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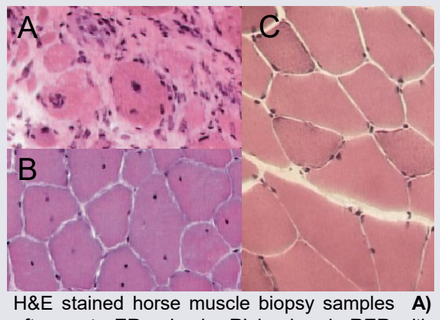
1. INTRODUCTION

- **Exertional rhabdomyolysis (ER)** is a muscle disease characterised by episodes of exercise-induced muscle stiffness and contractures
- **In humans**, ER typically affects athletes and military personnel[1,2], and **in horses** affects 7-10% of racehorses[3]
- **Recurrent ER (RER)** is a **heritable syndrome** in Thoroughbreds (TB) (h^2=0.44-0.49[4]), however the aetiopathogenesis is unclear and no causal genetic variants have identified[5,6]



H&E stained horse muscle biopsy samples **A)** after acute ER episode **B)** in chronic RER with myofibre regeneration **C)** unaffected by ER

- **Warmblood horses** (WBs) and **Connemara ponies** (CPs) are two diverse breeds predisposed to RER
- As a **metabolic disease**, **regulatory regions** of the genome may be implicated

1.1 Project Objectives

To identify **genomic regions (coding and regulatory) affecting RER susceptibility** in WBs and CPs using **genome wide association studies** (GWAS), **regional heritability mapping** (RHM), **F_{ST}** analyses and publicly available equine muscle **ChIP-seq** data.

2. METHODS

2.1 Genetic sampling & analyses

Method	No. of CPs	No. of WBs
WGS	19	19
DNA array: HD (670k) SNP	16	80
Total (unique after QC)	**33**	**94**

Connemara pony.

GWAS were carried out **within and across breeds** using linear mixed models in GEMMA. **RHM** was carried out **across breeds and in WBs** using REACTA. Sex, age and studbook were used as covariates in both analyses. The significance threshold was Bonferroni corrected p<0.05, and suggestive threshold of one false positive discovery per genome scan[7].

Warmblood horse.

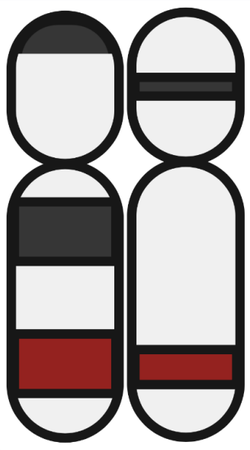
Sample QC	Threshold
Call rate	95%
MAF	1%
HWE	10^{-6}

2.2 Comparing genetic markers and histone marks

26 **previously identified[6] markers** on ECA16 for RER in **US TBs** were compared with the locations of histone marks identified in longissimus dorsi samples from two horses (A and B) from the Functional Annotation of Animal Genomes (FAANG) Consortium's data portal (ENA accession PRJEB35307).

Significant and suggestive markers from the GWAS, RHM and F_{ST} analyses in **CPs and WBs** were also compared with the FAANG ChIP-seq data.

The analysis was conducted using the full RHM regions, the SNP markers and windows of 10kb around SNP markers, and overlap was assessed using bedtools.



Overlapping genomic regions
(made in Biorender)

3. RESULTS

3.1 Genome-wide association studies

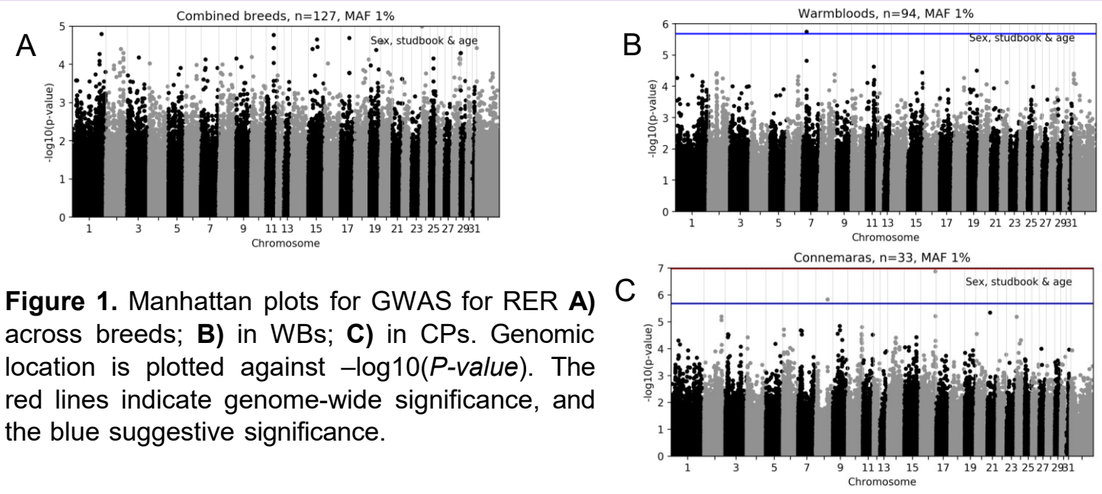
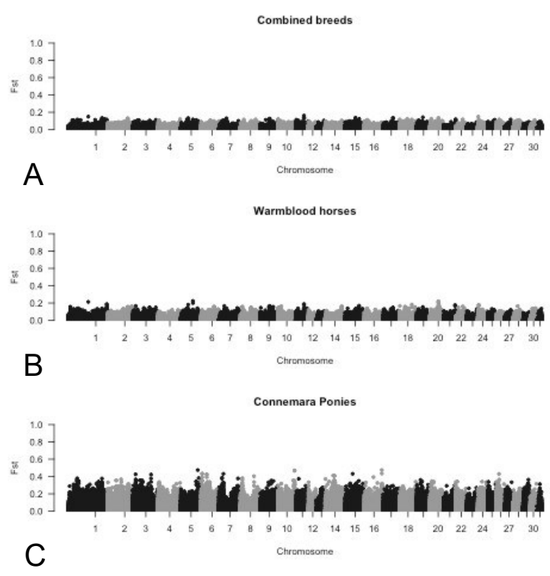


Figure 1. Manhattan plots for GWAS for RER **A)** across breeds; **B)** in WBs; **C)** in CPs. Genomic location is plotted against –log10(*P-value*). The red lines indicate genome-wide significance, and the blue suggestive significance.

3.2 Regional heritability mapping and F_{ST}

Figure 2 (right). Manhattan plots for RHM for RER **A)** across breeds; **B)** in WBs. Genomic location is plotted against –$\log_{10}$(*P-value*). The red lines indicate genome-wide significance, and the blue suggestive significance.



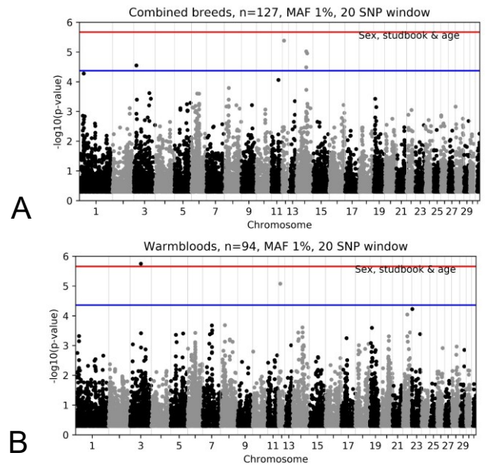


Figure 3 (left). Manhattan plots for F_{ST} for RER **A)** across breeds; **B)** in WBs; **C)** in CPs. Genomic location is plotted against F_{ST} value.

3.3 RER markers overlapping with histone marks

No ChIP-seq peaks directly overlapped previously identified **TB QTLs**, but 1 peak in the **combined breeds**, 6 in **CPs** and 2 in **WBs** overlapped with identified significant and suggestive significant **SNP markers for RER susceptibility**.

Within 10 kb of **TB QTLs** there were significantly **fewer H3K27me3** (promotor silencing) and **more H3K4me3** (TSS) peaks than expected, whilst the **combined breed and WB** RHM regions contained significantly **more H3K27me3** peaks and **fewer H3K4me1** (active enhancer) peaks than expected. No Bonferroni-corrected significant differences were identified in CPs alone.

Group	Marker type	H3K4me1	H3K4me3	H3K27ac	H3K27me3	X^2 p-value
US TB	40b QTL	0	0	0	0	-
	40b QTL + 10kb	25	12^	14	3*	**0.00511**
Joint CP & WB	SNP	1	0	0	0	-
	SNP +10kb	13	4	10	3	0.70280
	RHM region	340^	29*	123*	132^	**<0.00001**
CP	SNP	2	0	2	2	-
	SNP +10kb	32	8	27	27^	0.03046
WB	SNP	1	0	0	1	-
	SNP +10kb	23	8	7*	16^	0.01195
	RHM region	163*	51	163	108^	**0.00025**
Total Muscle A		100,999	25,123	86,524	40,566	-
Total Muscle B		137,322	30,428	78,047	55,955	-
Total		238,321	55,551	164,571	96,521	-

^ indicates higher number of histone marks than expected, with Chi-squared contributions of >2; * indicates fewer histone marks than expected, with Chi- squared contributions of >2; p-values in bold indicate significance at a Bonferroni-corrected threshold.

4. CONCLUSIONS

There are **multiple genomic regions** associated with RER susceptibility that differ by breed.

Candidate genomic markers for RER in **TBs** are close to multiple **histone marks**, predominantly associated with activator regions. In contrast, in **CPs and WBs**, a larger proportion of histone marks containing RER markers were downregulation peaks.

Overall, this indicates that the genomic architecture of RER susceptibility is likely **complex polygenic with possible epigenetic involvement,** and **the disease mechanisms** differ by breed. Future plans include generation of epigenetic data from RER cases and controls in order to verify these results.

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QR code for the RVC Comparative Neuromuscular Diseases Laboratory website (left)

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