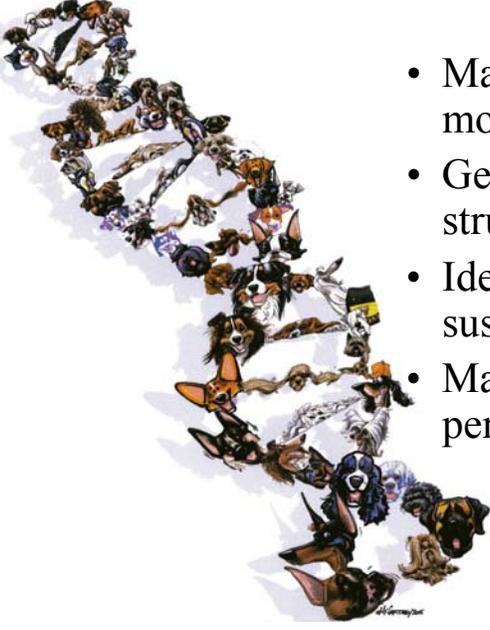


# *Dog Genes Tell Surprising Tales: Finding Genes for Complex Traits*

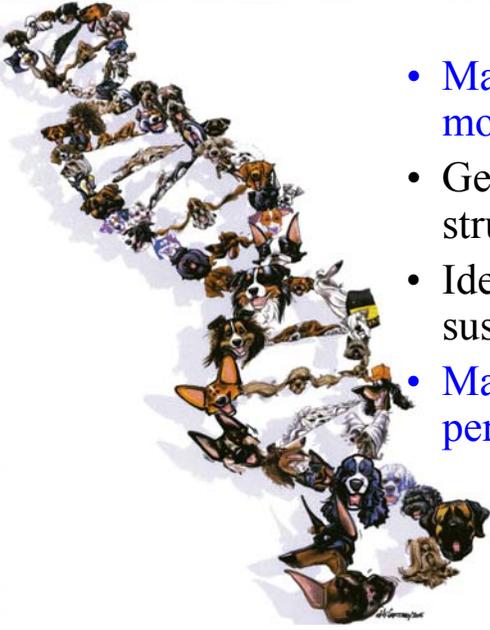
*Elaine A. Ostrander, Ph.D.  
Chief, Cancer Genetics Branch  
National Human Genome  
Research Institute*





### *Topics*

- Mapping genes for morphology
- Genetics of population structure
- Identification of cancer susceptibility genes
- Mapping genes for performance



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***Genetics of  
Morphology***

- Body Size
- Leg Length
- Snout length
- Head shape
- Leg width
- Fur texture
- Arch of Back

*Sutter et al., 2007, Science 316: 112-5*



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## ***Strategy for Finding Genes For Body Size***

- A genome-wide scan in a single breed, the Portuguese Water Dog (PWD) to map QTLs for body size
- Analysis of very large and very small PWD to narrow the associated interval
- Analysis of dogs from very large and very small breeds will identify shared haplotypes among size selected groups to reduce region to single gene



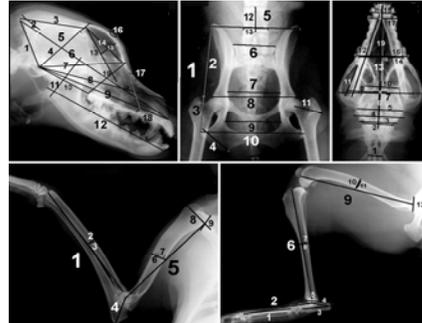
## ***Finding Quantitative Trait Loci (QTLs) for Size Using Portuguese Water Dogs***

- Variation in body size and shape within the breed
- 10,000 dogs that all descend from 30 dogs 50 years ago
- Suggests that few genes are segregating morphogenesis alleles



## ***Methods: Genotype, Measure Skeletal Phenotypes, and Perform Principal Component Analysis***

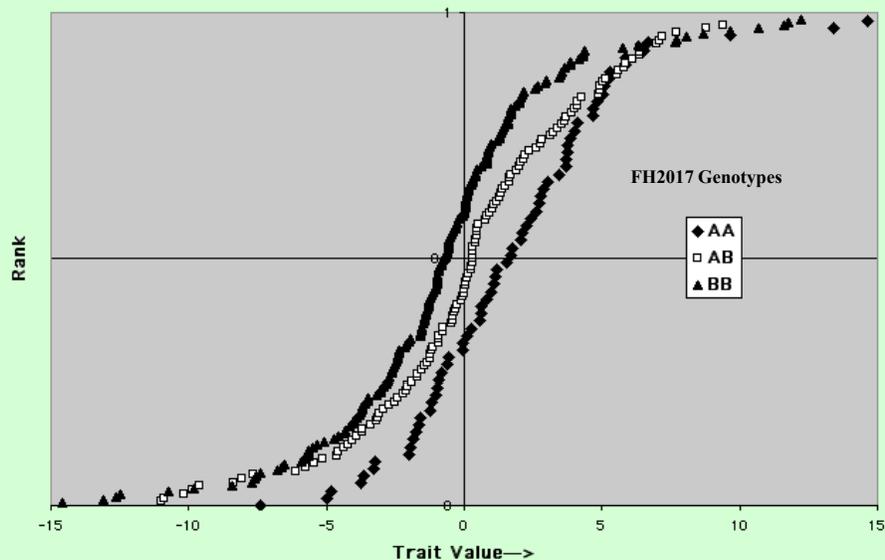
- 1000 Dogs are enrolled in the “Georgie Project”  
[www.georgieproject.com](http://www.georgieproject.com)
- Genotype 500+ pedigreed dogs at 500 microsatellite markers
- Collect 91 skeletal measurements from x-ray images



- Principal component (PC) analysis classifies phenotypic variation into independent systems of correlated traits
- PCS are phenotypes and thus amenable to genetic analysis

*Chase et al., 2002, PNAS 99: 9930-5*

### ***Marker FH2017 is Associated with Overall Body Size***



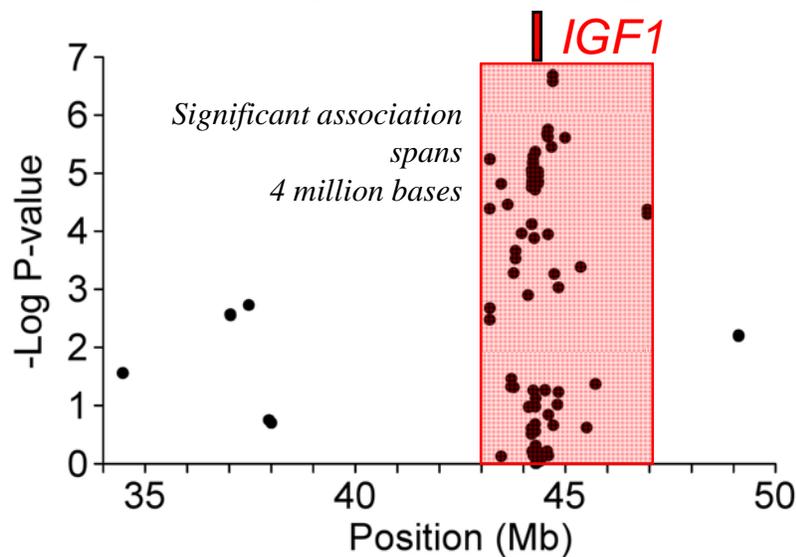
***Narrow the Associated Interval With Single Nucleotide Polymorphism (SNP)-Based Markers***

- SNP discovery on chromosome 15 over a 34-49 Mb interval that includes FH2017 and *IGF1*.
- Initially genotype large and small PWDs to find all useful SNPs
- Genotyped 497 PWD at 122 SNPs using SNPlex (ABI)



*Sutter et al., 2007, Science 316:112-5*

***Association With Skeletal Size In Portuguese Water Dogs***



*Sutter et al., 2007, Science 316:112-5*

***Insulin-like Growth Factor-1 is Important for Pre- and Postnatal Growth***

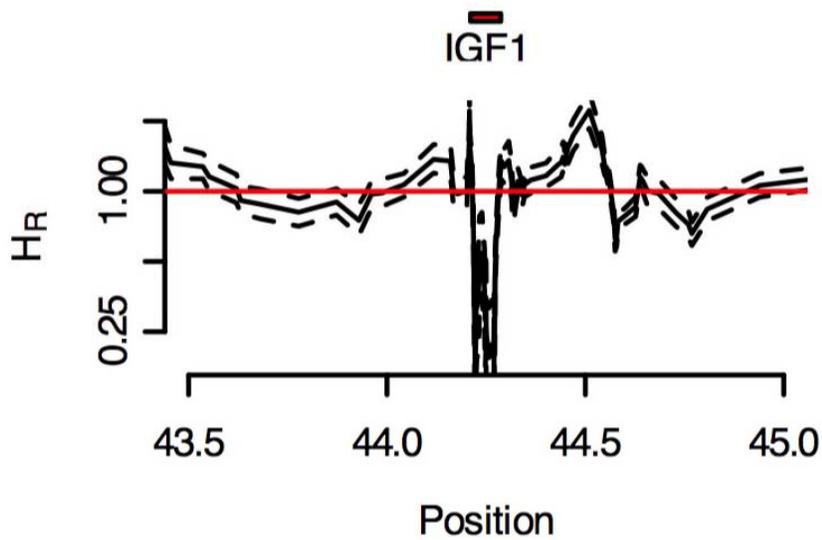
**Wild-type**

**IGF1(-/-)**



From Fig. 4A of Baker, J. et al, "Role of Insulin-like Growth Factors in Embryonic and Postnatal Growth." *Cell* 75:73-82. 1993.

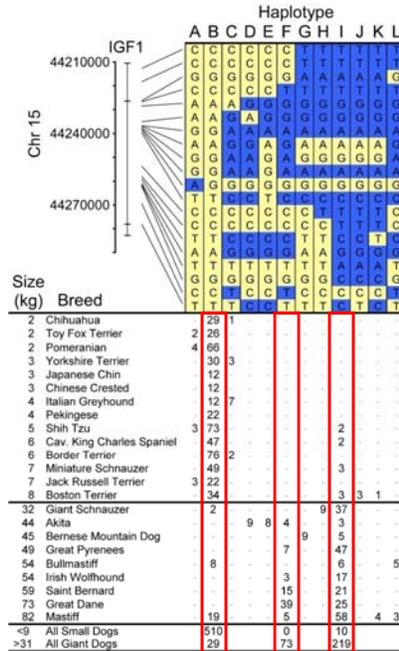
***Heterozygosity Ratio in 14 Small Breeds is Reduced Over IGF1 Gene on CFA15***



*Sutter et al., 2007, Science 316:112-5*

## Small Breeds Share the Same Sweep Haplotype

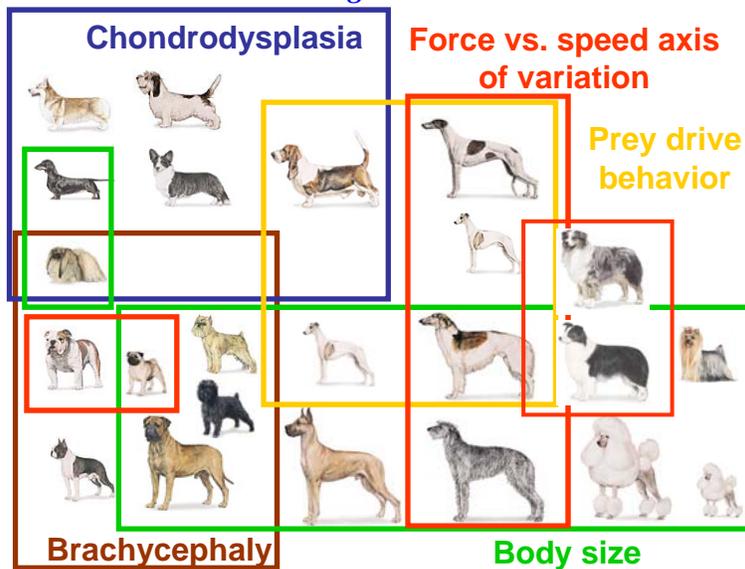
- Suggests a single ancient mutation for small dog breeds
- Giant breeds carry two haplotypes that are rare in small breeds



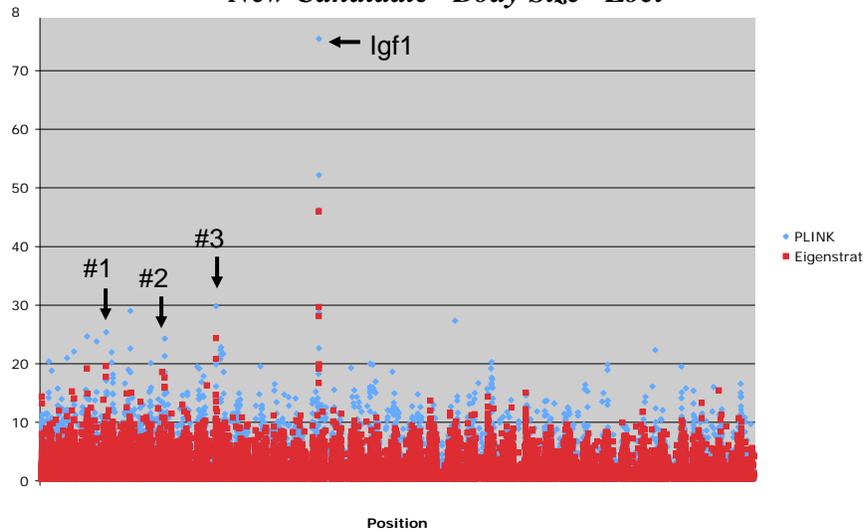
Sutter et al., 2007, Science 316:112-5

## CANMAP-Mapping Genes Controlling Breed-Fixed Morphology

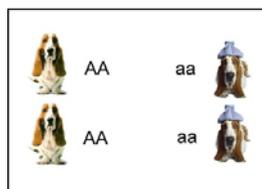
1000 Domestic Dogs and 500 Wild Canids



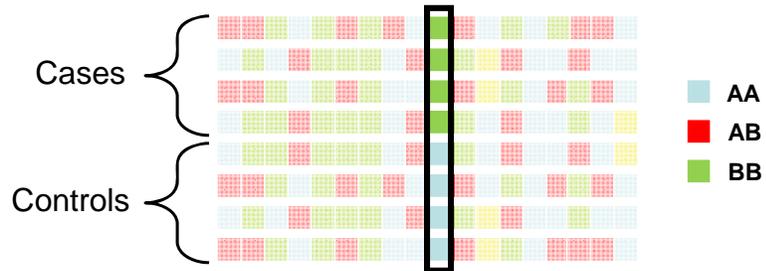
*CanMap Replicates IGF1 and Identifies Four New Candidate "Body Size" Loci*



*Whole Genome Association Study*



Look for alleles that are associated with phenotype



## Canine SNP Chip



127,000 SNPs selected from  
2.5million SNP's



- Filtered to exclude SNPs not typed in at least 95% of the samples
- Exclude SNPs with >60% het calls and <3% MAF
- Exclude loci with  $r^2 > 0.2$
- Left 50K SNPs

## Fixed Trait – Short Limbs

Chondrodystrophy, Achondroplasia, Chondrodysplasia, Asymmetrical Dwarfism  
61 “affected” (10-12 dogs/breed) and 392 controls dogs from 35 breeds



Cardigan Welsh  
Corgi



Pembroke Welsh  
Corgi



Pekingese



Basset Hound



Dachshund



Petit Basset  
Griffon Vendeen

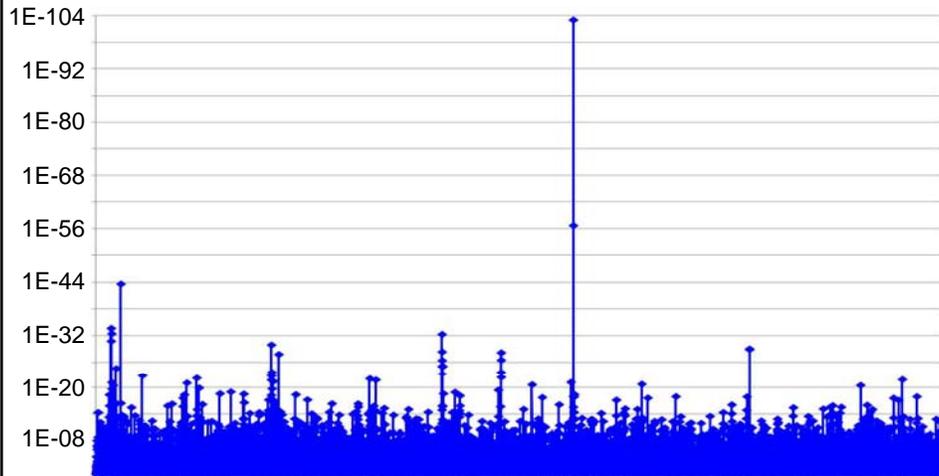
*Crosses suggest a single gene  
with dominant mutation*

*C.R. Stockard (1941)*

- Choose multiple breeds displaying the same fixed trait
- Possible outcomes
  - All breeds share the same mutation from common founder
  - All breeds have mutation in the same gene but not from common founder
  - Subsets of breeds have same mutation
  - Different breeds, different genes

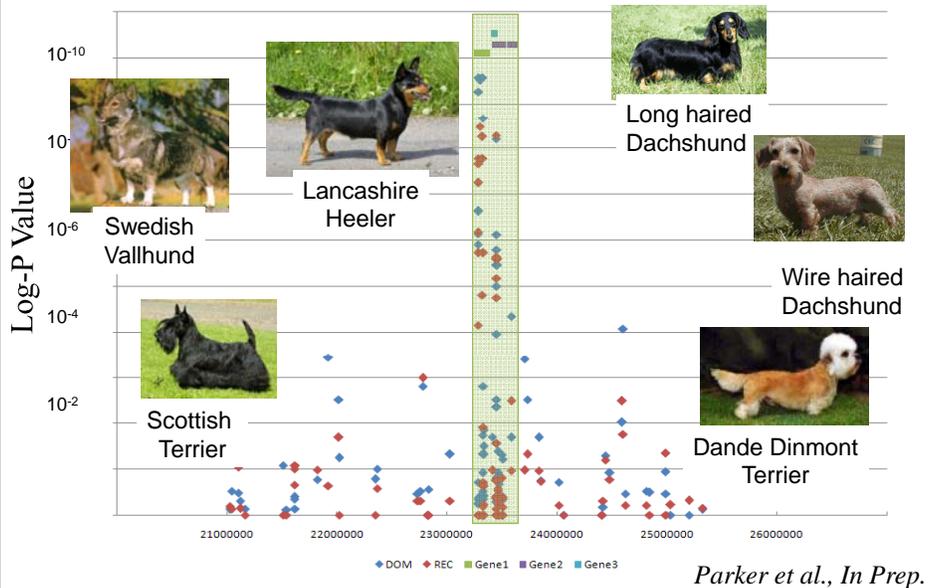
*Parker et al., In Prep.*

## Single Marker $\chi^2$ Association

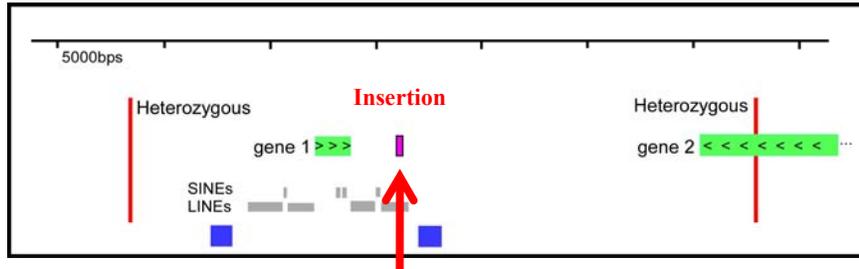


95 cases from 8 breeds versus 702 controls from 64 breeds.  
 Correction for population structure using EIGENSTRAT **p-value =  $1 \times 10^{-49}$** .  
 Repeat analysis with subsets of 2 and 3 affected breeds identified same region

## Additional SNPs Highlight a Region of 160 Kb Spanning Portions of Three Genes



## *Schematic of the Homozygous Region Associated with Chondrodysplasia*



- The putative regulatory regions are highly conserved in sequence and context from human through opossum.
- The insertion is 5-6 Kb and sits within a LINE.
- Second generation sequencing revealed a complete copy of a Fibroblast Growth Factor gene.**
- Mouse studies suggest that increased expression of this gene in the AER terminates limb bud growth through a negative feedback loop involving Grem1, BMP and SHH.

## *Variant Form of Gene Expressed in All Breeds with Asymmetrical Dwarfism*



DataMine™ and  
www.researchgate.net

*A Mutation in the Myostatin Gene Increases Muscle Mass and Enhances Racing Performance*



*Mosher et al., 2007, PLoS Genetics 3:(5)e79*

***Bully Whippets***

- Normal whippets appear very similar to greyhounds
- Owners recently began calling us to report a phenotype of extreme neck and chest musculature -- “Bully Whippet”



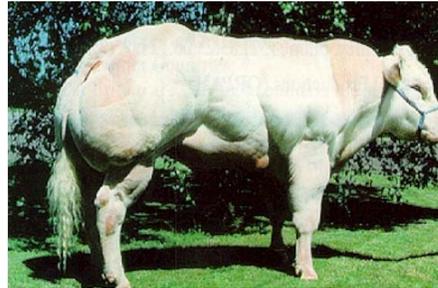
*Mosher et al., 2007, PLoS Genetics 3:(5)e79*

“Bully” whippets look qualitatively similar to other species showing the “double muscle” phenotype caused by mutations in the *myostatin* gene.

Bully Whippet

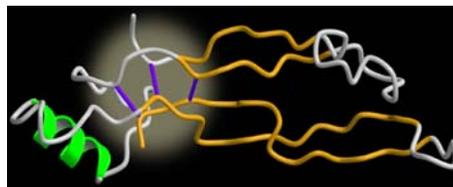


Belgian Blue Cattle



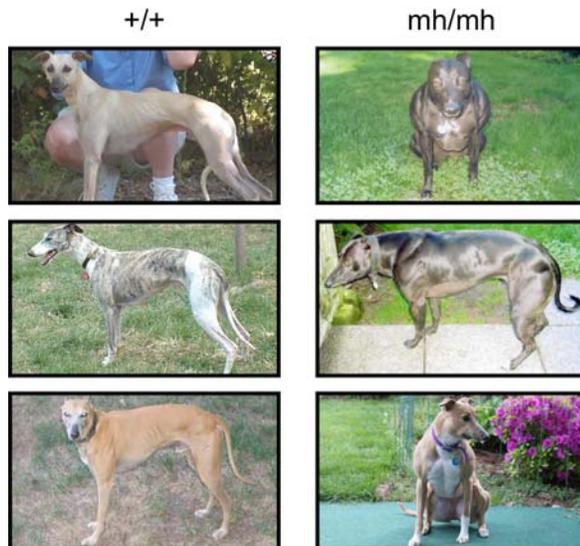
## Screening for Population Frequency

- Sequencing cases, obligate carriers and controls identified a 2 bp deletion that removes terminal 17% of protein and destroyed cysteine knot
- Sampled 146 additional dogs from racing and conformation events without regard to family history
  - 2 mh/mh, 20 mh/+ and 124 +/+
  - Overall frequency of the mutation in dogs sampled = 9%
  - Difference in number of heterozygous dogs in racing vs. conformation ( $p = 0.03$ , Fisher’s Exact Test)
- Appearance of the heterozygous carriers was interesting...



TGF- $\beta$  family member

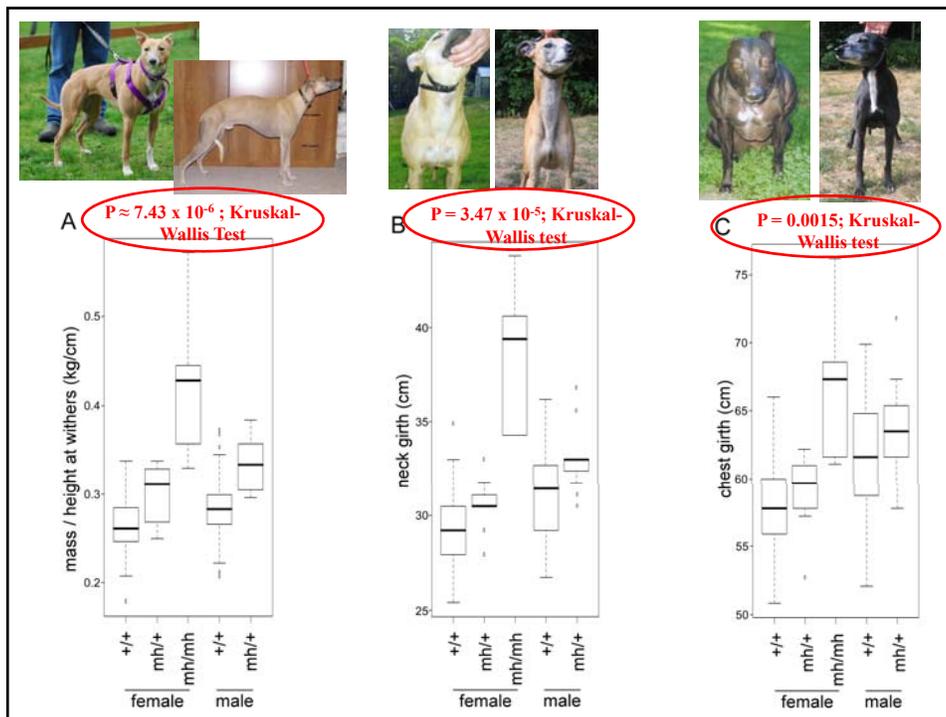
***Dogs Heterozygous for the MSTN Deletion Show an Intermediate Phenotype***



*Mosher et al., 2007, PLoS Genetics 3:(5)e79*

***Dogs Heterozygous for the MSTN Deletion Show an Intermediate Phenotype***





- Whippets race both straight and oval track
- Distances range from 200-400 yards
- Whippets are grouped into heats to race based on previous performance with each dog assigned a racing grade: A, B, C, or D
- We obtained racing grades for 85 whippets

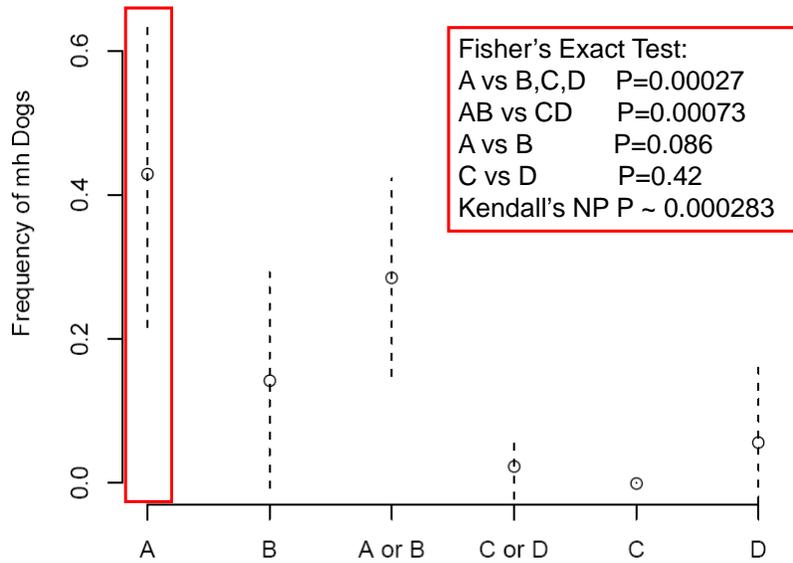
*Could this increased musculature affect a dog's speed?*



<http://www.whippetracing.org/Rules/2006/2006Chapter5.htm>

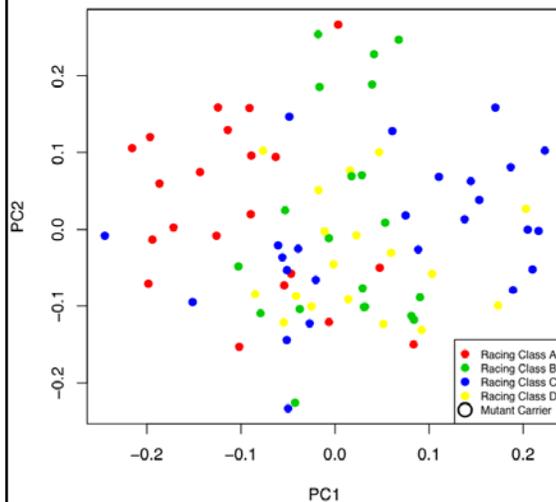
Mosher et al., 2007, PLoS Genetics 3:(5)e79

## *MSTN Deletion is Overrepresented in Grade A Racers*



Mosher et al., 2007, PLoS Genetics 3:(5)e79 Racing Grade

## *Principal Components Analysis (PCA)*

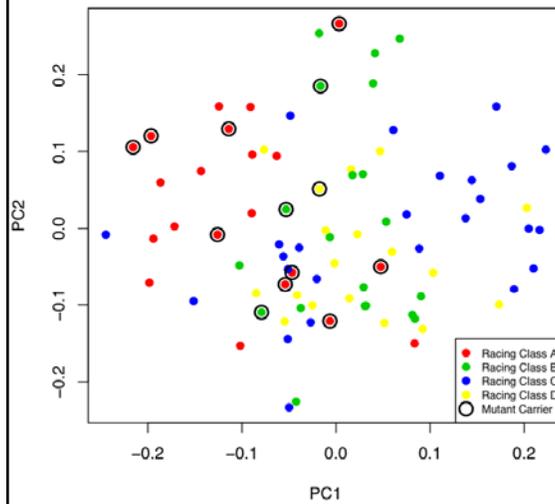


- Used microsatellite marker data for PCA analysis.

- Principle axis of variation separates “A” racers from the rest --particularly “C” racer.

Mosher et al., 2007, PLoS Genetics 3:(5)e79

## PCA Results



*Mh* deletion follows principle axes of variation

Not all *mh* deletion dogs cluster in one space of the PCA plot

Mosher et al., 2007, *PLoS Genetics* 3:(5)e79

## MSTN in Athletics

- Are similar mutations found in other competitive or high performing groups?
- Significant interest in this question for both humans and animals



Vogel. *Science* (2004)  
Sweeney. *Sci. Am.* (2004)



## Summary



- Identical 70 kb haplotype underwent a **selective sweep** in each of 14 small breeds
- Likely a **single IGF1** mutation contributes to small size in all breeds
- Asymmetrical dwarfism is controlled by **insertion** of a transcribed growth factor gene.
- Variants in **MSTN** contribute to racing performance.
- Mapping genes that contribute to a complex morphological traits is feasible using breeds **fixed** for the trait



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