GNOME
Genetic Profiling the future?

Breeding Symposium at
Osberton International Horse Trials

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www.buckinghamequinevets.com
Outline

• Traditional Techniques - Mendelian Genetics

• Genetics vs Genomics

• Equine Genome Project
  • In disease
  • In performance/conformation

• Practical application to horse breeding

The future
Definitions 1

- **Gene** – is locatable region of DNA, corresponding to a unit of inheritance.
- **Allele** – an alternative gene at any given site on chromosome.
- **Disease** – is a genotypic or phenotypic state, often influenced by environmental factors, that places individuals at risk for an internally derived adverse consequence.
Definition 2

- Phenotype – Horse’s observable traits
- Genotype – Inherited instructions
- Homozygous – Two alleles the same - NN
- Heterozygous – Two alleles different - NH
- Monogenetic Disease – Single gene mutation
- Complex Disease – Complex interplay of several genes and environmental factors
DNA
Two Distinct Topics

Health/Disease

Performance
Traditional Methods

Family Tree - HYPP
Mendelian vs non-mendelian traits

**Mendelian**
- Single gene mutations and segregate in a family
  - Autosomal recessive
  - Autosomal dominant
  - Co-dominant
  - X-linked
- **Eg:** HYPP, PSSM, MH, SCID

**Non-mendelian**
- Multifactorial, polygenic
- Complex interplay of several or many genes and environmental factors
- Complex diseases do not follow Mendelian segregation
- **Eg:** RAO (COPD), Navicular syndrome, OC/OCD
Genetics vs genomics

**Genetics**
- Study of genes and heredity; traditionally refers to classical Mendelian principles of inheritance

**Genomics**
- The branch of genetics concerned with the global characterisation of all genes and non-coding DNA sequence of organisms
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And coming around the track, looking promising ... The horse genome map
Equine Genome

- Diploid chromozone number within genus – 32-66
  - 32 – Zebra
  - 66 – Przewalski’s horses
  - 64 - Domestic horse
- Predicted number of genes – \( \approx 20,000 \)
- Size of haploid genome - \( 2.7 \times 10^9 \) base pairs
- Published in Science in 2009 – Wade et al 2009
Coat Colour – Simple Mendelian

• Red/Black Factor – Black dominant (EE) – base colour of all equines.
• Agouti (AA) – Black based Horse plus agouti gene - BAY
• Champagne Dilution (ChCh) – Coat lightening effect
• Cream Dilution – eg Chestnut – (nCr) Palomino – (CrCr) Cremello
• Silver Dilution – Light tail and manes in black based horses
• Grey - Gradual and progressive depigmentation.
• Frame Overo – white pattern gene – linked to LWOS
• Sabino 1 – White spotting gene – white border
• Tobiano – Pie/skewbald, tri-coloured
### TABLE 3: Characterised genetic diseases of the domestic horse

<table>
<thead>
<tr>
<th>Disease</th>
<th>Major breed(s)</th>
<th>Chromosome</th>
<th>Gene</th>
<th>Mutation</th>
<th>Inheritance</th>
<th>Testing*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperkalemic periodic paralysis (HYPP)</td>
<td>Quarter Horse</td>
<td>11</td>
<td>SCN4A</td>
<td>C to G substitution</td>
<td>Autosomal co-dominant</td>
<td>Y</td>
</tr>
<tr>
<td>(Rudolph et al. 1992a,b; Cannon et al., 1995; Naylor et al. 1999)</td>
<td>Paint</td>
<td></td>
<td></td>
<td>Phe to Leu substitution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polysaccharide storage myopathy (PSSM)</td>
<td>QH-related</td>
<td>10</td>
<td>GSY1,</td>
<td>G to A substitution</td>
<td>Autosomal dominant</td>
<td>Y</td>
</tr>
<tr>
<td>(McCue et al. 2008a,b, 2009)</td>
<td>QH-related Warmsbloeds Draughts</td>
<td></td>
<td>+/- others</td>
<td>Arg to His substitution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant hyperthermia</td>
<td>Quarter Horse</td>
<td>10</td>
<td>RYR1</td>
<td>C to G substitution</td>
<td>Autosomal dominant</td>
<td>Y</td>
</tr>
<tr>
<td>(Aleman et al. 2005, 2009)</td>
<td></td>
<td></td>
<td></td>
<td>Arg to Gly substitution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen branching enzyme deficiency (GBED)</td>
<td>Quarter Horse</td>
<td>26</td>
<td>GBE1</td>
<td>C to A substitution</td>
<td>Autosomal recessive</td>
<td>Y</td>
</tr>
<tr>
<td>Severe combined immunodeficiency (SCID)</td>
<td>Arabian</td>
<td>9</td>
<td>DNA-PKcs</td>
<td>5 bp deletion Unstable protein</td>
<td>Autosomal recessive</td>
<td>Y</td>
</tr>
<tr>
<td>Junctional epidermolysis bullosa (JEB)</td>
<td>American Saddlebred</td>
<td>8</td>
<td>LAMA3</td>
<td>6589 bp deletion Dysfunctional protein</td>
<td>Autosomal recessive</td>
<td>Y</td>
</tr>
<tr>
<td>(Liento and Cothran 2003; Graves et al. 2008)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junctional epidermolysis bullosa (JEB)</td>
<td>Belgian French draughts</td>
<td>5</td>
<td>LAMC2</td>
<td>C insertion Premature stop codon</td>
<td>Autosomal recessive</td>
<td>Y</td>
</tr>
<tr>
<td>(Spirito et al. 2002; Milenkovic et al. 2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereditary equine regional dermal asthenia (HERDA)</td>
<td>Quarter Horse</td>
<td>1</td>
<td>PPIB</td>
<td>Missense mutation Gly to Arg substitution</td>
<td>Autosomal recessive</td>
<td>Y</td>
</tr>
<tr>
<td>Overo lethal white syndrome (OLWS), ileocolonic aganglionosis</td>
<td>Paint</td>
<td>17</td>
<td>EDNRB</td>
<td>TC to AG substitution</td>
<td>Autosomal recessive</td>
<td>Y</td>
</tr>
<tr>
<td>(Metallinos et al. 1998; Yang et al. 1998; Santschi et al. 2001)</td>
<td></td>
<td></td>
<td></td>
<td>Ile to Lys substitution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grey horse melanoma</td>
<td>Many</td>
<td>25</td>
<td>STX17</td>
<td>Duplication in intron 6</td>
<td>Autosomal dominant</td>
<td>Y</td>
</tr>
<tr>
<td>(Pielberg et al. 2008)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lavender foal syndrome</td>
<td>Arabian</td>
<td>1</td>
<td>MYO5A</td>
<td>G138235715del Single bp deletion in exon 30</td>
<td>Autosomal recessive</td>
<td>N</td>
</tr>
</tbody>
</table>

*Y, testing available commercially; N, testing not yet available commercially, but expected soon.
Equus caballus Genome available on National Council of Biology Information

Fig 1: Map viewer of the Equus caballus genome available on National Council of Biology Information. View of the chromosome 10 where the glycogen synthase gene (GYS1) involved in 10 is located (McCue et al. 2008, Herszberg et al. 2009).
# Monogenetic diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Breed</th>
<th>Inheritance</th>
<th>Mutation</th>
<th>Clinical Signs</th>
<th>Diagnosis</th>
<th>Tx/Prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>HYPP</td>
<td>QH, Appo, Paint Horses</td>
<td>Autosomal</td>
<td>Phe→leuc α subNa channel</td>
<td>Muscle fasciculation</td>
<td>Hair with roots or</td>
<td>Reduce stress, carbo</td>
</tr>
<tr>
<td>Hyperkalaemic paralysis</td>
<td></td>
<td>Co-dominant</td>
<td>Resting potential reduced</td>
<td>weakness, dysphonia/dysphagia</td>
<td>whole blood. KCL chal</td>
<td>Acetazolamide, K intake</td>
</tr>
<tr>
<td>Type 1 PSSM</td>
<td>QH, Drafts, WBs</td>
<td>GYS 1 (chro 10)</td>
<td></td>
<td>ER, mild colic, gait abnormalities</td>
<td>CK and AST elevation</td>
<td>Diet and exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plus others</td>
<td>10 single base-pair sub</td>
<td>muscle wasting</td>
<td>Genetic Test/muscle biopsy</td>
<td></td>
</tr>
<tr>
<td>Malignant Hyperthermia</td>
<td>QH</td>
<td>Autosomal</td>
<td>RYR1 exon 46</td>
<td>Hyperthermia - halogenated ana</td>
<td>&gt;40°C, lactate acidosis</td>
<td>Pre-tx oral dantrolene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBED</td>
<td>QH and Paint Horses</td>
<td>Autosomal</td>
<td>CtoA pt mutation</td>
<td>Aborted or still born,</td>
<td>No glycogen on histo</td>
<td>PMs on aborted foals</td>
</tr>
<tr>
<td>Glycogen Branching enz</td>
<td></td>
<td>Recessive</td>
<td>→stop codon</td>
<td>weak hypothermic</td>
<td>Genetic Test</td>
<td>Test for carrier status</td>
</tr>
<tr>
<td>SCID</td>
<td>Arabian</td>
<td>Autosomal</td>
<td>Frameshift mut. - DNA-PKcs</td>
<td>No B or T lymphocytes</td>
<td>Absolute lymphopaenia</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>Severe combined immunodeficiency</td>
<td>Recessive</td>
<td>DNA-protein kinase</td>
<td>Present around 5mths once PT</td>
<td>Lymphoid hypoplasia</td>
<td>Transplantation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>recessive</td>
<td>catalytic subunit</td>
<td>wanes. Secondary infec</td>
<td>Genetic Test</td>
<td>Euthanasia</td>
</tr>
<tr>
<td>Condition</td>
<td>Breed/Disease</td>
<td>Gene/Location</td>
<td>Mutation/Change</td>
<td>Symptoms/Notes</td>
<td>Diagnostic Test</td>
<td>Treatment/Prognosis</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>--------------------------------------</td>
<td>---------------------------------</td>
<td>----------------------------------</td>
<td>--------------------------------------------------------------</td>
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</tr>
<tr>
<td>Ileocolonic aganglionosis</td>
<td>American Paint, QH Autosomal</td>
<td>Isoleucin→lysine (chrom 17)</td>
<td>Colic and does not pass meconium</td>
<td>Genetic Test, Euthanasia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overo lethal white foal (OLWS)</td>
<td>Rarely TBs, Recessive</td>
<td>Endothelin receptor B</td>
<td>Essential for enteric ganglia and melanocytes</td>
<td>White or nearly white foals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fell Immunodeficiency</td>
<td>Fell and Dale Ponies, Autosomal</td>
<td>Absence of B-cells</td>
<td>2-4 weeks old</td>
<td>Genetic Test, Euthanasia, Abs, blood transfusions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syndrome (FIS)</td>
<td>Recessive</td>
<td>Severe anaemia, immunodef</td>
<td>Lack normal lymphoid</td>
<td>Genetic Test - AHT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lavender Foal Syndrome</td>
<td>Egyptian Arabians, Autosomal</td>
<td>Frame shift mutation</td>
<td>Neurological signs - tetanic-like</td>
<td>Genetic Test, Euthanasia/death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellar abiotrophy</td>
<td>Arabians, Autosomal</td>
<td>UNKNOWN</td>
<td>6 wks - intention tremor, stiff</td>
<td>Genetic test - surrog markers, Paddock - pet</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td></td>
<td>uncoordinated gait.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td></td>
<td>Exaggerated actions often fall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>as unsafe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junctional Epidermolysis</td>
<td>Belgians and saddlebreds, Autosomal</td>
<td>Drafts - Premature stop codon</td>
<td>Irregular, reddened erosions</td>
<td>Skin biopsy, No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td>in Lamc2 - encodes laminin</td>
<td>&amp; ulcerations of skin and mouth</td>
<td>Genetic Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td>gamma 2 subunit</td>
<td>over pressure pts or trauma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bullosa (JEB)</td>
<td>QH and related horses, Autosomal</td>
<td>Chromo 1 G→A codin 115</td>
<td>1.5yrs assoc with initial saddling tr</td>
<td>Skin biopsy, Euthanasia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HERDA</td>
<td>QH and related horses, Autosomal</td>
<td>Equine cyclophilin B.</td>
<td>Seromas or haematomas, open</td>
<td>Genetic Test, common</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereditary Equine</td>
<td>Recessive</td>
<td>Gene involved in collagen</td>
<td>Wounds or sloughing skin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regional</td>
<td></td>
<td></td>
<td>Skin does not return to normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermal Asthenia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Complex Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Abbreviation</th>
<th>Affected Breeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Pastern Dermatitis</td>
<td>CPD</td>
<td>German Draft Horses</td>
</tr>
<tr>
<td>Recurrent Airway Obstruction</td>
<td>RAO, COPD</td>
<td>Warmblood</td>
</tr>
<tr>
<td>Navicular Disease</td>
<td>RAC</td>
<td>Hannoveranian</td>
</tr>
<tr>
<td>Guttural pouch tympany</td>
<td>GPT</td>
<td>Arabian, German WB</td>
</tr>
<tr>
<td>Osteochondrosis</td>
<td>OD, OCD</td>
<td>Hanoveranian</td>
</tr>
<tr>
<td></td>
<td></td>
<td>South German Cold Blood</td>
</tr>
<tr>
<td>Mutiple Cogenital Ocular Abnormalities</td>
<td>MCOA</td>
<td>Rocky Mountain Horse</td>
</tr>
</tbody>
</table>
Nature vs nurture

- The influence of genetics vs the environment
Heritability Estimates – \( h^2 \)

- Estimate the influence of genetic components and non-genetic (environment) effects on trait variability.

- Heritability is the ratio between genetic variance and total variance including genetic factors.

- E.g. \( h^2 > 0.4 \) indicate 40% of the trait could be transmitted to offspring by the mare or stallion.
Performance Heritability

- Flat Racing – 0.15-0.55
- Trot or pace racing – 0.17-0.26
- Showjumping and 3-day eventing - 0.05-0.28
- Dressage – 0.11

N.B. Little correlation between jumping ability and other sport performance and gait variables.

Heritability estimates are only valid for the breed they are calculated for. Re-calculate over time.
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The future
Pre-purchase examination – Genetic Testing

Is the horse of a breed or colour known for a genetic disease?

- Dominant
- Recessive

Does the seller have genetic test on sire/dam?

- Yes
- No

Test Horse

- Positive
- Negative
Pre-purchase examination – Genetic Testing

a) Prepurchase Examination

Is the horse of a breed, line or colour known for a genetic disease?

- Yes: No action is necessary.
- No: Proceed to the next question.

Is there a test available for the disease?

- Yes: Note that testing is available for most genetic diseases of the horse that have been characterised to date (see Table 3).
- No: Proceed to the next question.

Does seller have genetic test result on sire/dam?

- Yes: If sire and dam are proven free of disease, offspring (i.e. the horse to be purchased) should be free of disease. Request notarised copy of test results on sire/dam as part of transfer. Review breed-specific testing / registration requirements for offspring. If sire or dam possess deleterious allele, horse should be tested. Prior to testing, discuss who will pay for test and how disclosure will be handled.
- No: Proceed to the next question.

This horse should be tested.

- Negative: No further action required. Test results should become part of horse’s record.
- Positive: Proceed to the next question.

Is the disease dominant or recessive?

- Dominant (e.g. PSSM, HYPP): Discuss required husbandry, medications, prognosis, humane concerns, financial and emotional commitments for informed decision. Recommend full disclosure to breeder.
- Recessive (e.g. SCID, OLWS, JEB, HERDA, etc): In most instances, horse will be unaffected by clinical disease. If horse is positive for OLWS, recommend hearing evaluation as part of prepurchase. Recommend full disclosure to breeder.

Was animal intended to be breeding stock?

- Yes: Recommend full disclosure on future sales of the animal if purchase is completed.
- No: Proceed to the next question.

Go to C) if a stallion or D) if a mare

Veterinarian should document all test results and discussions (especially refusals to test) in the prepurchase report.
Mare Breeding Soundness Examination

d)

Mare Breeding Consultation

Is the horse of a breed, line or colour known for a genetic disease?

Yes

No action is necessary.

Is there a test available for the deleterious allele?

Yes

Note that testing is available for most genetic diseases of the horse that have been characterised to date (see Table 3).

Are test results available on sire/dam?

Yes

If sire and dam are proven free of the allele, mare should be free of the allele. Request notarized copy of test results on sire/dam to disclose to potential stallion owners. Review breed-specific testing/registration requirements for offspring. If sire or dam possess deleterious allele, mare should be tested.

No

Mare should be tested.

Mare should be tested.

Mare should be tested.

Positive

No further action required. Test results should become part of horse’s breeding record. Recommend breeding only to negative stallions to maintain a disease-free program.

Negative

Is the disease dominant or recessive?

Dominant (e.g. PSSM, HYPP)

Mare and 50% of offspring will be affected. Discuss required husbandry, medications, prognosis, humane concerns, financial and emotional commitments. Recommend removal from breeding program.

Follow up with testing on foal. Recommend full disclosure to purchasers. Veterinarian should consider the ethical and legal issues surrounding continued involvement with breeders unwilling to fully disclose the presence of genetic disease in a mare or foal.

Recessive (e.g. SCID, OLWS, JEB, HERDA, etc)

Recommend removal from breeding program unless mare is extremely valuable genetically. If mare is to be bred, recommend disclosure to all stallion owners and breeding only to negative stallions. Recommend that long-term breeding program include goal of replacement with a similar, disease free animal. If allowable, recommend assisted reproduction techniques to expand the pool of potential stallions.
Stallion Breeding Soundness Examination

c)

Stallion Breeding Soundness Examination

Is the horse of a breed, line or colour known for a genetic disease?

Yes → No action is necessary.

No

Is there a test available for the deleterious allele?

Yes → Note that testing is available for most genetic diseases of the horse that have been characterised to date (see Table 3).

Are test results available on sire/dam?

Yes → If sire and dam are proven free of the allele, stallion should be free of the allele. Request notarized copy of test results on sire/dam to disclose to potential mare owners. Review breed-specific testing / registration requirements for offspring. If sire or dam possess the deleterious allele, stallion should be tested.

No → Stallion should be tested.

Positive → No further action required. Test results should become part of horse’s breeding record.

Recessive (e.g. SCID, OLWS, JEB, HERDA, etc)

In most cases, animals homozygous for recessive disease will die or show clinical signs prior to breeding age. Breeding of heterozygotes should be undertaken with much consideration for the health of the offspring. If bred to another carrier, 25% of offspring will be affected, 50% will be carriers, 25% will be normal. If bred to a normal animal, 50% of offspring will be carriers.

Recommend removal from breeding program unless stallion is extremely valuable genetically. If stallion is to be bred, recommend disclosure to all mare owners and breeding only to negative mares. Recommend that long-term breeding program include the goal of replacement with a related animal.
Disease – Genetic Testing

Is there a known genetic disease consistent with signalment and observed clinical signs?

- Yes
  - Investigate other differential diagnoses. If no diagnosis can be made, or if affected animal is a foal (particularly one with an unusual color) contact the closest veterinary teaching hospital to investigate further.

- No
  - Is there a test available for the disease?
    - Yes
      - Note that testing is available for most genetic diseases of the horse that have been characterised to date (see Table 3).
      - Horse should be tested.
    - No
      - Investigate other differential diagnoses.

Is the disease dominant or recessive?

- Dominant (e.g. PSSM, HYPP)
  - Discuss required husbandry, medications, prognosis, humane concerns, financial and emotional commitments.
  - Recommend full disclosure to breeder.

- Recessive (e.g. SCID, OLWS, JEB, HERDA, etc)
  - Virtually all identified recessive diseases of the horse carry a poor prognosis so this animal, by definition a homozygote, likely will not survive.
  - Discuss progression of disease and humane concerns.
  - Recommend full disclosure to breeder.

Was animal intended to be breeding stock?

- Yes
  - Recommend full disclosure on future sales of the animal.
- No
  - Go to C) if a stallion or D) if a mare.
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The future
Future
• Genome-wide scan study
• Microsatellite markers
• Single nucleotide polymorphisms (SNPs)
• Identify genes linked to some performance traits or health issues
Potential for miss-use

- The increasing availability of biotechnologies’ will raise the possibility of illicit use – eg muscular transgenesis.

- Research is underway to detect this new type of genomic doping in man and horses.
Acknowledgements

• Osberton International Horse Trials
• Stewart and Jane Hastie
• Buckingham Equine Vets – www.buckinghamamequinevets.com
• The Royal Veterinary College
1. Review: Genetics and genomics in equine exercise physiology: an overview of the new applications of molecular biology as positive and negative markers of performance and health.
   Barrey E.
   PMID: 21059061 [PubMed - in process]

   Bailey E.
   PMID: 21322892 [PubMed - in process]

   Brosnahan MM, Brooks SA, Antczak DF.
   Review.
   PMID: 20840582 [PubMed - indexed for MEDLINE]