Breed-specific effects of inbreeding and artificial selection on susceptibility to equine exertional rhabdomyolysis

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Abstract

Background: Equine exertional rhabdomyolysis (ER) syndrome is a myopathy characterised by repeated episodes of muscle fibre damage induced by exercise. Whilst ER syndrome is heritable, previous studies have not identified causal variants nor pathophysiological mechanisms. The disorder has welfare and financial implications, but despite this, a performance advantage has been identified in susceptible racing Standardbreds. **Objectives:** To assess whether artificial selection and inbreeding are associated with increasing susceptibility to ER syndrome in horses. **Study design:** Case-control study. **Methods:** We used genetic data from 33 Connemaras (CP) and 94 Warmbloods (WB) to calculate Wright's fixation index (F_{ST}), runs of homozygosity (ROH), genomic inbreeding, and to model inbreeding depression both within and across the two breeds. **Results:** Signatures of selection were identified both in WB and across breeds; CP had elevated F_{ST} values across the genome, indicating a higher degree of differentiation. When using a hierarchical F_{ST} model, a greater degree of differentiation was captured when disease state was nested within breed. ROHs in ER WBs were associated with an overrepresentation of cyclic AMP signalling pathway genes, and a greater proportion of large (>16Mb) ROHs were identified in ER-susceptible WBs, indicative of recent inbreeding. ER WBs had significantly higher (U=1414.0, p=0.018) inbreeding coefficients (F_{ROH}) than controls, however inbreeding depression models did not have good predictive ability. **Main limitations:** Small sample size, particularly for inbreeding depression modelling and within CP, and results present associations rather than proven causality. **Conclusions:** In summary, ER appears to have different genetic background in different breeds, with potential contributing effects of artificial selection in CP and of inbreeding in WB. Although a difference in inbreeding between WB cases and controls was observed, a specific effect of inbreeding depression was not supported.

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Keywords: neuromuscular disease; horse genetics; myopathy; genetic disease; equine inbreeding; genetic selection

Summary

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of homozygosity (ROH), genomic inbreeding, and to model inbreeding depression both within and across the two breeds.**Results:** Signatures of selection were identified both in WB and across breeds; CP had elevated F_{ST} values across the genome, indicating a higher degree of differentiation. When using a hierarchical F_{ST} model, a greater degree of differentiation was captured when disease state was nested within breed. ROHs in ER WBs were associated with an overrepresentation of cyclic AMP signalling pathway genes, and a greater proportion of large (>16Mb) ROHs were identified in ER-susceptible WBs, indicative of recent inbreeding. ER WBs had significantly higher (U=1414.0, p=0.018) inbreeding coefficients (F_{ROH}) than controls, however inbreeding depression models did not have good predictive ability.**Main limitations:** Small sample size, particularly for inbreeding depression modelling and within CP, and results present associations rather than proven causality.**Conclusions:** In summary, ER appears to have different genetic background in different breeds, with potential contributing effects of artificial selection in CP and of inbreeding in WB. Although a difference in inbreeding between WB cases and controls was observed, a specific effect of inbreeding depression was not supported.

Introduction

Equine recurrent exertional rhabdomyolysis (RER) is a myopathic syndrome characterised by repeated episodes of muscle fibre damage induced by exercise. The frequency, severity, and clinical presentation of exertional rhabdomyolysis (ER) episodes varies both between individual horses and between episodes in the same horse, but clinical signs can include stiffness, muscle pain, fasciculations or cramping, elevated muscle enzyme activities (creatine kinase (CK) and aspartate aminotransferase (AST)) in serum, reluctance to move, myoglobinuria, recumbency, kidney damage and even death. RER as a syndrome is sometimes considered to be a disease of Thoroughbreds (TB), affecting 5-7% of racehorses[1-3]and with a significant economic impact in the industry racing[1-3] – but, recurring episodes of ER are also seen in other breeds of horse involved in harness racing[4], eventing[5], polo[6], endurance riding[7] and more, including breeds such as Warmbloods (WB) and Connemara ponies (CP), which may represent the same disease as seen in TBs.Whilst RER is heritable in TB (h^2=0.41-0.46) and Standardbred (STB) horses (h^2=0.39-0.49)[8], genome-wide association studies (GWAS) in TB[9,10], have failed to identify associated genetic markers or causative mutations, and aetiopathogenesis remains largely unclear. It was previously identified in 2010 that STB horses with RER susceptibility had faster race times than matched horses without RER[4]. This indicated a performance advantage for RER cases, despite the welfare and financial implications of the disorder: due to the heavy selection for racing performance in both TB and STB racehorses, this prompted the question of whether breeders are inadvertently selecting for RER susceptibility alongside performance in racehorses. Neither CP nor WB are racing breeds, but both are used for equestrian sport[11], so similar performance enhancing effects could apply to ER syndrome in these breeds. Inbreeding, and the subsequent accumulation of homozygosity and risk of recessive disease alleles, can affect fitness and reproductive traits – termed inbreeding depression[12]. In cattle, inbreeding depression on reproductive traits and resistance to disease such as mastitis is well established[13,14]. In horses, inbreeding depression on fertility traits is described[15,16], and one study identified inbreeding depression on racing performance in TBs[17], which might relate to the performance advantage in racing due to RER described previously[4]. It is possible that inbreeding depression plays a role in ER susceptibility in other breeds; indeed, both CP and WB have a moderate degree of inbreeding (mean F_{ROH} of 0.047 to 0.084 in different CP groups and 0.095 to 0.118 in different WB groups[11]). In order to investigate the hypothesis that artificial selection and inbreeding play a role in ER susceptibility, here we compared genotypic data from ER cases and controls, within WB and CP and across both breeds, using approaches such as Wright's fixation index (F_{ST}) , runs of homozygosity (ROH) [18], and genomic inbreeding (F_{ROH})[18]estimations.

Materials & methods

Ethical approval

All work was conducted with the approval of the XXX's Clinical Research Ethical Review Board (CRERB, reference 2018 1834-2) and Social Science Research Ethical Review Board (SSRERB, reference SR2018-1799).

Sample selection

WB and CP ER cases were identified from the XXX's XXX Laboratory's (XXX) muscle biopsy service biobank, or recruited as discussed below. Cases from the biobank were diagnosed with ER by a specialist (author XX) based on clinical history, signalment, and the presence of typical (non-specific) myopathic histological features in muscle biopsy samples. Horses with an active inflammatory histological presentation (defined by presence of endomyseal or sarcoplasmic inflammatory cellular infiltrates) were excluded. Recruited cases had displayed 2 or more episodes of ER detected clinically, with associated elevation (above laboratory reference range) in serum or plasma creatine kinase (CK) and aspartate aminotransferase (AST) activity by a veterinary surgeon. These samples were then referred to as the ER CP and ER WB groups. Control horses were defined as registered WB and CP aged 8 and over, with no history of ER, or any other muscle or hepatic disorders (the latter can sometimes be mistaken biochemically for muscle disorders), that had had previous or were in current athletic use. Residual blood samples from 7 suitable control horses (2 CP and 4 WB) that had been submitted to the RVC for unrelated diagnostic purposes were used. Moreover, sixty-three other control horses (16 CP and 46 WB) were recruited via the XXX website, social media and stakeholder groups, from across the UK and a range of different sporting disciplines, with a hair root sample provided for each one. Further metadata that included breed sub-type based on the relevant registered studbook, age, sex, use (such as showjumping, eventing, leisure riding, etc) and fitness were also gathered, either from submitted veterinary history in cases, or via an owner questionnaire where possible for cases, and for all controls.

DNA extraction, whole genome sequencing and DNA array genotyping

DNA was extracted using the manufacturers' protocols with the following three extraction kits: for muscle tissue, the Qiagen DNEasy Blood and Tissue kit; for whole blood, the Illustra Nucleon BACC kit; for hair root, the Qiagen Gentra Puregene kit. Subsequently, 17 CP and 79 WB were genotyped using the Affymetrix 670k HD Equine SNP array[19] and 19 CP and 19 WB were whole genome sequenced (WGS) at 20X coverage using Illumina HiSeqX 150bp paired-end sequencing (commercially by Edinburgh Genomics). Three randomly selected individuals' DNA were both sequenced using WGS and genotyped on the Equine Affymetrix array. The sequencing reads were mapped to EquCab3.0 and variants were called using the GATK4 Best Practices pipeline[20]. Biallelic single nucleotide variants (SNVs) from the WGS overlapping with those single nucleotide polymorphisms (SNPs) on the HD array were retained and filtered, and the WGS SNVs and HD array genotypes were then merged using bcftools[21] and GATK. This merged dataset then underwent the following quality control using PLINK 1.9 software[22]: 95% call rate per sample and SNP, 1% minor allele frequency (MAF), and a Hardy–Weinberg equilibrium test P-value of $>10e^{-6}$. After quality control, 127 samples (33 CP and 94 WB, see Supplementary Table S1) and 493,795 SNPs remained for further analysis, referred to hereafter as the genotypic data.

Principal components analysis

Based on the genotypic data, centred genetic relationship matrices (GRMs) were produced both within and across breeds, and principal components analysis (PCA) carried out using the algorithm GEMMA (*-eigen*)[23]. Principal components 1 to 3 (PCs) were then plotted in biplots Python 3.7 using seaborn[24] and matplotlib[25] packages.

Wright's fixation index (F_{ST}) analysis

F_{ST} values were calculated using *–fst* in PLINK v1.9[22] comparing RER cases and controls both across and within breeds, and plotted using *manhattan* from the R package qqman[26]. The top 0.5% SNPs with the highest F_{ST} values were extracted, and the genes within 1 Mb windows (as defined from previous linkage disequilibrium (LD) analysis[11]) around these SNPs were annotated using the BiomaRt package in R[27,28]. The lists of genes were then assessed using an over-representation test in DAVID[29] for significant curated database terms to indicate particular overrepresented pathways or processes that potentially are subject to selection[30-34], using the official gene symbols, the *Equus caballus* background, an EASE threshold of 0.1, and a Benjamini-Hochberg-corrected p-value of 0.05. Finally, overall F_{ST} and F_{STP}(F_{ST} weighted for group sample size) for disease state across and within breeds, and hierarchical F_{ST} both for disease state within breed and for breed within disease state were calculated using the genotypic data and commands *wc* and*varcomp.glob* from the hierfstat package[35] in R. By comparing overall F_{ST} with hierarchical F_{ST} estimates, we aimed to assess whether greater differentiation is seen by accounting for breed in a hierarchical model.

Runs of homozygosity

Runs of homozygosity (ROHs) were detected for each individual sample from autosomes using the detectRUNS package in R[36]. ROH detection settings were made equivalent to PLINK defaults, excepting minimum ROH length (1 Mb, derived from prior LD analyses[11]), minimum density (1 SNP per 60kb) and maximal gap (500kb) according to Meyermans et al[37], where the effects of various ROH detection parameters on animal genotypic data were examined. Plots were then produced using detectRUNS, seaborn[24], matplotlib[25] and statannotations[38]in R[36], and overlapping runs between individuals were identified using the *multiinter* command from bedtools[39,40]. ROHs present in [?]10% of individuals in CP or WB ER or control groups, or in at least 2 individuals within the group (whichever was the larger), were plotted using the PhenoGram web tool[41]. Genes within all of these ROHs were identified using Ensembl Biomart[42], and assessed using an over-representation test in DAVID[29] as described above to identify overrepresented gene ontology (GO) terms.

Genomic inbreeding

Inbreeding was then calculated as F_{ROH}[18], where:

$$F_{\mathrm{ROH}} = \frac{\sum \mathrm{ROH}_{\mathrm{length}}}{\mathrm{Length}_{\mathrm{genome}}}$$

Equation 1 F_{ROH} was calculated at both the chromosome-wide and genome-wide level and compared across and within breeds between ER and control horses using nonparametric Mann-Whitney U tests[43] and effect size calculated as Cliff's δ[44] where:

$$\delta = \frac{2U}{\mathrm{mn}} - 1$$

Equation $2U$ is the Mann-Whitney U statistic, where m and n represent the size of the two distributions. ROHs were also split into classes: from 1-2 Mb, 2-4 Mb, 4-8 Mb, 8-16 Mb and >16 Mb to assess recent versus ancient inbreeding[45], and recent inbreeding measures ($F_{ROH(>8Mb)}$ and $F_{ROH(>16Mb)}$) were calculated using Equation 1, but with the sum of all ROH lengths replaced with the sum length of all ROHs greater than 8 Mb and 16 Mb, respectively. Comparisons of ER and control horses within and across breeds for these recent inbreeding measures were then carried out as described above for F_{ROH}.

Modelling covariates for inbreeding depression on ER

Generalised linear models with a logit link function in R (using glm())[46]) were used to model effects of variables on the binary ER case-control outcome. Sex was previously identified as a risk factor for RER in TBs and STBs[1-4], and age as a risk factor in TBs[1,2], so these were included as fixed effects in all models due to possible biological relevance. A variable accounting for breed (whether breed, registered studbook or within-breed genetic group[11]) was also included in the final model - these variables were compared univariably. Moreover, the variables of horse use (e.g. eventing, dressage, leisure, etc), fitness, and platform (DNAarray or WGS) were also tested both in univariate models and then in a backwards elimination multivariable model. Furthermore, variance inflation factor (VIF) was calculated using the car package[47] in R as the ratio of the overall model variance to the variance of a model only containing the tested variable. VIF was calculated for each fixed effect in the final model, and fixed effects of registered studbook, genetic group and the first two PCs from the PCA separately and in combination. As including PCs from a PCA of the GRM is commonly used to correct for cryptic population structure in GWAS and other genetic studies[48], we tested for collinearity between studbook, within-breed genetic group and the first two PCs, as well as including sex and age for their biological relevance. Four models were run: Model 1.1, with all six variables; Model 1.2, with age, sex, studbook and genetic group; Model 1.3 with age, sex, studbook and the first two PCs; and Model 1.4, with age, sex and studbook.

Inbreeding depression

The effect of inbreeding on ER susceptibility was investigated using inbreeding depression models. Initially, outlier inbreeding values, defined as outwith 1.5 times the interquartile range within disease groups, were removed for each of the three inbreeding metrics (F_{ROH}, $F_{ROH(>8Mb)}$, and $F_{ROH(>16Mb)}$). Logistic regression models, both within and across breeds, were utilised to identify the effect of inbreeding measures F_{ROH}, $F_{ROH(>8Mb)}$, and $F_{ROH(>16Mb)}$ on disease state using statsmodels.Logit() function in Python 3.7[49]. A multivariable model including both inbreeding and the covariates identified from the above analysis (Model 2.1), and a univariable model for inbreeding alone (Model 2.2) was used across and within breeds for each inbreeding measure:

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4$$

Equation 3 Where p represents probability of being an ER case,β_0 represents the intercept, whilstx_i represents the explanatory variables andβ_i the regression coefficients of those variables.

Results

The mean age of all horses was 10.6 (ranging from 2 to 26 years; SD=4.3) years old. 51.2% of the samples were males (of which 6 were entire) and 42.0% were females (sex was not recorded in a small number of cases).

Principal components analysis

Decomposition of the genomic relationship matrix was carried out using PCA. The biplots of the PCs and kernel density estimator (KDE) plots of the distribution across PCs (Figure 1) showed that CP and WB separated well, with only the Anglo-European and British WBs separating out within the WB subtypes. However, there was no apparent separation of cases from controls.

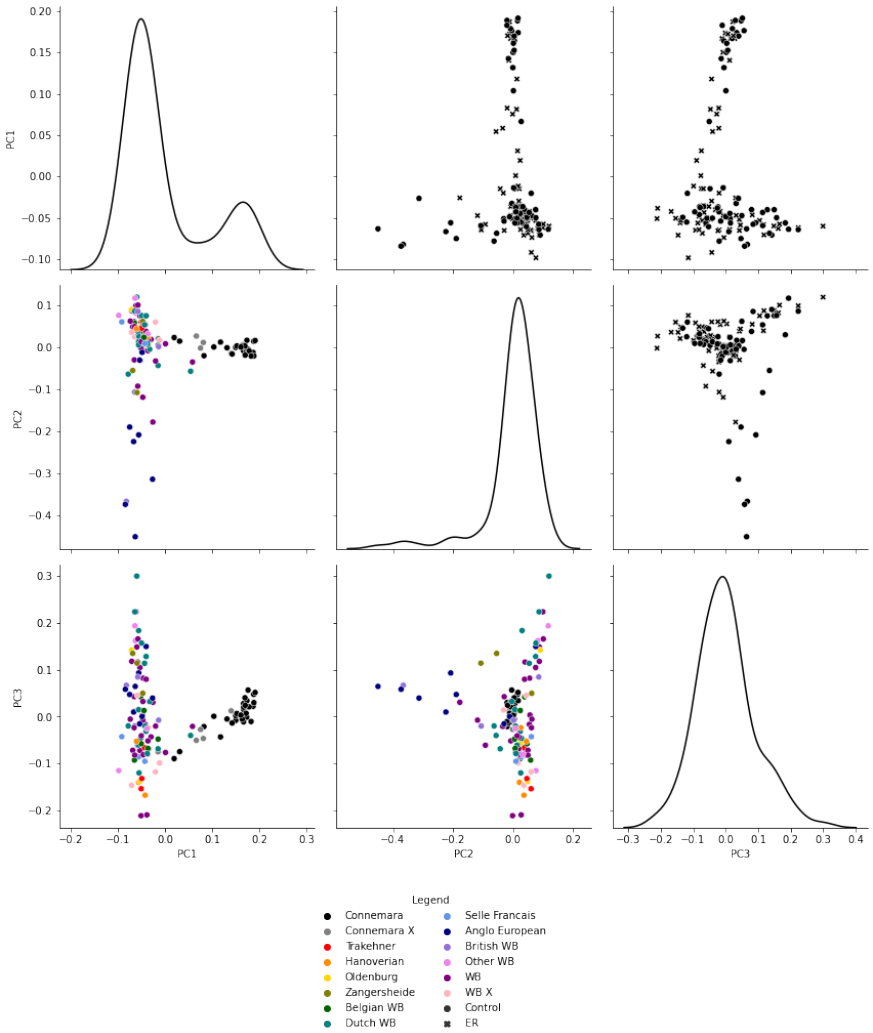

Figure 1: Biplots of the principal components analysis based on the genomic relationship matrix for 94 WB and 33 CP. Lower diagonal plots show PCA biplots with colour designating the breed subtype; and upper diagonal plots show PCA biplots with marker shape designating the disease phenotype (crosses represent ER cases and circles represent controls). Diagonal plots are kernel density estimator plots illustrating the distributions of the principal components.

PCA within each breed revealed similar results as noted in the biplots of the principal components (PCs) (Supplementary Figure S1). As seen previously in a larger sample of WBs and CPs[11], non-registered CPs and Anglo-European and British WBs did separate slightly from the rest of the registered studbooks. In both analyses, this separation was observed particularly along PC1.

Wright's fixation index (F_{ST}) analysis

F_{ST} values were calculated per SNP marker across and within breeds. F_{ST} values were higher when CP ER cases were compared to controls than in WBs or across breeds comparisons (Figure 3) suggesting there might be selection pressures on ER in CPs, which is not obvious in WBs. Manhattan plots displaying the F_{ST} values per marker are found in Figure 2.

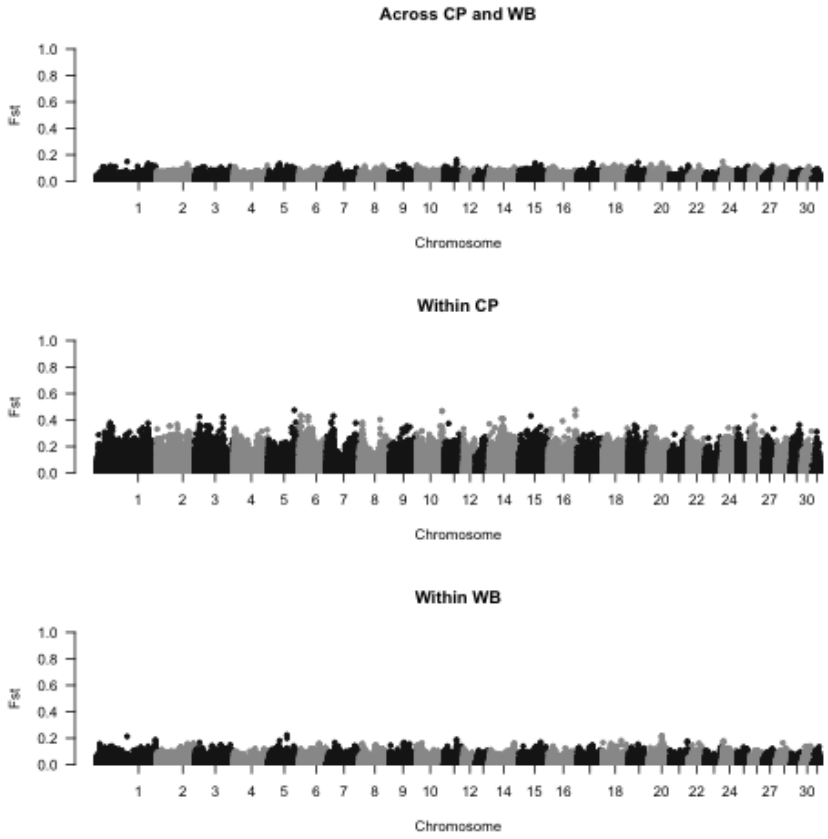

Figure 2: Manhattan plot demonstrating the results of the Wright's Fixation Index analysis for ER susceptibility. Y-axis shows F_{ST} values per genetic marker between ER cases and controls across both breeds (n=127, top), in CP (n=33, middle) and in WB (n=94, bottom), with the x-axis showing genomic location (by chromosome).

The top 0.5% of F_{ST} values (2,144 SNPs) from the genotyping data ranged from 0.061 to 0.162 across breeds, from 0.198 to 0.475 in CP, and from 0.081 to 0.224 in WB. This resulted in 7,157 (across breeds), 6,549 (CP), and 6,307 (WB) genes, respectively. From these, five, zero and two GO terms respectively were overrepresented in the gene lists according to DAVID analysis (Table 1), with ether lipid metabolism identified both across breeds and in WBs, with RNA transport also identified in WBs and muscle-related terms such as calcium ion binding and vascular smooth muscle contraction identified across breeds.

For both overall F_{ST} and F_{STP}, values were low both across and within breeds. This would be expected of a disease where natural or artificial selection has not been applied and hence there is not strong genetic differentiation between cases and controls into distinct subpopulations. However, both $F_{ST}$and F_{STP} were slightly higher in CP than in WB or across breeds. Overall F_{ST}, weighted F_{ST}(F_{STP}) and hierarchical F_{ST}(F_{STH}) are presented below in Table 2.

When assessed in a hierarchical model, F_{STH} for disease state within breed was slightly higher than in WB or across breeds, but slightly lower than in CP alone. When breed was within disease state, a negative F_{STH} value for disease state was calculated. Whilst F_{ST} is a 0 to 1 scale, the Weir and Cockerham[50] unbiased estimator does produce negative values, which reduces inflation of F_{ST} when differentiation is weak or non-existent. This can be interpreted as F_{ST} of 0, indicating no genetic differentiation based on disease state when breed was nested within disease state in a hierarchical model, although a slightly higher degree of

differentiation was apparent when disease state was nested within breed.

Runs of homozygosity

Whilst CP cases and controls were similar in the sum of the length of all ROHs (U=122, p=0.627), case WBs had higher median sum ROH than controls (U=1414, p=0.018). The total length of all ROHs is illustrated per disease state and breed in Supplementary Figure S2.

Moreover, when the average length of ROHs was compared with the average number of ROHs, it became clear that whilst CP cases and controls had very similar average number and length of ROHs, case WBs tended to have slightly more and slightly longer ROHs on average than WB controls (Supplementary Figure S3), although number of ROHs was not significantly different (U=600.5, p=0.140).

Overlapping ROHs within disease groups were identified, and ROHs present in 10% or more of horses within each group were plotted across the EquCab3.0 genome (Supplementary Figure S4). Notably, chromosomes 9, 24 and 25 had high coverage by ROHs.

CP had a greater proportion of runs >4Mb than WB, in both cases and controls, following splitting ROHs by size class (Figure 3). Control WB had approximately half the average number of ROHs number (ER CP=0.625; Control CP=0.647; ER WB=0.840; Control WB=0.450). This is indicative of a difference in degree of recent inbreeding between case and control in WBs, possibly due to selective pressure[51].

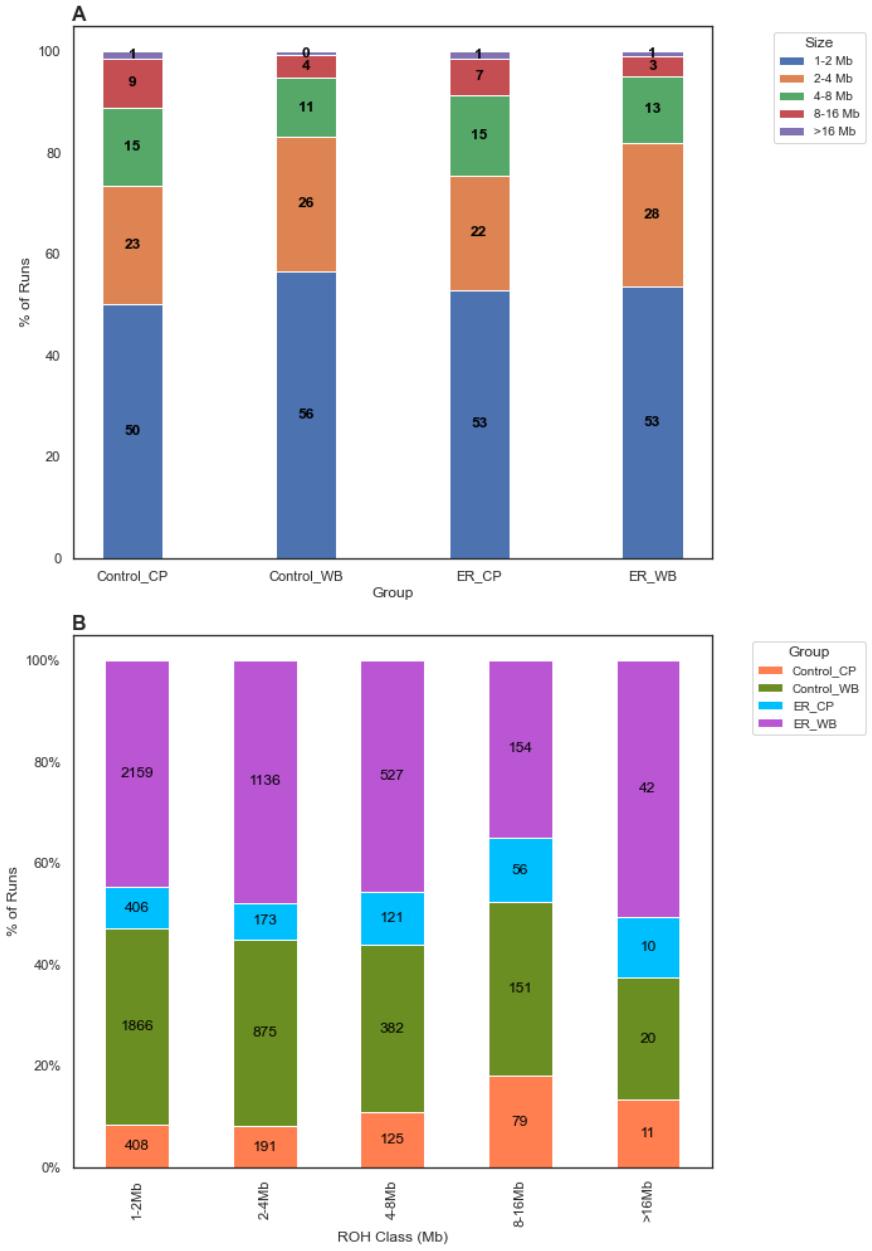

Figure 3: A: Stacked bar chart showing percentage (%) of ROHs per phenotype group (ER cases and controls in CP and WB) colourd by ROH size class (1-2Mb, 2-4Mb, 4-8Mb, 8-16Mb and >16Mb), with number of ROHs per group per class annotated. B: Stacked bar chart of percentage (%) of ROHs per ROH size class coloured by phenotype group (case and control CP and WB), with annotation of number of ROHs per group in each class.

Genes within common ROHs per disease group were extracted, and overrepresentation of ontology terms identified using the DAVID database. Significant overrepresentation (Benjamini-adjusted p-value of $1.5E^{-2}$) was identified for the cAMP signalling pathway only in ER WB horses.

The overlap between genes within common ROHs across the different groups are illustrated in the Venn diagram in Supplementary Figure S5. 105 genes were shared between ER CP and ER WB cases, whereas

only 50 were shared between control CP and WB. Notably, CP common ROHs included far more genes than WB common ROHs.

The number of genes unique to the two ER groups and the two control groups and the number of genes shared between controls and cases were compared using Chi-square test. The controls shared significantly fewer genes between CP and WB control groups than was the case in ER horses (χ=51.39, p=7.59E^{-13}).

Genomic inbreeding

Median F_{ROH} tended to be slightly lower in control horses than ER cases, with a median of 0.087 and 0.094 in control and RER horses, respectively (Supplementary Figure S6). $F_{ROH(>8Mb)}$ was also slightly lower in controls, with a median of 0.016 and 0.022, respectively, but the median of both case and control animals for $F_{ROH(>16Mb)}$ was 0. When compared with Mann-Whitney U tests, no across-breed case-control comparison was significant.

Median F_{ROH} tended to be slightly lower in ER CP cases than controls and higher in ER WBs than controls, with a median of 0.071 and 0.100 in ER CP and WB respectively, compared to 0.075 and 0.091 in Control CP and WB, respectively (Figure 4). However, in $F_{ROH(>8Mb)}$ the results were similar between the two ER groups, while the control WBs had a lower $F_{ROH(>8Mb)}$ than other groups (ER CP=0.020; Control CP=0.029; ER WB=0.022; Control WB=0.015). For $F_{ROH(>16Mb)}$, medians across all four groups were 0. Despite similar ROH genome coverage by large ROHs, the number of ROHs varied (as was illustrated in Figure 3 and Supplemenatry Figure S3) and therefore the average size of those large ROHs also varied.

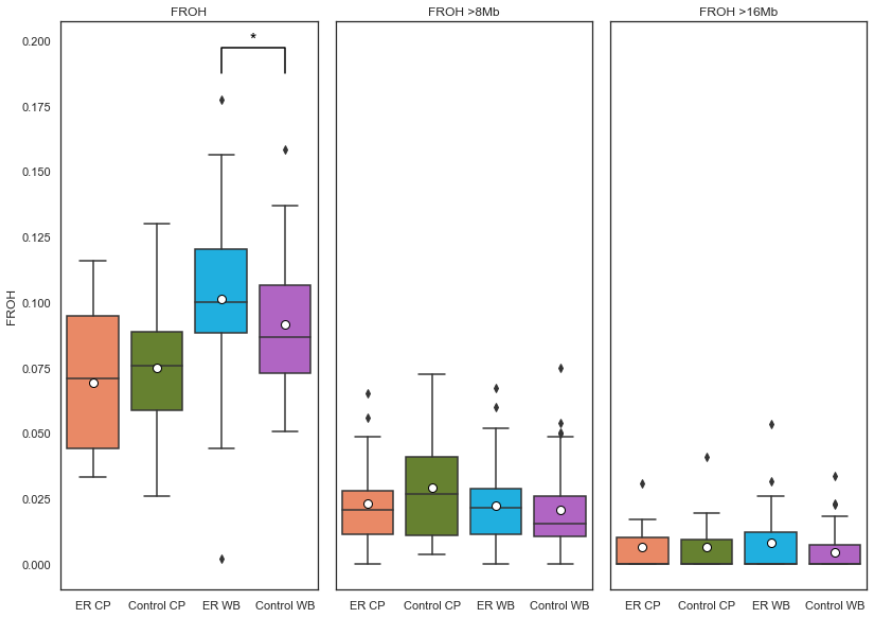

Figure 4: Boxplots of inbreeding in ER case and control CP and WB represented by F_{ROH}, with mean given in white circles and outlier values as diamonds. After removal of statistical outliers, within-breed case-control comparisons that were significant using a Mann-Whitney U test at p<0.05 are indicated by *. Whilst there were no significant differences between case and control F_{ROH} measures within breed at the >8Mb and F_{ROH} than control WB.

When inbreeding was compared with a Mann-Whitney U test, F_{ROH} was not significantly different between ER cases and controls across breeds (U=2409.0, p=0.056) or within CP (U=122.0, p=0.627), but ER WBs were significantly more inbred than WB controls (U=1414.0, p=0.018). This was not the case for $F_{ROH(>8Mb)}$

or $F_{ROH(>16Mb)}$, where all comparisons were non-significant. The effect size was small (δ between 0.147 and 0.33 as per Romano et al[52]) both in the case of WBs (δ=0.2855) and across breeds (δ=0.1967).

When F_{ROH} was broken down at chromosome level, distinct patterns began to emerge (Supplementary Figure S7). Inbreeding was more uniform across the genome in WB groups than CP groups, with increased inbreeding seen in ECA24 and ECA25 and decreased inbreeding in ECA12 and ECA13 in CP groups compared to WB groups.

Modelling covariates for inbreeding depression models of ER

Whenever any two or more of studbook, genetic group or PC1 were in the model a VIF above 2.5 was observed, with collinearity particularly between genetic group and PC1 (Supplementary Table S2), as might be expected due to the nature of the genetic group being based on principal components as described before[11]. In the univariable analysis, all variables except platform (DNA array or WGS) and breed (CP or WB) were significantly associated with disease state.

The initial multivariable model therefore included all significant variables except for genetic group, as this would correlate to some degree with studbook, but was less significant in a univariable model. The final reduced model consisted of sex, studbook and age. Results for all univariable models and the final multivariable model are presented in Table 3.

Inbreeding depression

Inbreeding depression was not observed, as no inbreeding metric was significant in any of the univariable or multivariable models (Supplementary Tables S3, S4 & S5), however notably the pseudo r^2 was not above 0.07 in any bar one model, indicating the lack of predictive ability. Despite the significant difference in F_{ROH} between WB cases and controls using a Mann Whitney U test (see above), this did not result in significant predictive ability of disease status in WBs.

Nevertheless, in WBs, sex and studbook were either significant or suggestive in the models for all 3 inbreeding measures, with age also suggestive for F_{ROH}.

Discussion

In this study, we investigated the potential role of inbreeding and selection pressure on equine ER susceptibility. We identified increased F_{ST} differentiation between ER cases and controls in CP, compared to WB, implying that in CP, indirect selection might exist for ER. On the other hand, a significant difference in F_{ROH}estimated between ER cases and controls in WB, indicating an increased level of inbreeding in ER cases that might suggest reduced fitness due to inbreeding depression; however, this difference was not reflected in significant inbreeding depression models for susceptibility to ER. F_{ST} analysis is typically used to assess differentiation of populations and subpopulations, or to identify signatures of selection between subpopulations at single markers or across the genome[53] – occasionally studies have used susceptible and resistant breeds or lines of animals as a method to identify regions under selection and thus genomic areas of association with disease[54]. However, in this study, we treated case and control horses from the same population as different subpopulations, looking for both population differentiation and single-marker signatures of selection between cases and controls. This approach has been used previously in resistance to Aleutian mink disease virus in American mink[55] and in German Shepherd dogs with inflammatory bowel disease[56]. Similarly to those studies[55,56], we found no evidence of overall differentiation in our subpopulations based on disease state. We also found similar low levels of differentiation between the breeds as described previously[11] – likely low due to the admixture in the overall WB population resulting is lower F_{ST} values when compared with CP than might be expected . Single-marker F_{ST} analysis comparing ER cases versus controls identified

significant ontology terms in the 'across breed' analysis: calcium ion binding; vascular smooth muscle contraction; Ras signalling; T-cell receptor signalling; and ether lipid metabolism. Whilst immune terms such as T cell receptor signalling are common signatures of selection in animals including between horse breeds[53], the other ontology terms might then be implicated in ER. Ca^{2+} regulation and vasodilation have been jointly implicated in bleeding abnormalities in human ER caused by *RYR1* mutations[57], and abnormalities in Ca^{2+} regulation have been previously proposed as a mechanism of disease for RER in horses[58-62], with 148 different calcium binding genes identified in our analysis, including voltage-sensitive calcium channel *CACNA1B* , calcium activated potassium channel *KCNN3* , and calcium homeostasis modulators *CALHM1* , *CALHM2* and *CALHM3* . Therefore these terms could be suggestive of across breed selection for performance that has contributed to disease susceptibility. Ether lipids are associated with the peroxisome and involved in antioxidant activity[63]which has been suggested as associated with RER[64], and enriched in skeletal muscle[65]. Ras regulates the cell cycle, cell survival and the reorganisation of the cytoskeleton, and in humans, developmental disorders known as RASopathies are characterised by cardiomyopathy and musculoskeletal disorders amongst other symptoms[66]. One such RASopathy is Costello syndrome, in which patients present with increased resting energy expenditure[67] as well as decreased muscle mass[68]. In CP, both the highest 0.5% of F_{ST} values (F_{ST}) and the overall F_{ST} and F_{STP} were higher than across breeds or in WB. This suggests more regional genetic differentiation between cases and controls within CP, a possible indicator of (likely inadvertent) selection for ER susceptibility – although importantly, at a whole-genome level there was little evidence of genetic differentiation between cases and controls. This interpretation is supported by previous research in Standardbreds (STB) with RER: specifically, Isgren et al[4] identified that RER-susceptible cases had significantly faster times from a standstill start, and higher winning and placing percentages than controls, indicating that RER susceptibility conferred a performance advantage. In breeds such as STB where there is considerable selective breeding for harness racing performance, if RER susceptibility is either causally related to or in linkage with performance traits, then there is likely inadvertent selection for RER susceptibility alongside improved performance. However, the top 0.5% of single-marker F_{ST} values in CP were not associated with any specific ontology terms, giving no indication as to what selected trait might be linked to ER susceptibility. Further supporting the role of selection in CPs, the highest r^2 model of inbreeding depression was the multivariable model of $F_{ROH(>8Mb)}$ in CPs, where $p<0.1$: considering the lower sample size in CP, this could yet be indicative of inbreeding depression, possibly due to the link between ROH islands and selection pressures as has been demonstrated previously in other species[51]. Whilst FROH in neither of our breeds reached the >0.2 reported in Thoroughbreds[69,70], the WBs in this study had higher mean inbreeding than reported previously[71](mean F from observed and expected heterozygosity was 0.059 in Swiss WBs and 0.052 in Hanoverians), with the ER WB having a mean F_{ROH} over 10%. Whilst CP had lower inbreeding than WB, previous work has indicated that they might have a higher degree of recent inbreeding[11], so management of genetic diversity might still be required. Moreover, in WB there was a significant difference in genomic inbreeding between ER cases and controls. In contrast to inadvertent positive selection for a disease trait, inbreeding depression is caused by an increasing degree of genome-wide homozygosity due to inbreeding, which leads to increased frequency of recessive deleterious variants and reduced fitness, survival and fertility[12,72]. In this instance, we saw significantly greater genomic inbreeding in ER WBs than controls, but F_{ROH} was not a significant predictor of disease state in an inbreeding depression model. This is most likely due to the low pseudo r^2 in the models, indicating poor predictive power. Exploration of this effect using a larger cohort would clarify further if inbreeding depression plays a role on ER susceptibility in WB horses. Enrichment for cyclic adenosine monophosphate (cAMP) signalling was identified in common ROHs appearing in WB ER cases. cAMP signalling pathways have a number of roles in skeletal muscle[73], including muscle development[74], regeneration[74], contractility[75-80], response to exercise[81-83], changes in myofibre size[73,74,84,85] and promotion of fibre-type shift towards glycolytic fibres[73,74,84]. In RER, exercise induces ER episodes, and glycolytic myofibres are those worst affected[86], so common ROHs containing an overrepresentation of cAMP signalling pathway genes in ER WB but not in controls offers another interesting potential pathophysiological insight, worthy of further study. In future, studies with a larger sample size are required to confirm these results. The sample size may also have reduced power to detect an effect in the inbreeding depression models. In future, ideally more environmental variables including diet and exercise levels[87-90]

would also be included in these models for all horses. In summary, there may be inadvertent selection for ER susceptibility, possibly alongside performance traits, in CPs, although cases and controls are not differentiated populations. In contrast, in WBs, there is evidence of differences in genomic inbreeding between cases and controls, which might or might not be linked to inbreeding depression on ER resistance. These results imply that different genetic effects could be contributing to ER in the two different breeds, further supporting that the genetic architecture of ER varies between horse breeds.

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Author contributions

The study was conceived and funding secured by XX, XX and XX. XX carried out initial diagnoses of RER cases, and XXX carried out study recruitment and prepared DNA samples. Genetic studies were carried out by XXX with guidancefrom XX. XXX, XX, XX and XX interpreted the results. XXX wrote the manuscript with input from XX, with all other co-authors providing manuscript editing and feedback prior to approval of the final manuscript.

Data availability statement

Sequencing data files will be available at ENA Accession PRJEB83318 after publication of the next paper on this data.

Research ethics statement

All work was conducted with the approval of the XXX's Clinical Research Ethical Review Board (CRERB, reference 2018 1834-2) and Social Science Research Ethical Review Board (SSRERB, reference SR2018-1799).

Competing interests

The authors declare that they have no competing interests.

References

https://CRAN.R-project.org/package=detectRUNS https://github.com/neilernst/cliffsDelta https://www.R-project.org/ https://doi.org/10.3168/jdsc.2020-0011 https://doi.org/10.1016/j.bbabio.2018.05.003 1 MacLeay, J. M., Sorum, S. A., Valberg, S. J., Marsh, W. E. & Sorum, M. D. Epidemiologic analysis of factors influencing exertional rhabdomyolysis in Thoroughbreds. *Am J Vet Res* **60** , 1562-1566 (1999).2 McGowan, C. M., Fordham, T. & Christley, R. Incidence and risk factors for exertional rhabdomyolysis in thoroughbred racehorses in the United Kingdom. *Veterinary Record* **151** , 623-626 (2002).3 Upjohn, M., Archer, R., Christley, R. & McGowan, C. Incidence and risk factors associated with exertional rhabdomyolysis syndrome in National Hunt racehorses in Great Britain. *The Veterinary Record* **156** , 763

(2005).4 Isgren, C. M. *et al.* Epidemiology of exertional rhabdomyolysis susceptibility in standardbred horses reveals associated risk factors and underlying enhanced performance. *PLoS One* **5** , e11594, doi:10.1371/journal.pone.0011594 (2010).5 Singer, E. R., Barnes, J., Saxby, F. & Murray, J. K. Injuries in the event horse: training versus competition. *The veterinary journal* **175** , 76-81 (2008).6 McGowan, C. M., Posner, R. E. & Christley, R. M. Incidence of exertional rhabdomyolysis in polo horses in the USA and the United Kingdom in the 1999/2000 season. *Veterinary record* **150** , 535-537 (2002).7 Wilberger, M. S., Mckenzie, E. C., Payton, M. E., Rigas, J. & Valberg, S. J. Prevalence of exertional rhabdomyolysis in endurance horses in the Pacific Northwestern United States. *Equine Veterinary Journal* **47** , 165-170 (2015).8 Norton, E. M. *et al.* Heritability of recurrent exertional rhabdomyolysis in standardbred and thoroughbred racehorses derived from SNP genotyping data.*Journal of Heredity* **107** , 537-543 (2016).9 Fritz, K. L., McCue, M. E., Valberg, S. J., Rendahl, A. K. & Mickelson, J. R. Genetic mapping of recurrent exertional rhabdomyolysis in a population of North American Thoroughbreds. *Anim Genet* **43** , 730-738, doi:10.1111/j.1365-2052.2012.02351.x (2012).10 Tozaki, T. *et al.* A genome-wide scan for tying-up syndrome in Japanese Thoroughbreds.*Anim Genet* **41** , 80-86 (2010).11 Lindsay-McGee, V.*et al.* Genetic characterisation of the Connemara pony and the Warmblood horse using a within-breed clustering approach. *Genetics Selection Evolution* **55** , 60-82, doi:10.1186/s12711-023-00827-w (2023).12 Charlesworth, D. & Willis, J. H. The genetics of inbreeding depression. *Nature Reviews Genetics* **10** , 783-796, doi:10.1038/nrg2664 (2009).13 Doekes, H. P. *et al.* Inbreeding depression due to recent and ancient inbreeding in Dutch Holstein–Friesian dairy cattle. *Genetics Selection Evolution* **51** , 54, doi:10.1186/s12711-019-0497-z (2019).14 Gutiérrez-Reinoso, M. A., Aponte, P. M. & García-Herreros, M. A review of inbreeding depression in dairy cattle: current status, emerging control strategies, and future prospects. *Journal of Dairy Research* **89** , 3-12, doi:10.1017/S0022029922000188 (2022).15 Perdomo-González, D. I. *et al.* Genetic inbreeding depression load for fertility traits in Pura Raza Española mares. *Journal of Animal Science* **99** , skab316 (2021).16 Perdomo-González, D. I.*et al.* Genetic inbreeding depression load for fertility traits in Pura Raza Española mares. *Journal of Animal Science* **99** , doi:10.1093/jas/skab316 (2021).17 Todd, E. T. *et al.* Founder-specific inbreeding depression affects racing performance in Thoroughbred horses. *Scientific reports* **8** , 1-10 (2018).18 McQuillan, R. *et al.* Runs of homozygosity in European populations. *The American Journal of Human Genetics* **83** , 359-372 (2008).19 Schaefer, R. J. *et al.* Developing a 670k genotyping array to tag˜ 2M SNPs across 24 horse breeds.*BMC genomics* **18** , 1-18 (2017).20 DePristo, M. A. *et al.* A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics* **43** , 491-498, doi:10.1038/ng.806 (2011).21 Danecek, P. *et al.* Twelve years of SAMtools and BCFtools. *Gigascience* **10** , doi:10.1093/gigascience/giab008 (2021).22 Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American journal of human genetics* **81** , 559-575 (2007).23 Zhou, X. & Stephens, M. Genome-wide efficient mixed-model analysis for association studies. *Nature genetics* **44** , 821-824 (2012).24 Waskom, M. L. seaborn: statistical data visualization. *Journal of Open Source Software* **6** , 3021, doi:10.21105/joss.03021 (2021).25 Hunter, J. D. Matplotlib: A 2D graphics environment. *Computing in Science & Engineering* **9** , 90-95 (2007).26 Turner, S. D. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots.*Journal of Open Source Software* **3** , doi:10.21105/joss.00731 (2018).27 Durinck, S. *et al.* BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. *Bioinformatics* **21** , 3439-3440 (2005).28 Durinck, S., Spellman, P. T., Birney, E. & Huber, W. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nature protocols* **4** , 1184-1191 (2009).29 Dennis, G. *et al.* DAVID: database for annotation, visualization, and integrated discovery. *Genome biology* **4** , R60 (2003).30 Ashburner, M. *et al.* Gene ontology: tool for the unification of biology. *Nature genetics* **25** , 25-29 (2000).31 Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research* **28** , 27-30 (2000).32 Kanehisa, M. Toward understanding the origin and evolution of cellular organisms. *Protein Science* **28** , 1947-1951 (2019).33 Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M. & Tanabe, M. KEGG: integrating viruses and cellular organisms. *Nucleic acids research* **49** , D545-D551 (2021).34 Gene Ontology Consortium. The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Research* **49** , D325-D334 (2021).35 Goudet, J. Hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular ecology notes* **5** , 184-186 (2005).36 detectRUNS:

Detect Runs of Homozygosity and Runs of Heterozygosity in Diploid Genomes v. R package version 0.9.6 (, 2019).37 Meyermans, R., Gorssen, W., Buys, N. & Janssens, S. How to study runs of homozygosity using PLINK? A guide for analyzing medium density SNP data in livestock and pet species. *BMC Genomics* **21** , 94, doi:10.1186/s12864-020-6463-x (2020).38 Statannotations v0.6 (Zenodo, 2022).39 Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features.*Bioinformatics* **26** , 841-842 (2010).40 Quinlan, A. R. BEDTools: the Swiss-army tool for genome feature analysis. *Current protocols in bioinformatics* **47** , 11.12. 11-11.12. 34 (2014).41 Wolfe, D., Dudek, S., Ritchie, M. D. & Pendergrass, S. A. Visualizing genomic information across chromosomes with PhenoGram. *BioData Mining* **6** , 18, doi:10.1186/1756-0381-6-18 (2013).42 Smedley, D.*et al.* BioMart – biological queries made easy. *BMC Genomics* **10** , 22, doi:10.1186/1471-2164-10-22 (2009).43 Vallat, R. Pingouin: statistics in Python. *Journal of Open Source Software* **3** (2018).44 cliffs_delta (, 2015).45 Schiavo, G.*et al.* Comparative evaluation of genomic inbreeding parameters in seven commercial and autochthonous pig breeds. *animal* **14** , 910-920 (2020).46 R: A Language and Environment for Statistical Computing v. 4.0.4 (2021-02-15) – "Lost Library Book" (, 2021).47 Fox, J. & Weisberg, S. *An R companion to applied regression* . (Sage publications, 2018).48 Price, A. L. *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nature genetics* **38** , 904-909 (2006).49 Seabold, S. & Perktold, J. in *Proceedings of the 9th Python in Science Conference.* 61 (Austin, TX).50 Weir, B. S. & Cockerham, C. C. Estimating F-statistics for the analysis of population structure. *evolution* , 1358-1370 (1984).51 Rodriguez-Ramilo, S. T., Reverter, A. & Legarra, A. Islands of runs of homozygosity indicate selection signatures in Ovis aries 6 (OAR6) of French dairy sheep.*JDS Communications* **2** , 132-136, doi: (2021).52 Romano, J., Kromrey, J. D., Coraggio, J., Skowronek, J. & Devine, L. in*annual meeting of the Southern Association for Institutional Research.* 1-51 (Citeseer).53 Gurgul, A. *et al.* A genome-wide scan for diversifying selection signatures in selected horse breeds.*PLOS ONE* **14** , e0210751, doi:10.1371/journal.pone.0210751 (2019).54 Xu, L. *et al.* Genetic assessment of inbred chicken lines indicates genomic signatures of resistance to Marek's disease.*Journal of Animal Science and Biotechnology* **9** , 65, doi:10.1186/s40104-018-0281-x (2018).55 Karimi, K., Farid, A. H., Myles, S. & Miar, Y. Detection of selection signatures for response to Aleutian mink disease virus infection in American mink. *Scientific Reports* **11** , 2944, doi:10.1038/s41598-021-82522-8 (2021).56 Peiravan, A. *et al.* Genome-wide association studies of inflammatory bowel disease in German shepherd dogs. *PLOS ONE* **13** , e0200685, doi:10.1371/journal.pone.0200685 (2018).57 Lopez, R. J. *et al.* An RYR1 mutation associated with malignant hyperthermia is also associated with bleeding abnormalities. *Science Signaling* **9** , ra68-ra68, doi:doi:10.1126/scisignal.aad9813 (2016).58 Dranchak, P. K. *et al.* Exclusion of linkage of the RYR1, CACNA1S, and ATP2A1 genes to recurrent exertional rhabdomyolysis in Thoroughbreds. *Am J Vet Res* **67** , 1395-1400 (2006).59 Dranchak, P. K. *et al.* Inheritance of recurrent exertional rhabdomyolysis in thoroughbreds.*Journal of the American Veterinary Medical Association* **227** , 762-767 (2005).60 Mlekoday, J. A. *et al.* Calcium sensitivity of force production and myofibrillar ATPase activity in muscles from Thoroughbreds with recurrent exertional rhabdomyolysis.*Am J Vet Res* **62** , 1647-1652 (2001).61 Ward, T. L., Valberg, S. J., Gallant, E. M. & Mickelson, J. R. Calcium regulation by skeletal muscle membranes of horses with recurrent exertional rhabdomyolysis. *Am J Vet Res* **61** , 242-247 (2000).62 Aldrich, K., Velez-Irizarry, D., Fenger, C., Schott, M. & Valberg, S. J. Pathways of calcium regulation, electron transport, and mitochondrial protein translation are molecular signatures of susceptibility to recurrent exertional rhabdomyolysis in Thoroughbred racehorses.*PLOS ONE* **16** , e0244556, doi:10.1371/journal.pone.0244556 (2021).63 Jimenez-Rojo, N. & Riezman, H. On the road to unraveling the molecular functions of ether lipids. *FEBS letters* **593** , 2378-2389 (2019).64 Piercy, R. J. & Rivero, J. L. L. in *Equine Sports Medicine and Surgery* (eds K. W. Hinchcliff, A. Kaneps, & R. Geor) Ch. 7, 109-143 (Saunders Ltd, 2014).65 Dean, J. M. & Lodhi, I. J. Structural and functional roles of ether lipids. *Protein & cell* **9** , 196-206 (2018).66 Dard, L., Bellance, N., Lacombe, D. & Rossignol, R. RAS signalling in energy metabolism and rare human diseases. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1859** , 845-867, doi: (2018).67 Leoni, C. *et al.* Understanding growth failure in Costello syndrome: increased resting energy expenditure. *The Journal of pediatrics* **170** , 322-324 (2016).68 Van der Burgt, I. *et al.* Myopathy caused by HRAS germline mutations: implications for disturbed myogenic differentiation in the presence of constitutive HRas activation.*Journal of medical genetics* **44** , 459-462 (2007).69 McGivney,

B. A. *et al.* Genomic inbreeding trends, influential sire lines and selection in the global Thoroughbred horse population.*Scientific Reports* **10** , 466 (2020).70 Lawson, J. M.*et al.* Does inbreeding contribute to pregnancy loss in <scp>Thoroughbred</scp> horses? *Equine Veterinary Journal* **56** , 711-718, doi:10.1111/evj.14057 (2024).71 Petersen, J. L. *et al.* Genetic diversity in the modern horse illustrated from genome-wide SNP data.*PLOS ONE* **8** , e54997, doi:10.1371/journal.pone.0054997 (2013).72 Doekes, H. P., Bijma, P. & Windig, J. J. How Depressing Is Inbreeding? A Meta-Analysis of 30 Years of Research on the Effects of Inbreeding in Livestock. *Genes* **12** , 926 (2021).73 Berdeaux, R. & Stewart, R. cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. *American Journal of Physiology-Endocrinology and Metabolism* (2012).74 Minetti, G. C. *et al.* Gαi2 signaling promotes skeletal muscle hypertrophy, myoblast differentiation, and muscle regeneration. *Science signaling* **4** , ra80-ra80 (2011).75 Van Der Heijden, H., Zhan, W., Prakash, Y., Dekhuijzen, P. & Sieck, G. Salbutamol enhances isotonic contractile properties of rat diaphragm muscle. *Journal of Applied Physiology* **85** , 525-529 (1998).76 Letts, L., Richardson, D., Temple, D. M. & Williams, L. The selectivity of beta-adrenoceptor antagonists on isoprenaline-induced changes in heart rate, blood pressure, soleus muscle contractility and airways function in anaesthetized cats. *British journal of pharmacology* **80** , 323 (1983).77 Hansen, A. K., Clausen, T. & Nielsen, O. B. Effects of lactic acid and catecholamines on contractility in fast-twitch muscles exposed to hyperkalemia. *American Journal of Physiology-Cell Physiology* (2005).78 Ha, T. N., Posterino, G. S. & Fryer, M. W. Effects of terbutaline on force and intracellular calcium in slow-twitch skeletal muscle fibres of the rat. *British journal of pharmacology* **126** , 1717-1724 (1999).79 Ha, T. N. & Fryer, M. W. Inhibitory effects of (±)-propranolol on excitation-contraction coupling in isolated soleus muscles of the rat. *British journal of pharmacology* **122** , 463-468 (1997).80 Cairns, S. P. & Dulhunty, A. F. β-Adrenergic potentiation of E–C coupling increases force in rat skeletal muscle. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine* **16** , 1317-1325 (1993).81 Catoire, M. *et al.* Pronounced Effects of Acute Endurance Exercise on Gene Expression in Resting and Exercising Human Skeletal Muscle. *PLOS ONE* **7** , e51066, doi:10.1371/journal.pone.0051066 (2012).82 Ropka-Molik, K. *et al.* Transcriptome profiling of Arabian horse blood during training regimens.*BMC Genetics* **18** , 31, doi:10.1186/s12863-017-0499-1 (2017).83 Ropka-Molik, K., Stefaniuk-Szmukier, M., Z. ukowski, K., Piórkowska, K. & Bugno-Poniewierska, M. Exercise-induced modification of the skeletal muscle transcriptome in Arabian horses.*Physiological genomics* **49** , 318-326 (2017).84 Chen, M.*et al.* Gsα deficiency in skeletal muscle leads to reduced muscle mass, fiber-type switching, and glucose intolerance without insulin resistance or deficiency. *American Journal of Physiology-Cell Physiology* **296** , C930-C940 (2009).85 Hinkle, R. T. *et al.* Activation of the CRF 2 receptor modulates skeletal muscle mass under physiological and pathological conditions. *American Journal of Physiology-Endocrinology And Metabolism* **285** , E889-E898 (2003).86 McEwen, S. & Hulland, T. Histochemical and morphometric evaluation of skeletal muscle from horses with exertional rhabdomyolysis (tying-up). *Veterinary Pathology* **23** , 400-410 (1986).87 Valentine, B., Hintz, H., Freels, K., Reynolds, A. & Thompson, K. Dietary control of exertional rhabdomyolysis in horses. *Journal of the American Veterinary Medical Association* **212** , 1588-1593 (1998).88 MacLeay, J. M. *et al.* Effect of diet on Thoroughbred horses with recurrent exertional rhabdomyolysis performing a standardised exercise test. *Equine Veterinary Journal* **31** , 458-462 (1999).89 McKenzie, E. C. *et al.* Effect of dietary starch, fat, and bicarbonate content on exercise responses and serum creatine kinase activity in equine recurrent exertional rhabdomyolysis. *Journal of veterinary internal medicine* **17** , 693-701 (2003).90 Finno, C. J., McKenzie, E., Valberg, S. J. & Pagan, J. Effect of fitness on glucose, insulin and cortisol responses to diets varying in starch and fat content in Thoroughbred horses with recurrent exertional rhabdomyolysis. *Equine Veterinary Journal* **42** , 323-328 (2010).

Table 1: Significant terms associated with genes within 1 Mb of top 0.5% F_{ST} markers between ER cases a

Group	
Across breeds	

Table 1: Significant terms associated with genes within 1 Mb of top 0.5% F_{ST} markers between ER cases a

Within CP
Within WB

GO: Gene Ontology term [30,34]; KEGG: Kyoto Encyclopaedia of Genes and Genomes pathway term [31-33]

Table 2: F_{ST}, F_{STP} and F_{STH}, across and within breeds between ER cases and controls

	F_{ST}	F_{STP}	Di
Across breeds	0.0002	0.0004	0.0
CP	0.0006	0.0011	-
WB	0.0004	0.0008	-

Table 3: Results from univariable and multivariable generalised linear models for ER across breeds

Fixed effect	**Univa p-valu**
Sex	4.854E-
Studbook	1.087E-
Age	3.513E-
Use	1.400E-
Fitness	1.306E-
Genetic group	2.985E-
Platform	1.351E-
Breed	6.416E-

indicates p-value <0.05; ** indicates p-value less than the Bonferroni-corrected threshold of p<0.00625