A study of the genetic basis of fracture risk in Thoroughbreds: Field data collection and single nucleotide polymorphism marker design

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Introduction

- Fractures due to repetitive exercise are a considerable problem in the racing industry, affecting approximately 10% of horses.
- In humans, case-control association studies have identified genetic associations between single nucleotide polymorphisms (SNPs) and an increased risk of osteoporotic fracture in the elderly.
- The way bone responds to exercise has also been shown to be genetically determined in humans and experimental animals.
- This led to our **hypothesis that risk of fracture in racehorses is associated with polymorphisms in specific candidate genes.**

Aims

- **To conduct prospective case-control studies of fracture in 2 populations of Thoroughbred racehorses: those trained for flat racing and those trained for National Hunt (NH) racing (racing over jumps).**
- **To collect DNA samples from 200 fracture cases and matched controls in each of the 2 populations.**
- **To develop a panel of SNP markers that will be used to establish the association between selected candidate genes and fracture in the Thoroughbred.**

Materials and Methods

Study Design

- Two prospective case control studies: NH and flat racehorses.
- 34 NH, 27 flat and 5 dual purpose training yards across the UK were recruited onto the study, providing around 1850 NH horses and 1800 flat horses in training at any one time.
- Case information is being collected retrospectively and will be ongoing until July 2008.
- Mane hair or blood samples are collected for DNA extraction.

Case definition: Any fracture confirmed by diagnostic imaging, excluding traumatic fractures and chips smaller than 5mm in diameter.
Controls: 3 per case selected at random and matched on yard, being in training on date of fracture and having no known history of fracture.

Genetics

- 19 candidate genes known to be associated with fracture or bone mineral density in humans were selected. These included Estrogen receptor alpha (ER α), Interleukin-6 (IL-6), Collagen type 1 alpha chain (COL1A1).
- Approximately 10 kilobases of DNA for each gene were assembled using comparative genomics. Assembled sequences were amplified in 6 stallions by PCR and DNA sequenced.
- Sequences were screened for SNPs and, based on their location within each sequenced genomic region, between 3 and 6 SNPs were selected for assay design. **Figure 1** illustrates the position of SNPs selected in ERα.
- Allele frequencies were then calculated for each assay in 48 Thoroughbreds.

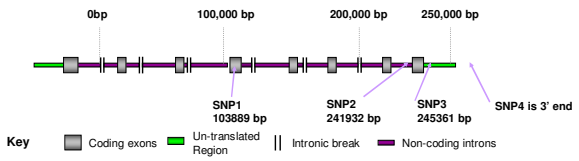


Figure 1: Equine ER alpha gene constructed using comparative genomics. The location of SNPs validated for genotyping are illustrated.

Results

Fractures

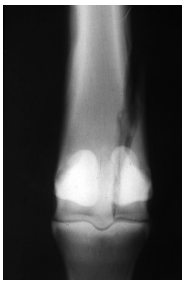
NH:

- 2332 horses sampled.
- 144 confirmed fractures to date.
- 6.2% fracture prevalence.
- 41% associated with racing.
- >45% stress fractures.
- Most commonly affected sites were the pelvis (26%), metacarpal/metatarsal III (21%) and tibia (14%).

FLAT:

- 1866 horses sampled.
- 218 confirmed fractures to date.
- 12% fracture prevalence.
- 16% associated with racing.
- >35% stress fractures.
- Most commonly affected sites were the metacarpal/metatarsal III (23%), tibia (19%) and pelvis (18%).

(A)



(B)

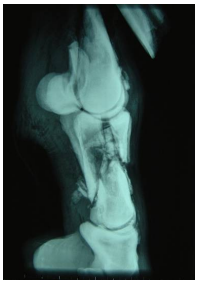


Figure 2: (A) Condylar fracture of the Metacarpal/metatarsal III, (B) Condylar fracture with comminuted fracture of the proximal phalanx.

Genetics

- 19 genomic sequences were assembled across the equine genome.
- 148 SNPs across 120kb of sequence were identified (average 7.8 SNPs per gene), ~1.2 SNPs per kilobase.
- 82% of SNPs had a minor allele frequency >10%; the remaining SNPs were excluded from the study.
- 45.5% of SNPs were in linkage disequilibrium (LD) with other adjacent SNPs in the same region. Only one SNP from each LD block was selected for inclusion in the final panel.
- A final panel of 49 SNPs is now being used for genotyping of cases and controls.

Conclusions

- **Fracture prevalence, types of fracture and their skeletal location are similar to what has been described previously.**
- **A panel of 49 robust and informative genetics markers have been designed and validated for use in the Thoroughbred.**
- **This panel will be used in case-control studies to determine whether there is a significant genetic component to the risk of fracture in Thoroughbred racehorses.**
- **Some genes in our study play a role in multiple tissues (e.g. type I collagen), SNPs in these genes can also be used to investigate the genetic basis of other musculoskeletal diseases.**

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