic inflation factors were estimated in SVS v8.9.0 (Golden Helix) as: Pseudo-Lambda = $\log_{10}(\text{median observed } P\text{-value}) / \log_{10}(\text{median expected } P\text{-value})$.

For comparison to the EMMAX GxE approach (Kang et al. 2010; Vilhjalmsjon 2012; Smith et al. 2019) utilizing 1,170 WTD, we also perform individual region-specific (i.e. Northeast, Midwest, South) GWAA for differential susceptibility to CWD using

EMMAX (Kang et al. 2010; Segura et al. 2012), and thereafter, utilize a meta-based approach employing Cochran's Q-test for heterogeneity of SNP effects (Cochran 1954; Willer et al. 2010). Briefly, SNP filtering for WTD from each US region (i.e. Northeast, Midwest, South), with additive recoding and gender correction, was performed as described above; thereby resulting in the following data sets for regional EMMAX GWAA: Northeast 124,977 SNPs ($n=116$ CWD positive, $n=170$ CWD nondetect); Midwest 125,446 SNPs ($n=208$ CWD positive, $n=114$ CWD nondetect); South 125,120 SNPs ($n=88$ CWD positive, $n=474$ CWD nondetect). Regional EMMAX GWAA were performed as described above within SVS 8.9.0 (Golden Helix) as follows: GWAA with no fixed effect covariates; GWAA with sex and age as fixed effect covariates; GWAA with sex, age, and US farm of origin as fixed effect covariates. The results of the regional EMMAX GWAA's were utilized to conduct a sample-size (Z-score based) meta-analysis, as specified in the program METAL (Willer et al. 2010), and implemented in SVS 8.9.0 (Golden Helix). For every regional EMMAX GWAA, the SVS implementation of METAL (Willer et al. 2010) utilizes SNP marker P-values, the effect direction (SNP Predictor Beta), and sample sizes (for weighting purposes) to compute a Z-score and overall P-value, but also implements Cochran's Q-test with P-values for identifying heterogeneity of SNP effects (Cochran 1954; Willer et al. 2010). Briefly, suppose that N_i is the WTD sample size from study i , while p_{ij} is the P-value from study i for SNP j , and Δ_{ij} is the direction of effect for study i at SNP j ; the SVS v8.9.0 implementation of METAL (Willer et al. 2010) uses a normally distributed intermediate statistic z_{ij} , defined as $z_{ij} = \Phi^{-1}(p_{ij}/2)\text{sign}(\Delta_{ij})$, to describe the effect, where Φ^{-1} denotes the inverse of 1 minus the cumulative distribution function of the normal distribution (the inverse survival function). Thereafter, using $w_{zi} = \sqrt{N_i}$ to represent the Z-score weight for WTD study i , the overall Z-score for SNP j is computed as $Z_j = \frac{\sum z_{ij}w_{zi}}{\sqrt{\sum w_{zi}^2}}$, and the overall P-value is estimated as $P_j = 2\Phi(|Z_j|)$, where Φ represents 1 minus the probability density function of the normal distribution (the survival function). Manhattan plots for the METAL-based meta-analysis ($-\log_{10}$ Overall P-value; $-\log_{10}$ Cochran's Q P-value) were constructed and visualized in SVS v8.9.0 (Golden Helix). For all analyses (i.e. EMMAX, METAL), we employed a nominal significance threshold (P-value $\leq 5E-05$) for polygenic traits (Wellcome Trust Case Control Consortium 2007; Neibergs et al. 2014; Seabury et al. 2017, 2020).

Results and discussion

A GWAA was conducted using a mixed linear model with GRM and variance component analysis, thereby producing a marker-based heritability estimate (GRM heritability) for differential susceptibility to CWD, as implemented in EMMAX (Kang et al. 2010; Segura et al. 2012), for a cohort of 1,170 farmed US WTD diagnostically classified (see *Materials and Methods*) as CWD positive ($n=412$) and CWD nondetect ($n=758$) from three US geographic regions (Northeast, Midwest, South). Notably, despite a 45% increase in overall sample size from our previous report (Seabury et al. 2020), including a more balanced sampling from each US geographic region (see *Materials and Methods*), the GRM heritability estimate remains comparatively high in this study (i.e. $h^2 = 0.611 \pm 0.056$; previously: $h^2 = 0.637 \pm 0.070$); with the codon 96 missense variant (G96S) again displaying the largest genome-wide effects on differential susceptibility to CWD (Fig. 1, Supplementary Table 2). Likewise, heritability estimates on the

liability scale (Lee et al. 2011; Yang et al. 2011) were also similarly high when CWD prevalence was ≥ 0.05 (i.e. h^2 from Sum of $V(G)_L/Vp = 0.557 \pm 0.053$), and these estimates only increased with increasing CWD prevalence, thereby suggesting that our current and previous report (Seabury et al. 2020) likely provide conservative heritability estimates; particularly since the weighted mean CWD prevalence across all farms included in the present study was 0.26 (Supplementary Table 2). However, it is also interesting to note that given a much larger and more regionally diverse sample in this study, the proportion of phenotypic variance explained (PVE) by PRNP codon 96 is markedly lower (PVE ≤ 0.026) than previously reported (PVE ≤ 0.052) for 807 farmed US WTD (Seabury et al. 2020). Moreover, in the present study, we noted 20 CWD-positive WTD that possessed the codon 96SS genotype, including one that was both lymph node and obex positive. Collectively, for an EMMAX GWAA with 1,170 farmed US WTD, only eight SNPs met a nominal significance threshold (P-value $\leq 5E-05$) for polygenic traits (Fig. 1, Supplementary Table 2) (Wellcome Trust Case Control Consortium 2007; Neibergs et al. 2014; Seabury et al. 2017, 2020), thereby confirming the CWD trait architecture previously described, where very few large or moderate-effect regions exist; but together with many small-effect regions, a significant proportion of the phenotypic variance can be explained (Seabury et al. 2020). Nevertheless, it should also be noted that EMMAX is known to produce conservative P-values (Zhou and Stephens 2012). In addition to PRNP, an investigation of nominally significant SNPs (P-value $\leq 5E-05$) revealed positional candidate genes previously implicated in aspects of prion disease (TPH2; PDE4DIP), including scrapie (ACSL4), regulation of the central nervous system (ADGRB3), neuroprotection (EN1), Alzheimer's (ASCL1, AMOTL2, RYK), and Parkinson's disease (EN1, ASCL1, RTL9) (Ide et al. 2005; Roffé et al. 2010; Filali et al. 2014; Nishizawa et al. 2014; Alleaume-Butaux et al. 2015; Rekaik et al. 2015; Dunn et al. 2019; Meyer et al. 2019; Scuderi et al. 2019; Feng et al. 2020; Gallart-Palau et al. 2020; Le Guen et al. 2020). Additional missense variants encoded by PRNP codons 37, 95, and 226 did not meet the nominal significance level (P-value $\leq 5E-05$) for polygenic traits (Wellcome Trust Case Control Consortium 2007; Neibergs et al. 2014; Seabury et al. 2017, 2020). Importantly, EMMAX mixed model solutions for the binary CWD case-control trait were robust to the inclusion of additional fixed effect covariates (i.e. sex, age, US region of origin; and/or sex, age, farm); as the majority of the significant SNPs (P-value $\leq 5E-05$) detected were shared across all analyses, including PRNP codon 96, which consistently displayed the largest genome-wide effects on differential susceptibility to CWD (Fig. 1, Supplementary Table 2 and Supplementary Fig. 1). Detailed summary data for all EMMAX GWAA's, including PVE, the direction of all SNP effects, Supplementary Manhattan plots, genomic inflation factors (Pseudo-Lambda), and PP-Plots are provided in Additional Files 1–7 in DRYAD (<https://doi.org/10.5061/dryad.wh70rxwnt>).

To investigate the potential for significant GxE interactions with respect to differential susceptibility to CWD, we conducted a GxE GWAA using EMMAX (see *Materials and Methods*). Collectively, 27 SNPs met the nominal significance level (P-value $\leq 5E-05$) for polygenic traits (Wellcome Trust Case Control Consortium 2007; Neibergs et al. 2014; Seabury et al. 2017, 2020), with no significant SNPs noted within or proximal to PRNP (Fig. 1, Supplementary Table 2). Notably, the largest-effect GxE signal detected for differential susceptibility to CWD was in ARSB (intron 4); a gene that encodes a lysosomal enzyme (Arylsulfatase B) required for the catabolism of

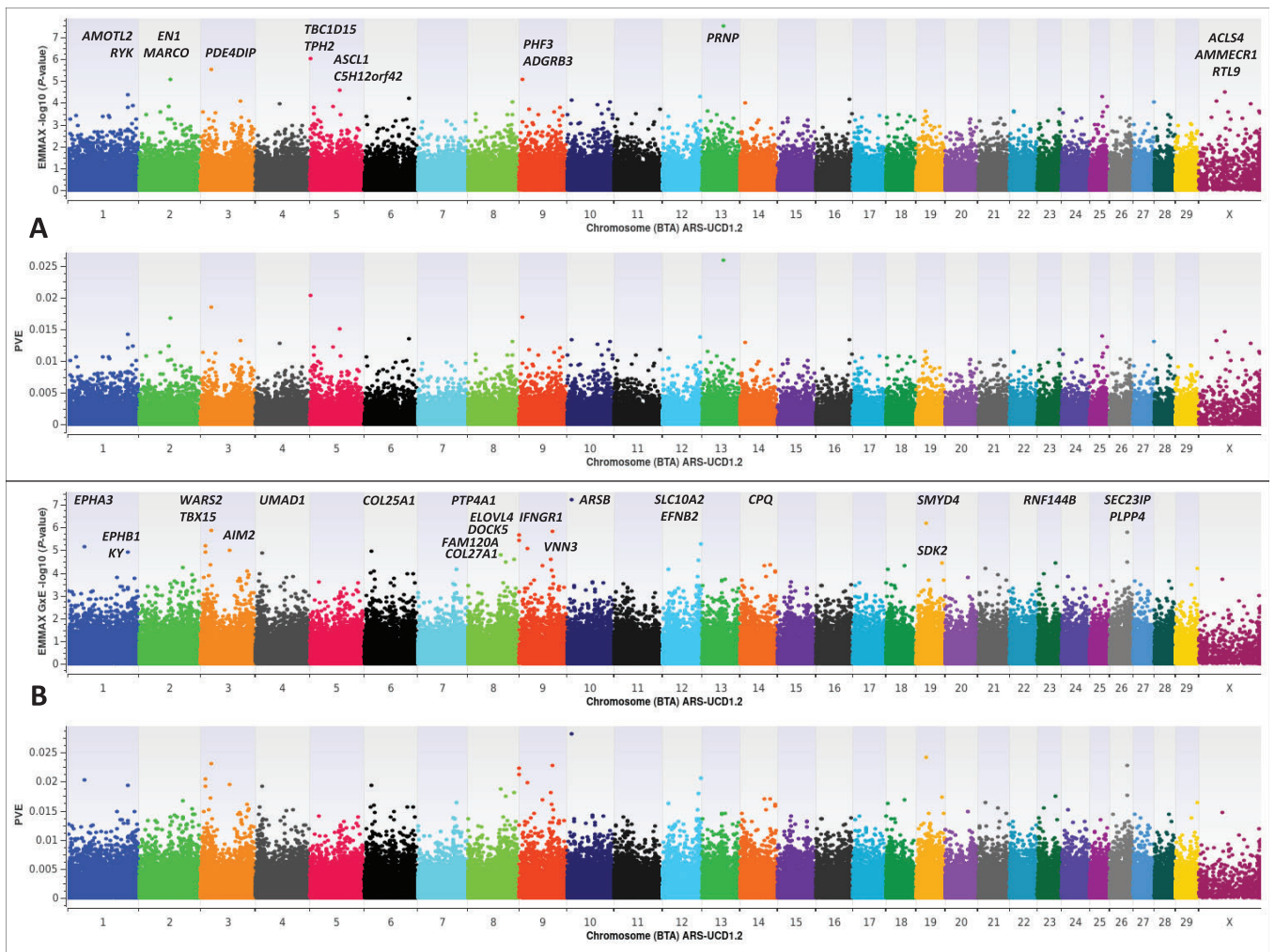


Fig. 1. EMMAX binary case-control (0, 1) GWAA for CWD in farmed US white-tailed deer (*Odocoileus virginianus*; hereafter WTD). All dual-panel Manhattan plots depict $-\log_{10}$ P-values and the proportion of phenotypic variance explained (PVE) by white-tailed deer marker-effects on the y-axis, and the comparative position of all SNPs on the x-axis, as inferred by blastn alignment with the bovine genome (ARS-UCD1.2) (Seabury et al. 2020). All analyses include diagnostically confirmed CWD positive ($n = 412$) and CWD nondetect ($n = 758$) WTD. a) EMMAX GWAA for CWD with no fixed-effect covariates, high GRM heritability estimates ($h^2 = 0.611 \pm 0.056$) (Kang et al. 2010; Segura et al. 2012; Seabury et al. 2020), and relevant positional candidate genes. Genomic inflation factor (Pseudo-Lambda) = 1.007. b) EMMAX GxE GWAA for CWD with US WTD region of origin (Northeast, Midwest, South) as the environmental interaction term, and relevant positional candidate genes. Genomic inflation factor (Pseudo-Lambda) = 1.140.

glycosaminoglycans (GAGs), including N-acetyl-D-galactosamine, dermatan sulfate, and chondroitin sulfate. Mutations in ARSB that result in a defective protein (i.e. enzyme deficiency) are known to be causal for the lysosomal storage disease known as Mucopolysaccharidosis (MPS) Type VI; with the concentration of urinary GAGs generally presenting as 5-100 times higher in patients with various forms of MPS (Pastores and Maegawa 2013; Sun et al. 2015; Vairo et al. 2015; Malekpour et al. 2018; Wang et al. 2018). Interestingly, the metabolism of GAGs is also known to be impaired in both humans and animals suffering from prion disease; with the degradation of GAGs disrupted by their interaction with PrP^{Sc}, thus resulting in their accumulation and secretion in urine (Mayer-Sonnenfeld et al. 2005). Moreover, hexosaminidase is known to be one of the last enzymes functioning in the degradation cascade for several GAGs (i.e. chondroitin sulfate, dermatan sulfate, and keratan sulfate), and its enzymatic activity is significantly elevated in the brains of scrapie-infected mice, as compared to controls (Mayer-Sonnenfeld et al. 2005). However, the relationship between GAGs and prion diseases in humans

and animals is somewhat complex, as the presence of GAGs (i.e. heparan sulfate; chondroitin sulfate) enhances PrP^{Sc} biogenesis and accumulation in cells, but the opposite has also been well postulated; where the accumulation of PrP^{Sc} may somehow cause an increase in GAG accumulation, particularly in lysosomes (Ben-Zaken et al. 2003; Mayer-Sonnenfeld et al. 2005). To the authors' best knowledge, this is the first report to ever demonstrate a direct genetic association between a lysosomal enzyme gene involved in GAG catabolism (i.e. dermatan sulfate; chondroitin sulfate), and prion disease (CWD); yet the presence of weakly and strongly sulfated GAGs (i.e. chondroitin, heparan, keratan, and/or heparin) have been known to colocalize with amyloid plaques in CWD-affected captive mule deer for more than 30 years (Guiroy et al. 1991). However, amyloid plaques were not uniformly found in all CWD positive mule deer (Guiroy et al. 1991). Notably, a more recent study in mice demonstrates that MPS can lead to amyloidosis, synucleinopathy, and an apparent prion encephalopathy; with the accumulation of misfolded proteins generally considered to be an indirect result of progressive failure of lysosomal function

in inbred mice (Naughton et al. 2013). Therefore, this raises the possibility that CWD may potentially present diagnostically in the absence of an infectious exposure (i.e. sporadically), and that future research should focus on the pathophysiological timing and potentially complex biochemical mechanisms of disease, as well as variation in PrP^{CWD} trafficking, including quantification of live-animal shedding given different genomic backgrounds (Seabury et al. 2020). Interestingly, in the present study, the ARSB SNP displaying significant GxE effects was observed to increase susceptibility to CWD in both the Northeast and the Midwest, but had the opposite direction of effect in the South; thereby underscoring the overall trait complexity. Beyond ARSB and its association with MPS, we also noted 24 positional candidate genes related to 26 additional EMMAX GxE signals (P -value $\leq 5E-05$; Supplementary Table 2, Fig. 1); the majority of which have previously been associated with Parkinson's disease (SMYD4, WARS2, IFNGR1, PLPP4, ASCL1, FAM120A), Alzheimer's disease (TBX15, IFNGR, PTP4A1, AIM2, SLC10A2, COL25A1, ASCL1, EPHB1, UMAD1, VNN3, COL27A1, RNF144B, SDK2), and various prion diseases (IFNGR, SEC23IP, EPHA3, EFN2, ELOVL4, DOCK5, COL27A1) including scrapie, bovine spongiform encephalopathy, and Creutzfeldt-Jakob disease (Ide et al. 2005; Julius 2008; Hashioka et al. 2009; Tong et al. 2010; Tian et al. 2013; Woodling et al. 2014; Majer 2015; Freeman and Ting 2016; Vélez et al. 2016; Watson et al. 2016; Mez et al. 2017; Su et al. 2018; Choubey 2019; Dabin 2019; Hirsch et al. 2019; Liu et al. 2019; Majer et al. 2019; Meyer et al. 2019; Thatra 2019; Bellenguez et al. 2020; Dabin et al. 2020; Donaldson et al. 2020; Martinelli et al. 2020; Wang et al. 2020; Vastrad and Vastrad 2021). Notably, the EMMAX GxE mixed model solutions were also robust to the inclusion of additional fixed effect covariates (i.e. sex, age; Supplementary Table 2 and Supplementary Fig. 2); as the majority of the significant SNPs (P -value $\leq 5E-05$) detected were shared across all analyses, including ARSB, which consistently displayed the most significant genome-wide GxE interactions related to CWD susceptibility. Detailed summary data for all EMMAX GxE GWAA's, including SNP-based regional interactions and

directions of effect, a Supplementary Manhattan plot (Supplementary Fig. 2), genomic inflation factors (Pseudo-Lambda), and PP-Plots are provided in Additional Files 8–12 in DRYAD (<https://doi.org/10.5061/dryad.wh70rxwnt>).

For comparison to our EMMAX GxE analysis with WTD region of origin as the interaction term, we performed individual EMMAX GWAA's for each US region (Northeast, Midwest, South), and used the corresponding regional results (Additional Files 13–15 in DRYAD; (<https://doi.org/10.5061/dryad.wh70rxwnt>) to conduct a meta-analysis, as previously described and implemented in the program METAL (Willer et al. 2010). Collectively, the majority of the significant EMMAX main effect SNPs were also detected by METAL (P -value $\leq 5E-05$); with the codon 96 missense variant (G96S) displaying the most significant genome-wide effects on differential susceptibility to CWD across all US regions (Fig. 2, Supplementary Table 2). However, the METAL-based approach also identified a significant main-effect SNP in ARSB (intron 6) which was not significant by EMMAX GWAA (Fig. 1, Supplementary Table 2), but nonetheless, was among the top 13 ranked SNPs (Additional Files 1–4 in DRYAD: (<https://doi.org/10.5061/dryad.wh70rxwnt>)). Five additional main-effect SNPs not detected by EMMAX were also detected by METAL; with positional candidate genes previously associated with Parkinson's disease (CDYL, NT5C2), Alzheimer's disease (CDYL), pathological inclusions of neuronal intermediate filaments (INA), and scrapie (NT5C2, TSR2) (Cairns et al. 2004; Filali et al. 2014; Nalls et al. 2014; Majer 2015; Lo et al. 2020; Aslam et al. 2021). Relevant to our EMMAX GxE analysis, SNPs displaying evidence of significant heterogeneity of effects, as evidenced by Cochran's Q-test, included ARSB (intron 4) as the most significant GxE interaction with respect to differential susceptibility to CWD (Fig. 2, Supplementary Table 2). In addition to ARSB, METAL-based analysis also identified eight additional SNPs with significant heterogeneity of effects across US regions; seven of which were also detected by EMMAX GxE GWAA (Fig. 2, Supplementary Table 2). One significant SNP that was detected in our METAL-based analysis via Cochran's Q-test for heterogeneity was intergenic between PLS3 and DACH2; with DACH2 previously implicated in the

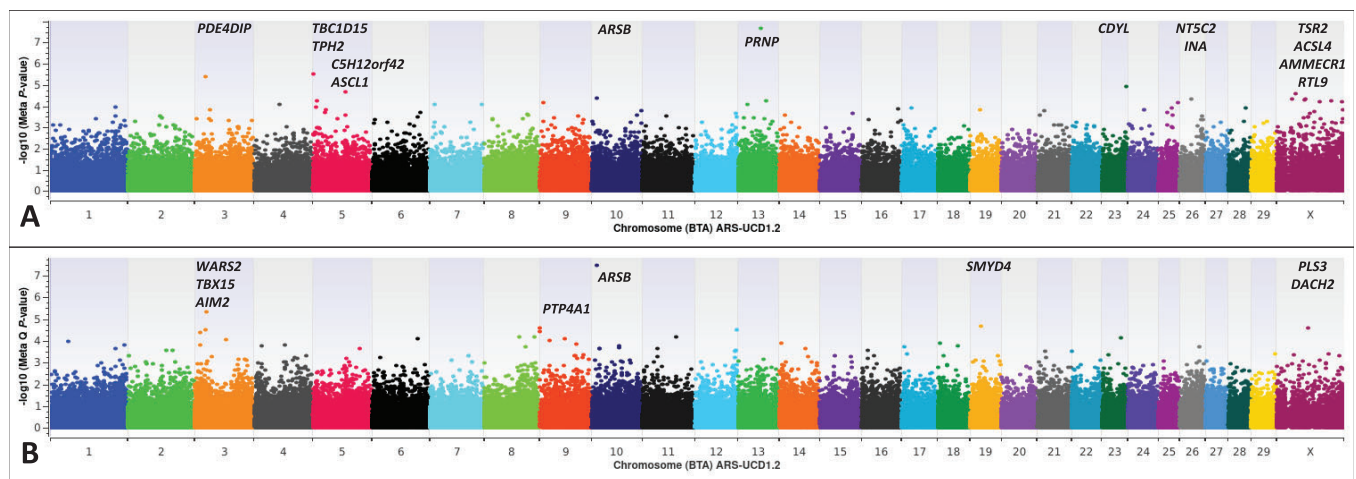


Fig. 2. Binary case-control (0, 1) meta-analysis for differential susceptibility to CWD in farmed US white-tailed deer (*Odocoileus virginianus*; hereafter WTD) from the Northeast, Midwest, and South. Individual EMMAX GWAA's (Kang et al. 2010; Segura et al. 2012; Seabury et al. 2020) for each US region were used in conjunction with the METAL-based approach to conduct a meta-analysis (Willer et al. 2010). METAL-based analyses included diagnostically confirmed CWD positive ($n = 412$) and CWD nondetect ($n = 758$) WTD. a) METAL-based Z-score analysis of shared WTD SNP effects and positional candidate genes influencing differential susceptibility to CWD across 3 US regions (Northeast, Midwest, South). Genomic inflation factor (Pseudo-Lambda) = 1.015. b) METAL-based Cochran's Q-test for heterogeneity of SNP effects (Cochran 1954; Willer et al. 2010) across 3 US regions (Northeast, Midwest, South) and relevant positional candidate genes. Genomic inflation factor (Pseudo-Lambda) = 0.997.

pathophysiology of scrapie (Gossner and Hopkins 2015). However, it should also be noted that the same SNP implicating DACH2 was also among the top 39 ranked SNPs in a EMMAX GWAA (Fig. 1, Additional File 1), and the most significant SNP in a regional EMMAX GWAA for farmed WTD in the US South (Additional File 15; DRYAD: <https://doi.org/10.5061/dryad.wh70rxwnt>). Altogether, these results are intriguing considering that the molecular phenotype of experimentally passaged CWD in sheep is known to be indistinguishable from some strains of scrapie in sheep (Cassmann et al. 2021). Application of the METAL-based meta-analysis approach to regional EMMAX GWAA's with and without additional fixed effect covariates (i.e. sex and age; sex, age, and farm) demonstrated that the majority of the significant main effect SNPs, and those displaying significant heterogeneity of effects across three US regions, were shared across all analyses. Thus, the mixed model solutions for various US regional model fits consistently implicate an overlapping set of the same significant SNPs and corresponding positional candidate genes. Detailed summary data for all METAL-based meta-analyses, including the EMMAX mixed model solutions from all regional model fits, and all corresponding METAL-based meta-analysis results with PP-Plots are provided in Additional Files 13–30 in DRYAD (<https://doi.org/10.5061/dryad.wh70rxwnt>). Collectively, our analyses of these data are compatible with several prior studies; where aspects of prion disease presentation were largely influenced by a genetic architecture independent of PRNP (Kingsbury et al. 1983; Stephenson et al. 2000; Iyegbe et al. 2010; Seabury et al. 2020).

Conclusions

Herein, we perform the largest GWAA to date for CWD in WTD, thereby further confirming that differential susceptibility to CWD is a highly heritable, polygenic trait in farmed US WTD, but with greater overall complexity than previously postulated or reported; as evidenced by significant GxE interactions, the general paucity of moderate or large-effect SNPs, and conversely, the large number of SNPs displaying small effects on risk. We also confirm PRNP codon 96 as the largest-effect region of the WTD genome across 3 US regions (Northeast, Midwest, South). **However, the proportion of phenotypic variance explained (PVE) by PRNP SNPs alone cannot be expected to facilitate a successful CWD eradication program, as further evidenced by 20 CWD positive WTD possessing the codon 96SS genotype enrolled in the present study; including one that was both lymph node and obex positive.** Finally, we provide the first evidence linking naturally occurring genetic variation in a lysosomal GAG catabolism gene (ARSB) to differences in CWD susceptibility in farmed US WTD, but also further confirm the involvement of genes underlying other neurodegenerative diseases such as Parkinson's, Alzheimer's, and various prion diseases of mammals, including scrapie and sporadic Creutzfeldt-Jakob disease.

Data availability

Accession codes are as follows: Data (DRYAD: <https://doi.org/10.5061/dryad.wh70rxwnt>).

Supplemental material is available at G3 online.

Author Contributions

CMS designed the research. MAL and TAN provided CWD diagnostic data, other relevant animal metadata, and biological samples for DNA isolation from existing agency repositories. CMS

performed quality filtering of genotypes, GWAA with marker-based heritability estimates, meta-analysis, and alignment with positional candidate genes. CMS wrote the manuscript; incorporating input from MAL, and TAN. All authors edited and approved the manuscript.

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Conflicts of interest

None declared.

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