Distichiasis in Friesian Horses

What is it? What is known and what is unknown? How can genetic testing help?

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FHANA

FRIESIAN HORSE ASSOCIATION OF NORTH AMERICA

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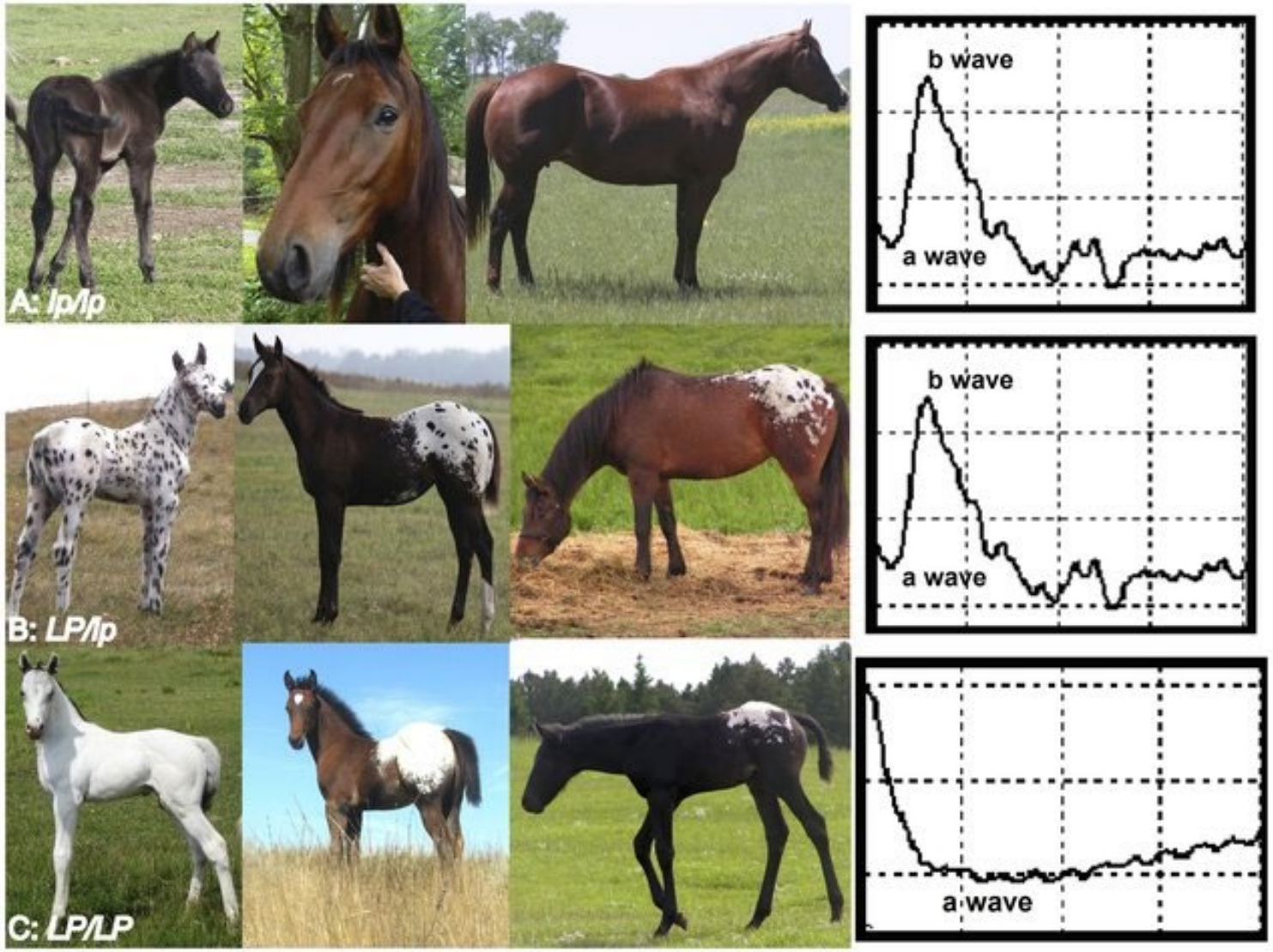
Why horses, genetics, and eyes?

Leopard Complex Spotting and CSNB

- Homozygotes (*LP/LP*) affected with Congenital Stationary Night Blindness[1]



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[1]Sandmeyer, L.S., Breaux, C.B., Archer, S. and Grahn, B.H. (2007), Clinical and electroretinographic characteristics of congenital stationary night blindness in the Appaloosa and the association with the leopard complex. Veterinary Ophthalmology, 10: 368-375. https://doi.org/10.1111/j.1463-5224.2007.00572.x

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Outline

- Distichiasis
  - What is it?
  - Prevalence?
  - What are signs/symptoms?
  - What are treatment options?
  - What is the prognosis?
- Genetics
  - What research was done to identify variant?
  - What does the research mean?
  - What is the genetic test for distichiasis evaluating?
  - How do I interpret the genetic test results?
  - What is left to understand?

What is Distichiasis?

- 1/3 Eyelash (cilia) disorder
- Normal eyelash function
- Extra eyelashes
- Exiting the meibomian gland

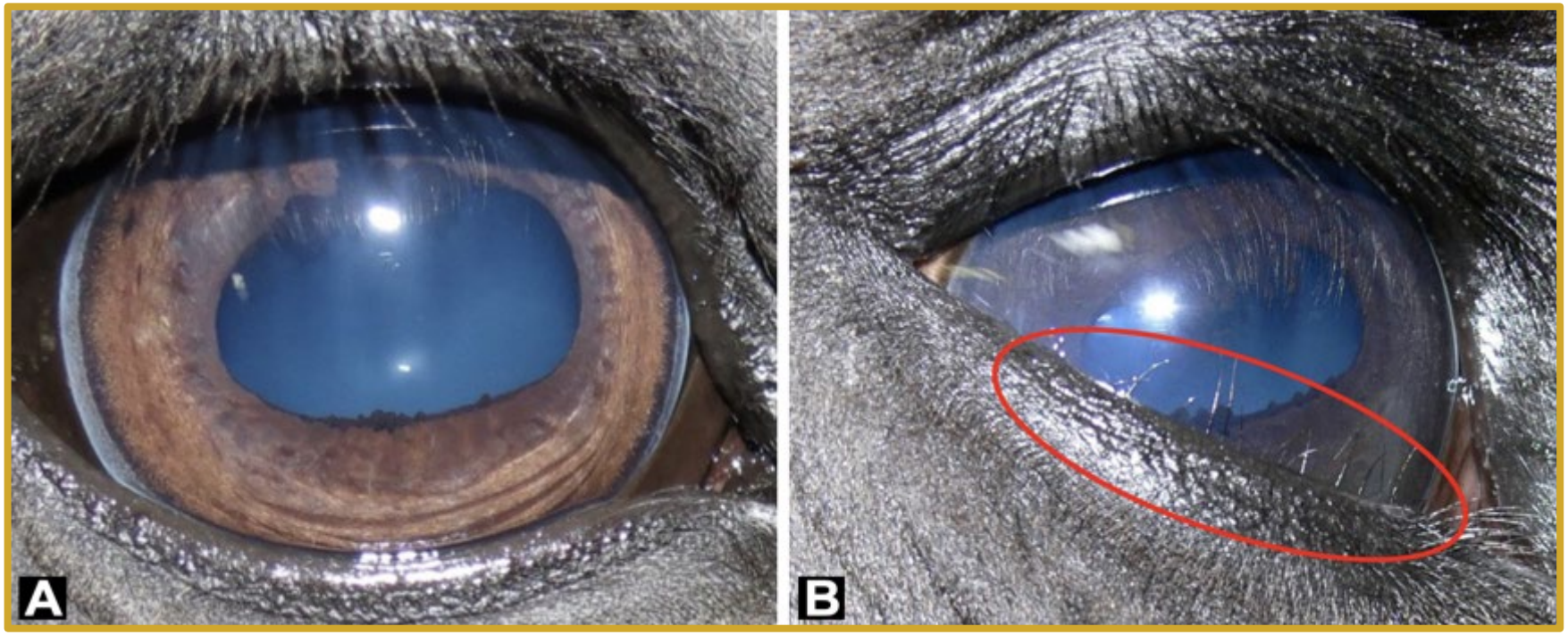
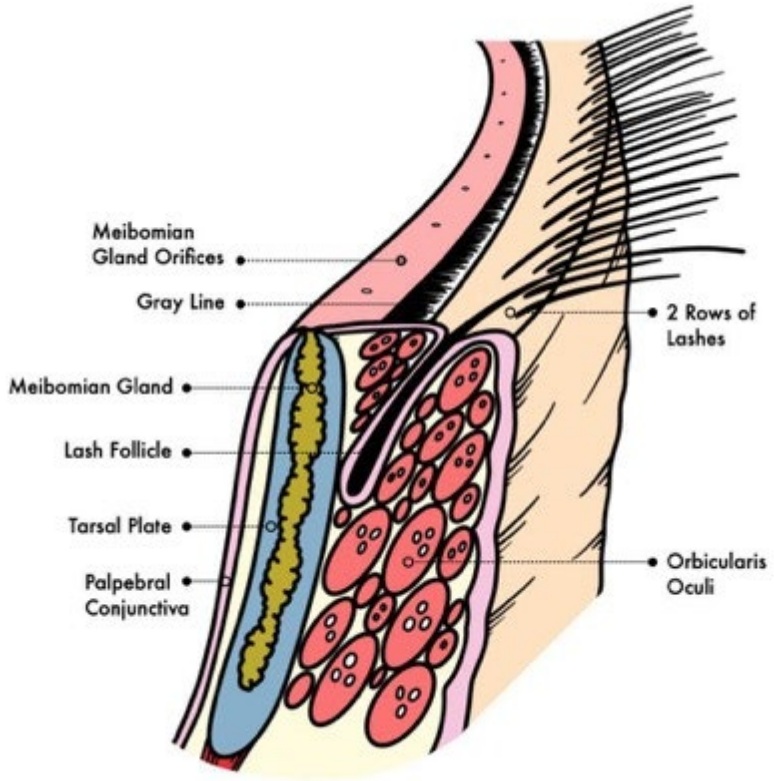
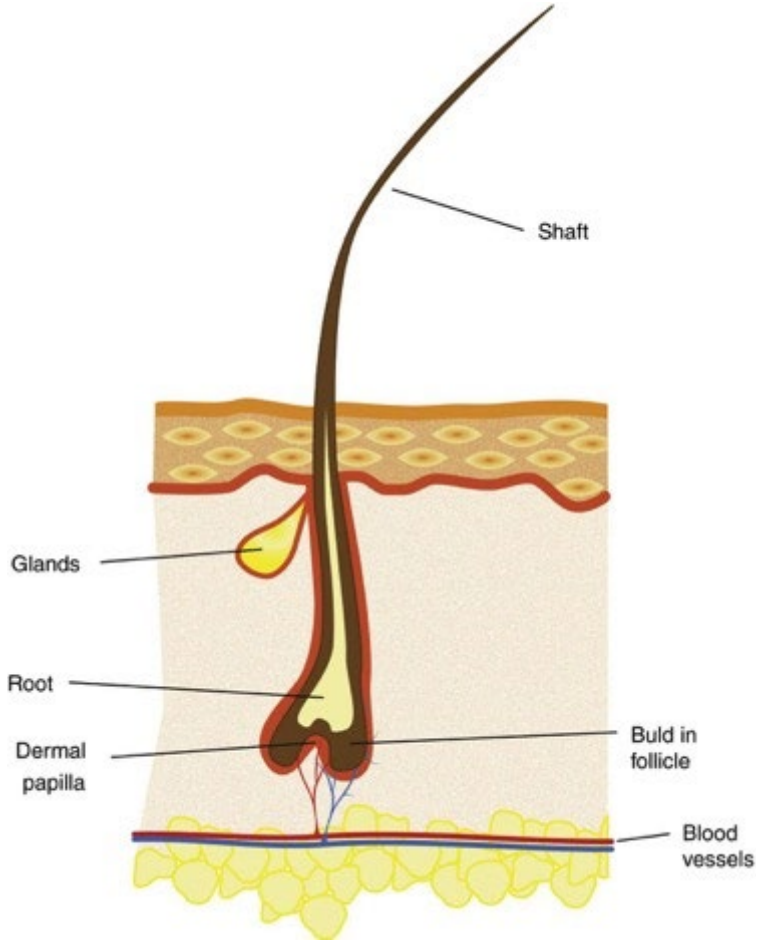


Photo Credit: Dr. H. Hermans Utrecht University

What is Distichiasis?



https://www.oculistprime.com/anatomy/c/0/i/36574690/eyelashes



Aumond, S. & Bitton E. 2018
https://doi.org/10.1016/j.optom.2018.05.003

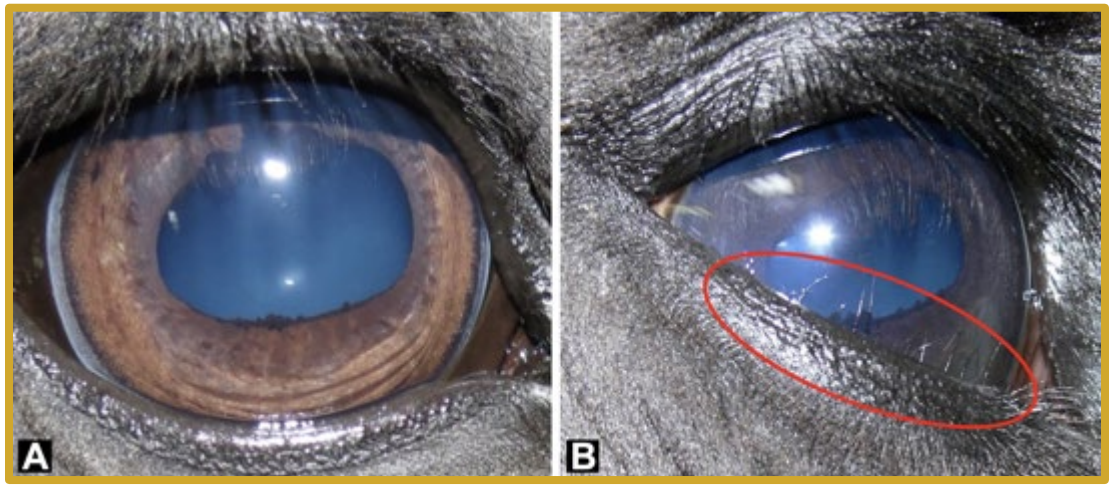
Prevalence

- Inherited distichiasis
  - Humans: dominant -*FOXC2*
    - (Zhang, L. *et al. J AAPOS.* 2016)
  - Dogs: dominant with incomplete penetrance no known cause
    - (Stiles, J. and Townsend, W. M. 2007)
  - Horses: only two reports
    - 2 cases in Friesians
      - (Utter, M. E. and Wotman K. L. *Equine vet. Educ* 2012)
    - Retrospective study: 17 of 18 cases were Friesians
      - (Hermans, H. and Ensink, J.M. *Equine Vet J* 2014.)

Photo credit: Dr. K. Knickelbein

Signs/Symptoms/Secondary problems

- Aberrant cilia (sometimes unnoticed until chronic ocular irritation)
- Blepharospasm (squinting)
- Epiphora (excessive tearing)
- Corneal scarring (opacification from the aberrant cilia)
- Corneal ulceration (wound on the clear outer window of eye) -> infection/impaired vision



Lassaline-Utter et al., 2014

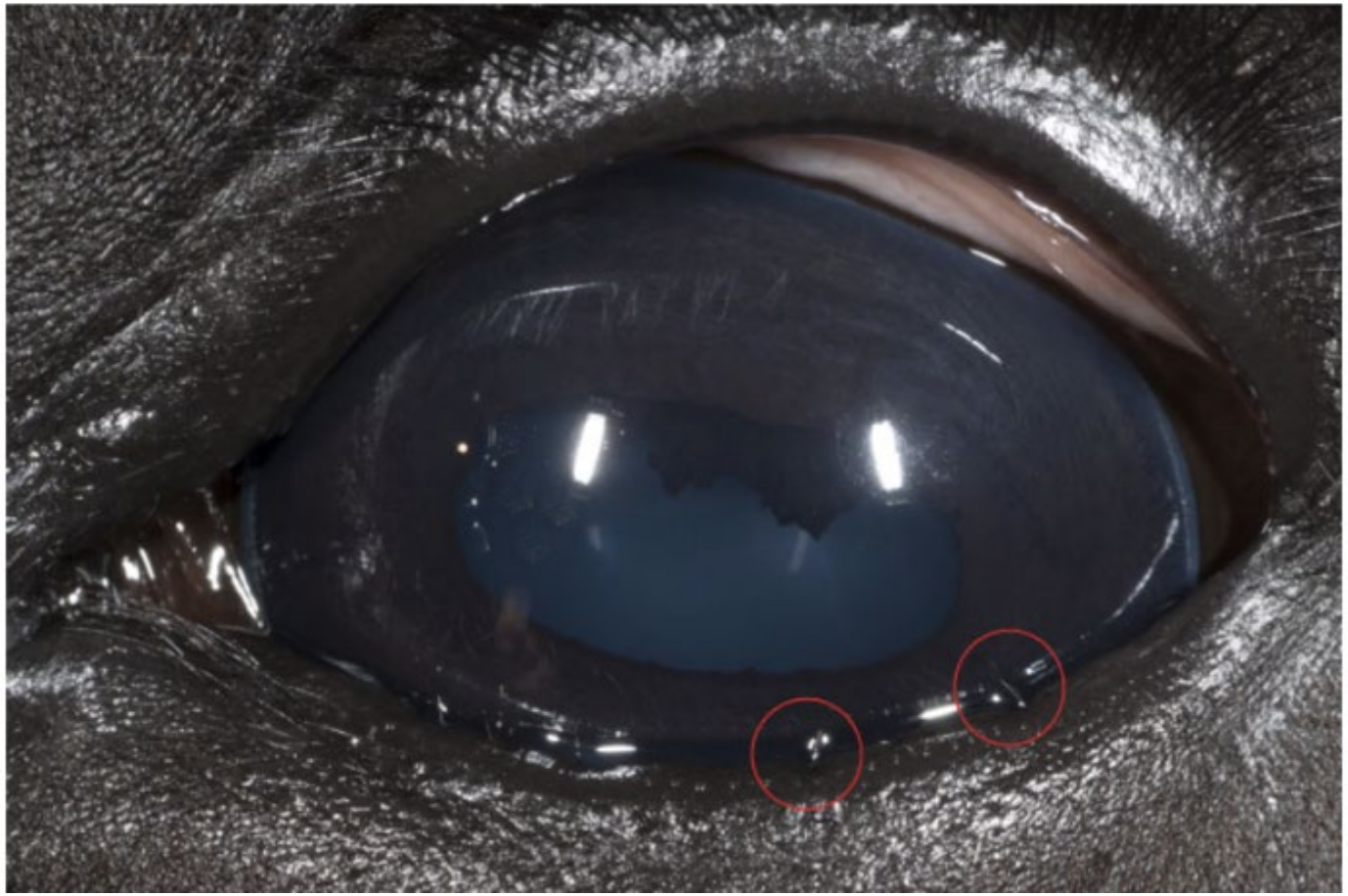
Treatment options

- Removal of cilia/destroy hair follicle
  - Epilation (pluck)
  - Cryoepilation (freeze)
  - Electroepilation (electrical current)
  - Surgical excision of follicles

HERMANS, H. and ENSINK, J.M. 2014
DOI:10.1111/evj.12157458

Complications

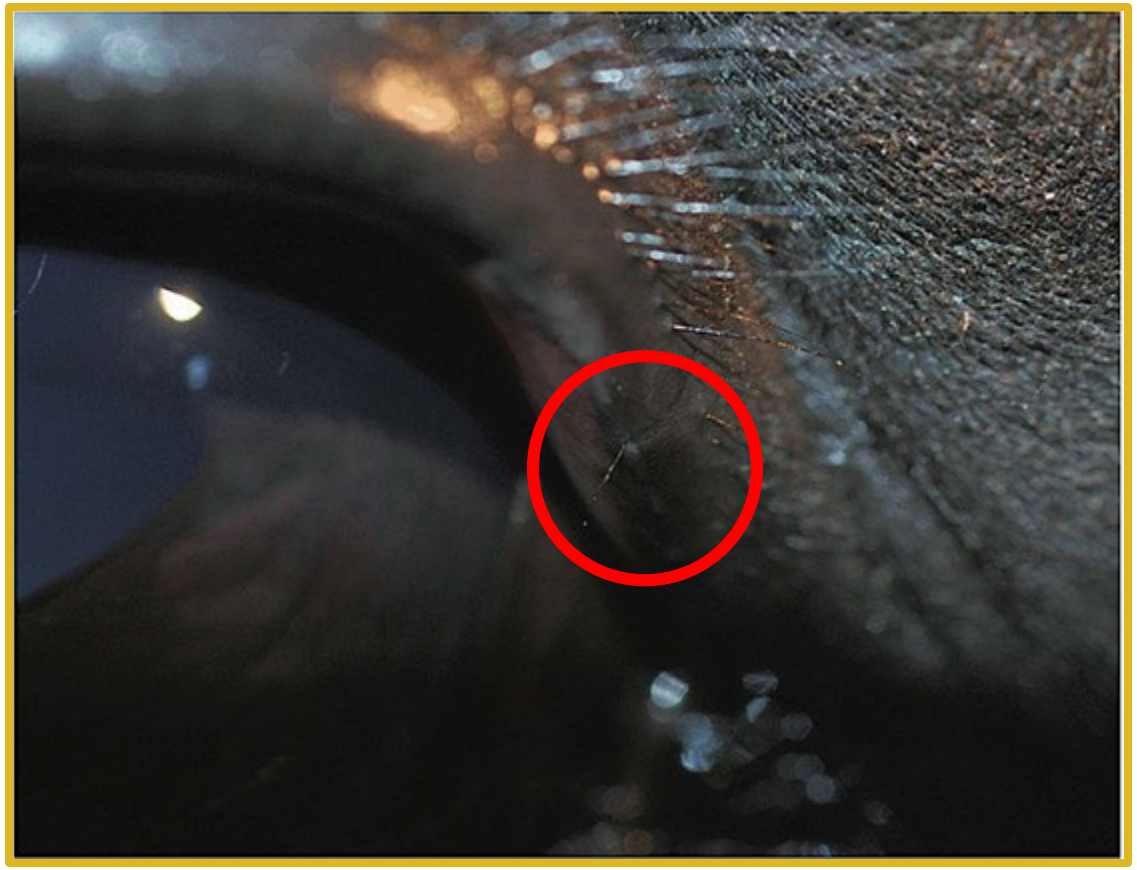
- Eyelid scarring
- Depigmentation of eyelid margin
- Meibomian gland dysfunction leading to corneal disease
- Regrowth of distichia
- Secondary problems



HERMANS, H. and ENSINK, JM 2014 DOI:10.1111/evj.12157458

Genetic Investigation

- Uncommon in horses but reported in a single breed (Friesian)
- Bilateral
- Hypothesis: Distichiasis in Friesian horses is a simple recessive inherited disorder.
- Aim: Perform a genome-wide association study (GWAS) followed by whole genome sequencing to identify a causal variant.

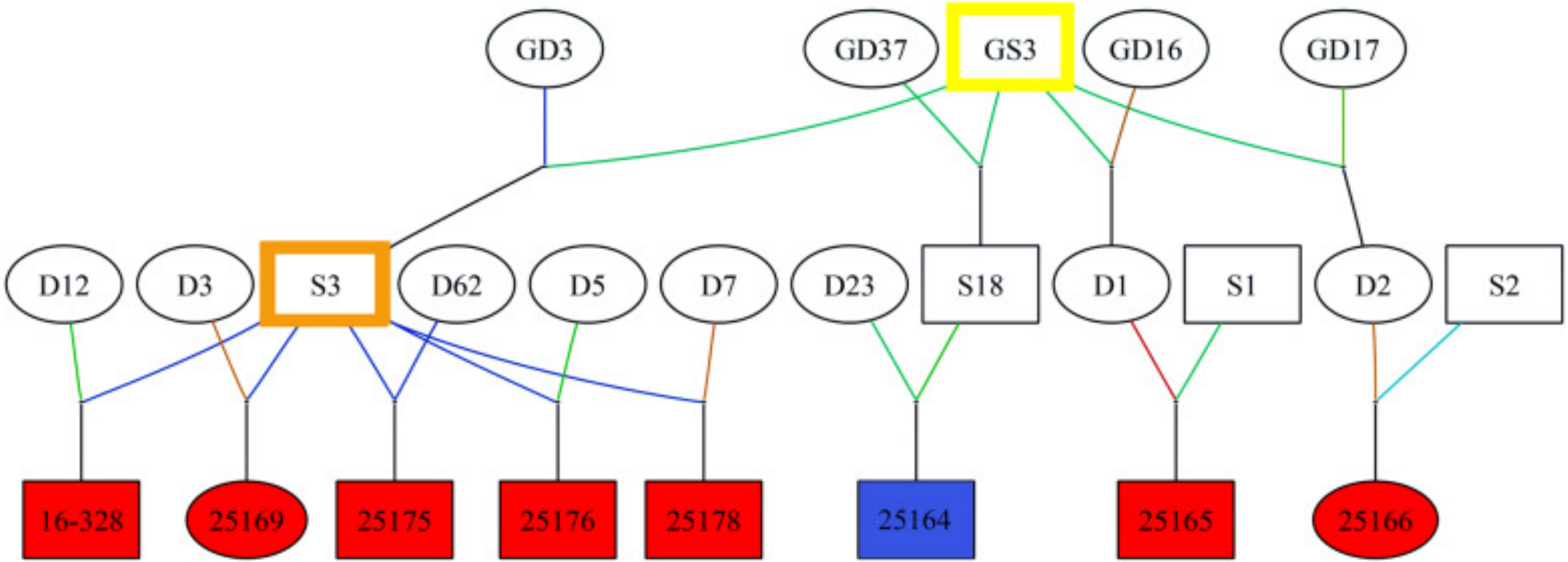


Methods

- Phenotyping:
  - Complete ocular exam by diplomate of ECVO or ACVO
- Samples:
  - 19/24 affected Friesians
  - 75 unaffected Friesians
  - DNA isolated from blood/hair

Photo Credit: UC Davis School of Veterinary Medicine

Pedigree Analysis



Methods: GWAS

- Genome Wide Association Study (GWAS) using Axiom MNEc670K and 52 Horses (14 cases and 38 controls)

	G	T
CASES		25
CONTRO	25	25

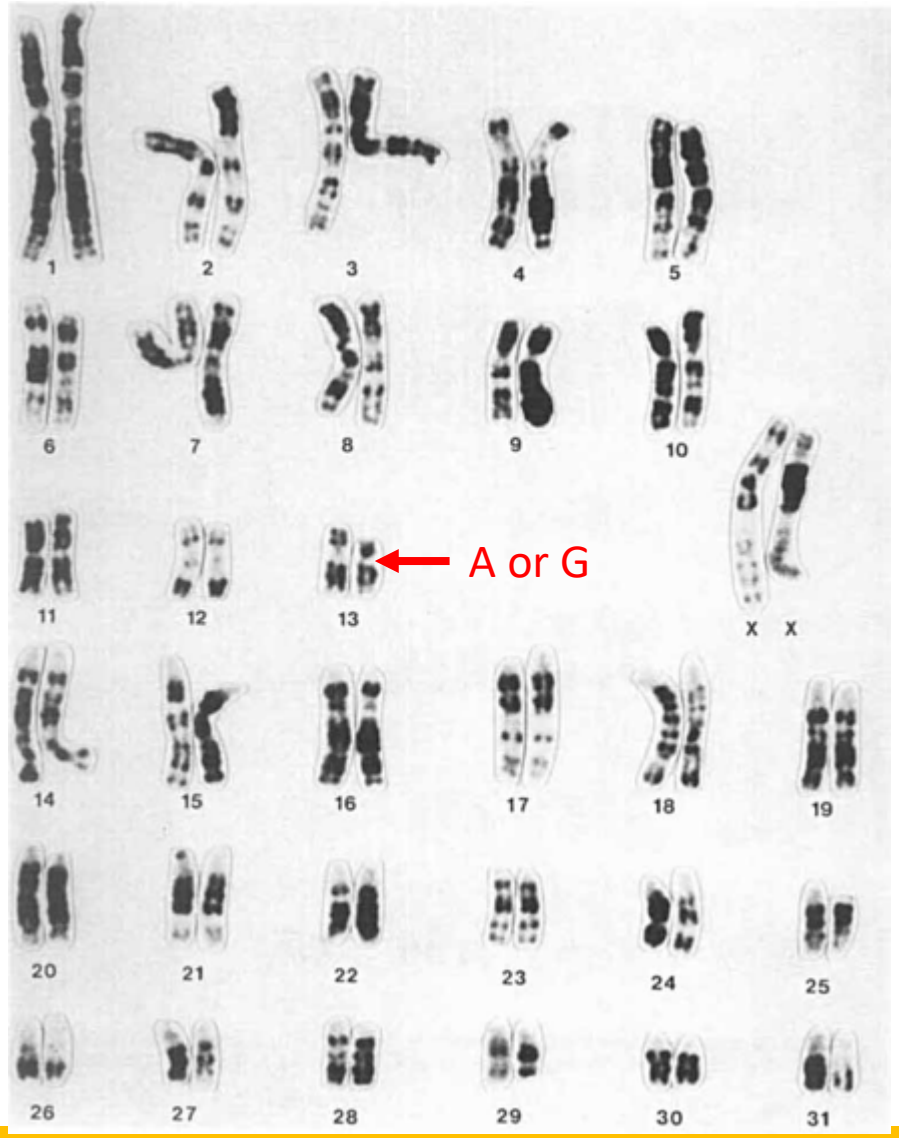
Not Associated

Methods: GWAS

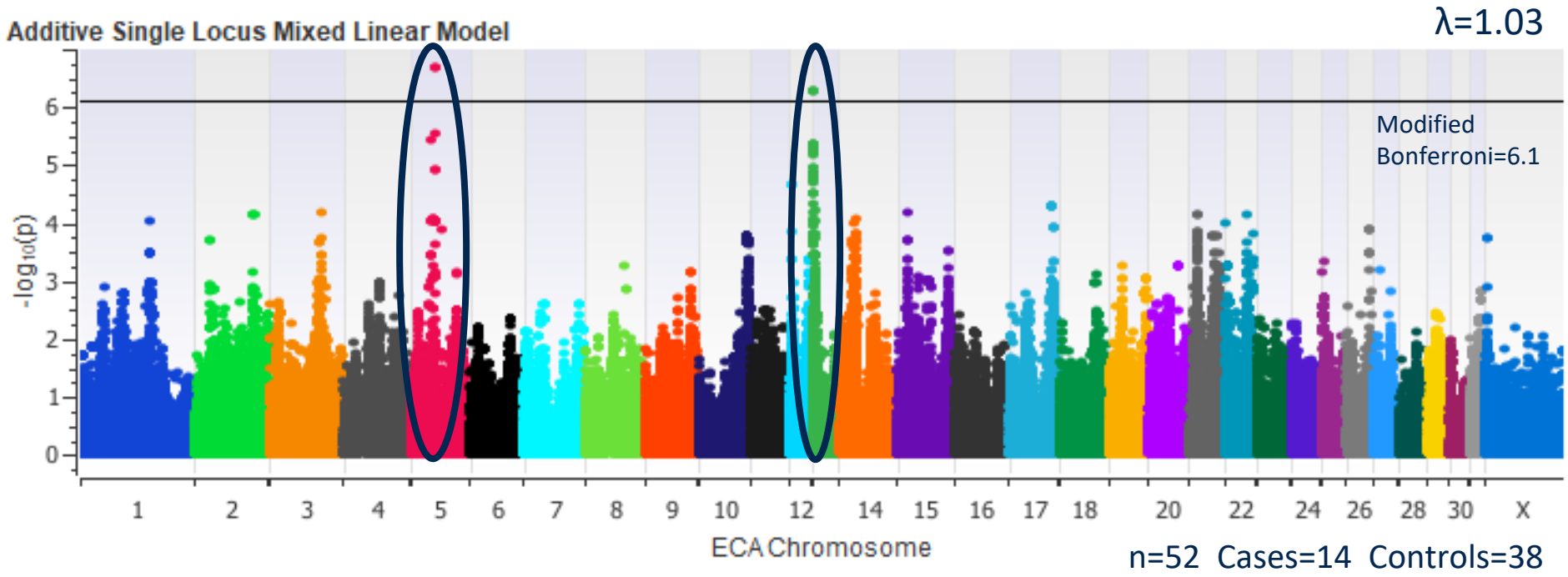
- Genome Wide Association Study (GWAS) using Axiom MNEc670K and 52 Horses (14 cases and 38 controls)

	A	G
CASES	50	0
CONTROL	0	50

Associated -Perfectly concordant



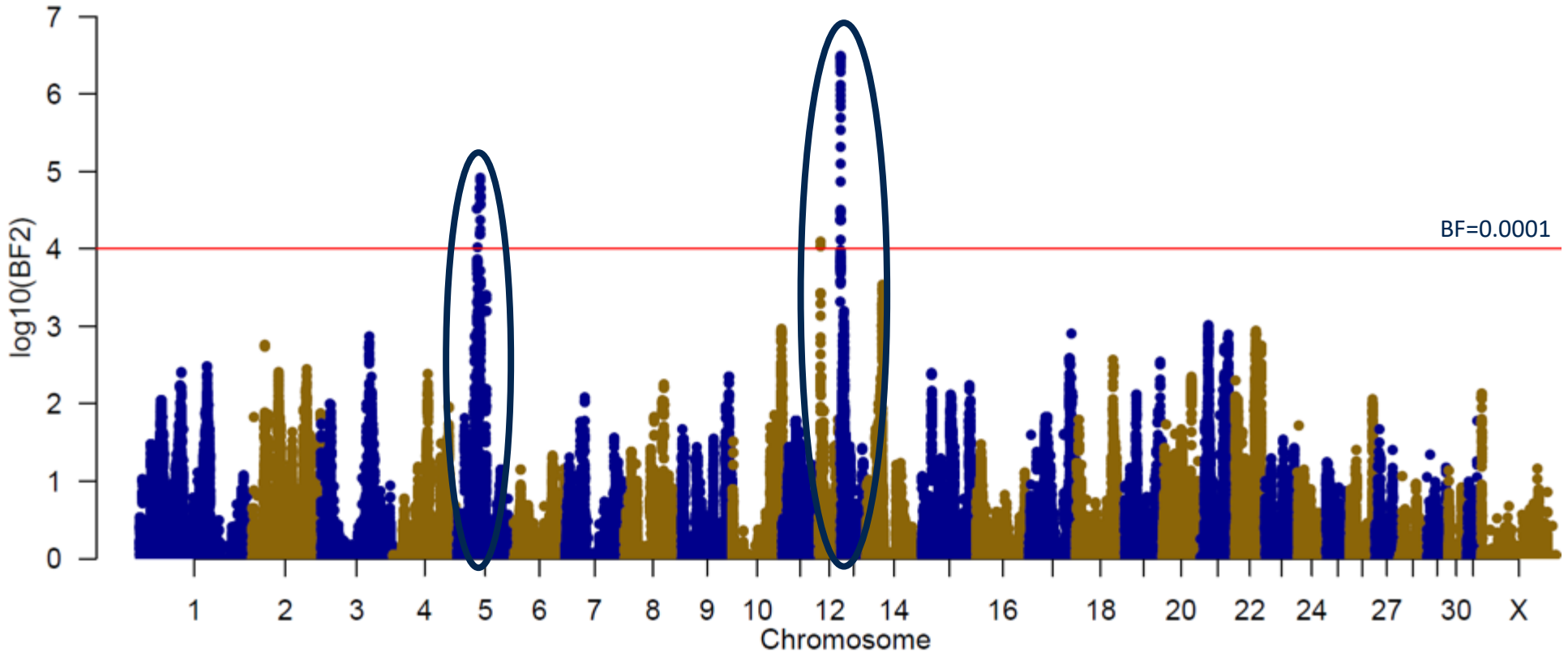
GWAS Results



ECA5 and 13 :

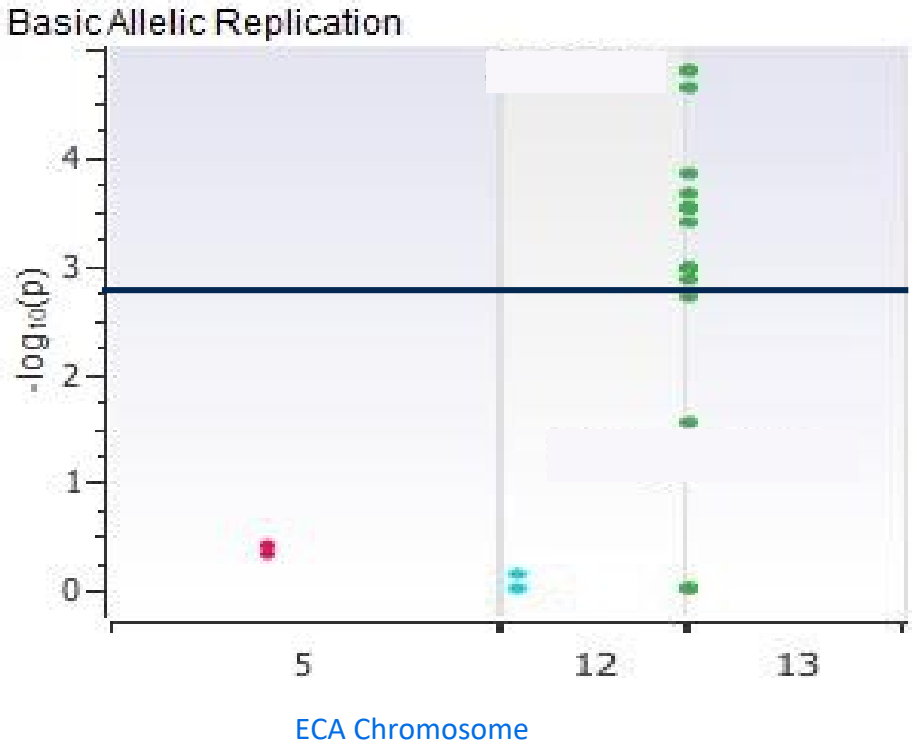
- Reach significance ($p_{corrected}$= 0.016 and 0.032, respectively)

HapQTL Analysis



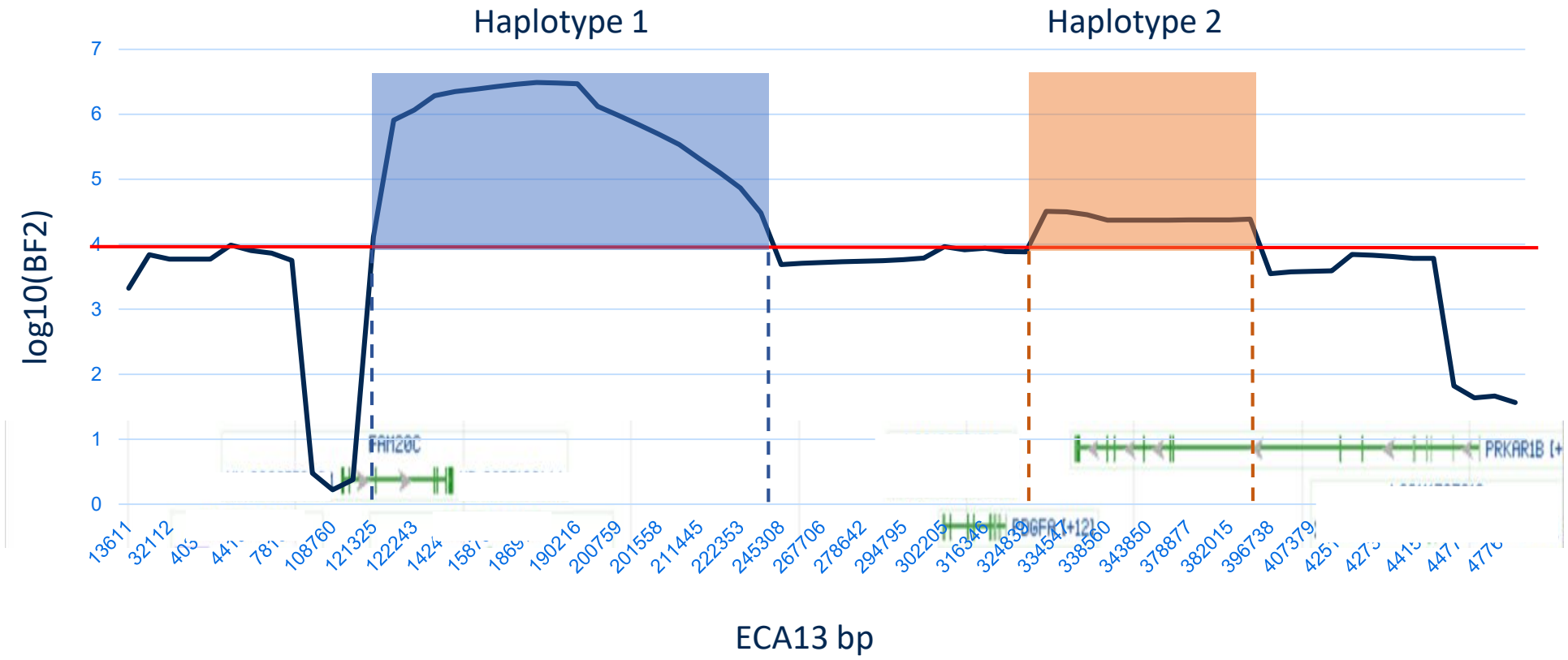
Five haplotypes identified 2 on ECA 5, 1 one ECA12, and 2 on ECA13

GWAS Replication Testing



- 32 SNPs N=42 5 cases 37 controls
- ECA5 locus no longer significant (p=0.40).
- ECA13 locus significant ($p=1.6x10^{-5}$)

HapQTL Analysis: ECA13

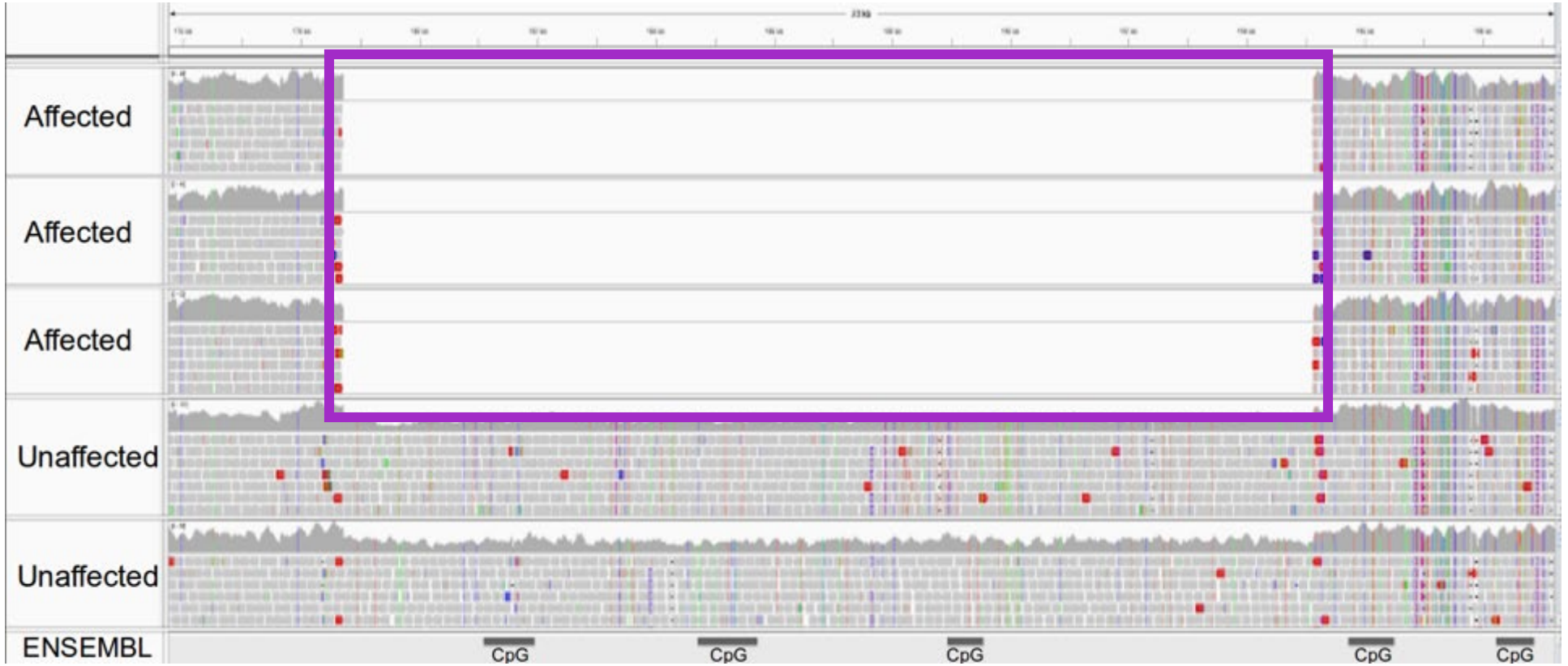


- Haplotype 1: contains *FAM20C* Haplotype 2: contains *PRKAR1B and PDGRA are located between the two*

Whole Genome Sequencing

- Whole genome sequencing to investigate associated region on ECA13 from GWAS for novel variant (30X coverage) ~2.7 billion bases make up the 32 chromosomes of the horse.
  - 3 cases and 2 controls

ECA13 WGS Coverage



16 kb deletion identified on ECA13

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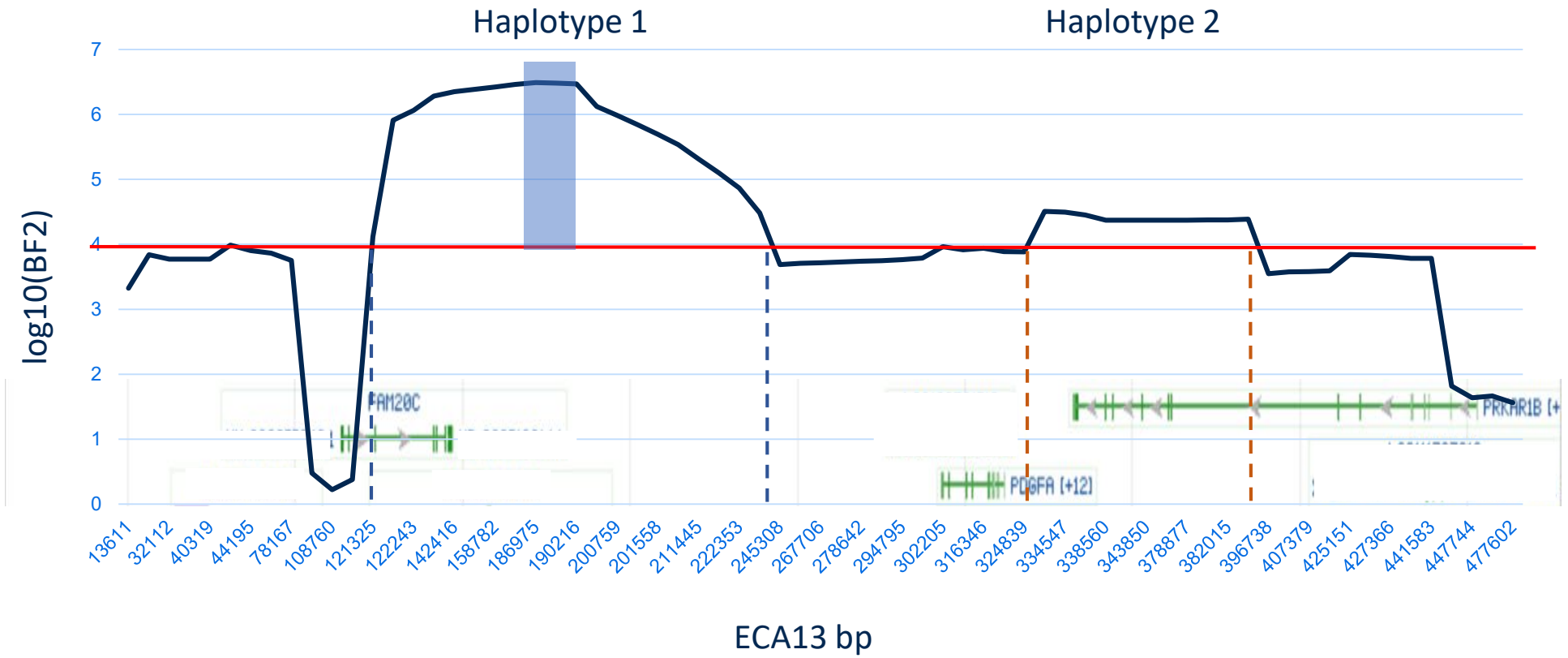
HapQTL Analysis: ECA13



- Haplotype 1: contains *FAM20C* Haplotype 2: contains *PRKAR1B and PDGRA are located between the two*

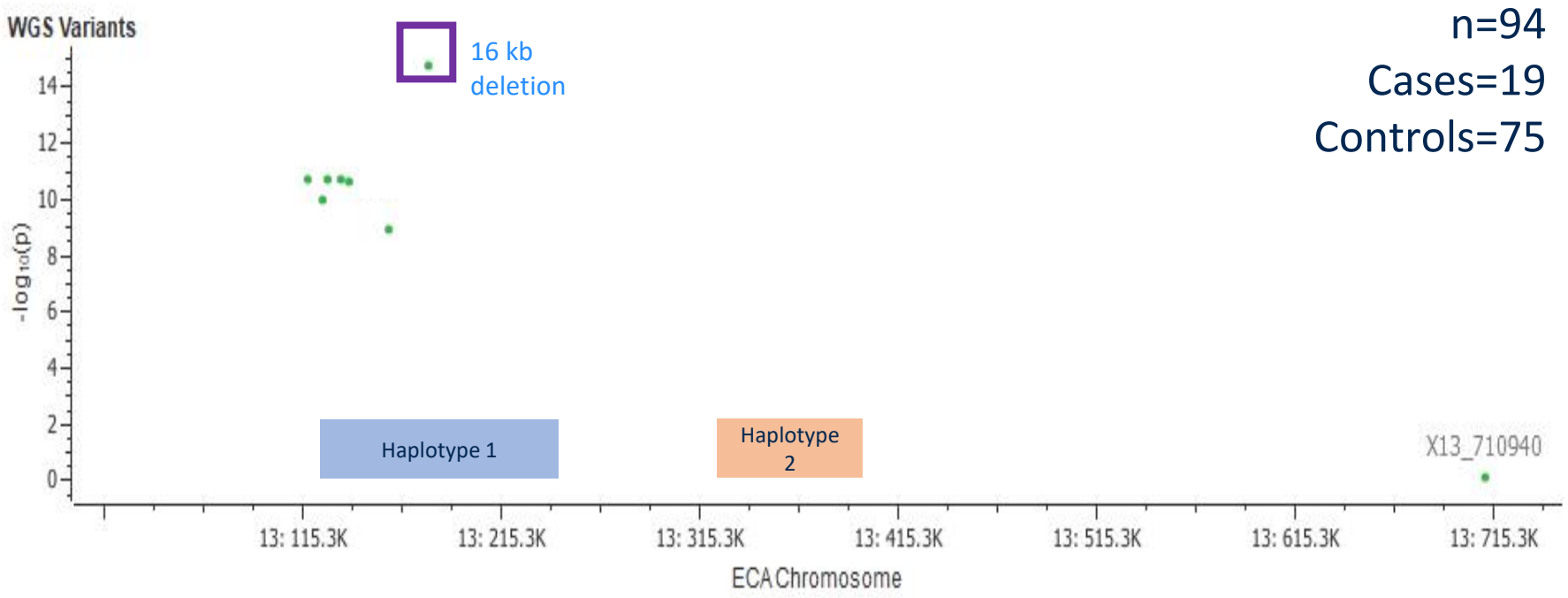
Deletion Genotyping: Friesians

Chi-squared $p=4.8 \times 10^{-13}$

	Del/Del	Ref/Del	Ref/Ref	Total
Cases	18	1	0	19
Controls	7	30	38	75
Total	25	31	38	94

Variants from WGS

- 7 SNPs from this region identified from WGS for further investigated
- None as concordant with case status as the deletion.



n=94
Cases=19
Controls=75

Deletion Genotyping: Other Breeds

- Out of 955 individuals from other breeds (closely and distantly related) identified in only 11 (1.15%) as heterozygous.
- Providing support that this is likely causal

Table 5 Number of Horses from Additional Breeds Identified with the 16 kb Deletion on ECA13 Based on Evaluation of 955 Samples

Breed	Horses
Native Mongolian Chakouyi Horse	1
Mangalarga Marchador Horse	1
Sorraia	1
Lipizzaner	4
Unknown Breed	4
Total	11

Hisey et al., 2020 BMC Genomics

Deletion Genotyping: Friesians

	Del/Del	Ref/Del	Ref/Ref	Total
Random Friesian population	21	88	92	201

Population allele Frequency= **high 32.34%**

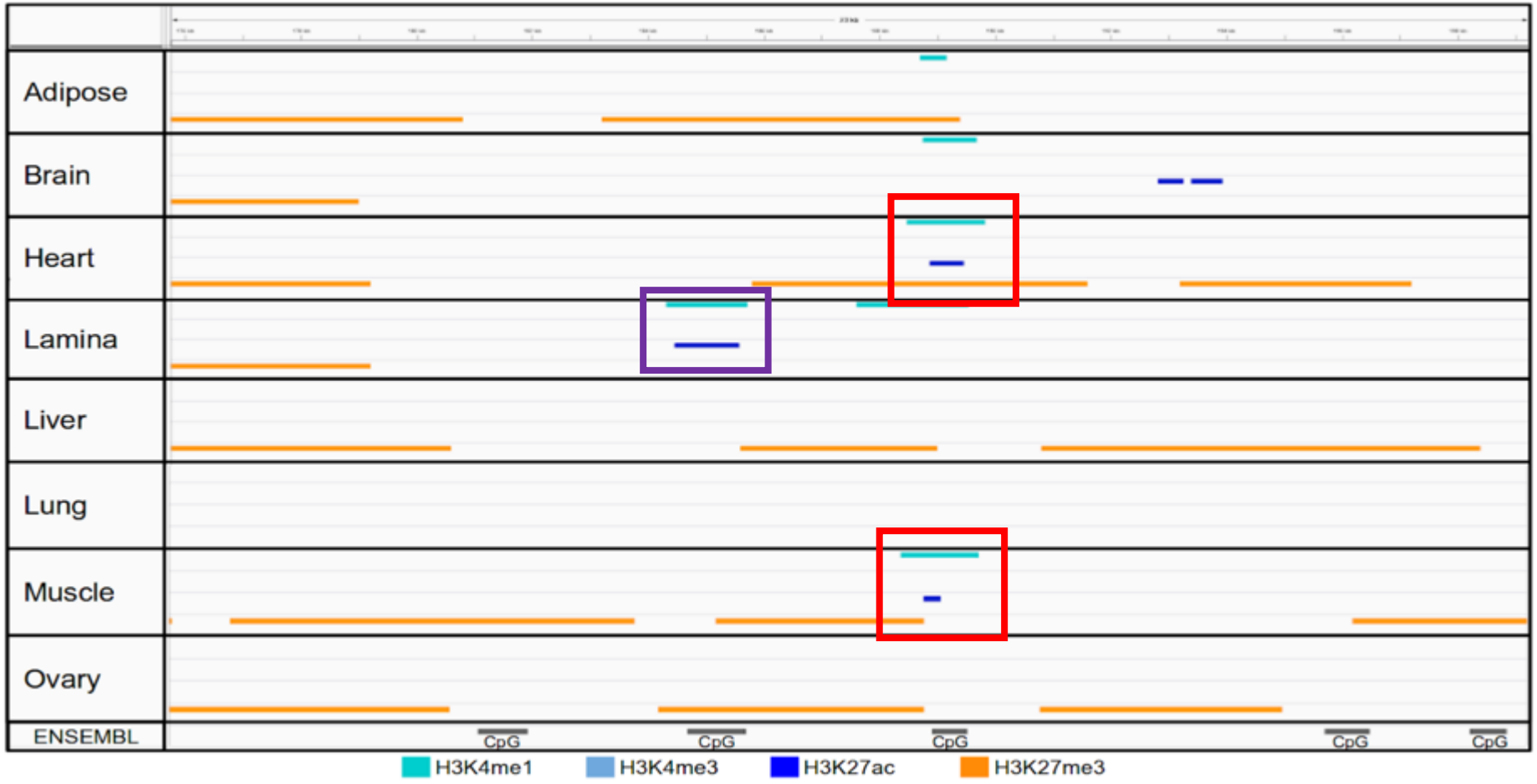
Hydrocephalus =7.5%

Dwarfism=6.2%

Region under positive selection?

Or other variant under positive selection and deletion is hitch-hiking?

Functional evidence



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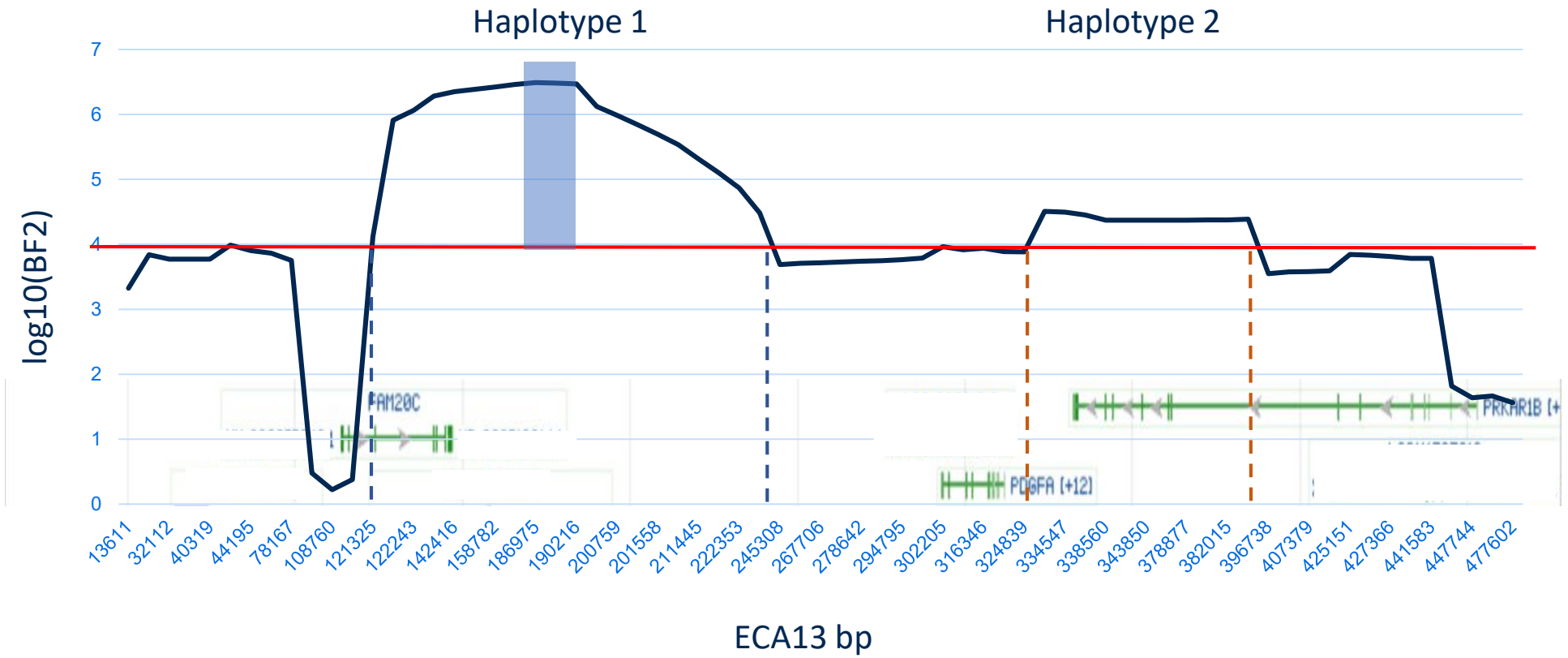
16 kb deletion on ECA13

Functional Hypothesis



Incomplete Penetrance?

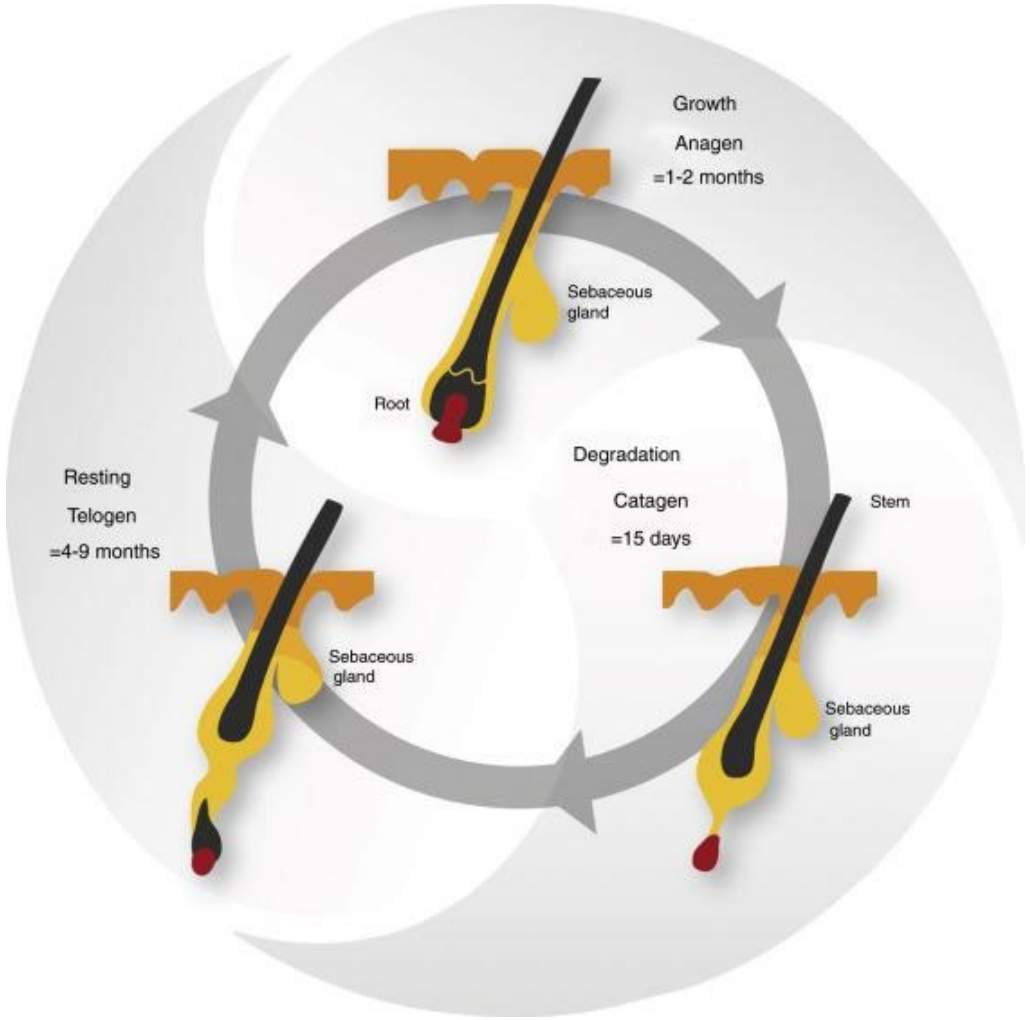
1 Ref/del case atypical presentation 1 single unilateral aberrant lash

	Del/Del	Ref/Del	Ref/Ref	Total
Cases	18	1	0	19
Controls	7	30	38	75
Total	25*	31	38	94

Penetrance?

- Little is known about eyelash hair cycle in horses
- Possible 7 horses homozygous for deletion with no evidence of distichiasis were
  - Not in anagen so aberrant lash not detected on single exam

  Or
  - Incomplete penetrance (similar to dogs)



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*In this study 72% of De//Del had evidence of disease. A prospective randomized study should be conducted to determine penetrance.

Genetic Testing

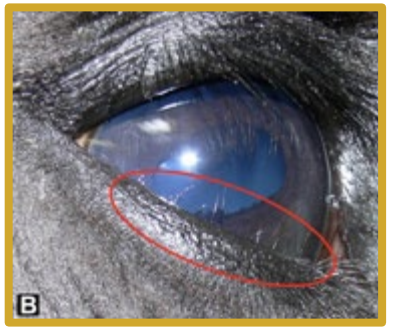
- Evaluating for absence/presence and number of copies of deletion
- **Deletion** is coded by UC Davis VGL as **Dis**
- **No deletion =N**

Genetic Testing Recommendations

- As a tool for breeding (for example avoid breeding dis/dis to dis/dis).
- But given the high frequency and in absence of other genetic diversity data I would not advise to eliminate from population.
- As a tool to identify horses that should be examined by veterinary ophthalmologist

Breeding Decisions

Dis/Dis



Dis/Dis

100% of offspring Dis/Dis *

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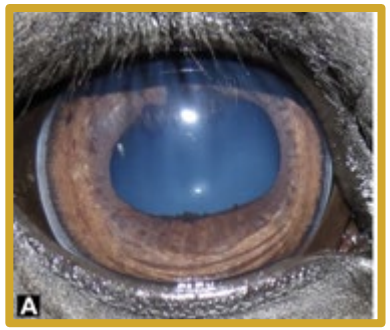
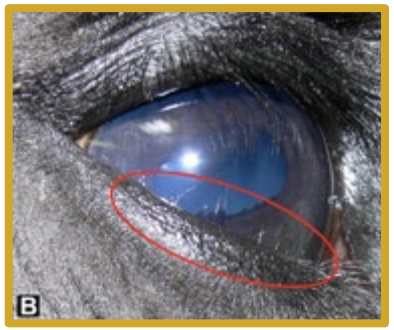
*** Penetrance rate of Dis/Dis currently unknown**

Breeding Decisions

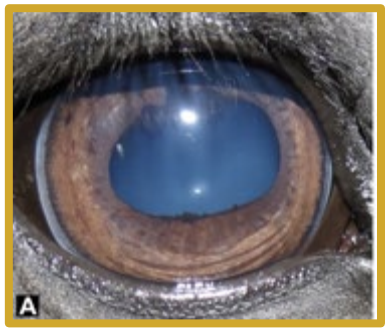
Dis/Dis



N/Dis



50% chance of Dis/Dis*



50% chance of N/Dis

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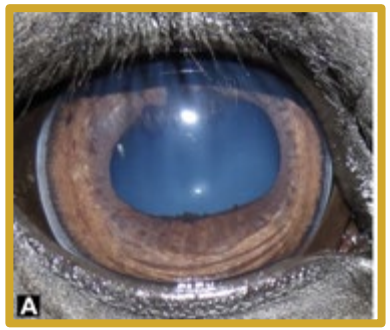
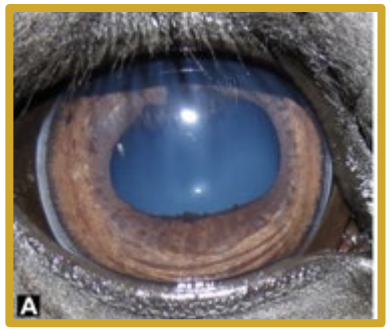
Breeding Decisions



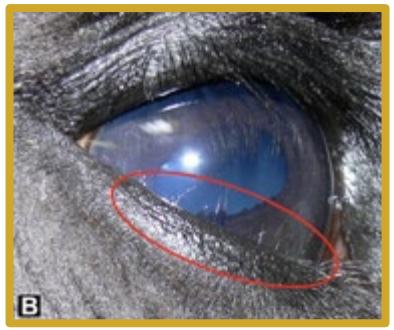
N/Dis



N/Dis

25% chance of N/N

50% chance of N/Dis



25% chance of Dis/Dis*

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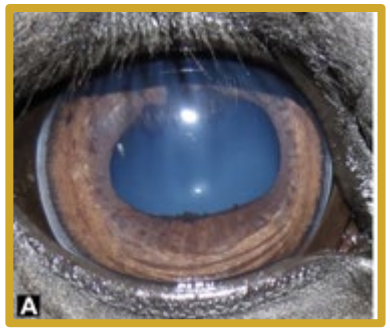
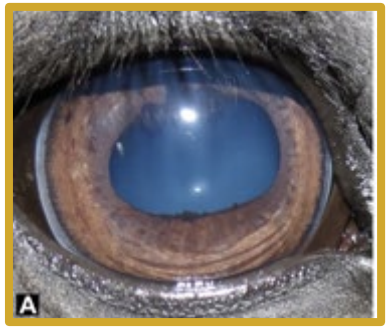
*** Penetrance rate of Dis/Dis currently unknown**

Breeding Decisions

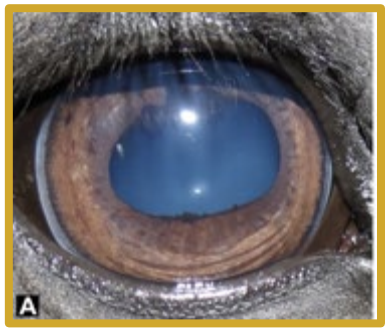
N/Dis



N/N

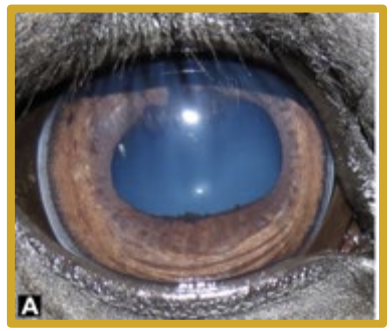


50% chance of N/N

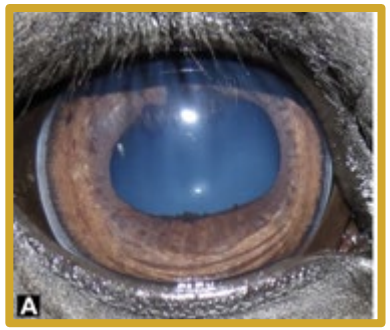


50% chance of N/Dis

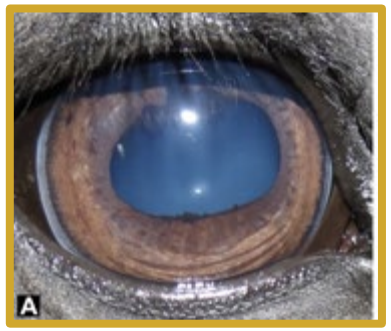
Breeding Decisions



N/N



N/N



100% of offspring N/N

Genetic Testing Results VGL

Test Result	Distichiasis Interpretation
N/N	**Normal.** Horse does not have the distichiasis associated variant.
N/Dis	**Carrier**. Horse has one copy of the distichiasis associated variant.
Dis/Dis	**Affected.** Horse has two copies of the distichiasis associated variant and should be clinically evaluated **by a veterinary ophthalmologist** for signs of disease.

Conclusions

What we know and what we want to study.

- **16 kb deletion identified on ECA13 that is strongly associated with distichiasis**. We don't yet understand the functional mechanism
- **Deletion is at a high frequency in the population**: We don't know why?
- **Data supports incomplete penetrance:** We don't know why or what other genetic variants (if any) allow for this?
- **Genetic testing is available and can be used to guide breeding and clinical evaluation decisions.**

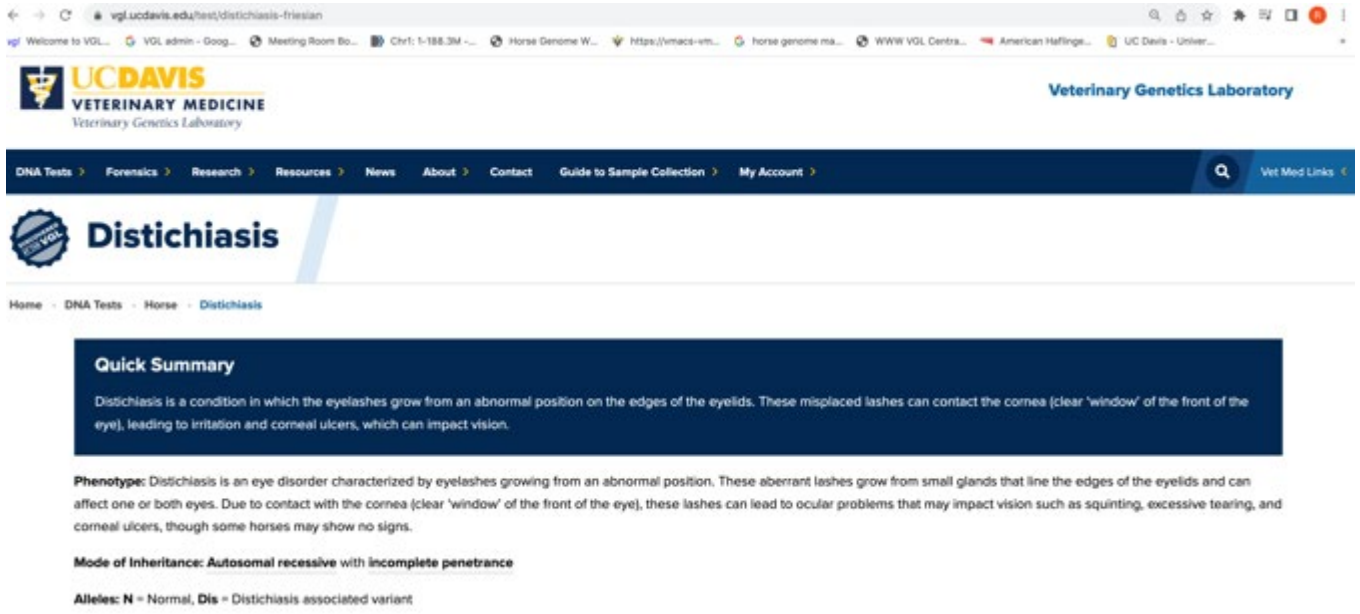
Ordering the Test at the VGL

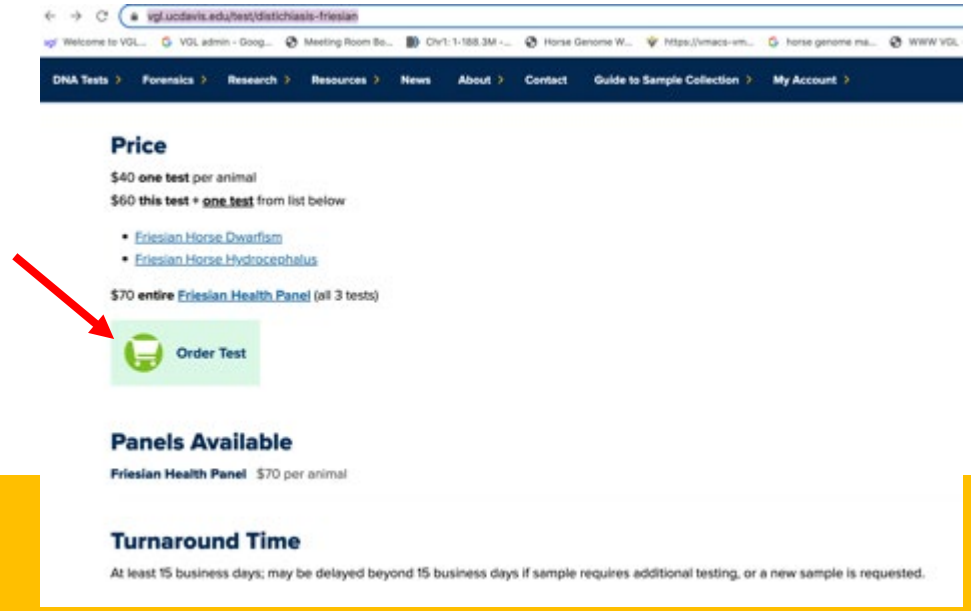
Information on test:

- https://vgl.ucdavis.edu/test/distichiasis-friesian

Create account if you don't have one or log in

- https://my.vgl.ucdavis.edu/myvgl/login.htm





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    - School of Veterinary Medicine

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Questions?

Hisey, E.A., Hermans, H., Lounsberry, Z.T. *et al.* Whole genome sequencing identified a 16 kilobase deletion on ECA13 associated with distichiasis in Friesian horses. *BMC Genomics* 21**,** 848 (2020). **https://doi.org/10.1186/s12864-020-07265-8**