767. Haplotype blocks and heterozygosity rich regions on ECA2 in Swedish Warmblood horses

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Abstract

The Fragile Foal Syndrome (FFS) is a disease caused by a recessive lethal missense mutation in the *PLOD1* gene (ECA2). Despite its harmful effect, a relatively high frequency of the FFS allele carriers has been found in the Swedish Warmblood (SWB), suggesting a heterozygous advantage. Balancing selection can be further studied in haplotype blocks and increased heterozygosity around the target of selection. In this study we evaluated the presence of haplotype blocks and Runs of Heterozygosity on ECA2 in 380 SWB horses divided by sport discipline. In horses mainly bred for dressage a haplotype block comprising the FFS variant was found. On average 11.7 Runs of Heterozygosity were identified per horse on ECA2, with no significant difference in numbers between the sport disciplines. This study provides a preliminary characterization of haplotype blocks and heterozygosity rich regions on ECA2 which may further corroborate the potential presence of balancing selection for the FFS allele.

Introduction

Fragile Foal Syndrome (FFS) is a monogenetic disease caused by a recessive lethal missense point mutation in the *PLOD1* gene located on ECA2. Most FFS recessive homozygous foals are assumed to be lost by abortion during late gestation, whereas liveborn affected foals show severe skin fragility, joint hyper-elasticity, and need to be euthanized (Aurich *et al.*, 2019). Despite its harmful effect, a relatively high frequency of FFS carriers (7.4%) was found in the Swedish Warmblood breed (SWB), suggesting the presence of a heterozygous advantage. The presence of balancing selection in SWB was corroborated in a recent study where significant positive effects of the FFS allele were found mainly on movement related traits (Ablondi *et al.*, 2022). Balancing selection causes various types of signatures in the genome such as: increased diversity around the target of selection, differentiation between populations departing from the same genome wide background, and increased linkage disequilibrium around the target of selection (Fijarczyk and Babik, 2015). The advances in high throughput genotyping procedures permit further investigation of the presence of such signatures within animal species. In this study we aimed to explore the presence of haplotype blocks and heterozygosity rich regions on ECA2 in SWB horses to investigate if there is balancing selection acting on the FFS variant or on another closely located variant.

Materials & methods

A total of 380 SWB horses born 2010-2011 were genotyped using the 670 K Affymetrix® Axiom® Equine Genotyping Array. These horses were also genotyped for the XM_001491331: c.2032G > A variant in the *PLOD1* gene by a TaqMan genotyping assay on a StepOnePlus™ instrument (Applied Biosystems, Foster City, CA, USA). For the scope of this work, only SNPs on ECA2 (52,075 SNPs) were retrieved. The quality control (QC) was performed in PLINK v1.90 (Purcell *et al.*, 2007), based on the criteria: minor allele frequency (MAF) (<0.05), missing genotype per single SNP (GENO) (>0.10) and missing genotype per individual (>0.10). Haplotype blocks were defined as in Gabriel *et al.* (2002), considering the region including the *PLOD1* gene ± 1 Mbp, and detected using the Haploview software.

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Runs of Heterozygosity (ROHet) were detected using the settings suggested by (Santos *et al.*, 2021) in the R package 'detectRUNS' (Biscarini *et al.*, 2019). ROHet within the ECA2 were considered as indicator of heterozygosity rich regions if they were shared by over 25% of the analysed horses. Haplotype blocks and ROHet detection was performed separately in two subgroups, defined as in Ablondi, *et al.* (2019), where horses with higher or lower EBV for show jumping than the reference population (mean EBV of 100) were classified as show jumping (SJ) and non-show jumping (NS) horses, respectively. Most NS horses had a dressage index above average. The Ensembl gene annotations EquCab3 was used to identify genomic elements extending 250 kb up- and downstream of the potential heterozygosity rich regions.

Results

A total of 31,122 SNPs and 380 horses passed the QC (189 NS and 191 SJ). Overall, 47 and 50 haplotypes were found in the NS and SJ horses, respectively, considering the region including the *PLOD1* gene ± 1 Mbp. These haplotype blocks were between 2 Kb to 115 Kb long. The haplotype with the greatest number of SNPs in the NS horses comprised 18 SNPs (40 Kb long) and was located 836 Kb upstream the FFS variant. For SJ horses, the highest number of SNPs within a haplotype was 19 (115 Kb long) and located 349 Kb downstream the FFS variant. In the NS, but not in the SJ, the FFS variant was in strong LD (D' equals to 1) with the closest downstream SNP (7 Kb apart), located within the *MFN2* gene (Figure 1).

On average 11.7 ROHet per horse were identified on ECA2, which was equal in both groups (SJ and NS horses). The ROHets had a length ranging between 12.6 Kb and 324 Kb and contained from 15 to 30 SNPs. On average ROHets were 69.5 Kb long and included 15.8 SNPs. A total of three ROHets were shared among

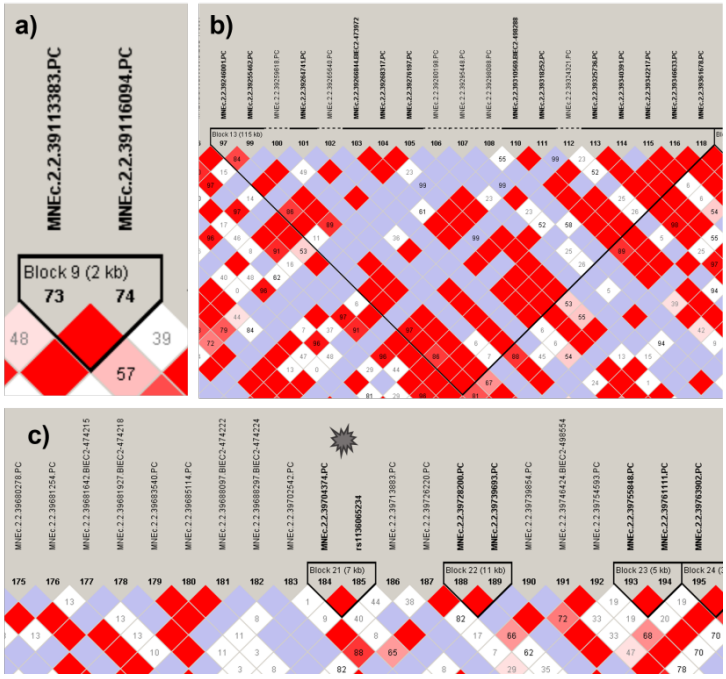

Figure 1. Examples of haplotype blocks in SWB within the *PLOD1* gene ± 1 Mbp: (a) haplotype block with minimum block size of 2 Kb, (b) haplotype block with maximum block size of 115 Kb in SJ horses, (c) haplotype block between FFS variant and the closest downstream SNP in NS horses which is highlighted with a grey star.

25% of the SWB horses: one in the SJ subgroups and two in the NS subgroup (Figure 2 and Table 1). In the case of NS horses, six lncRNA, five protein coding genes and one snRNA were found within the two heterozygosity rich regions shared in over 25% of the horses. In contrast in SJ, seven protein coding genes, two lncRNA and one miRNA were found.

Discussion

In NS horses, we found a haplotype block containing the FFS variant which was not detected in the SJ horses. A potential reason behind the failure to detect the haplotype in the SJ may be the lower frequency (5.8% in SJ vs 14.6% in NS) of the FFS variant in SJ horses as previously found by Ablondi *et al.* (2022) or that the selection pressure for heterozygosity in *PLOD1* is lower in SJ horses. In NS horses, the FFS variant was in strong LD with a SNP located within the Mitofusin 2 (*MFN2*) coding gene. The *MFN2* is involved in the regulation of vascular smooth muscle cell proliferation and has been found associated with disorders of the peripheral nervous system (Engelfried *et al.*, 2006), but is not known to be associated with joint

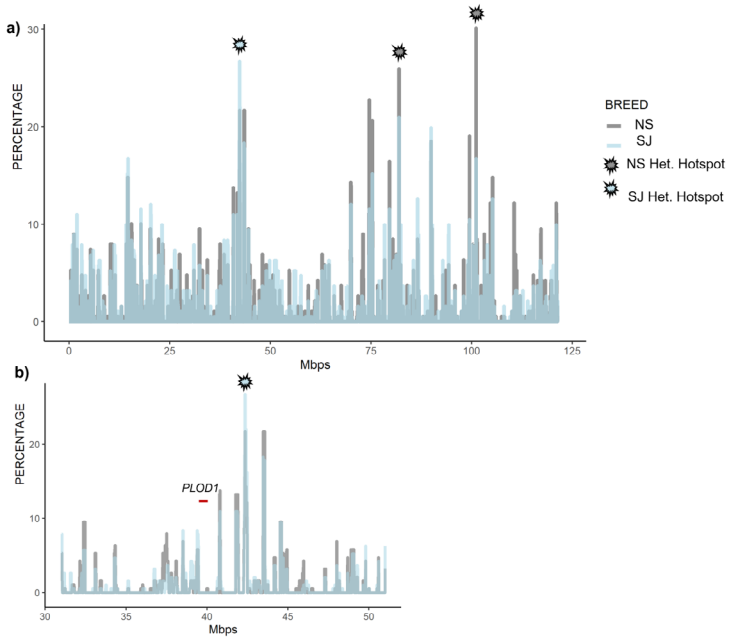

Figure 2. Proportion of times each SNP falls inside a ROHet in the two subgroups (SJ and NS) plotted against their position along the ECA2, with (a) showing the whole ECA2 and (b) showing a zoom-in representation on the *PLOD1* region ±10 Mb is shown.

Table 1. Heterozygosity rich regions on ECA2 shared in over 25% of the SWB horses divided into subgroups (SJ and NS) based on sport discipline.

Subgroup	Start (bp)	End (bp)	N. SNPs	Kb length	Percentage shared
NS[1]	81,932,632	81,972,128	16	39.5	25%
NS	101,054,766	101,088,692	12	33.9	32%
SJ[2]	42,333,297	42,360,263	3	26.9	27%

[1] Non-show jumping SWB horses.
[2] Show jumping SWB horses.

elasticity or fragile skin. Interestingly, although for the *MNF2* SNP, the reference allele is the most common allele in the SWB, all FFS carriers horses among the NS horses carried the variant allele.

Compared to the length of runs of homozygosity (ROH) segments found in the SWB breed by Ablondi, *et al.* (2019) the ROHet investigated in this study were much fewer and detected more scarcely. This result was expected since polymorphic sites only account for a small portion of the whole genome. Nevertheless, the average number of ROHet found in the SWB ECA2 was more than three times higher compared to the one found in Mangalarga Marchador horse breed (Santos *et al.*, 2021). We did not find any clear signs of heterozygous rich regions next to the FFS variant. This can be expected if strong selection pressure is present around this locus, and heterozygosity advantage is only present for the FFS variant itself. We will further compare our findings on SWB ECA2 with the rest of the SWB genome to evaluate the potential role of balancing selection for the FFS variant.

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