

Introduction

Equine familial isolated hypoparathyroidism (EFIH) is a fatal condition in Thoroughbred foals. It is characterized by muscle contractions and seizures due to hypocalcemia. Originally, the condition was termed idiopathic hypocalcemia in five Thoroughbred foals in 1997.

- 4 to 35 days old
- Severe hypocalcemia and hyperphosphatemia
- Seizure activity, tetany, muscle rigidity
- Death from severe hypocalcemia or euthanasia

An underlying genetic etiology was suspected as only Thoroughbred foals were affected.

Genetic basis identified in 2019 at UC Davis

- RAPGEF5 nonsense variant (c.2624C>A p.Ser875)

- Highly expressed in parathyroid tissue
- Results in loss of function of parathyroid hormone
- Dysregulation of calcium homeostasis
- Direct mechanism unknown
- Role in early embryonic development

Autosomal recessive mode of inheritance

- Identified in Thoroughbred breed
- Allele frequency previously estimated at 1.8%
- 82 Thoroughbreds
- Small non-random sample population

Economic Impact

- Estimated 20,000 Thoroughbred foals registered annually
- Average yearling price in 2020 was $62,208
- Even a single loss can result in substantial economic impact.

An accurate estimate of allele frequency and date of origin for EFIH will inform genetic counseling.

Hypothesis

The RAPGEF5 c.2624C>A genetic variant has a low allele frequency Thoroughbred population and dates back to the first archived DNA sample of the breed.

Specific Aims

1. Determine the allele frequency of the RAPGEF5 variant in a large random cohort of Thoroughbred horses
   - Across the 7 geographical regions of the US

2. Determine if this is a recent variant in the Thoroughbred population and if this variant is undergoing positive selection by comparing the allele frequency of these two time points

Methods

Random generation of sample population
- Samples randomized and selected from each of the seven geographical regions of the United States (Fig. 1)
  - 4 males
  - 4 females
  - 56 samples per year

Genotyping of samples through Agena mass array platform
- 1988-2000 (n=728)
- 2001-2019 (n=1064)
- DNA from hair or purified serum samples
- 3 positive controls and 1 negative control
- Genotyped based on extension product that is classified based on molecular weight difference of base (C or A)

Genotyped
- Homozygous unaffected (N/N)
- Heterozygous carrier (N/H)
- Homozygous affected (H/H)

Analysis
- Allele and carrier frequencies calculated
- 95% Confidence Intervals calculated
- Statistical analysis by Fischer’s exact test
- Significance at P<0.05

Results

Table 1: Allele and carrier frequencies between 1988-2000 and 2001-2019

<table>
<thead>
<tr>
<th>Year</th>
<th>Allele Frequency</th>
<th>95% CI</th>
<th>Carrier Frequency</th>
</tr>
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<tbody>
<tr>
<td>1988-2000</td>
<td>0.83%</td>
<td>0.46% to 1.47%</td>
<td>1.65%</td>
</tr>
<tr>
<td>2001-2019</td>
<td>0.77%</td>
<td>0.92% to 2.90%</td>
<td>1.5%</td>
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Discussion

- RAPGEF5 c.2624C>A variant is present at low frequency in the United States Thoroughbred Population (Table 1)
- Allele frequency of 0.77% estimates that annually 308 out of 20,000 foals are carriers
- No significant difference in allele frequencies observed between timepoints examined (P=0.84)
- First sample detected with mutation was born in 1992 (Fig. 2). This is not a recent mutation but exact origin remains unknown.
- No EFIH homozygotes detected as expected for a lethal condition
- While not significant, noted differences in allele frequencies in 1997 and 2014 suggest trends should continue to be monitored over time (Fig. 2)
- While not statistically significant, changes in geographical distribution of carriers between time periods warrants further study (Fig. 3)

Recommendations

- Genetic testing within breed
- Continue to monitor trend of variant frequency over time
- Avoid producing affected foals by not mating carriers

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References