Novel variant in Burmese cats with Ehlers-Danlos syndrome

Veterinary Research Scholars Program
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Introduction:

- Ehlers-Danlos syndrome (EDS) is a group of congenital disorders of connective tissue characterized by hyper-extendible skin, joint hypermobility and tissue fragility[1].
- EDS type VIIC or dermatospraxis, characterized by hyper-extendible skin and tissue fragility, has been described clinically and genetically in multiple species, including humans, cattle and sheep[1,2].
- EDS in cats (dermatosparaxis or feline cutaneous asthenia) found primarily in the Burmese breed (**Figure 1a**) is suspected to have an autosomal recessive inheritance[3].
- EDS in the Burmese breed has a unique presentation, with hyper-extendible, extremely fragile skin, small areas of alopecia, and large ischemic skin lesions, (**Figure 1b and 1c**) that result from minor trauma, especially scratching.
- Previous studies[4] have shown that the collagen of cats affected with EDS displayed disrupted collagen fibrils (**Figure 2**) and irregularly shaped collagen fibers.
- Due to poor quality of life, cats affected with EDS are frequently euthanatized.
- The objective of this study is to identify the gene and causal variant(s) of EDS in Burmese cats.
- Whole genome sequencing (WGS) was performed on the affected Burmese cats to evaluate polymorphisms in known EDS candidate genes and to detect variants in novel loci.

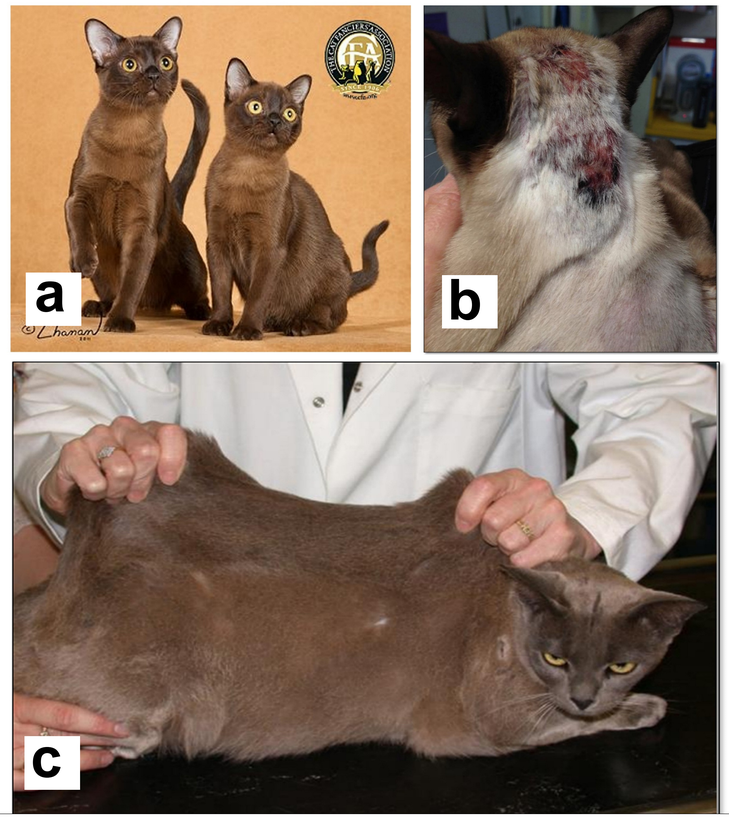

Figure 1: Unaffected and affected Burmese cats. a: Unaffected sable Burmese cat. **b**: Affected Burmese cat displaying hyper-extendible skin and small areas of alopecia. **c**: Affected Burmese cat displaying large, ischemic skin lesions on the base of the head. Such lesions occur often during induction of anesthesia due to scruffing the cat.

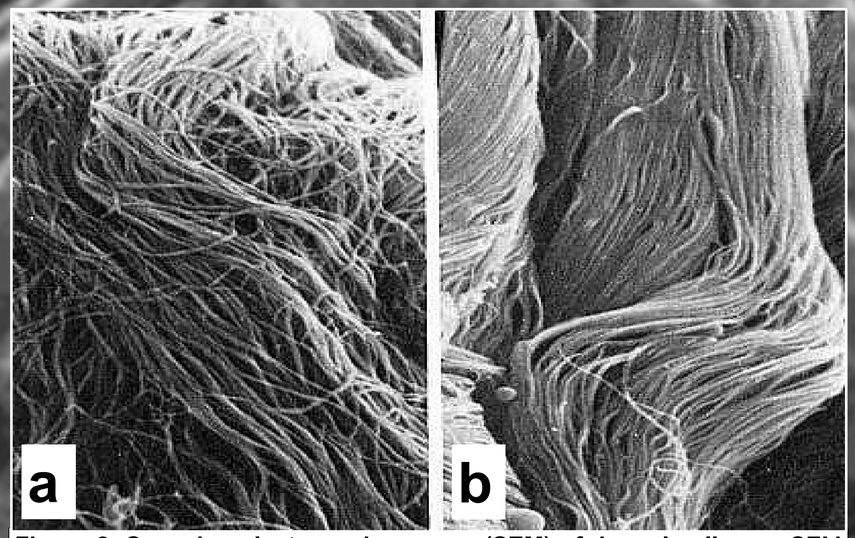

Figure 2: Scanning electron microscope (SEM) of dermal collagen: SEM dermal collagen fibrils of an affected (a) and an unaffected cat (b). Affected Burmese cats have highly disarrayed fibrils.

Materials and Methods:

- DNA from two affected Australian Burmese cats was collected and extracted using the phenol-chloroform method
- WGS was performed on two affected Burmese using two PCR free libraries with insertion sizes of 350 bp and 550 bp.
- 100 bp paired end reads were generated with Illumina HiSeq 2500 to 30x depth of coverage.
- Reads were aligned to the publically available feline reference genome (ver6.2 assembly).
- Variants were complied using the program Platypus and were filtered based on expected genotype using Maverix Biomics variant tools.
- Variants were prioritized based on impact to gene transcript and function.
- 54 additional healthy cats were with WGS were used to exclude polymorphisms.
- Primers were designed and the variant in *CCDC80* was tested by Sanger sequencing in 5 affected Burmese, 4 related individuals and 31 unaffected Burmese from the Australian Burmese population.

Table 1: 10 Variants in 11 genes concordant with the EDS phenotype

Chromosome	Position	Effect	Severity	Gene Name
chrA1	197759995	Non synonymous coding	Moderate	*ADRB2*
chrA1	199981400	Non synonymous coding	Moderate	*ITGA2*
chrA2	12430969	Non synonymous coding	Moderate	*MYO9B*
chrA3	76502204	Non synonymous coding	Moderate	*CCDC104*
chrB3	49303339	Non synonymous coding	Moderate	*TCF12*
chrB4	13818049	Non synonymous coding	Moderate	*RPP38*
chrC2	**58790528**	**Frame shift**	**High**	****CCDC80***
chrC2	59087082	Non synonymous coding	Moderate	*C3orf17*
chrC2	60025448	Non synonymous coding	Moderate	*KIAA1407*
chrC2	60026318	Non synonymous coding	Moderate	*KIAA1407*
chrD1	109719672	Non synonymous coding	Moderate	*VPS51*
chrE1	22607144	Non synonymous coding	Moderate	*GAS2L2*

***CCDC80** was concordant in the 54 cats tested.

Results:

- Burmese affected with EDS had a skin extensibility index (SEI) between 22-28%, while unaffected cats had a SEI of less than 19%, with an average of 11%.
- 14,014 exonic variants were identified in the two affected Burmese, 9,702 were homozygous for the alternative allele.
- The inclusion of 54 WGS that do not have EDS, reduced the number of variants concordant with EDS to 10 variants in 9 different genes.
- Of the 10 variants, a variant in *CCDC80* **(Figure 4),** a gene involved in collagen synthesis, was predicted to produce a frameshift mutation, resulting in a severely truncated protein.

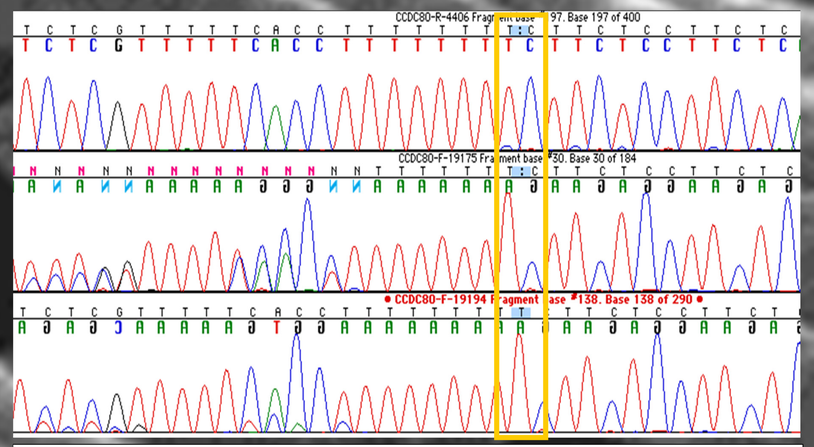

Figure 4: Sanger sequencing of Burmese cats for *CCDC80*: Sanger sequencing of an unaffected (top), carrier (middle), and affected Burmese cat (bottom) for the gene *CCDC80.*

Conclusion:

- **Based on the function of *CCDC80* and the severity of the alteration, the variant in *CCDC80* may be a novel variant resulting in Ehlers-Danlos Syndrome in Burmese cats**
- Understanding the underlying genetics of Burmese EDS offers a novel animal model and allows a genetic test to be developed to achieve a better general health of the breed.
- Further population screenings are necessary to confirm this variant as causal for Burmese EDS.
- Further research into the role of *CCDC80* in collagen synthesis is necessary to better understand the pathogenesis and etiology of EDS in Burmese cats.

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