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SCREENING GENETIC DISEASES OF HORSES

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Summary
Increasingly, genetic screening tests will become available to determine whether individual horses carry mutations that predispose them to have, or to pass on, inherited diseases. Several genetic tests are already available and their development, use, potential advantages and future possibilities will be discussed. Where the disease is inherited as a simple Mendelian trait their application to selective breeding is relatively simple. Where the condition is inherited in a genetically complex manner the use of DNA-based tests will require a more sophisticated decision making process.

Introduction
The genetic information of an individual has been described as its 'book of life', containing as it does all the instructions needed to produce an animal as complicated as a horse from the initial fusion of a sperm and an egg. Sometimes there are errors in these DNA instructions that can lead to problems, which are called as inherited diseases. Many similar diseases occur in different mammalian species and a large number of mutations have been identified that are responsible for inherited diseases in man. Using the power of comparative genetics it is possible to identify genes that cause diseases similar to those in horses. These genes are called candidate genes. The molecular genetic basis for several inherited diseases in horses is now known, and the successful identification of the mutations responsible has been largely reliant on the candidate gene approach. In this strategy, the DNA sequences of the candidate genes are compared between normal and affected individuals to search for mutations that could be responsible for the disease. In the future it is likely that novel mutations that are not known in other species will be identified through the power of genetic linkage mapping.

Inherited diseases in the horse for which the mutation has Been identified hyperkalaemic periodic paralysis (HYPP)

HYPP was the first genetic diseases in horses that was characterised at the molecular level. HYPP is characterised by sporadic muscle tremors that can result in collapse. The disease is inherited as an autosomal dominant trait, with a single copy of the gene mutation being sufficient to produce disease. The disease is present in American Quarter horses and all cases have been traced back to a single popular sire, Impressive. The disease mutation appears to have been actively selected by breeders because it resulted in increased musculature, which is a desirable trait in the show ring.

The mutation is in a muscle sodium channel gene, one of the genes that controls muscle fibre contraction, making the muscle contract involuntarily. The sodium channel is affected by the amount of potassium in the blood; hyperkalemia means excessive potassium, so that consumption of feeds with high potassium can trigger muscle tremors or paralysis. There exists individual variability in the severity of attacks, though the basis for this is currently unknown. The condition can be controlled by maintaining a low potassium diet and clinical episodes can be treated with drugs such as acetazolamide or hydrochlorothiazide.

The gene containing the mutation responsible for HYPP was identified using the candidate gene approach. A mutation responsible for HYPP in humans was identified within a skeletal muscle sodium channel gene and researchers initially determined whether this gene was linked to the disease in Quarter horses (Rudolph et al., 1992a). The precise mutation responsible for the defect was then rapidly identified as a Phe to Leu amino acid substitution within the transmembrane domain, IVS3, in the adult skeletal muscle sodium channel alpha subunit gene (Rudolph et al, 1992b) and a DNA test established.

Severe combined immunodeficiency disease (SCID)

SCID is a disease of Arab horses that is inherited as a recessive condition. A copy of the mutation must
be inherited from both the stallion and the mare. If a single copy of the mutation is inherited the animal will be clinically normal, but will be a carrier capable of passing on the mutation, and of producing affected offspring. Both parents of an affected foal are obligate carriers of the SCID mutation. The disease is lethal, affecting both the T- and the B-lymphocytes of the immune system, and most foals die from infections within 5 months regardless of the veterinary care they receive (McGuire et al., 1974).

SCID occurs in several mammalian species and different gene mutations have been identified. These include DNA-dependent protein kinases (DNA-PK) and interleukin receptors. In humans and some dog breeds the disease, linked to the gamma chain of the interleukin 2 receptor, is X chromosome linked. In Arab horses there is no evidence that the disease is sex-linked. The SCID mutation in Arab horses was identified in the DNA-PK gene which maps to horse chromosome 9. The disease is caused by a five base pair deletion that results in a frameshift mutation and a premature stop codon (Shin et al., 1997). Mutations in the same gene cause SCID in mice and in Jack Russell Terrier dogs. Horses heterozygous for the SCID mutation are thought to be at greater risk of developing sarcomas, suggesting that DNA-PK may be a tumour suppressor gene (Ding et al., 2002). The frequency of SCID carrier horses was reported as being about 8% in randomly tested US Arab horses (Bernoco and Bailey, 1998), whilst a slightly lower frequency was found in the UK (Swinburne et al., 1999). The DNA-based test for SCID in Arab horses has been patented.

**Lethal White disease**

A syndrome involving foals born as blue eyed whites (or with very few coloured hairs) that lack nerve cells controlling the peristaltic muscle movements of the gut (intestinal aganglionosis) is known as Lethal White Foal Syndrome (LWFS). The condition is similar to Hirschsprung disease in man, for which endothelin-B receptor (EDNRB) gene mutations have been identified as responsible for some forms. This gene, which plays a role in the development of neural crest cells that become enteric ganglia and melanocytes, was investigated in LWFS (Santschi et al., 1998, Metallinos et al., 1998 and Yang et al., 1998). A dinucleotide missense mutation leading to an Ile to Lys amino acid substitution in the first transmembrane domain of this protein was found to be associated with the disease.

**Glycogen storage disease IV (GBE1)**

Recently, a recessive fatal fetal and neonatal glycogen storage disease was identified in American Quarter horses. Comparative biochemical and histopathological evidence suggested that a defect in the glycogen branching enzyme encoded by the GBE1 gene was responsible. A substitution resulting in the formation of a stop codon was subsequently identified in exon 1 (Ward et al., 2004). A DNA-based test for the disease has been developed and extensive testing of the breed is underway.

**Junctional epidermolysis bullosa (JEB)**

JEB, also known as epitheliogenesis imperfecta (EI), is inherited as a recessive disease and affected individuals do not survive to breeding age. The disease is characterised by moderate to severe skin lesions at pressure points and is also known as 'red foot disease' through the loss of the hoof in newborn foals. Secondary infection frequently results in affected individuals having to be euthanized. The mutation responsible for the disease has been identified in Belgian Draft horses (Spirito et al, 2002), two French draft breeds, Trait Breton and Trait Comtois (Milenkovic et al., 2003) and a DNA test to detect the mutation in these breeds is commercially available. The mutation is an insertion of a single extra nucleotide cytosine base in exon 10 of the LAMC2 gene, which leads to a premature termination codon in the gamma 2 chain of the laminin 5 protein, thereby blocking the expression of this gene. The LAMC2 gene maps to equine chromosome 5. The disease in horses is analogous to the severe Herlitz form of JEB in humans in which three laminin genes LAMA3, LAMB3 and LAMC2 have been implicated. Interestingly, the laminin gamma 2 chain amino acid sequence is nearly identical between horses and humans suggesting a strong degree of functional constraint in protein structure. Recently, the disease in American Saddlebred horses has been mapped to equine chromosome 8 (Lieto and Cothran, 2003), the location of the LAMA3 locus in horses, strongly implicating this gene as responsible for the disease in this breed.
Other diseases

Most mammalian species have abnormalities in the number of sex chromosomes. Females with only one X chromosome rather than the normal two copies in humans have Turner’s syndrome. These individuals are infertile. A similar condition is seen rarely in different horse breeds. There are other rarer chromosome abnormalities such as females with three X chromosomes, or males with two X chromosomes and a Y chromosome, or even three X chromosomes and a Y chromosome. These individuals are all likely to have reproductive problems. Simple genetic tests can determine whether sex chromosome abnormalities are present in mares who appear to be infertile or have reduced fertility (Breen et al., 1997).

Using the results of genetic screening tests

DNA-based tests are particularly useful for recessive diseases where it is not possible to detect clinically whether an animal is genetically clear or a carrier of a disease mutation. The important goal is to avoid mating a carrier to a carrier and risking the production of an affected foal. Only breeding animals need to be tested. The tests results allow management of most situations. For example, if a “perfect” horse is found to be a carrier for SCID, should it be removed from the breeding population? If it is an excellent horse it could be bred to individuals which are tested clear of the SCID mutation. In this scenario, no affected foals are produced and no foals will die. From such a mating on average half genetically clear and half SCID carrier offspring will be produced. If the foals produced are tested, half will be completely clear and may have inherited the other good properties from the “perfect” horse. The carriers produced could again be bred to a tested clear individual if they had desired traits. Over time the frequency of the mutation will be reduced through careful selective breeding. There is a temptation to try and remove all the carriers from breeding quickly, but it is not always the best strategy, especially if the disease allele frequency is high.

Future developments

Several other conditions in horses have a genetic component, though for many of them the genetics are complex and the diseases often have strong environmental components. About 10% of Thoroughbred horses suffer some level of fracture, often a minor stress fracture, during a year in training. Laryngeal hemiplegia (roaring) is a defect in a nerve that affects the windpipe. At least 5% of Thoroughbreds have a severe problem and many more have some degree of laryngeal problem. Tying up, (recurrent exertional rhabdomyolisis), a dominant muscle disease is also thought to affect between 5-10% of Thoroughbreds.

Most of these diseases are complex. We and others are working on fracture and tendonitis, as orthopaedic problems in the Thoroughbred are the biggest cause of wastage to the industry. We are trying to find the mutations in the genes which underlie the susceptibility to fracture. Markers from candidate genes for human osteoporosis and other bone conditions are being tested in Thoroughbreds who have had a fracture and compared to age and sex-matched individuals who are sound, in an approach called an association study. We have already found one gene marker that appears to double the risk of fracture and are actively searching for more. In the future it may be possible to test Thoroughbreds before they enter training and identify horses who are particularly susceptible to fracture. The trainer could then change the training routine and the management of that horse, and the veterinary surgeon can look at the horse more carefully and identify problems before they become serious. Whilst the research involves Thoroughbreds the results are likely to be applicable to many horse breeds.

There is a real momentum behind genetic studies on diseases in horses and it is clear that many useful new DNA-based screening tests will become available in the coming years.

References


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