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Spongiform leucoencephalomyelopathy (SLEM) in Border Terriers is associated with a single nucleotide variant in the *OPA1* gene

Introduction

Spongiform leucoencephalomyelopathy (SLEM) in Border Terriers is a canine juvenile-onset neurodegenerative disease of suspected genetic origin with an autosomal recessive mode of inheritance. Puppies of a few weeks of age typically show a coarse whole-body tremor and dysmetria that progresses during the first months of life. Deafness has also been reported.[1,2] Presentation, diagnostic tests and histopathological features raise the suspicion of a mitochondrial condition.[1,2] An unpublished prior analysis found an intronic single-base substitution in the *OPA1* gene associated with SLEM, but the reference genome used included several gaps near the *OPA1* gene and the pathophysiology associated with an intronic variant is unclear. This study replicated the genomic analysis using an updated canine reference genome assembly to identify a likely causative variant.

Material and Methods

Whole genome sequencing (WGS) of 12 Border Terriers (BoT) and 125 dogs of different breeds:

SLEM affected	SLEM obligate carrier	SLEM unaffected
5 BoT	2 BoT parents of affected puppies	5 BoT and 125 dogs of multiple breeds (healthy or with unrelated diseases)

The WGSs were aligned to the CanFam4 canine reference assembly and variants were filtered to retain the ones with the appropriate segregation pattern: **homozygous** in all SLEM affected cases, **heterozygous** in obligate carriers and **heterozygous or absent** from all clinically unaffected BoT and **absent** from dogs of other breeds.

Results

After filtering variants using an in-house bioinformatics pipeline, only one of the candidate variants segregated with the expected mode of inheritance - a single nucleotide variant (SNV) in the *OPA1* gene - Chr23:52653183G>A (figure 1).

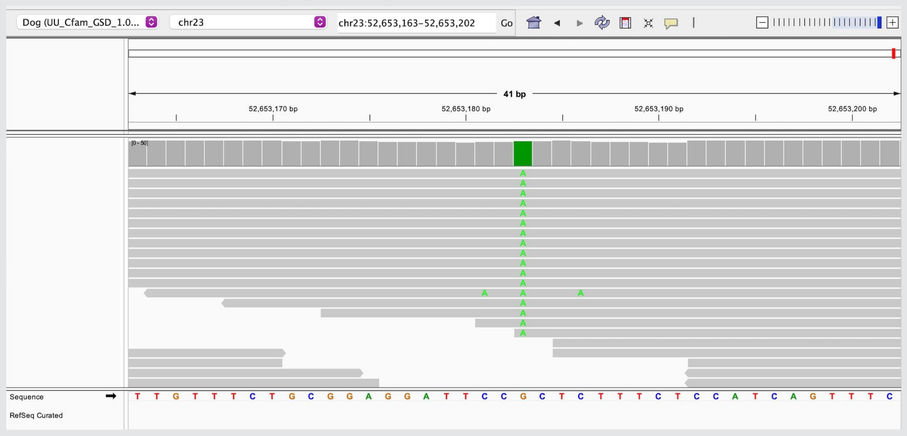

Figure 1: WGS read on Integrative Genomic Viewer (IGV) of a SLEM affected case at the site of the candidate polymorphism, compared to the reference genome (canine CanFam4 UU Cfam GSD 1.0 reference genome). A SNV is illustrated as the green base 'A' compared to the base 'G' from the reference genome.

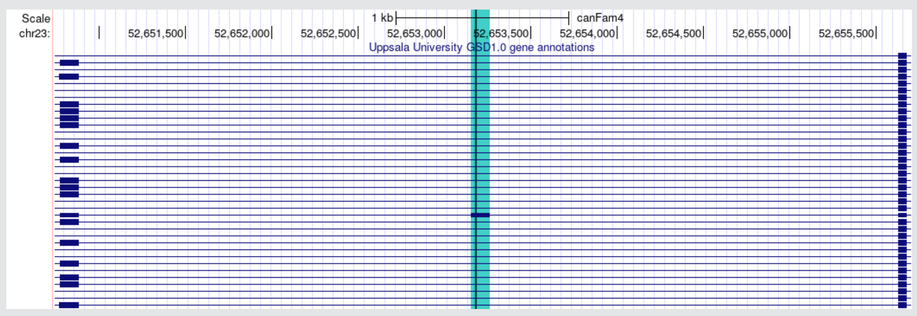

Figure 2: Uppsala University GSD1.0 gene annotations CanFam4 at the level of the area of interest in the OPA1 gene – in light blue the site of ambiguous annotation, with the vertical dark blue line marking the site of the SNV.

The single base substitution seems to be in an untranslated region (5'UTR) of one transcript, although potentially incomplete annotation of *OPA1* in CanFam4 means there is still a possibility the site might be in an unannotated exon (figure 2), causing a missense mutation (Ala>Thr change). RNA extracted from the brain of two SLEM cases revealed the polymorphism in some, but not all *OPA1* transcripts.

Discussion and Future Plans

OPA1 encodes a mitochondrial dynamin-like GTPase (Guanosine-Triphosphate hydrolase) protein, which has an important role as a regulator of mitochondrial inner membrane fusion and cristae remodeling.[3] Changes in the mitochondrial dynamics triggered by mutations in this gene can lead to impairment of the main functions of the organelle, such as respiration, ROS production, calcium homeostasis and apoptosis.[3] It is still not completely clear if the polymorphism found is in an unannotated exon or a UTR. If the former, leading to a nonsynonymous substitution, the change in polarity associated with the amino acid swap could affect the protein folding and stability, and therefore have functional consequences. If present in a UTR (5'UTR), it could impact mRNA stability, translation and post-transcriptional processes. SLEM has clinical, diagnostic and histological features of a mitochondrial encephalomyelopathy[1,2], and the *OPA1* variant is therefore a compelling candidate for the disease pathophysiology. In humans, *OPA1* polymorphisms, in both coding and non-coding sites, are associated with the development of dominant optic atrophy (DOA).[4,5,6] SLEM affected puppies have not been reported to present optic nerve/retinal ganglionic cell changes. However, multisystem neurological disease can affect up to 20% of human *OPA1* mutation carriers, including deafness and abnormal myelination[7,8], both of which are seen in our canine affected population.

Next steps

Further studies are necessary to characterize the association between the variant and disease. Investigation of structural variants, western blots, immunohistochemistry and functional testing could help investigate causality of disease.

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