Genetic Investigation of Equine Occipitoatlantoaxial Malformation in Four Foals

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Occipitoatlantoaxial Malformation (OAAM)

- Developmental disorder of the craniovertebral junction (CVJ) **(Fig. 1)** associated with **highly variable** craniofacial and sternal defects, most commonly:
  - Fusion of **occiput (skull base)** with **1st cervical vertebra (atlas)**
  - Absent dens process on **2nd cervical vertebra (axis) (Fig. 2)**
- Clinical signs:
  - Stiff gaits and posture
  - Abnormal CVJ extension
  - Pain localized to CVJ
- Signalment:
  - Neonates (congenital)
  - Highest incidence in **Arabian horses**

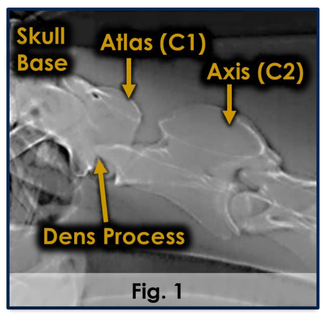

Fig. 1

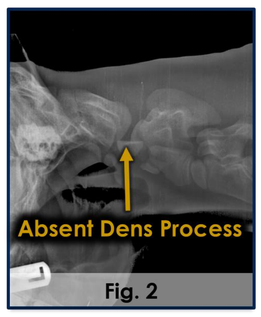

Fig. 2

Project Background & Genetic Etiology of OAAM

- OAAM caused by a homozygous recessive **2.7 kb DNA deletion between *HOXD3* and *HOXD4* on ECA18 in one Arabian (Fig. 3)**
  - Homeobox (HOX) genes control embryonic development according to specific body plans in higher animals
  - *HOXD3* and *HOXD4* direct development of the mammalian skull base and cranial spine **(Fig. 4)**
- *HOXD3/4* deletion carried by 4 healthy Arabians in the population (n = 162), but **absent in other Arabian cases (n = 2) and other breeds (n = 2 cases, 371 controls)**

Fig. 3 DNA sequence on ECA18 from the OAAM-affected Arabian possessing the *HOXD3/4* deletion and a healthy Arabian control

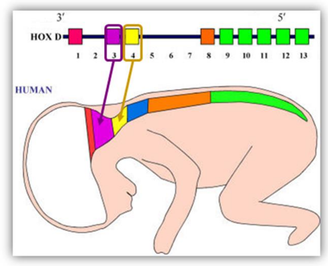

Fig. 4 Mammalian homeobox D genes mapped to the anatomic regions that develop under their control

Research Question

Is genetic heterogeneity in the region of *HOXD3* and *HOXD4* responsible for the phenotypic variation of OAAM within and across horse breeds?

Specific Aim 1

Use whole genome sequence of 4 OAAM-affected foals (2 Arabians, 1 Arabian/Appaloosa cross, 1 Thoroughbred) to fully evaluate the *HOXD3/4* region for putative functional variants.

HYPOTHESIS:
One or more novel variants in the *HOXD3/4* gene cluster is associated with OAAM in these foals.

Specific Aim 2

Use whole genome sequence of an OAAM-affected Thoroughbred foal and its unaffected live-born twin to exclude prenatal intrauterine environment as a cause of OAAM in a non-Arabian breed.

HYPOTHESIS:
Any variants associated with OAAM in the affected Thoroughbred foal will be absent, or present only in the heterozygous condition, in its healthy twin.

Study Design & Progress

- **Isolated Genomic DNA from 4 Affected Foals** (2 Arabians, 1 Arabian/Appaloosa, 1 Thoroughbred) **and 7 Healthy Arabians** ✓
- **Sequenced Whole Genomes** on *Illumina® HiSeq Platform* and **Trimmed Low Quality Data** with *Trimmomatic* ✓
- **Mapped Sequence Data to the Equine Reference Genome** (EquCab2.0) with *Burrows-Wheeler Aligner* ✓
- **Identified Genetic Variants in Study Population** with *Freebayes* (small polymorphisms) and *Delly* (structural variants) ✓
- Labeled Cases and Controls and **Filtered the Most Significant Variants after Contingency Testing** with *SnpSift* ✓
- **Predicted the Effects** (e.g. amino acid changes, premature stop codons, etc.) **of the Most Significant Variants** with *SnpEff* ✓
- **Evaluate Putative Variants for OAAM** and **Assess Variant Prevalence Within and Across Horse Breeds** using the *NCBI Sequence Read Archive*

Filtered Variant Counts

Different case-control labeling permutations were utilized when filtering variants, to better detect those unique to breeds and individuals. In the leftmost column, **colored individuals represent OAAM-affected foals labeled cases** while **grey individuals represent those excluded** from a given analysis. **Each analysis included 7 Arabian controls**.

Filtering Permutation	Tool	Total Variants	*P* < 0.01	*P* < 0.001	*P* < 0.0001	*P* < 0.00001	Of the shaded variants, were any…	
							Coding?	Located Near *HOXD3/4*?
All Affected Horses	Freebayes	18,412,660	144,736	13,483	1,428	385	No	No
	Delly	179,648	1,221	50	8	2	No	No
Affected Arabians Only	Freebayes	18,412,660	160,657	36,175	0	0	Yes (112)	No
	Delly	179,648	602	131	0	0	No	No
Affected Arabian #1 Only	Freebayes	18,412,660	34,608	0	0	0	Yes (102)	No
	Delly	179,648	104	0	0	0	Yes (1)	No
Affected Arabian #2 Only	Freebayes	18,412,660	34,349	0	0	0	Yes (100)	No
	Delly	179,648	103	0	0	0	No	No
Affected Arabians & Arabian/Appaloosa Only	Freebayes	18,412,660	131,656	19,956	4,912	0	Yes (2)	No
	Delly	179,648	524	88	14	0	No	No
Affected Arabian/Appaloosa Only	Freebayes	18,412,660	33,560	0	0	0	Yes (101)	No
	Delly	179,648	101	0	0	0	Yes (2)	No
Affected Thoroughbred Only	Freebayes	18,412,660	40,742	0	0	0	Yes (122)	No
	Delly	179,648	145	0	0	0	No	No

Project Status & Next Steps

- Currently **reviewing the type, location, and predicted effects of the most significant variants under each filtering permutation** to evaluate their likelihoods as causative for OAAM in the affected foals in this study
  - Based on the data presented, the *HOXD3/4* gene cluster is not a strong candidate region for causing OAAM in this study population **(Specific Aim 1)**
- **Putative functional variant(s) in cases vs. controls** will be screened for within the NCBI Sequence Read Archive to **assess variant prevalence within and across breeds**
  - Variant(s) present in the affected Thoroughbred in this study will also be screened for in its unaffected twin and dam to potentially exclude prenatal intrauterine environment as a cause of OAAM in this breed **(Specific Aim 2)**

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