Genetic Testing and your Friesians

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When considering the growing needs of the Friesian breeders, owners, and future owners, the use of modern science has begun to play a much greater role. Through genetic testing of stallions, mares, and foals, the Friesian breed moves forward with greater knowledge to protect and preserve the breed, aid in the selection of breeding pairs to maintain the greatest diversity in the breed, and with the hope of reducing the risk of known detrimental traits appearing in the offspring while benefiting from the genetics available even within carrier individuals. To gain a better of the genetics of the Friesian and the information conveyed by genetic test results The goal of this article is cover both general genetics of the horse, including what is meant by carrier status, the range of genetic disorders currently known in the Friesian, as well as information studies that may result in future biochemical or genetic tests.

Friesians as with all breeds of modern domesticated equine have 64 chromosomes, known as ECA. Half of these chromosomes are contributed by the sire and half by the dam, resulting in 31 pairs of non sex chromosomes (autosomes), plus 1 pair of sex chromosomes (XX for females, or XY for males). As of 2017, these chromosome pairs are currently estimated to carry approximately 20,000 to 26,000 protein-coding genes within each cell's nuclear genome. In addition, the dam also provides heritable genetic information to her offspring through mitochondria (300-600 per cell). The mitochondria are important cellular structures (organelles) which are intimately involved in energy production in muscles, eyes, and all other organs and tissues. Not all of the mitochondria are the same within a cell, and typically have several different types per cell depending on the specific various mutations carried in its DNA that can have dramatic effects on any structure or tissue that requires high energy utilization.

As of 2017, there are 233 known traits or disorders of the horse listed in the Online Mendelian Inheritance in Animals (OMIA) database. Only 57 of these are considered to be well-characterized and classified as Mendelian traits or disorders, and therefore likely candidates for genetic test development. The variant forms of several of these genes and others are still under investigation. As part of the International Horse Genome Project, the focus has been on major breeds of horses but the Friesians, because of their uniqueness and the high-level of pedigree documentation through KFPS, have been able to make significant specific contributions. See the *Table 1: General genetics FAQs on the horse* for additional information on the genes of the horse.

For any single gene, an individual horse typically will have two very similarly functioning versions of a particular gene or two different versions of the gene. When considering Mendelian traits or disorders, a key aspect is the relationship between these different versions of one particular gene. If one version can mask or control the expression of the gene, then it is considered to be the *dominant form*. The alternate version in this situation is considered to be the *recessive form*. If the detrimental or undesirable version of the gene is *recessive*, then a with a combination of both a recessive form and dominant form is considered to be a "Carrier" of that trait or disorder. In all Friesians, the horses carry two identical recessive copies of the *Agouti* (*A*) gene, but they are either non-carriers or carriers of the recessive form of the *Extension* (*E*) gene. Therefore, in Friesians a specific example would be the *Extension of Color* gene (or *Extension gene*, *E*), that results in the chestnut or red coat color trait. Available *versions of the gene*, are "*E*" and "*e*", where the recessive form "*e*" allows for the development of chestnut color but only if both copies are the recessive form or "ee". So, if the following gene combinations are considered:

Non-carrier: (*aaEE*) Black Friesian
Carrier : (*aaEe*) Black Friesian, Carrier for Red *factor*
RED (Affected): (*aaee*) Chestnut Friesian

Case A: If a Friesian non-carrier for the *Extension* gene is bred to a Friesian carrier for *Extension*, then the possible outcomes have the probabilities:

aaEE x aa*Ee*
→ $^1/_2$ Black, non-carrier (*aaEE*)
or $^1/_2$ Black, carrier (*aaEe*)

Therefore, if a non-carrier is bred to a carrier, the risk of the offspring being a non-carrier is 50% and a carrier is 50%.

Case B: If a Friesian carrier for the *Extension* gene is bred to another carrier for the Extension gene, then the possible outcomes have the probabilities:

aaEe x aa*Ee*
→ $^1/_4$ Black, non-carrier (*aaEE*)
or $^2/_4$ Black, carrier (*aaEe*)
or $^1/_4$ RED (*aaee*)

Therefore, if a carrier is bred to a carrier, the risk of the offspring being a non-carrier is 25%, a carrier is 50%, and a Chestnut Friesian is 25%.

Knowledge of KFPS Stamlines provides the means to trace the mitochondrial contributions across generations within the Friesian breed through the dam, who is the sole source of mitochondrial DNA which is inherited only from the egg which the dam provides. Thus, dam selection in breeding programs is crucial since dams provides greater genetic contributions to each subsequent generation via larger of the two sex chromosomes and as the sole source of mitochondrial DNA. Therefore, it is important to maintain available registry information on Stamlines in order to make informed breeding selections. And, the stallions known as producers of many of the important dams in the Stamlines are actually the contributors of one of the two possible sex (X) chromosomes (50%) in the next generation of stallions. Therefore, the ability to predict potential risks to offspring related to the inheritance of specific genes, and in particular genes on the sex (X) chromosomes, can only be established within the breed through the maintenance of the careful documentation afforded by the KFPS and FHANA registries. Prior to the development of gene specific DNA tests, a willingness to provide information and or participate in focused genetic studies will help identify more genetic traits and the underlying genetic contributions to disorders of interest in the Friesian.

Genetic Traits, Disorders, & Diseases Affecting the Friesian

Friesians as a breed originated from a limited gene pool after the devastation of the World Wars, particularly World War II. Founder effects come along with the limited initial gene pool. However, through the dedication and careful selective breeding of owners and breeders who recognized the uniqueness of the specific characteristics of the breed that sets it apart from others, the breed was able to recover and flourish.

Efforts continue to be made to offset the impact of the effects of the limited genetic base of the Friesians, and the set of specific undesirable traits and detrimental genetic disorders/ diseases have been identified in the Friesian breed.

Research has been able to reveal the specific genes associated with those conditions. For information on the key traits and genes of interest in the Friesian breed, see *Table 2: Genetic Traits, Disorders, & Diseases Affecting Friesians*. This increase in knowledge has subsequently allowed for the development of genetic tests which have been adopted by the KFPS and FHANA. In the cases of those genes associated with dwarfism and hydrocephaly, the only way to reduce the incidence of its occurrence in future generations is through the careful genetic monitoring of all breeding individuals in order to benefited the entire population. For information on the research and development of test for a range of other traits and disorders/ diseases known to occur within the Friesian breed, see the full-length article on the FHANA website.

Importance of Testing in KFPS and FHANA

KFPS and FHANA are encouraging owner/breeders to be proactive with regards to the most significant identified genetic challenges currently known in the Friesian breed. To this end, KFPS now requires testing, in addition to lineage DNA verification, tests for Dwarfism (*B4GALT7* gene), Hydrocephaly (*B3GALNT2* gene), and the Red factor (Chestnut coat color or *Extension* [E] gene)

tests. Of the tests currently being performed in the USA within the Friesian breed, the two specific genetic tests for Dwarfism and Hydrocephalus are offered by both the University of California – Davis, and the University of Kentucky, Gluck Equine Center.

The KFPS website updates any new testing requirements for both stallions, mares, and foals (KFPS News, 2017).

1. *All KFPS approved stallions to be used for breeding must be DNA tested for dwarfism and hydrocephaly.*

2. *In addition to lineage verification, all mares that are declared Star and all mares with a foal accepted for registration also need to undergo DNA testing for hydrocephaly, dwarfism and the chestnut factor.*

Note, the chestnut factor is the same as the Extension gene [E] used as an example previously. In addition, aberrant semen morphology testing is no longer used as a basis for ruling out young stallions, where previously this test would have been considered valid reason for elimination.

With respect to Lineage testing, as of February 2018 (KFPS News, 2018), mares can be exempted from testing based on lineage if BOTH sire and dam have been tested and determined to be non-carriers for dwarfism and or hydrocephaly. In addition, ALL stallions will continue to be tested regardless of carrier status of both parents. From a genetics perspective, continued monitoring allows for possible early detection of new mutations but only if they occur in the same DNA sequences used in the specific tests. For a breakdown of the required testing see *Table 3: Required Testing as of February 2018,* and for a summary of testing on the Approved stallions see *Table 4: KFPS Studbook Stallions 2018 positive test results*

Accuracy of tests

The accuracy of any of the tests used depends first and foremost on the quality of the starting material. If the samples to be drawn are blood, or other types of tissues, it will be critical that they are sampled as cleanly as possible, stored (such as prior to shipment or analysis), and shipped, where the least amount of degradation of the essential elements to be analyzed.

For many genetic DNA tests, the following materials are used:

- 20-40 *pulled* hairs from mane or tail, do not use shed hairs, do not use hair from brushes. *See the resources reference at the end of this article for the podcast by Dr. Bailey for an excellent step-by-step guide.*
- Root bulb is the important part, not the hair shaft.
- Laboratories suggest using tail hair for foals or horses under 1 yo, unless otherwise instructed, because of the fineness of foal hair.

Sampling hair for genetic analysis of a horse is actually dependent on the collection of hair root bulbs. Mane hairs are usually appropriate for sampling with adult horses, depending on the specific test. The full length of the hair shaft can be trimmed or cut, as long as the sample that is sent for analysis contains hair root bulbs which are the cells needed for the actual analysis. If the owner or breeder simply provides a folded length of several hair shafts, then the sample may be inadequate to for testing because the number of hair root bulb cells would yield an insufficient amount of DNA.

Similarly, the mane hairs of foals can be very fine and are unlikely to provide as many hair root bulb cells when the hairs are pulled for analysis. Therefore, when collecting a sample from a foal or young horse under the age of 1 year, it is often advisable to collect a tail hair sample instead of the mane. In any case, always confirm the specific type of sample, method of collection, method of storage until shipped to the testing facility or on-site analysis is performed, with the laboratory performing the test (usually via their website or by phone), or with your veterinarian.

Suggested Questions to Consider - when preparing to test:

A List of testing facilities is provided in *Table 5: Laboratory Locations and Contact information.* An example of the KFPS DNA testing form is also provided as an example.

1. *When to test?*
2. *How to test?*
3. *Who does the tests, or draws the sample?*
4. *Who does the analysis? (on-site analysis, veterinarian analysis, mail-in analysis)*
5. *What is the accuracy or possibility of a false positive (or false negative)?*
6. *Are further consultations usually required to interpret the results?*
7. *What are the costs for each test versus combinations of tests, or is there a discount through the breed association?*

Summary

The Friesian community of owners and breeders has made great strides to guard against propagating any sort of health problems within the breed. Their care with selective breeding, and participation in research demonstrates their on-going efforts to not only benefit the current state of the breed but to improve the breed with an eye to the future. The key will be knowledge - pedigree analysis, genetic testing, performance testing, and finally expertise in selective breeding. And, by far, the single

MOST important factor in the health and welfare of the Friesian breed is the depth of appreciation for the beauty of the breed and the tireless dedication of the efforts of Friesian owners and breeders worldwide.

For a more in-depth version of this article, please go to the FHANA website.

Additional Resources

Ayala-Valdovinos, MA, J Galindo-García, D Sánchez-Chiprés, and T Duifhuis-Rivera. 2017. Genotyping of Friesian horses to detect a hydrocephalus associated c.1423C>T mutation in B3GALNT2 using PCRRFLP and PCRPIRA methods: Frequency in stallion horses in México. Mol Cell Probes 32:69-71.

Bailey, E. 2017. Collecting a Horse DNA Sample. *In* The Horse: Your Guide to Equine Health, published online Aug 22, 2017.

Ducro, BJ, A Schurink, JWM Bastiaansen, IJM Boegheim, FG van Steenbeek, M Vos-Loohuis, IJ Nijman, GR Monroe, I Hellinga, BW Dibbits, W Back, and PAJ Leegwater. 2015. A nonsense mutation in *B3GALNT2* is concordant with hydrocephalus in Friesian horses. BMC Genomics. 16: 761. doi: 10.1186/s12864-015-1936-z Published online 2015 Oct 9.

KFPS News. 2017. Lineage Verification and Application DNA Tests Dwarfism and Hydrocephaly. August 11, 2017. URL: http://english.kfps.nl/Actueel/News/newsdetail.aspx?newsid=4161

KFPS News. 2018. Carrier Status on the Basis of Lineage Added to MyKFPS. February 9, 2018. URL: http://english.kfps.nl/Actueel/News/newsdetail.aspx?newsid=4277

Leegwater, PA, M Vos-Loohuis, BJ Ducro, IJ Boegheim, FG van Steenbeek, IJ Nijman, GR Monroe, JW Bastiaansen, BW Dibbits, LH van de Goor, I Hellinga, W Back, and A Schurink. 2016. Dwarfism with joint laxity in Friesian horses is associated with a splice site mutation in B4GALT7. BMC Genomics 17:839.

Ploeg, M, V Saey, G van Loon, and C Delesalle. 2017. Thoracic aortic rupture in horses. Equine Vet J. 49(3): 269-274.

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Table 1: General genetics FAQs on the horse.

Genus species name: *Equus ferus caballus*	
Total number of chromosomes (ECAs)	64
Total nuclear DNA	2.7×10^9 bases
Est. number of protein coding genes	20-26,000
Average number of mitochondria/cell	300-600
Est. number of protein coding genes	37
Total traits/disorders (2017)	233
Mendelian traits/disorders	57
Mendelian trait/disorder; likely causal variant(s) known	44
Dwarfism	4
Congenital heart disease	8

Reference: Online Mendelian Inheritance in Animals (OMIA), as of April 27, 2018, *http://omia.org/browse/*

Table 3: Required Testing, as of February 2018.

Classification	**Lineage Verification Test**	**Dwarfism Carrier Test**	**Hydrocephaly Carrier Test**	**Red (Extension gene) Carrier Test**
Ster Mares *(not previously tested)**	Test	Test	Test	Test
Ster Mares *(lineage verified)*	n/a	Test	Test	Test
Ster Mares *(carrier status tested)*	Test	n/a	n/a	n/a
Ster Mare *(carrier status based on lineage)*	Test	Exempt	Exempt	Exempt
Foals *(with Inspected Mare)*	Test	n/a	n/a	n/a

* Exception to testing are those mares who are non-carriers based on test results of BOTH sire and dam test results and therefore are exempt based on lineage.

Table 2: Genetic Traits, Disorders, & Diseases Affecting Friesians.

Category **Trait or Disorder**	**Test Available**	**Gene/ Chromosome & Mutation**	**Clinical Features & Notes**
Coat Color			
Chestnut Coat Color (Extension gene [E])	DNA	E (Extension)/ ECA Autosomal recessive	Red foals can be entered in Foalbook Black offspring from Red mare bred to an approved stallion can entered in registry
Bone & Cartilage			
Dwarfism Occurrence: 1/4000 births Prevalence: 12% of all Friesians are carriers	DNA	*B4GALT7*/ ECA14 Mutation: nonsense type Autosomal recessive	Clinical Growth plates involved Suppression of osteoblasts As compared to Normal: 50% less weight 25% shorter limbs Fetlocks hyperextend Dysplasia in the metacarpus and metatarsals Round belly and chest areas Weak musculature Friesian form thought to be similar to Osteochondrodysplasia
Hydrocephaly, congenital Occurrence: >2% of births Prevalence: 13.3% of stallions 17.3% of broodmares Carrier Estimate: 17% for all Friesians	DNA	*B3GALNT2*/ ECA1 Mutation: nonsense type Autosomal recessive	Usu. stillborn foals Caused by "stenosis of the jugular foramen" Cerebral cortex is very thin Skull height is abnormal Skull width is expected to be 3x larger Can cause dystocia in mare Identical to muscular dystrophy-dystroglycanopathy with hydrocephalus in Humans
Collagen Disorders			
Megaesophagus	None	Acquired or Congenital condition	Hypothesized to be hereditary Caudal esophageal muscular hypertrophy chronic dilation of the esophagus Young foals have presence of abnormal collagen 2 Types: obstruction v. dysfunction Obstructions = *acquired* disorder Dysfunction = *congenital* or inherited disorder Dysfunctional Type: lack of neurons near esophagus or from muscle atrophy Less elastin around the esophagus Most often in horses aged 0-2 yrs
Aortic Rupture	None	UNKNOWN Evidence suggests: localized hereditary defect	Sudden Death or Slow death with colic-like symptoms Median age of death = 4 yrs (range 1-20 yrs) Collagen or elastin shown to be affected in aortic media Collagen percentage at rupture site is higher than normal Medial fibrosis seen at rupture

Table 4: KFPS Studbook Stallions 2018 positive test results.

Country	Dwarfism	Hydrocephaly	Red (Extension gene)	Total Stallions Tested***
France	0	1	0	1
Germany	0	0	0	1
Hungary	0	0	0	1
Netherlands	9	9	0*	67**
South Africa	3	1	0	6
Sweden	0	0	0	1
USA/Canada	0	3	0***	19
Totals	**12**	**14**	**0**	**96**

* 12 tested negative; includes all 10 stallions tested beginning with Stallion #494 through #503, and 2 additional stallions prior to #494
** 1 tested positive for both Dwarfism and Hydrocephaly
*** 7 tested, all negative

Table 5: Laboratory Locations and Contact information

Location	Website	Contact	Phone
Cornell Univ.	https://ahdc.vet.cornell.edu/test/	Cornell University College of Veterinary Medicine P.O. Box 5786	607/253-3935
UC-Davis	http://www.vgl.ucdavis.edu/services/horse.php **Email Form:** https://www.vgl.ucdavis.edu/contact/email.php	University of California, Davis PO Box 1102w Davis, CA 95617-1102 https://www.vgl.ucdavis.edu/contact/email.php	530-752-2211
Univ. Ky.	http://getgluck.ca.uky.edu/	Department of Veterinary Science Gluck Equine Research Center University of Kentucky Lexington, KY 40546	859-218-1165 Graves, Ph.D. ktgraves@ky.edu
Univ. Minn.	https://www.vdl.umn.edu/services-fees/equine-neuromuscular/submission-guidelines	University of Minnesota 1333 Gortner Avenue St. Paul, MN 55108-1098 vdl@umc.edu	612-625-8787
Animal Genetics Inc.	https://www.animalgenetics.us/ **Forms & Kits:** https://www.animalgenetics.us/Equine/Equine_Test_Now.asp	Animal Genetics Inc. 1336 Timberlane Road Tallahassee, FL 32312 contact@animalgenetics.us	800-514-9672
Maxxam Analytics	http://maxxam.ca/services/dna-testing/animal-dna-testing/	335 Laird Road, Unit 2 Guelph, Ontario N 1 H 6J3 Canada	519-836-2400