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ORIGINAL RESEARCH

Genetic Testing and the Future of Equine Genomics

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ABSTRACT

Genetic tests are now available for most coat color traits and many simple Mendelian diseases in the horse. The horse genome sequence was completed in 2006, with sequence available online to researchers in June of that year. This wealth of new data has already been exploited to provide some very powerful tools that can be used to define more simply inherited diseases in horses at the molecular level, as well as potentially more complex diseases. With the continued availability of genetic testing in horses, veterinarians, and particularly reproduction experts, need to have a good basic understanding of these tests to assist their clients in making informed breeding decisions.

Keywords: DNA; Gene; Inherited; Genomic; single nucleotide polymorphism (SNP)

GENETIC BASICS

Mammalian genetics follows simple rules of inheritance named after the monk, Gregor Mendel, who discovered them. Each animal carries two copies of each chromosome, with the exception of the sex chromosomes (males carry one X and one Y chromosome and females carry two copies of the X chromosome). Two copies of each chromosome means that there are two copies of each gene as well. The two copies of the genes are not always identical to each other. DNA sequence differences within and near genes cause the many phenotypic differences seen between individuals like coat color, disease state, and body size. The different versions of the genes that exist within a species are called alleles and are defined by the DNA sequence (genotype) or the physical manifestation of the genotype (phenotype). An individual is said to be homozygous when the two alleles are identical, and heterozygous when the two alleles are different.

The mode of inheritance of a disease or trait is the way that the phenotype is manifest from the genotype. If a phenotype is only seen when an animal has two copies of an allele, it is called a recessive trait. Carrier animals are animals that do not display a phenotype for a recessive trait since they are heterozygous (have one normal allele and one mutated allele). For diseases or traits with a dominant mode of inheritance, a phenotype is seen in the heterozygous state. Although a classic dominant mode of inheritance implies that the phenotypes of homozygotes and heterozygotes are identical, many times that is not the case. If heterozygotes have an intermediate phenotype and homozygotes have a more severe phenotype, the mode of inheritance is called semi-dominant.

In addition to the basic Mendelian modes of inheritance, there are a few other factors that can influence the disease status of an individual. The mode of inheritance is not always simple. Multiple genes can interact with each other to create a phenotype (multigenic mode of inheritance) or many genes of small individual effect can cause a phenotype (polygenic mode of inheritance). In addition, there can be gene–environment interactions that can complicate the mode of inheritance. Penetrance, the proportion of animals that display a phenotype when they have the appropriate genotype, is also a factor. The simplest scenario is one where the penetrance is 100%, but sometimes it can be less than 100%, confounding inference of the genotype of an animal based on its phenotype.

Another genetic term that is used to describe what is seen in nature is *variable expressivity*. This means that animals with the same genotype can display differences in phenotype severity. This can be distinguished from penetrance issues because reduced penetrance means that the animal does not display the phenotype at all, whereas expressivity means that the phenotype is displayed, but to varying degrees. One last genetic term is *epistasis*; this means that one gene's phenotype masks the manifestation of another gene. There are many coat color examples of this phenomenon. For example, if a 10-year-old horse is grey in color, then the underlying coat color that the horse was born with has been masked, since grey is epistatic to other colors in adults. The horse may have been bay, black, or chestnut as a foal but as an adult one cannot detect the original color. Epistasis is an important genetic interaction that probably affects many traits in mammals.

The mode of inheritance is important to understand to make predictions about breeding combinations and what they will produce, whether it is for coat colors or disease states. If two carrier animals are bred to each other for a recessive trait, there is a 25% chance that the foal will be affected with the disease, a 50% chance that it will be

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Table 1. Outcomes for various crosses for a recessive trait

Cross	Outcome
Unaffected X unaffected	100% unaffected
Carrier x Carrier	25% unaffected, 50% carrier, 25% affected
Affected X affected	100% affected
Unaffected X carrier	50% unaffected, 50% carrier
Carrier X affected	50% carrier, 50% affected
Unaffected X affected	100% carrier

Assuming that affected animals can breed. Carrier implies heterozygous, affected implies homozygous for the mutation and unaffected implies homozygous for the normal allele.

a carrier, and a 25% chance that it will be a non-carrier. In the case of a dominant mode of inheritance, half of foals out of an animal heterozygous for the mutation will be affected and all foals out of an animal homozygous for the mutation will be affected. The outcomes for various crosses for both dominant and recessive traits are show in Tables 1 and 2.

These tables are written in a general fashion so that they could be used for either a desirable trait or an undesirable trait. In the case of a disease, breeding goals should be centered on reducing the disease allele frequency, as well as elimination of the occurrence of the disease itself entirely. Toward this end, a reduction in allele frequency can be obtained by not breeding animals with dominant diseases as well as not breeding carriers of recessive diseases. In a breed with low diversity, the goal might be to reduce the allele frequency of the disease allele more slowly to avoid a reduction in diversity within the breed. In that case, a strategy of breeding an individual, testing its genotype and replacing that individual with an unaffected offspring can be used where carriers are bred but non-carrier offspring are retained for future breeding. In the case of a dominant trait, heterozygotes are bred and unaffected progeny are retained in the breeding program.

GENETIC TESTS

The first genetic test offered in the horse was for hyperkalemic periodic paralysis (HYPP).[1] This disease is inherited as a semi-dominant trait, meaning that heterozygous animals (carriers) have a phenotypic effect of the mutation, but animals homozygous (two copies of the mutation) for the mutation have a more pronounced phenotype.[2] The mutation was identified as a result of comparative analysis with humans. A candidate gene was selected based on a similar phenotype that occurred in people with mutations in this gene. DNA sequence analysis confirmed a mutation in the sodium channel gene (SCN4A). The mutation was confirmed to segregate with the disease phenotype in a family of horses. This type of approach is called the candidate gene approach. Researchers compare phenotypes between humans and horses and develop a list of potential candidate genes. Severe combined immunodeficiency (SCID) in Arabians,[3] lethal white foal syndrome (LWFS),[4-6] glycogen branching enzyme deficiency (GBED) and junctional epidermolysis bullosa (JEB)[7] were also determined using this methodology. Some of the coat color genes identified in horses were determined using comparative genetics (chestnut,[8] black,[9] dominant white,[10] sabino,[11] and silver[12]), whereas others were determined using basic linkage mapping approaches (tobiano,[13,14] cream dilution,[15,16] gray,[17-19] appaloosa pattern,[20,21] and grey[22]).

Basic linkage analysis is performed by collecting samples from informative families that segregate the trait under study (ideally 3 generations or large half-sibling families). Molecular markers (microsatellite markers) that display differences between individuals are genotyped in the families and the segregation of the marker and the disease is tested.[23] Statistical analysis for linkage is called a logarithm of the odds of linkage/log of the odds of non-linkage (LOD) score. LOD scores of ≥ 3 are considered significant.

Because traditional horse breeding practices in which a single offspring is produced do not lend themselves well to families that are large enough, and therefore powerful enough, for linkage analysis, other methodologies have been used to identify disease genes. An association analysis followed by homozygosity mapping was used to identify the mutation that causes the recessive disorder hereditary regional dermal asthenia (HERDA).[24] A genome-wide association analysis of microsatellite markers was also used to identify the dominant disorder polysaccharide storage myopathy (PSSM).[25] These approaches relied on the fact that markers close to the disease loci have alleles that are more likely to occur in diseased individuals than in healthy ones. Association analysis will become the most common way to map traits and diseases in horses with the new technology that is based on the horse genome sequence (highlighted in the next section). The diseases and coat colors that have been defined at the molecular level are listed in Tables 3 and 4 with their inheritance patterns.

GENOME SEQUENCE OF THE HORSE (SNPS, SNP ARRAYS, POTENTIAL APPLICATIONS)

The horse was selected for full genome sequencing in 2005. Within one year, the 6.8X horse genome sequence was made available on the internet to researchers worldwide. The DNA sequence was obtained in small pieces and then assembled into longer stretches called contigs. Eventually, the contigs were linked into supercontigs and then assigned chromosomal locations. This is a fairly long process and the