Determination of the genetic etiology of bilateral anterior hemimelia in the Chihuahua

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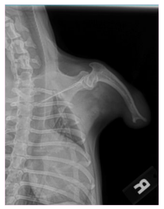
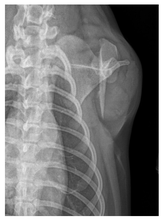
Introduction

Bilateral anterior hemimelia (BAH) is the congenital absence of the majority of the bones of the thoracic limbs. This disease has been reported in the Chihuahua breed in Mexico in 1982 [1]. Since affected dogs were the result of inbreeding it was hypothesized to have a recessive mode of inheritance. The identification of the variant responsible will enhance our understanding of the developmental difference between thoracic and pelvic limbs.

Phenotype





Dogs affected with BAH have variable length thoracic limbs. The most severe have absent humeri and all distal bones. The most mildly affected have normal humeri and radius and ulna but missing carpal, metacarpal and/or phalanges [1]. The genetic term for this is variable expressivity.

An affected dam and offspring were relinquished to a local shelter and adopted by a veterinary student at UC Davis. 11 additional cases were identified through social media from rescue organizations. All 13 cases were missing all bones distal to the radius and ulna.

Methods

DNA Sample Collection
- 13 dogs collected that were affected with BAH
- 366 control dogs with 4 limbs from Bannasch laboratory DNA database

GWAS
- 10 samples, 61 controls genotyped on Illumina canine HD SNP array–220,853 markers
- Case-Control X^2 analysis of genotypes
- Population structure analysis- GEMMA

Whole Genome Sequencing
- 6 cases, 161 controls
- Filtering variants: PLINK VCFs association, WEBGQT [3], homozygosity analysis

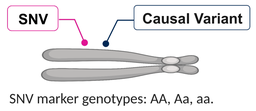
Sanger Sequencing & Genotyping
- 13 cases, 200 controls

Genome-Wide Association Study

1. Phenotype data and DNA samples

2. Collect unrelated cases and controls

3. Markers are single nucleotide variants (SNV) that are a proxy for the causal variant

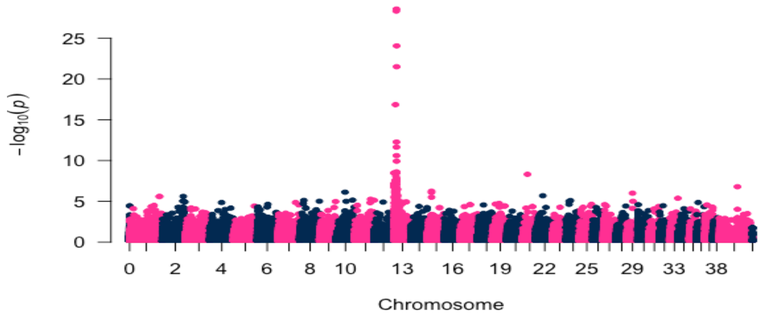


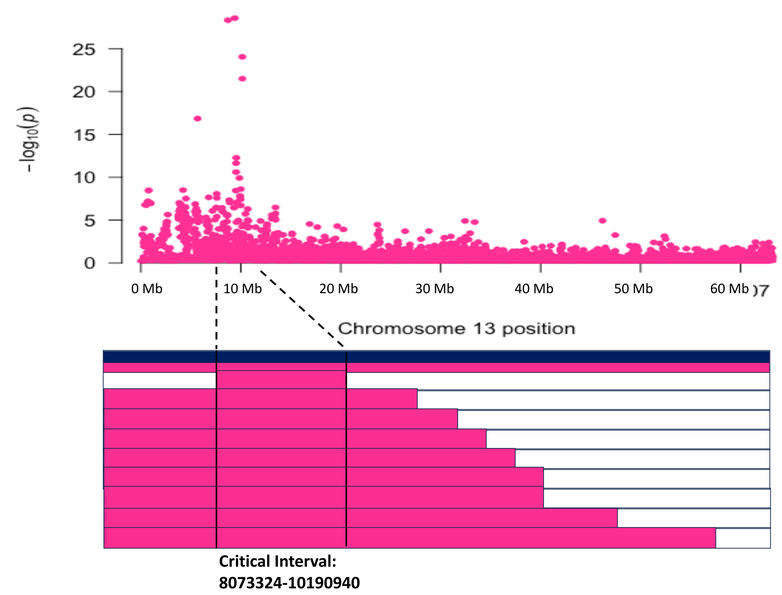
SNV marker genotypes: AA, Aa, aa.

4. GWAS use evenly-spaced SNVs across the genome and asks if the allele frequency of "a" VS "A" is significantly different between cases and controls for each SNV.

a=0.9, A=0.1  a=0.1, A=0.9 

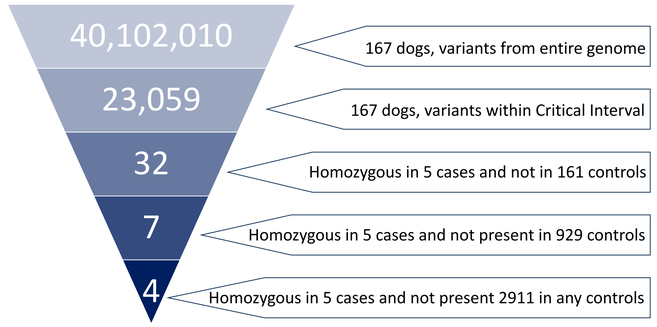
Results are displayed on a Manhattan plot, where the $-\log_{10}(P)$ for the allele frequency difference for every SNV is displayed by chromosome.





Homozygosity was used to define the critical interval. One of the nine cases was heterozygous, which can be seen by the pink and blue line. In the critical interval, there are 9 genes: *ANGPT1, RSPO2, EIF3E, EMC2, TMEM4, TRHR, NUCD1, ENY2, and PKHD1L1.*

Variant Filtering



Future Directions

Limb deformities affecting all four limbs in mice [2,4], humans [5,6], and Holstein cattle [3] have been linked to *RSPO2* . In other species, these diseases cause tetramelia, loss of all four limbs, or other limb deformities. We suspect that the variant causing the disease may be in a regulatory region of *RSPO2* so we hope to further explore the regulation of *RSPO2* and its effect on the limb bud in development. We will continue to genotype four candidate SNVs in 7 cases and 200 control chihuahuas to see if the SNVs segregate in cases and controls. In addition to the SNVs of interest, we will continue to search visually for structural variants in the cases in the genome browser program IGV. We will also look for variations in coverage between our cases and controls. There are two gaps in the genome assembly canfam4 in the region that will be Sanger sequenced in cases.

Acknowledgments

Thank you to Dr. Bannasch and Julia Vo for all of their help and support this summer with my STAR project! Financial support was provided by the Students Training in Advanced Research (STAR) program through the NIH T35-OD010956 grant. I would also like to acknowledge the Maxine Adler Endowed Chair Fund for funding my research. I would like to thank the dog owners who provided samples for this project.

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