Genome-wide Association Study of Type A Pulmonic Stenosis in Bulldogs

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Pulmonic Stenosis

- Pulmonic stenosis (PS) is the most common congenital heart defect in dogs.[1]
- Clinical signs include: exercise intolerance, collapse, and congestive heart failure.[2]
- Type A PS (fused pulmonic valve leaflets) is the most frequent form observed (Fig 1).[2]
- Bulldogs are overrepresented for PS suggesting a genetic cause.[2]
- **Hypothesis:** PS in Bulldogs is associated with genetic variants that disrupt cardiac development.

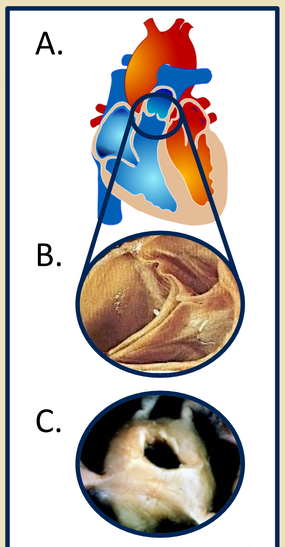

Figure 1. A. Location of pulmonary valve
B. Normal valve anatomy
C. Type A valve anatomy with dome shape and commissural fusion

Result: Pulmonic Stenosis Incidence

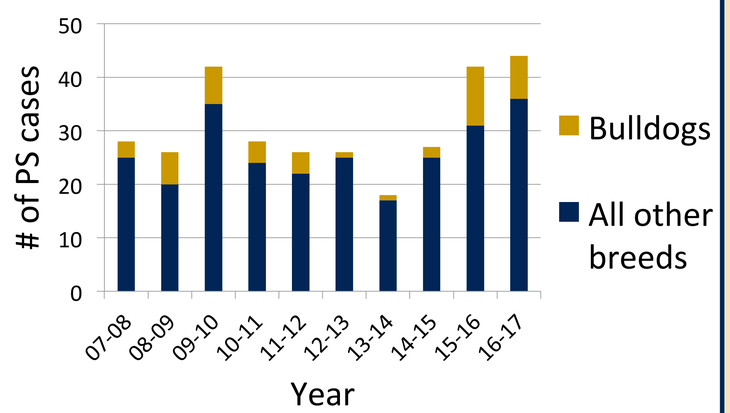

Figure 2. Incidence of PS each year for 10 years at the UC Davis Veterinary Medical Teaching Hospital (VMTH).

The top five breeds affected with PS as a percent of total PS cases were:

1. Bulldogs – 15%
2. Pitbull terrier – 11%
3. French Bulldog – 6%
4. Chihuahua – 4%
5. German Shepherd – 3%

4% of all Bulldogs that were seen at VMTH between 2007-2017 were diagnosed with PS.

Methods: Pulmonic Stenosis Incidence

Reviewed canine VMTH records for PS cases over a 10 year period

Signalment for each case was recorded along with severity and type of PS

Analyzed the confirmed PS cases for affected breeds by year and overall (Fig 2)

Methods: GWAS

Phenotyped and collected DNA on 13 cases and 62 controls

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Genotyped samples on Illumina 230K Canine SNP Array

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Golden Helix Software:

1. Quality control and filtering of SNPs
2. Removal of sample outliers on a PCA plot
3. Additive Case-Control X^2 association test
4. EMMAX to correct population stratification
5. Bonferroni correction for multiple testing
6. 100,000 permutations testing

↓

Identification of candidate genes

Result: GWAS

- 121,832 markers remained after quality control and filtering (call rate >0.90; MAF <0.05).
- 12 cases and 45 controls were included in the analysis after PCA outliers were removed.
- There was mild population stratification present with an inflation factor (λ) of 1.18.
- A Bonferroni significant association was present on chromosome 7 after both EMMAX ($p_{adjusted}$ =9.89x10^{-3}) and 100,000 permutation testing($p_{adjusted}$ =6.1x10^{-3}) **(Fig 3).**
- The extent of population stratification after mixed linear model analysis (EMMAX) correction (**Fig 4**).
- There is one gene located over the three significant SNPs, PTPN2.

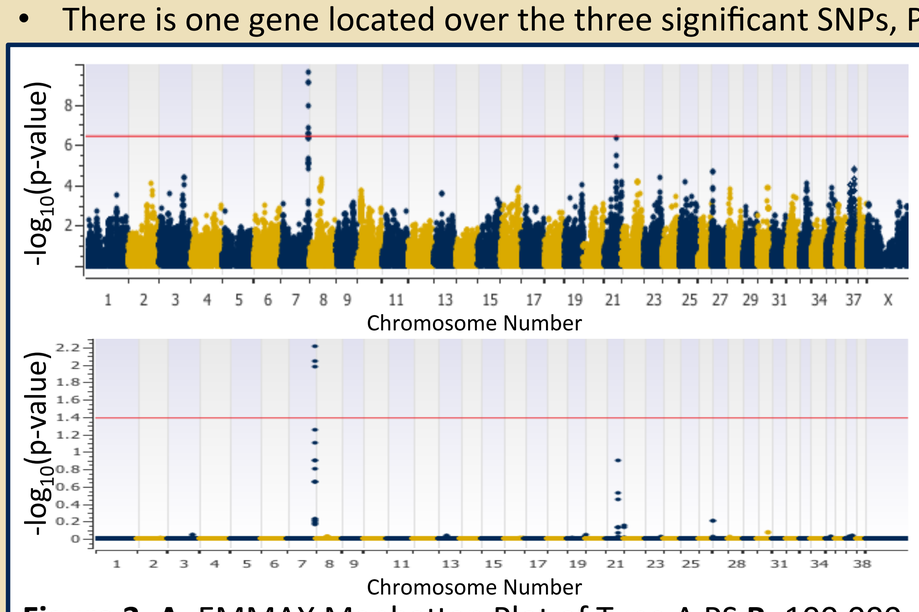

Figure 3. A. EMMAX Manhattan Plot of Type A PS **B.** 100,000 permutation testing. SNPs are color-coded by chromosome. Red line indicates Bonferroni genome-wide significance.

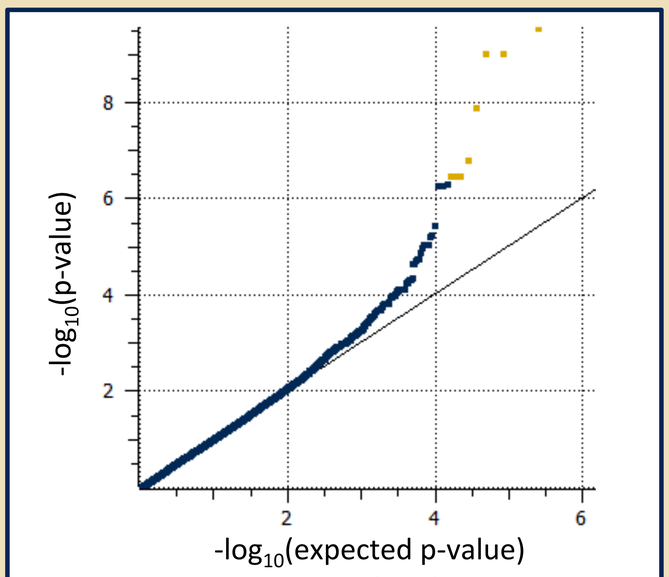

Figure 4. EMMAX QQ plot demonstrating the remaining population stratification. Gold dots are Bonferonni significant SNPs.

Discussion

Pulmonic stenosis is a significant disease in Bulldogs. At the UC Davis Veterinary Medical Teaching Hospital 4% of all Bulldog patients were diagnosed with PS. They were the most affected breed accounting for 15% of all PS cases. A genetic investigation into the cause of PS in Bulldogs revealed a statistically significant region on chromosome 7 (CFA 7) and a suggestive region on CFA 21. On CFA 7 a likely candidate gene is PTPN2. PTPN2 is in the same family as PTPN11, which causes Noonan syndrome in humans.[3] It has been hypothesized previously that Bulldogs may be afflicted with a canine form of Noonan syndrome, but the genetic evidence has yet to be found.[4] Further sequencing in the region is necessary to identify a possible causal variant.

Acknowledgements
Student Funded by: AVMA/AVMF Second Opportunity Research Scholarship
National Heart, Lung, and Blood Institute NIH T32 HL086350
Research Funded by: AKC Canine Health Foundation Grant #0419 (Stern PI)
UC Davis School of Veterinary Medicine - Center for Companion Animal Health
The Bulldog Club of America Charitable Fund

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