

# A Mutation in a Novel Transcription Factor Affects the Pattern of Locomotion in Horses



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## Gaits in Horses

Walk



4-beat

Trot



2-beat, diagonal

Canter



3-beat



Tölt  
4-beat



Pace  
2-beat,  
lateral

## Gaited Horse Breeds

- \* Gaited horses have more than the three basic gaits
- \* They are more common in South and North America than in Europe

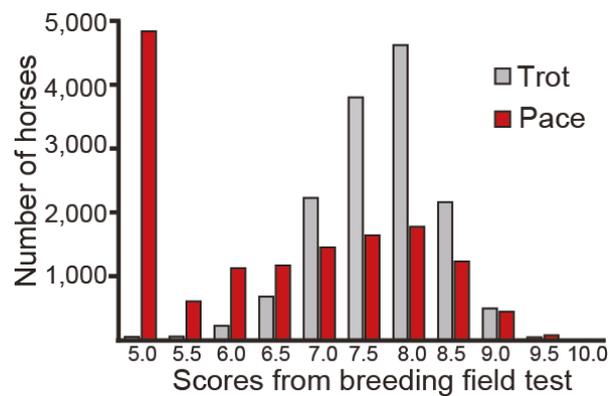


Example of some gaited breeds:

|                      |                      |                           |
|----------------------|----------------------|---------------------------|
| Icelandic Horse      | Peruvian Paso        | Pacing Standardbred       |
| American Saddlebred  | Tennessee Walker     | Kentucky Mountain Saddle  |
| Campolina            | Racking Horse        | Paso Fino                 |
| Mangalarga Marchador | Rocky Mountain Horse | Virginia Highlander Horse |
| Missouri Foxtrotter  | Marwari Horse        | Albanian Horse            |

## The Icelandic Horse

- \* An Icelandic horse can be four- or five-gaited (with or without pace)
- \* The heritability of pace in Icelandic horses is estimated at 0.60

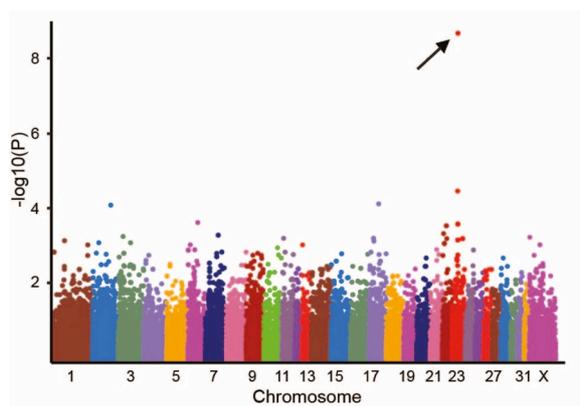


## Genome Wide Association Study

- \* Illumina EquineSNP50 Genotyping BeadChip
- \* Genotypic data from 209 Icelandic horses
- \* Questionnaire - Is your horse capable of pace?
- \* 30 without pace (four-gaited) and 40 with pace (five-gaited)



## Genome Wide Association Study



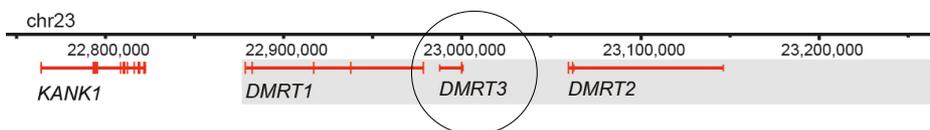
|          | CC/CT | TT |
|----------|-------|----|
| pace     | 1     | 39 |
| not pace | 21    | 9  |

Pace has a recessive mode of inheritance

$$p = 1.74 \times 10^{-9}$$

$$EMP2 = 2.00 \times 10^{-4}$$

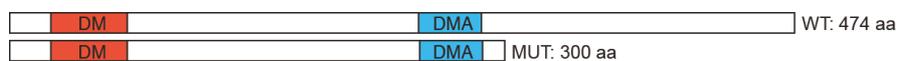
## Mutation Identification



- \* Established a 438 kb IBD region
- \* Whole genome Illumina sequencing on one four-gaited and one five-gaited horse
- \* Identified one nonsense mutation (C/A) within the critical interval
- \* Transcription factor (*DMRT3*) with unknown function

## The *DMRT3* Nonsense Mutation

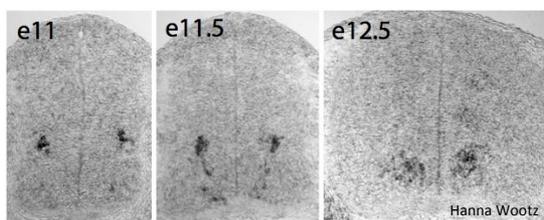
- \* The truncated protein is predicted to lack 174 amino acid residues of the full length protein
- \* 92.5 % of these are identical between human and horse *DMRT3*



|            | 280         | 300                   | 320  |
|------------|-------------|-----------------------|--|
| Horse WT   | LELILKGC    | DLVSAVEVLLSSRSSASAA   | •DRTSA•EPESLVLPSNGHIFEHTL                        |
| Horse MUT  | -----       | -----                 | •*-----  |
| Cattle     | -----       | -----S-----           | -----G-----L-----                                |
| Human      | -----       | -----VTG-----         | -----E-----A-----                                |
| Chimpanzee | -----Q----- | -----G-----C-VTG----- | -----E-SC-----L-PR-----                          |
| Dog        | -----       | -----G-----A-----     | -----SE-A-----A-----P-----L-----G-----           |
| Mouse      | -----       | -----                 | -----AGT•E-----AE•••-----S-----                  |
| Rat        | -----       | -----                 | -----AG•E-----AE•••-----S-----                   |
| Chicken    | -----       | -----                 | -----G-----VAGG•E-----E•SDG-----                 |
| Zebrafish  | -----       | -----                 | -----G-I-I-----TMKPE•KIL-E•SSDA-----L-----P----- |

### Expression of *DMRT3* in Mouse

- \* The gene is expressed in a subset of inhibitory neurons in the spinal cord
- \* These neurons cross the dorsal midline of the spinal cord and connect the right and left hand sides of the horse
- \* Connect to motor neurons



### Distribution of the Mutation in Icelandic Horses

| Phenotype   | CC | CA  | AA  | Total |
|-------------|----|-----|-----|-------|
| Five-gaited | 0  | 1   | 65  | 66    |
| Four-gaited | 2  | 83  | 39  | 124   |
| Total       | 3  | 105 | 149 | 257   |

$p = 2.4 \times 10^{-14}$

- \* One probable misclassification
- \* 31% of four-gaited horses should be able to pace according to the genetic model (modifier loci or training)
- \* Low number of homozygous wild type - traditions in breeding

## Examination of Icelandic Horse Pedigrees

Data collected from the data base WorldFengur is concordant with a recessive mode of inheritance

| Mating              | Number of offspring | Expected non-pace (%) | Observed non-pace (%) |
|---------------------|---------------------|-----------------------|-----------------------|
| non-pace x non-pace | 336                 | 75                    | 75                    |
| non-pace x pace     | 1098                | 50                    | 46                    |
| pace x pace         | 948                 | 0                     | 9                     |

## Distribution of the Mutation Across Breeds

| Breed                          | CC | CA  | AA  |
|--------------------------------|----|-----|-----|
| <u>Gaited</u>                  |    |     |     |
| Icelandic Horse                | 3  | 105 | 149 |
| Rocky Mountain Horse           | 0  | 0   | 17  |
| Kentucky Mountain Saddle Horse | 0  | 2   | 20  |
| Missouri Fox Trotters          | 0  | 0   | 40  |
| Peruvian Paso                  | 0  | 0   | 19  |
| Paso Fino                      | 0  | 0   | 45  |
| Standardbred Pacers            | 0  | 0   | 37  |
| Tennessee Walkers              | 0  | 1   | 32  |
| <u>Non-Gaited</u>              |    |     |     |
| Gotland Pony                   | 28 | 0   | 0   |
| Swedish Ardenne                | 22 | 0   | 0   |
| Swedish Warmblood              | 35 | 0   | 0   |
| Arabian                        | 29 | 0   | 0   |
| Thoroughbred                   | 35 | 0   | 0   |
| Shetland Pony                  | 20 | 0   | 0   |
| North Swedish Draft            | 31 | 0   | 0   |

## High Frequency of the Mutation in "Trotters"

| Breed             | CC | CA | AA  | Total |
|-------------------|----|----|-----|-------|
| Standardbred (SB) | 3  | 31 | 304 | 338   |



The high frequency of the A-allele in the Standardbred strongly indicates that this gene variant is favorable for performance.

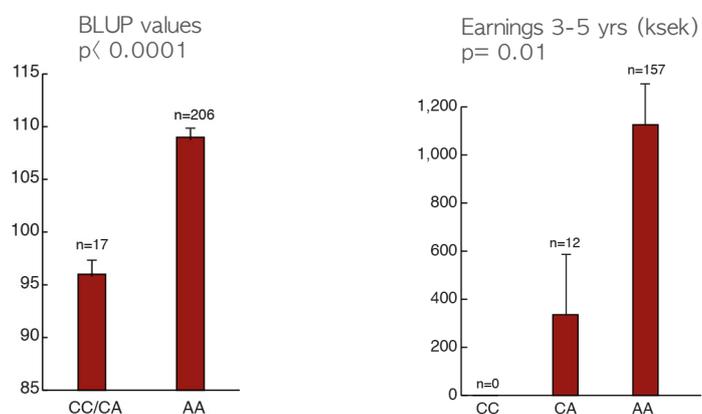
## The Mutation Influence Trotting Ability

- \* Blind study in a stable with 61 Standardbred horses
- \* Two horses were reported to have difficulties in sustaining trot
- \* These two horses were heterozygous (CA)
- \* The remaining 59 horses were homozygous for the mutation (AA)



The mutation enables the horse to trot at fast speed without proceeding into gallop. This has been crucial for the development of the trotting harness racing sport.

## The Mutation Affects Performance



| Breed        | Performance                     | Freq (CA) |
|--------------|---------------------------------|-----------|
| Standardbred | 50 best competition horses      | 0.00      |
|              | 50 horses that have not started | 0.08      |

P<sub>one sided</sub> = 0.06

## The Mutation Affects Performance

- \* Famous Standardbred stallion was CA
- \* Tested 92 of his offspring

|                                    | CA     | AA      | p     |
|------------------------------------|--------|---------|-------|
| Average earnings (life):           | 85,652 | 195,363 | 0.062 |
| Average earnings (3-5 yrs):        | 20,174 | 78,618  | 0.002 |
| # entered races (3-5 yrs)          | 9      | 17      | 0.004 |
| Offspring entering races (3-5 yrs) | 49%    | 80%     | 0.001 |

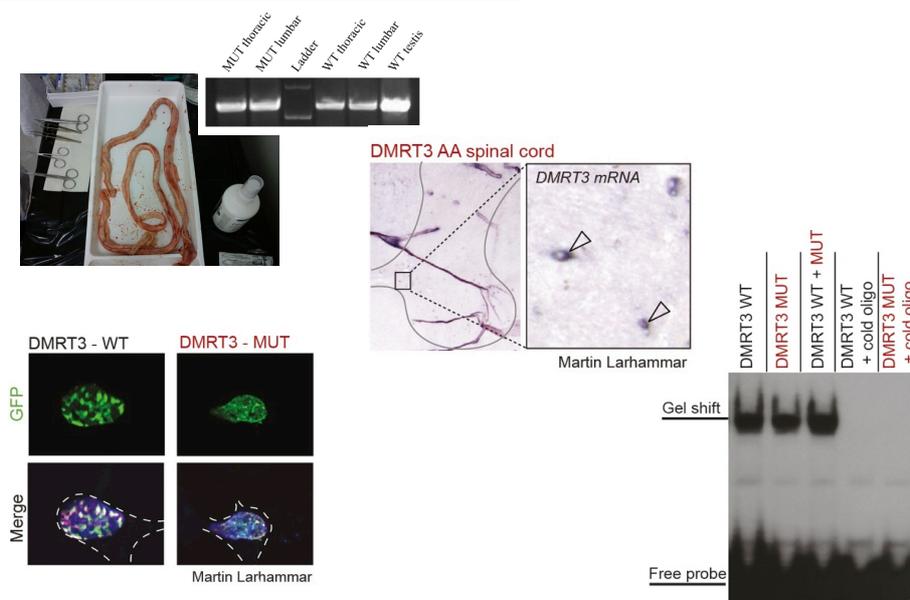
## Introgressed into the North Swedish Trotter

| Breed                   | CC | CA  | AA  |
|-------------------------|----|-----|-----|
| Standardbred (trotting) | 3  | 31  | 304 |
| North Swedish Trotter   | 74 | 168 | 48  |
| North Swedish Draft     | 31 | 0   | 0   |



*DMRT3* genotype correlates significantly with trotting technique

## Functional Characterizations



## Selection against A in some breeds

- \* High frequency in gaited breeds and trotters but almost completely absent in most other breeds
- \* Seem to be disadvantageous for horses used for show jumping, dressage, heavy drafting and gallop racing
- \* Icelandic four-gaited horses inferior scores for trot and canter
- \* The single base change has had a major impact on the diversification of the domestic horse

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## Summary

This study reveals a previously unknown molecule required for setting up the control centre coordinating limb movements in vertebrates.

The nonsense mutation in *DMRT3*:

- Causes the recessive trait pace in Icelandic horses
- Is permissive for a variety of alternate gaits across gaited breeds
- Has a favorable effect on high speed trot in horses used for harness racing by delaying the transition to gallop



## A genetic test - SynchroGait



- \* Genotyping of *DMRT3* nonsense mutation will be valuable for breeders and trainers of trotters and gaited horses
- \* A patent application has been filed for the commercial use of this test
- \* The genotyping test is called SynchroGait
- \* The test will be licensed to DNA typing laboratories worldwide
- \* For more information email: [info@capiletgenetics.com](mailto:info@capiletgenetics.com) (Lisa Andersson)

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