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A Genome-wide association study of osteochondritis dissecans in the Thoroughbred

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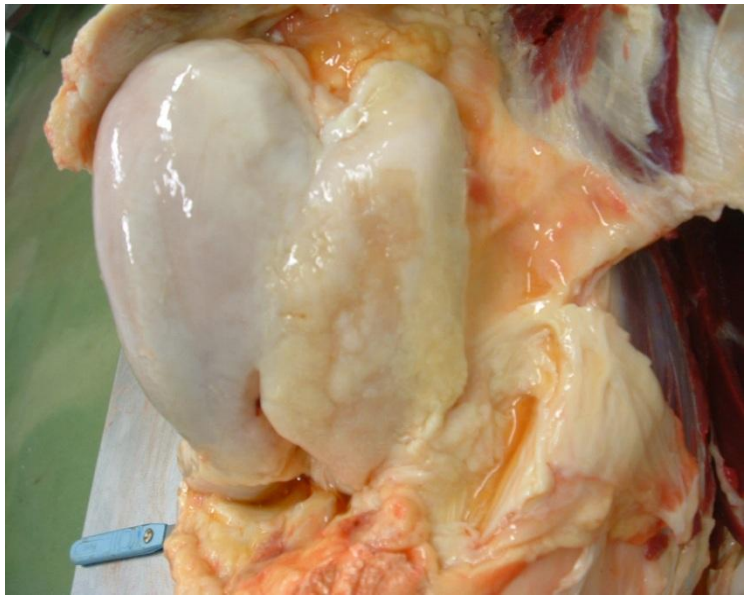
Prj: EGP

Background: Osteochondrosis 1

A clinical perspective...

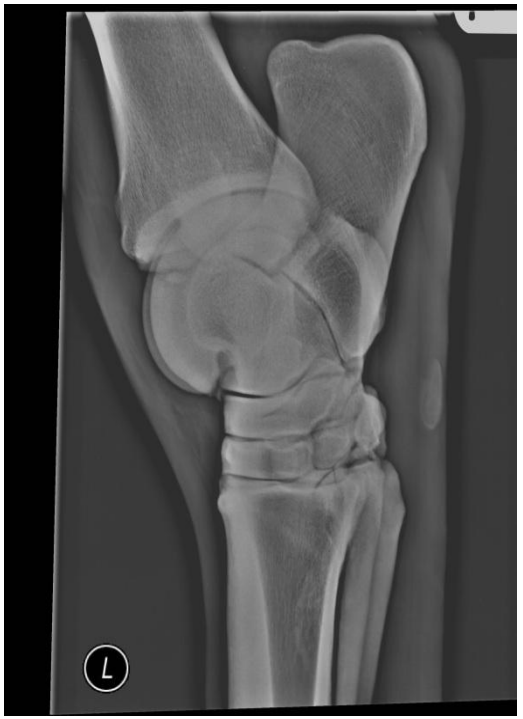
- Osteochondrosis is:
  - A joint disease
  - A developmental orthopaedic disease (DOD)
  - A disturbance of the normal process of endochondral ossification (where cartilage turns to bone during growth)



- The diseases may be classified by location and severity.

Background: Osteochondrosis 2

- Clinical signs vary. The disease may be subclinical (no outward signs) or may cause lameness and swelling in the affected joint.
- Diagnosis is usually by radiography although ultrasonography may also be used.
- Treatment ranges from conservative measures, such as enforced rest, to arthroscopic surgery in more severe cases.



Background: Osteochondrosis 3

- Prevalence varies by breed and definition, but it has been estimated that ~25% of Thoroughbred horses are affected (Lepeule *et al.* 2009).

- Osteochondrosis therefore represents a significant issue in the Thoroughbred industry, both from a welfare perspective and in economic terms.

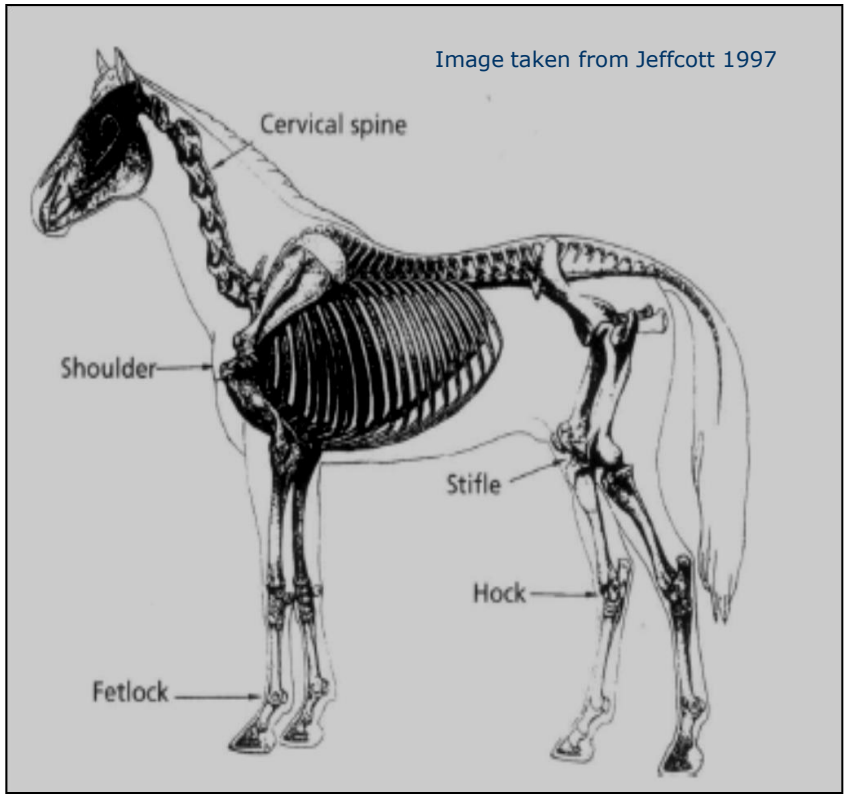
Background: Osteochondrosis 4

Classification based on:

joint affected

In horses, osteochondrosis most commonly affects the fetlock, hock and stifle joints. However, the shoulder and cervical spine may also be affected.

The condition is often bilateral (affects both the right and left sides).



Background: Osteochondrosis 5

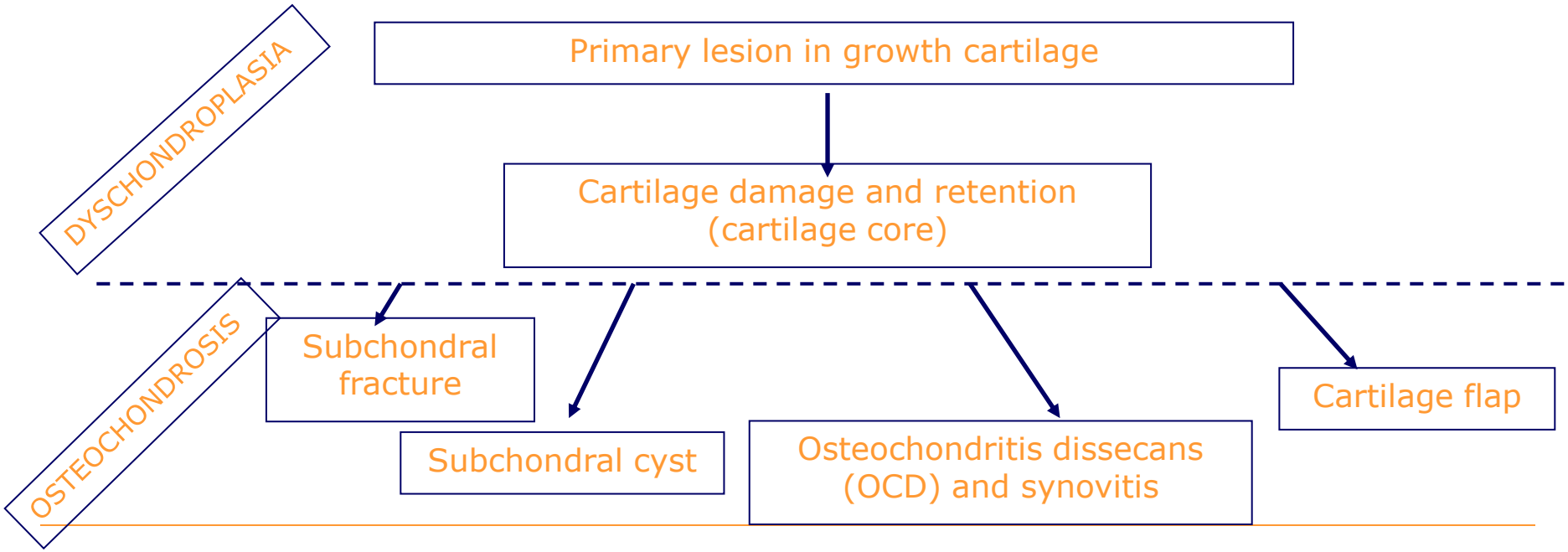
The disease begins with a primary lesion in the growth cartilage. In some foals these may simply disappear, causing no further problems.

However, some lesions will develop further to cause cartilage cores within the bone, causing a weak spot to develop on the surface of the joint.

These weak spots go on to cause a range of problems of varying severity. In the most severe cases, pieces of cartilage and bone break away from the joint surface and are left suspended with the joint cavity itself causing inflammation and potentially further damage.

Background: Osteochondrosis 6

In such cases the disease is commonly referred to as osteochondritis dissecans or OCD – 'itis' for the inflammation of the cartilage or bone and 'dissecans' for the separation of the cartilage from the underlying bone.



Background:
Genes and disease 1

- Whether or not a horse develops a particular disease may be determined by its genes – its 'genotype', its environment, or a combination of its genotype and environment.

- Diseases which are partly or wholly determined by genes are said to be heritable, which means they are passed on by the sire and/or dam to the foal.

Background: Genes and disease 2

- Foal Immunodeficiency Syndrome (previously known as Fell Pony Syndrome) is an example of a disease which is wholly determined by genes – in this case, a mutation in a single gene is responsible for causing this fatal condition.

- Osteochondrosis is a more complex disease. It is thought to be heritable, but there are also many environmental factors which have been linked to its development, such as: nutrition, exercise (especially at a young age) and growth rate. Therefore, there are likely to be many genes involved in the development of osteochondrosis.

Reason for this study

The reason for undertaking this study was to identify genetic variants associated with osteochondrosis

How did we do this? 1

- In order to understand which genes are involved in passing on a susceptibility to osteochondrosis, we compared the DNA of horses with and without the disease.

- The comparison was done using many thousands of genetic variants or markers spread across the whole equine genome which were read simultaneously to create a DNA profile for each horse.

- Blood samples from Thoroughbred cases and controls were used in this study.

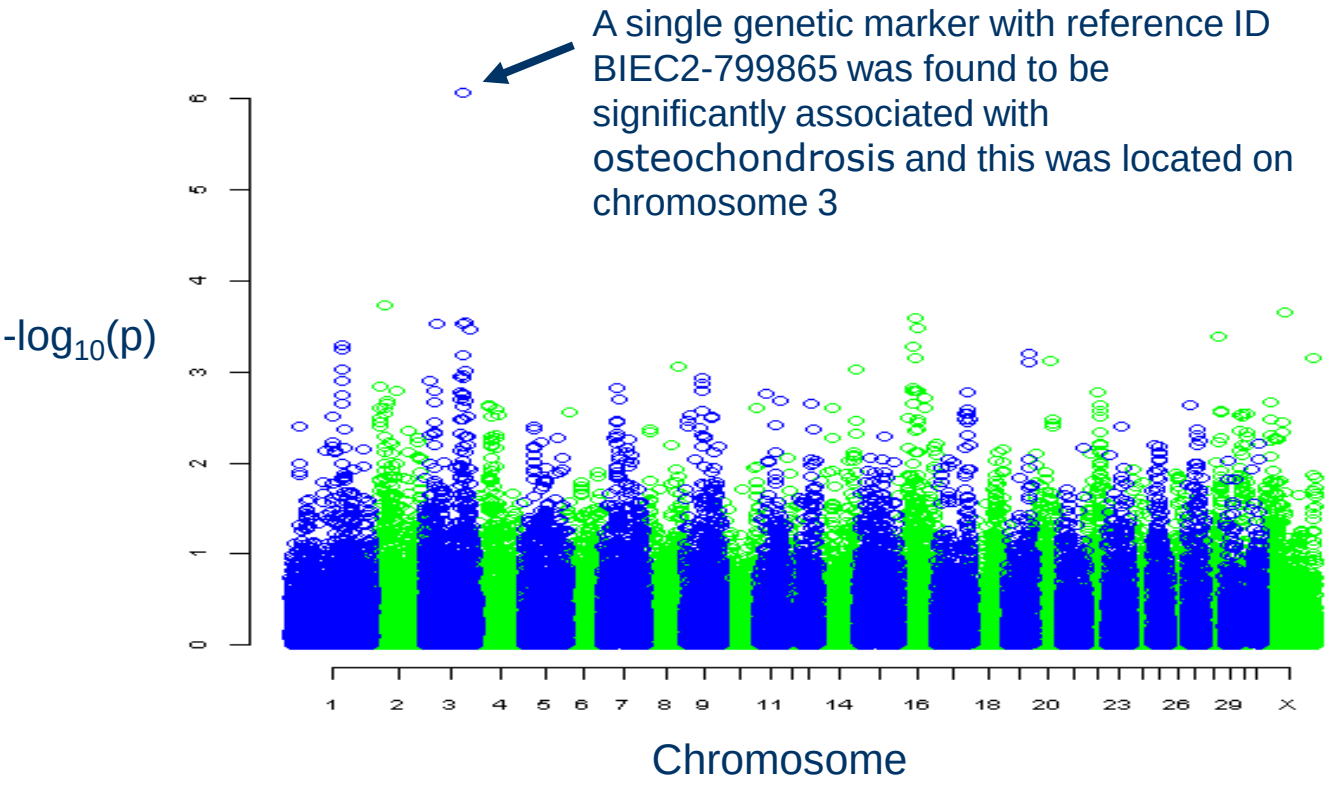
How did we do this? 2

  - Case horses were admitted to the clinic for osteochondrosis surgery of the fetlock, hock and/or stifle.
  - Control horses were admitted to the clinic for surgery for conditions other than osteochondrosis.

- A mathematical model was fitted to the data in order to account for non-genetic effects on disease such as the sex of the horse.

- An analysis was then carried out in order to identify genetic markers associated with osteochondrosis.

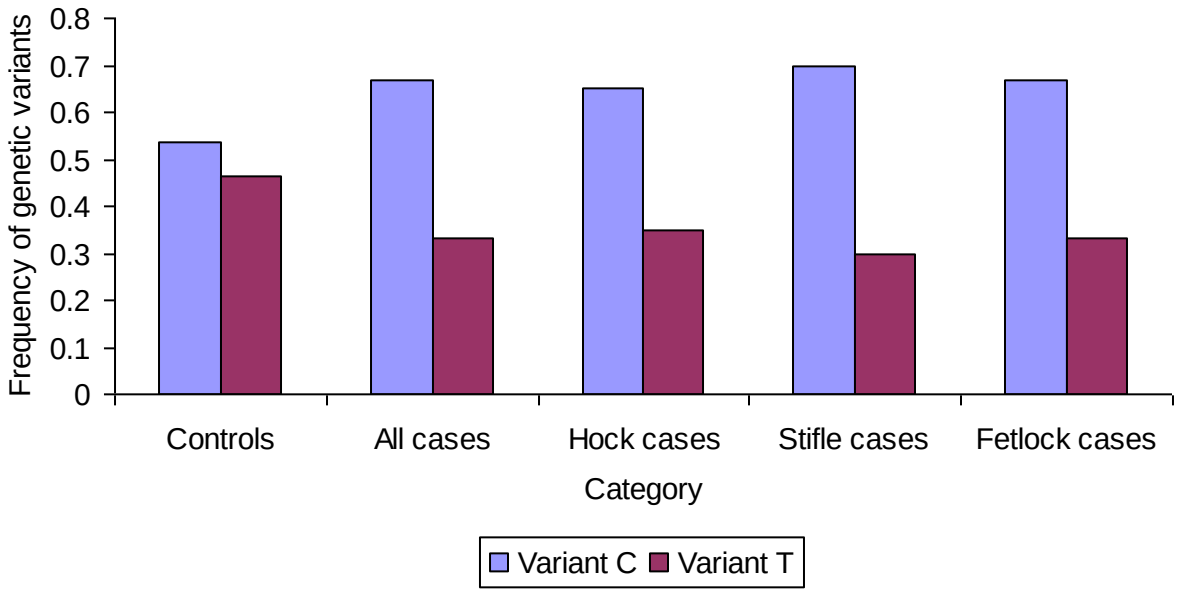
What did we find? 1



This plot, known as a Manhattan plot, shows the results of the analysis. Each circle represents one of around 40,000 genetic markers. The circles are positioned on the x-axis according to their location on the genome, with alternating colours according to the chromosome on which they lie. On the y-axis, circles are positioned according to the degree to which they were found to be associated with osteochondrosis.

What did we find? 2

The graph below shows the relative frequency of the two different versions of the marker identified. It shows that the C variant is more common in horses with osteochondrosis than in horses without the disease. This appears to be true regardless of the joint in which the horse is affected by osteochondrosis.



What did we know about this genetic marker? 1

BIEC2-799865 in a biological perspective:

- The genetic marker BIEC2-799865 is not in a gene and has no obvious function. Therefore, we looked in the region of the genome surrounding the marker for genes of interest.

- In the 2Mb region that surrounds BIEC2-799865, there are 22 labelled genes.

What did we know about this genetic marker? 2

- The closest gene to the marker is similar to a human gene called *kruppel-like factor (KLF) 3 (basic)*, which has been shown, in studies of mice and worms, to be involved in the generation of fat cells.
- There was no clear functional evidence linking most of the remaining genes to OCD.
- The most relevant gene in the region was *UDP-glucose dehydrogenase (UGDH).*

What did we know about this genetic marker? 3

UDP-glucose dehydrogenase (UGDH)

- UGDH is thought to be involved in the regulation of glycosaminoglycan (GAG) synthesis in cells lining the articular cartilage surface.

- GAGs are biological substances that play a crucial role in the generation of cartilage cells and natural lubrication of joints. Chondroitin sulphate is an example of a GAG and is a common ingredient in joint supplements for horses.

- Other researchers have demonstrated a potential link between GAG and osteochondrosis.

Potential next steps 1

- Despite representing one of the largest equine cohorts genotyped in this way, our study may benefit by repeating with increased statistical power.

- Factors which influence the power of the analysis include:
  - Sample size (sample size used was small relative to human studies of similar diseases)
  - The way in which horses are diagnosed and categorised for OCD, ie. Improved phenotyping
  - Number of markers tested

Potential next steps 2

- Some differences within the study population could be caused by non-genetic factors, e.g. different management practices

Potential benefit

- If an association is confirmed subsequent research could:

  a) Help researchers to better understand what causes OCD and how best to treat and/or prevent the disease

  b) Help breeders to select horses in such a way that the risk of OCD is reduced