# Prevalence of the *RAPGEF5* c.2624C>A Variant Associated with Equine Familial Isolated Hypoparathyroidism (EFIH) in the Thoroughbred Population

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## Introduction

**Equine familial isolated hypoparathyroidism (EFIH)**

- Fatal condition in Thoroughbred foals
- Characterized by muscle contractions and seizures due to hypocalcemia
- Originally termed idiopathic hypocalcemia in five Thoroughbred foals in 1997[1]
  - 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)[2]
  - Highly expressed in parathyroid tissue
  - Results in loss of function of parathyroid
    - Dysregulation of calcium homeostasis
    - Direct mechanism unknown
  - Role in early embryonic development
- Autosomal recessive mode of inheritance
  - Only 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[3]
- 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
   - Between 1988-2000 and 2001-2019

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

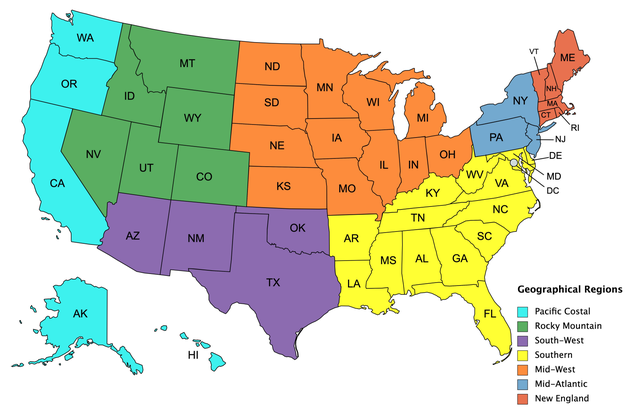


**Fig. 1** Geographical regions of the United States

**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

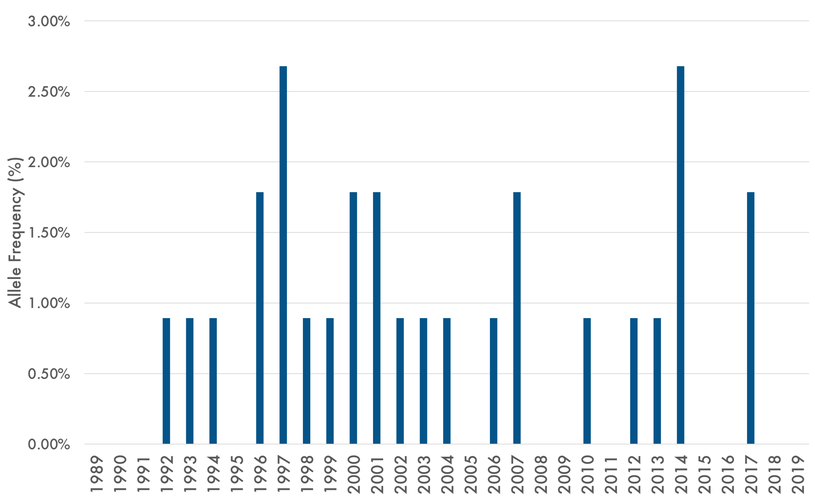


**Fig. 2** Allele frequencies by year, 1988-2019

| | 1988-2000 | 2001-2019 |
|---|---|---|
| Allele Frequency | 0.83% | 0.77% |
| 95% CI | 0.46% to 1.47% | 0.92% to 2.90% |
| Carrier Frequency | 1.65% | 1.5% |
| 95% CI | 0.91% to 2.90% | 0.92% to 2.47% |

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

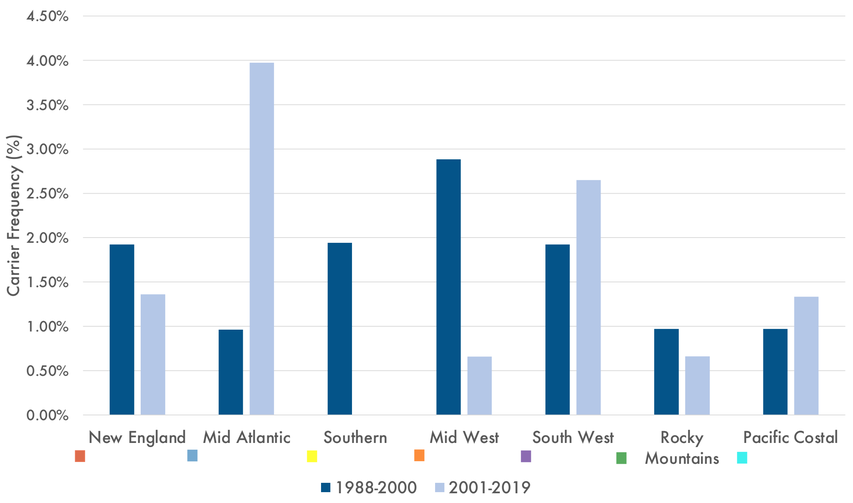


**Fig. 3** Geographical distribution of carriers between 1988-2000 and 2001-2019

## 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)**. Thus, 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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