

PCR Genotyping of a Novel FGF4 Retrogene in Pit Bull **Terriers and Mixed Breed Dogs Affected by Disc Disease**

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Introduction

Intervertebral Disc Disease (IVDD) is an incredibly devastating and painful disease that affects a wide variety of dog breeds. Chondrodystrophic dog breeds such as Dachshunds and French Bulldogs exhibit the highest frequency of IVDD [1]. Chondrodystrophy is characterized by shortened long bones and prematurely calcified intervertebral discs [2]. IVDD is the result of herniation of this intervertebral disc into the spinal canal often causing pain and paralysis. A recently discovered fibroblast growth factor 4 (FGF4) retrogene on chromosome (Chr) 12 is responsible for canine chondrodystrophy and a leading contributor to a majority of IVDD cases [3]. However, not all dogs affected with IVDD have the Chr 12 retrogene [Brown et al. and K. Batcher and D. Bannasch unpublished results]. Recently the laboratory identified a second FGF4 retrogene on Chr 13 in two IVDD affected Pit Bull Terriers that did not have the Chr 12 retrogene. The purpose of this study was to determine if this FGF4 retrogene may be a contributing cause of IVDD in dogs.



Phenotype

A classic example of IVDD is shown in Figure 1 and Figure 2 below. The CT images show different angles of the same calcified disc that has ruptured and is compressing the spinal canal dorsally. The yellow arrow points to the herniated degenerative disc material. Herniated intervertebral discs can cause clinical signs ranging from sudden onset paralysis to chronic back pain. Treatment includes

The FGF4 retrogene on Chr 13 came from the endogenous FGF4 gene on chromosome 18. FGF4 is composed of three exons and two introns. When FGF4 is transcribed the introns are removed and a poly-A tail is added. This entire transcript is approximately 3.2kb long. Retrogene formation is a rare event that can occur when RNA is reverse transcribed into DNA and integrated back elsewhere in the genome [4]. Several FGF4 retrogenes have been identified in the dog genome [3,5]. The focus of this study was a FGF4 retrogene on chromosome 13 identified in two Pit Bull Terriers affected by IVDD.



surgery to reduce the spinal compression and crate rest.





Figure 1: sagittal CT image of herniation at T11-T12; SC= spinal canal

Figure 2: cranial-caudal CT image of herniation at T11-T12; SC= spinal canal

Materials and Methods

Sample Identification

Screened Bannasch Lab DNA database and the William R. Pritchard Veterinary Medical Teaching Hospital records for IVDD surgical cases from mixed breeds, Pit Bull Terriers and related breeds.

N = 217 N = 83 dogs with Non IVDD IVDD

In order to identify dogs containing the FGF4 retrogene on chromosome 13 a three primer PCR reaction was used as shown above. One primer pair was designed flanking the retrogene insertion site and an additional forward primer was designed within the retrogene. When no retrogene insertion is present, the product between the forward genomic (F1) and reverse genomic primer (R1) is 451bp in length. When the retrogene insert is present, the product between the forward primer within the retrogene (F2) and R1 produces a product 626bp in length.

Frequency of FGF4 Retrogene on Chr 13

	Pit Bull Terrier		Staffordshire Terrier		Mixes	
Chr 13 Retrogene	Pos	Neg	Pos	Neg	Pos	Neg
IVDD	2	20	0	0	0	61
Not IVDD	0	179	0	14	0	24

Only two copies of the retrogene on Chr 13 were found, and both were in Pit Bull Terriers. As Pit Bull Terrier is not a well defined breed we expanded the search to include Staffordshire Terriers and other mixed breeds that were both affected and unaffected by IVDD. The novel Chr 13 retrogene was rare and appeared in only 0.995% of the Pit Bull Terriers tested. The only dogs that had the Chr 13 retrogene had IVDD and did not have any other identified retrogenes.

dogs

Genotyping

Used a three primer PCR to amplify retrogene and non retrogene DNA (see next box).

Evaluated PCR product sizes using agarose gel electrophoresis

References

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Conclusion

The rare Chr 13 FGF4 retrogene appears to cause IVDD in dogs.

Future Directions

- Look at the dogs that have the previously identified retrogene on Chr 12 and see if there are any dogs that have both Chr 12 and Chr 13 retrogenes.
- Identify more FGF4 retrogenes that could cause IVDD in other unexplained cases.