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FACULTY OF VETERINARY MEDICINE AND ANIMAL SCIENCE

# Phenotypic and genotypic characterization of a myelopathy in pugs

A journey from 'wobbly pugs' to pug dog myelopathy

CECILIA ROHDIN





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# Phenotypic and genotypic characterization of a myelopathy in pugs

## Abstract

Spinal cord disease, myelopathy, in dogs have several aetiologies, all characterised by their clinical presentation. Pugs with a thoracolumbar myelopathy (PDM) differ from most dogs by developing a form of myelopathy that shows little clinical variability. Most pugs with PDM show slowly progressive paraparesis and ataxia, absence of obvious spinal pain and a high prevalence of incontinence.

The overall aims of the thesis were to investigate the prevalence of PDM, to clinically and pathologically characterize this unique disorder in pugs and to explore underlying aetiologies. The importance of congenital vertebral malformations (CVMs) was examined using advanced diagnostic imaging. A potential contribution of an autoimmune aetiology was studied by analysing biomarkers in blood and cerebrospinal fluid. The necrotizing meningoencephalitis (NME) susceptibility dog leucocyte (DLA) class II risk was assessed and a genetic contribution to PDM was investigated by molecular genetics.

In summary, gait abnormalities were common in Swedish pugs and the character of the abnormal gait suggesting an underlying spinal cord disorder. The lack of association between presence, or type, of CVMs in the thoracolumbar vertebral column and neurological deficits, paraparesis and ataxia, suggested that other or additional factors were important for the development of PDM. Neuropathological examination showed that the clinical disease, PDM, was caused by the formation of profound meningeal fibrosis that interfered with the vascular supply to the spinal cord as well as caused obstruction of cerebrospinal fluid (CSF) flow with subsequent parenchymal spinal cord destruction. In addition a considerable number of PDM pugs presented with inflammation in the central nervous system. A contribution of the immunological system on the development of PDM was supported by the finding of anti-GFAP autoantibodies in the CSF of pugs with PDM. Our results also indicated that the DLA class II risk haplotype for necrotizing meningoencephalitis (NME), even in a heterozygous state, may cause a more severe PDM phenotype. The potential relevance of multiple candidate genes to the pathogenesis of PDM, including those implicated with bone homeostasis, the

differentiation of cartilage, inflammation and fibrotic scar tissue formation, should be confirmed in future studies.

*Keywords:* pug, ataxia, constrictive myelopathy, subarachnoid diverticula, caudal articular process, meningeal fibrosis, lymphohistiocytic inflammation, central nervous system, DLA class II

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# Beskrivning av fenotyp och genotyp för mopsmyelopati

## Sammanfattning

Ryggmärgssjukdom, myelopati, har många olika etiologier hos hund och kännetecknas av skillnader i kliniska sjukdomstecken. Mopsar med en myelopati i bröst-ländryggen (PDM) utmärker sig genom att uppvisa en tämligen likartad sjukdomsbild. Mopsar med PDM utvecklar i regel progressiva neurologiska bortfall med parapares och ataxi i frånvaro av uppenbar ryggsmärta och med en hög förekomst av inkontinens.

De övergripande målen med avhandlingen var att fastställa förekomsten av PDM, att via klinisk och patologisk undersökning karakterisera detta unika sjukdomstillstånd hos mops, samt att utreda bakomliggande orsaker till PDM. Betydelsen av medfödda kotanomalier (CVM) studerades med hjälp av avancerad bilddiagnostik. Immunsystemets potentiella relevans för utvecklandet av PDM undersöktes med hjälp av biomarkörer i blod och ryggmärgsvätska (CSF). Genetisk bedömning av nekrotiserande meningoencefalit (NME) DLA klass II (hundens MHC klass II) risk och molekylär genetiska studier genomfördes.

Sammanfattningsvis så var det vanligt att svenska mopsar rörde sig avvikande på ett sätt som indikerade ryggmärgssjukdom. Utifrån att ett samband mellan förekomst, eller typ, av CVM i bröst-ländryggen och neurologiska bortfall, parapares och ataxi, inte kunde påvisas behövdes andra eller ytterligare bakomliggande orsaker övervägas till PDM. Den kliniska sjukdomsbilden vid PDM motsvarades av karakteristiska förändringar vid neuropatologisk undersökning. Omfattande fokal fibros av meninger (ryggmärgshinnor) påverkade blodcirkulationen och hindrade CSF flödet med åtföljande destruktion av motsvarande ryggmärgssegment. Utöver dessa patologiska förändringar sågs tecken på inflammation i det centrala nervsystemet hos ett stort antal PDM-mopsar. Förekomst av anti-GFAP autoantikroppar i ryggmärgsvätskan på mopsar med PDM stöder ytterligare immunsystemets betydelse för uppkomst av PDM.

Den potentiella relevansen av ett flertal kandidatgener för patogenesen av PDM, inklusive de som är inblandade i att upprätthålla ben-homeostas, i

differentiering av brosk, samt i inflammation och fibros, ärrbildning, bör undersökas i framtida studier.

*Keywords:* mops, ataxi, konstriktiv myelopati, araknoidal divertikel, facettleader, meningeal fibrosis, lymfocytär inflammation, centrala nervsystemet, DLA klass II

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# Dedication

To all pugs

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## List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I. Rohdin C, Jäderlund KH, Ljungvall I, Lindblad-Toh K, Häggström J (2018). High prevalence of gait abnormalities in pugs. *Vet Rec.* 182(6):167
- II. Rohdin C, Häggström J, Ljungvall I, Nyman Lee H, De Decker S, Bertram S, Lindblad-Toh K, Hultin Jäderlund K (2018). Presence of thoracic and lumbar vertebral malformations in pugs with and without chronic neurological deficits. *The Vet J.* 241:24-30
- III. Rohdin C, Ljungvall I, Häggström J, Leijon A, Lindblad-Toh K, Matiasek K, Rosalti M, Wohlsein P, Hultin Jäderlund K (2020). Thoracolumbar meningeal fibrosis in pugs. *J Vet Intern Med.* 34(2):797-807
- IV. Rohdin C, Ljungvall I, Jäderlund KH, Anna Svensson, Lindblad-Toh K, Häggström J. Concentrations of GFAP, anti-GFAP autoantibodies and genetic characterization of NME susceptibility in pug dog myelopathy (manuscript)
- V. Brander G, Rohdin C, Bianchi M, Andersson G, Ljungvall I, Hultin Jäderlund K, Häggström J, Bergvall K, Hedhammar Å, Lindblad-Toh K, Tengvall K. Multiple genetic loci associated with pug dog thoracolumbar myelopathy (submitted)

Paper I-III are reproduced with the permission of the publisher

The contribution of Cecilia Rohdin to the papers included in this thesis was as follows:

- I. Took major part in planning the study, performed the data analysis, interpreted results together with supervisors and had the main responsibility for writing the manuscript.
- II. Took major part in planning the study, performed computed tomography studies, participated in data analysis, interpreted results together with colleagues (veterinarians) and supervisors and had the main responsibility for writing the manuscript.
- III. Took major part in planning the study, performed the diagnostic work-up, participated in the autopsies and in the histopathological evaluation, interpreted results together with colleagues and supervisors and had the main responsibility for writing the manuscript.
- IV. Took major part in planning the study, collected blood and cerebrospinal fluids, participated in data analysis, interpreted results together with supervisors and had the main responsibility for writing the manuscript.
- V. Took part in planning the study, performed phenotypic characterization of dogs, collected blood, interpreted results together with colleagues and supervisors and took part in writing the manuscript.



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## Abbreviations

BBB	blood brain barrier
BOAS	brachycephalic obstructive airway syndrome
CAP	caudal articular process
CNS	central nervous system
CSF	cerebrospinal fluid
CM	constrictive myelopathy
CVM	congenital vertebral malformation
CT	computed tomography
FSCP	focal spinal cord pathology
GFAP	glial fibrillary acidic protein
IVD	intervertebral disc
IVDH	intervertebral disc herniation
IVDD	intervertebral disc disease
IQR	interquartile range
LMN	lower motor neuron
MRI	magnetic resonance imaging
NME	necrotizing meningoencephalitis
NSAID	non-steroidal anti-inflammatory drug
PDE	pug dog encephalitis
PDM	pug dog myelopathy
PNS	peripheral nervous system
SAD	subarachnoid diverticula
SLU	Swedish University of Agricultural Sciences
SD	standard deviation
UMN	upper motor neuron



# 1. Introduction

This section includes the background information, provides the foundation and contributes the necessary context for the work of this thesis. This is where we start our journey from ‘wobbly pugs’ to Pug Dog Myelopathy (PDM).



‘The pug emperor’ by Sean Lewis (around year 2010)

Used with permission from the artist

## 1.1 History of the pug

The pug is one of the most ancient breeds of dogs. Originally bred as lap dogs for Chinese emperors, the pug was imported to Europe by Dutch traders in the 16-17th century. The personality of the pug soon made them popular among aristocracy and royalties. According to the legend, Napoleon's wife Joséphine owned a pug called 'Fortune' and Marie Antoinette a pug called 'Mops'. Later research however found that 'Mops' actually never existed. Whether the pug resting in Princess Ekaterina Dmitrievna Golitsyna's lap, adorning the front of this dissertation, was real or not is unknown. However, the portrait by Louis Michael van Loo, hanging at the famous Pushkin museum in Moscow, illustrates how the pug, through history, has been under aristocratic and royal patronage.

In modern times, the status of the pug is maintained by actors and influencers with some pugs having their own social media accounts with millions of followers (e.g. Doug The Pug. "Posts". *Instagram*, 2022, [www.instagram.com/itsdougthepug/](http://www.instagram.com/itsdougthepug/)).

Although it has been shown that the infant face of pugs and similar breeds is attractive to us humans (Archer & Monton 2011) their popularity suggests these dogs appeal to people in ways beyond their looks.

*'As a house dog the pug admits no superior  
I certainly claim that there is no breed that can compete  
with the pug-dog in question of general intelligence,  
especially in understanding the moods of his owner and  
accommodating himself to them- for either a tramp or a  
siesta in front of the fire. He is ready when you are'  
(Swainston-Goodger 2013)*

Although the pug has maintained its attractive character over the centuries, breeding has changed the breed's anatomical features. The distinctive form of the pug has been exaggerated with the head becoming bigger, the neck and back shorter and the face flatter (figure 1). The original pug was fawn coloured with a black face. The black coloured pug was not introduced until the late 19<sup>th</sup> century (Swainston-Goodger 2013).

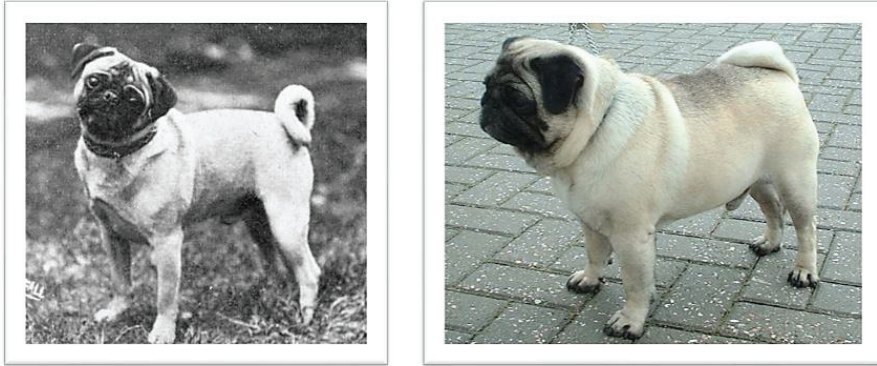


Figure 1.

To the left the historical pug (1915) and to the right the modern pug (2003)

[https://commons.wikimedia.org/wiki/File:Pug\\_from\\_1915.JPG](https://commons.wikimedia.org/wiki/File:Pug_from_1915.JPG)

[https://commons.wikimedia.org/wiki/File:Pug\\_600.jpg](https://commons.wikimedia.org/wiki/File:Pug_600.jpg)

Whilst the pug largely shares the same anatomic features as other flat-faced brachycephalic dog breeds; including the French and the English bulldog, they are in fact not very closely related (Parker *et al.* 2017). However, due to their similar constitution these breeds share several common health conditions (O'Neill *et al.* 2021; O'Neill *et al.* 2022) including a high prevalence of thoracolumbar vertebral malformations (Ryan *et al.* 2017; Lackmann *et al.* 2022).

## 1.2 Health of the pug

The pug is a breed with multiple health problems. It has even been suggested that the pug has diverged so substantially from mainstream dog breeds that they no longer can be considered typical dogs from a health perspective (Packer *et al.* 2019; O'Neill *et al.* 2022). Overweight/obesity, ophthalmological- and dermatological problems, were the most commonly reported health disorders of pugs under primary veterinary care, with spinal cord disorders only reported in 1.4 % (O'Neill *et al.* 2016). Conversely, pugs showed a seven-fold mortality risk compared to all breeds of dogs for

signs of spinal cord disease in data from Sweden's largest insurance company (AGRIA Pet Insurances, 2006-2011).

Pugs are considered a breed with extreme brachycephaly. The word brachycephaly derives from the Greek “*brakhu*” meaning short and “*cephalos*” meaning head (<https://en.wiktionary.org/wiki/brachycephaly>) (figure 2). Brachycephaly, with the short muzzle, predispose the pug to conformation-related respiratory disorders, the so called brachycephalic obstructive airway syndrome (BOAS) (Packer *et al.* 2015; Liu *et al.* 2017). The clinical consequence of BOAS includes dyspnoea, exercise intolerance, vomiting, regurgitation and sleep apnoea (Lodato & Hedlund 2012; Ekenstedt *et al.* 2020). In other breeds of dogs, brachycephaly is a known risk factor for the development of specific spinal disorders (Rusbridge *et al.* 2009; Mitchell *et al.* 2014).



Figure 2. The skull of a normocephalic dog (left) and of a pug, a breed with brachycephaly (right). Notice the different shapes with evident shortening and rounding of the skull of the pug. Photo by the author.

In addition to the well-known health problems associated with the pugs' brachycephalic constitution and BOAS, pugs also present with diseases affecting the central nervous system (CNS).

Necrotizing meningoencephalitis (NME), an encephalitis caused by an aberrant immune response directed against the CNS, occurs in some dogs including pugs (Cantile *et al.* 2001; von Praun *et al.* 2006; Timmann *et al.* 2007; Higgins *et al.* 2008; Talarico & Schatzberg 2010; Cooper *et al.* 2014; Flegel 2017). Necrotizing meningoencephalitis in the pug is also called pug



dog encephalitis (PDE) (Kobayashi *et al.* 1994; Kuwabara *et al.* 1998; Flegel *et al.* 2008; Levine *et al.* 2008; Greer *et al.* 2009). Although NME in pugs usually presents as an acute, fulminant disorder, with a poor prognosis, chronic presentations have been described (Cordy & Holliday 1989). The histopathology is characterized by lymphohistiocytic, plasmacytic, inflammation of the meninges and brain and cerebrocortical necrosis with or without cavitations, in the absence of detectable infectious agents (Cordy & Holliday 1989; Kuwabara *et al.* 1998; Flegel *et al.* 2008; Uchida *et al.* 2016). The potential diagnostic usefulness of biomarkers in cerebrospinal fluid (CSF) and serum, in pugs with NME has been reported (Uchida *et al.* 1999; Shibuya *et al.* 2007; Toda *et al.* 2007; Matsuki *et al.* 2009; Miyake *et al.* 2013). Genetic studies have identified risk loci for NME and commercial testing is available (Greer *et al.* 2010; Barber *et al.* 2011; Pedersen *et al.* 2011; Safra *et al.* 2011; Schrauwen *et al.* 2014).

In 2013 Fischer (Fisher *et al.* 2013) reported a condition not previously described or documented in pugs. Affected pugs (n=11) presented with a history of nonpainful, chronic, progression of ataxia and paraparesis, but with intact nociception. Urinary and faecal incontinence were common coexisting signs in affected dogs. Based on imaging and histopathological findings, the clinical signs were attributed to constriction of meningeal fibrous tissue. The excessive fibrous tissue was considered to develop as a consequence of chronic instability of the vertebral column secondary to the presence of thoracolumbar caudal articular process (CAP) malformations. The retrospective study by Fisher, of pugs with constriction of fibrous tissue and thoracolumbar CAP malformation, included dogs from two referral institutions in the USA, spanning over a 16 year period proposing the condition to be uncommon, even rare (Fisher *et al.* 2013).

However, Fischer's study also included pugs with CAP malformations without neurological signs seen, suggesting that the presence of aplastic or hypoplastic CAPs in the thoracolumbar region did not always cause clinical signs. Clinical signs associated with CAP malformations had previously only rarely been described in dogs other than pugs (Nykamp *et al.* 2001; McDonnell *et al.* 2003; Penderis *et al.* 2005).

### 1.3 The anatomy of the spine



Figure 3. Three-dimensional (3D) computed tomography of the skull, the vertebral column and pelvis of a pug. Photo credit Tove Nielsen

The spine includes the vertebral column, the spinal cord, the meninges, the cerebrospinal fluid (CSF) and the associated vasculature. The vertebral column is composed of the vertebrae, the intervertebral discs (IVD) and the articular processes. The vertebral column supports the head and provides attachment for muscles (Evans & de Lahunta 2013b; Bouma 2016). The spinal cord, a cylindrical extension of the CNS, essentially forms a highway between the brain and the body (Thomson & Hahn 2012). The main function of the spinal cord is to receive and distribute information to the peripheral nervous system (PNS) and to relay sensory information from the body to the brain. The spinal cord is a delicate structure with the consistency of toothpaste and is therefore in large need of protection. This protection is provided by the vertebrae, the meninges and the CSF.

### 1.3.1 The vertebrae

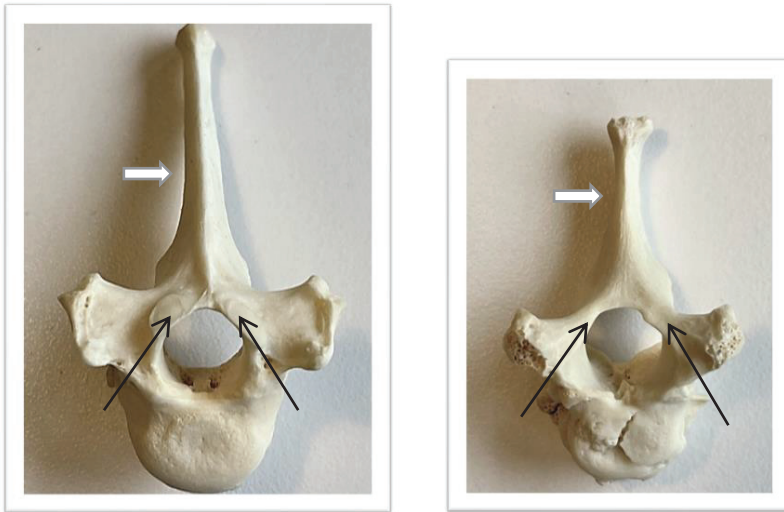


Figure 4. The shape of the cranial aspect of the 8<sup>th</sup> thoracic vertebra of a large breed normocephalic dog (left) and a pug (right). The thoracic vertebral bodies are small with large spinous processes (white arrow). Thin black arrows point at the cranial articular processes of different size in the large breed dog and at the corresponding location of the pug vertebra (without recognizable cranial articular processes). It is the author's experience that the surface of the vertebral body of pugs may present with the "fracture-like" appearance shown in the figure. Photo by the author.

The vertebrae are bony structures interconnected by the cartilaginous intervertebral discs and the facet joints. The facet joint is formed by the articular processes connecting the vertebrae (Evans & de Lahunta 2013b; Bouma 2016). In addition to the bony structures, ligament and muscles add further stability and support to the vertebral column and govern body movement. Each vertebra contains an opening, a hole, and together the vertebrae form a tube where the spinal cord is contained (Evans & de Lahunta 2013b).

There has been great conservation of the vertebral column formula through evolution, with the dog having seven cervical, thirteen thoracic, seven lumbar, three fused sacral vertebrae and a number of coccygeal vertebrae (Galis *et al.* 2014). The vertebrae along the vertebral column

come in different shapes and their unique features highly depend on their position in the spine (Evans & de Lahunta 2013b) (Figure 4).

Bone is a dynamic tissue and continuous remodeling of the bone is required throughout life to preserve the vertebrae's (and all skeletal structure's) ability to provide mechanical support and to protect the spinal cord. This process of bone remodeling, where old and damaged bone is removed by osteoclasts and replaced by new bone formation by osteoblasts, is an essential mechanism for providing bone homeostasis (Clarke 2008; Feng & McDonald 2011).

### 1.3.2 The intervertebral disc

The intervertebral discs (IVD) are fibrocartilaginous structures located between the vertebral bodies providing mechanical support and allowing movement of the spine. The IVD is composed of an outer annulus fibrosus, a central nucleus pulposus and cartilaginous endplates (Bergknut *et al.* 2013). The major route of nutrient supply into the avascular disc is via capillary beds of the endplates and the peripheral annulus fibrosus (Maroudas *et al.* 1975; Holm *et al.* 1981; Urban *et al.* 2004; De Geer 2018). As a result, nutrients supplied by the capillaries have to penetrate the dense hyaline cartilage of the endplate before reaching the disc. Metabolic waste products are removed from the tissue by the reverse route (Urban *et al.* 2004).

Degeneration of the IVD is a common phenomenon in dogs and occurs with normal aging. Disturbances to blood supply with the subsequent failure of nutrient supply is thought to be a cause of disc degeneration (Urban *et al.* 2004). During the process of intervertebral disc degeneration, the disc loses some of its stability and biomechanical properties, which can lead to intervertebral disc disease (IVDD) (Hansen 1952; Brisson 2010; Bergknut *et al.* 2013).

### 1.3.3 The articular processes

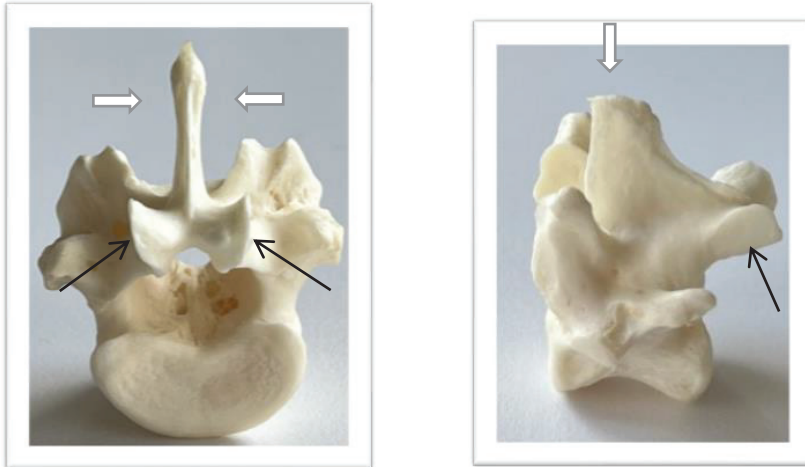


Figure 5. The shape of the caudal articular processes (CAPs) (black arrow) of the 11<sup>th</sup> thoracic vertebra of a large breed normocephalic dog. Caudal view (left) and lateral view (right). Whilst in most dogs CAPs are still prominent in this region, the facet joints begin to diminish in size cranial to the 10<sup>th</sup> or 11<sup>th</sup> thoracic vertebra. Notice the vertical spinous process (white arrow) representing the transition from the caudally directed spinous processes in the cranial thoracic region and the cranially directed spinous processes in the thoracic vertebrae caudal to the transition. Photo by the author.

The vertebrae are interconnected by articular processes that form synovial joints (facet joint) coated with cartilage. The paired articular processes are present at both the cranial and the caudal surface of the vertebra (Bouma 2016) (Figure 5). The shape and orientation of the facets help to guide spinal motion and show regional differences throughout the vertebral column (Bouma 2016). In the cranial thoracic spine the articular processes also play a role in weight bearing (Breit 2002). The articular processes, in addition to the intervertebral discs, belong to the structures contributing the greatest to the biomechanical stability of the thoracolumbar spine (Smith & Walter 1988; Penderis *et al.* 2005; Bouma 2016).

#### 1.3.4 The spinal cord

The spinal cord enables sensory and motor information to be communicated between the body and the brain. The spinal cord consists of central grey matter (primarily neuronal cell bodies and their synaptic connections) and peripheral white matter (primarily myelinated and unmyelinated axons). The white matter is divided into major bundles, called funiculi, which contain tracts, groups of nerve fibres, that carry sensory information dorsally and motor information ventrally in the spinal cord (de Lahunta *et al.* 2021b). The spinal cord can be divided into functional segments based on where the nerves that supplies the limbs branch off. The functional segments in the dog include the; C1-C5, C6-T2, T3-L3, L4-S3 and the Cd1-5 areas of the spinal cord (de Lahunta *et al.* 2021c). A lesion in any of the functional segments will present with a predictive loss of neurological function.

The spinal cord is supplied by spinal arterial branches arising from the vertebral artery and aorta. Within the vertebral canal, each branch divides into a smaller dorsal and larger ventral branch. The arterial branches need to perforate the dura mater and arachnoid meninges to enter the subarachnoid space (SAS), and supply the spinal cord via the pia mater (Evans & de Lahunta 2013a; Skerritt 2018c) (Figure 6). The spinal cord has an internal vertebral venous plexus located on the floor of the spinal canal, with paired vessels that diverge to drain into intervertebral veins (Skerritt 2018a).

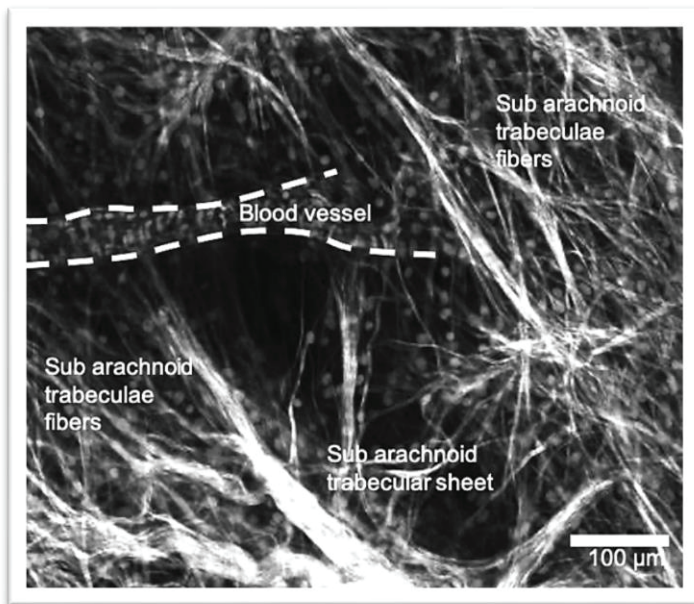


Figure 6. Scanning electron microscopy of the canine subarachnoid space (SAS). The structural components of the SAS consist of subarachnoid (SA) trabeculae fibres and subarachnoid membranes (sheets). SA trabecular fibres and sheets form fine spider-web structures that extend across the SAS from the arachnoid to the pia and enclose the small blood vessels travelling the SAS. Original magnification X 140. Photo credit Martin M. Mortazavi. DOI: [10.1016/j.wneu.2017.12.041](https://doi.org/10.1016/j.wneu.2017.12.041)

### 1.3.5 The meninges

In addition to the bony construction of the vertebral column, the delicate spinal cord is protected by the meninges and the CSF (Evans & de Lahunta 2013a; de Lahunta *et al.* 2021a). The meninges are the connective tissue coverings consisting of the dura- the arachnoid- and the pia mater. The outermost resistant dura forms a loose sheath around the spinal cord. The innermost pia mater is joined to the arachnoid membrane of the arachnoid mater by numerous arachnoid fibres and trabeculae, which traverse the subarachnoid space (Evans & de Lahunta 2013a; Mortazavi *et al.* 2018) (Figure 7). The pia mater and the arachnoid mater are together called the leptomeninges. The cranial aspect of the dura mater is circumferentially

attached to the base of the foramen magnum and to the floor of the vertebral canal of the first and second cervical vertebrae before it separates from the periosteum (Evans & de Lahunta 2013a). Unlike the cranial part in the skull, the meninges surrounding the spinal cord are not attached to the bony walls of the vertebral canal. The separation of the meninges from the periosteum at the foramen magnum therefore allows some freedom of the spinal cord within the vertebral canal (Evans & de Lahunta 2013a).

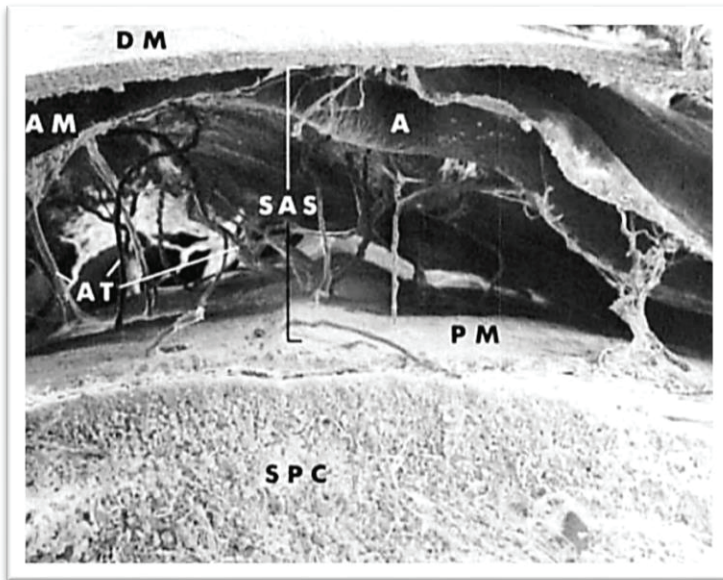


Figure 7. Scanning electron microscopy of the canine meninges. SPC; spinal cord, SAS; subarachnoid space, PM; pia mater, AM; arachnoid mater, AT; arachnoid trabeculae, DM; dura mater, A; artery. Original magnification X 140. Photo credit Martin M. Mortazavi. DOI: [10.1016/j.wneu.2017.12.041](https://doi.org/10.1016/j.wneu.2017.12.041)

### 1.3.6 The cerebrospinal fluid

Cerebrospinal fluid is derived from blood. Cerebrospinal fluid fills the ventricular system in the brain, the central canal in the spinal cord and surrounds the entire CNS in the subarachnoid space. The cerebrospinal fluid offers protection, shock absorption, allows for movement and



nourishes and removes waste products of the CNS (Skerritt 2018b; de Lahunta *et al.* 2021a). Although challenged, the traditional hypothesis is that the CSF is mainly formed by secretions from the choroid plexuses of the brain ventricles. It flows inside the ventricular cavities to enter the subarachnoid spaces, to be reabsorbed into venous sinuses across arachnoid villi (Chikly & Quaghebeur 2013; Atchley *et al.* 2022). Both the cardiac and the respiratory cycle drive CSF flow with forced inspiration identified as a dominant regulator of CSF dynamics (Dreha-Kulaczewski *et al.* 2015; Lloyd *et al.* 2020) With every cycle of forced inspiration, there is an increase in the venous outflow from the skull and an influx of CSF into the skull. Conversely, with every forced expiration there is a corresponding reduction in venous outflow and an efflux of CSF from the skull (Dreha-Kulaczewski *et al.* 2015)

## 1.4 The development of the vertebrae

During development, mesenchymal cells differentiate into a cartilage model for each vertebra. Bone then gradually replaces the cartilage through enchondral ossification (Angevine 1973; Bailey 1975; Kaplan *et al.* 2005). The growth of the vertebra takes place at the ossification centres in each vertebra (Angevine 1973; Bailey 1975). There are three primary ossification centres, one on the vertebral body and one on each side of the vertebral arch (Bailey 1975). The secondary ossification centres include the cranial and caudal epiphysis, the transverse processes, the dorsal spinous process and the cranial and the caudal articular processes (Bailey 1975).

## 1.5 Vertebral malformations

Vertebral malformations occur frequently in the canine vertebral column and in the majority of cases they are considered incidental findings (Morgan 1968; Bailey & Morgan 1992; Westworth & Sturges 2010). Vertebral malformations are generally considered congenital (CVM). However, there is little evidence of CVMs actually being present at birth in dogs and they could therefore, at least in part, arise during postnatal development (De Rycke *et al.* 2016). Most clinically significant congenital

anomalies result from abnormalities at one of the three primary ossification centres. Abnormalities arising from the large number of secondary ossification centres are less frequently described in the veterinary literature (Penderis *et al.* 2005).

Congenital vertebral malformations in dogs as well as in people are aetiologically heterogeneous with poorly understood environmental and genetic factors; contributing to their occurrence (Giampietro *et al.* 2003; Giampietro *et al.* 2013; Schlensker & Distl 2013; Schlensker & Distl 2016). Particularly, maternal exposure to various environmental conditions during gestation including hypoxia and hyperthermia, have in people been implicated to play significant roles in the development of CVMs (Hensinger 2009; Sparrow *et al.* 2012; Hou *et al.* 2018).

### 1.5.1 Hemivertebrae



Figure 8. Computed tomography image of the thoracic vertebral column of a pug (sagittal view). Hemivertebra of the 7<sup>th</sup> thoracic vertebra (white arrow) with secondary kyphosis (spinal curvature abnormality). Illustration by the author.

Hemivertebrae are considered defects of formation and are most frequent in the midthoracic region of dogs (Done *et al.* 1975). They can affect single or multiple vertebrae in the same dog and can be associated with spinal curvature abnormalities, such as kyphosis and scoliosis (Guevar *et al.* 2014; Dewey *et al.* 2016).

Although in general considered incidental findings, hemivertebrae have the potential to cause clinical signs of progressive spinal cord dysfunction. When clinical signs occur they are considered to be a consequence of progressive vertebral canal stenosis and vertebral column instability (Aikawa *et al.* 2007; Moissonnier *et al.* 2011; Schlensker & Distl 2013; Charalambous *et al.* 2014; Schlensker & Distl 2016; Ryan *et al.* 2017; Wyatt *et al.* 2018).

The angular magnitude of a spinal deformity is usually quantified using the Cobb angle. In dogs a kyphotic angle of  $>35^\circ$  has been shown to be highly associated with the presence of neurological deficits (Guevar *et al.* 2014; Brown *et al.* 2021; Lackmann *et al.* 2022). The origin of hemivertebrae is not known but unilateral congenital absence of vascularization has been suggested (Bailey 1975; Done *et al.* 1975). A hereditary component to the development of hemivertebrae is suggested based on its high prevalence in certain breeds (Done *et al.* 1975; Kramer *et al.* 1982; Schlensker & Distl 2013; White 2013; Schlensker & Distl 2016).

### 1.5.2 Hypo-and aplasia of the caudal articular processes

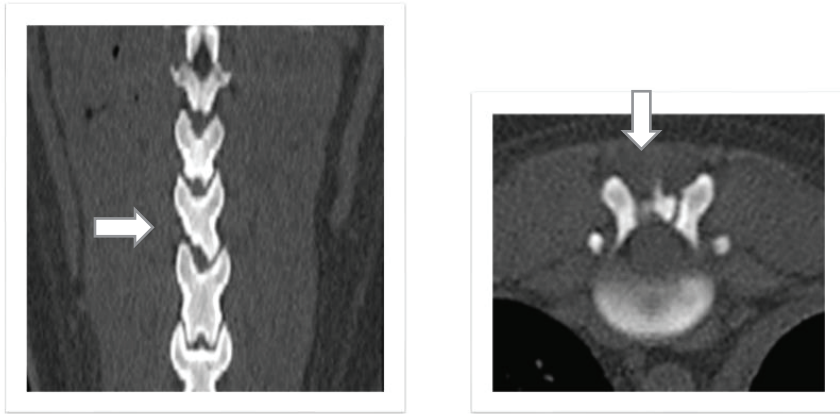


Figure 9. Computed tomography images of the thoracic vertebral column of a pug (dorsal view to the left and transverse view to the right). Unilateral aplasia of the CAPs of the 12<sup>th</sup> thoracic vertebra (white arrow) and bilateral hypoplasia of the CAPs of the 11<sup>th</sup> thoracic vertebrae. Illustration by the author.

Articular process dysplasias encompass a spectrum of articular process anomalies and include absent (aplasia); incomplete (hypoplasia) as well as enlarged (hyperplasia) process formation (Bouma 2016).

Hypo- and aplasia of the CAPs are common and present as an incidental finding in almost all small breed dogs (Morgan 1968; Breit 2002). It has been suggested that hypo-and aplasia of the CAPs in people and dogs develop as a consequence of abnormal development of the secondary ossification centres, potentially associated with lack of vascularisation (Rowe & Roche 1953; Ikeda *et al.* 1992; Shim & Oh 2008; Bouma 2016). Although facet aplasia could be related to congenital factors it has also been suggested that biomechanical factors may play a role in their development (Breit 2002).

### 1.5.3 Transitional vertebrae

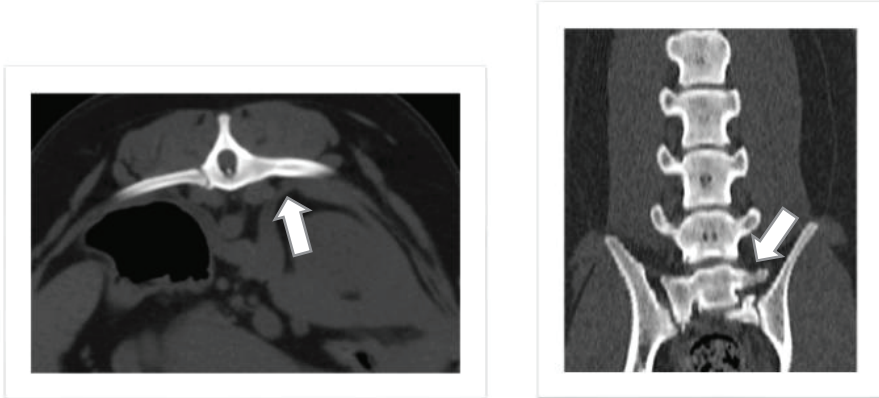


Figure 10. Computed tomography images of the thoracolumbar (left) and the lumbosacral (right) area of the vertebral column of pugs. In the transverse view of the thoracolumbar area a rib is present on the left and a process resembling a lumbar process is present on the right (white arrow). In the dorsal view of the lumbosacral area a lumbar transverse process is present on the right (white arrow) and the left transverse process is superimposed on the ileum resembling a sacral vertebra. Illustration by the author.

Transitional vertebrae at any region junction are characterized by features retained from two adjacent regions in the vertebral column (Brocal *et al.* 2018). In dogs the lumbosacral transitional vertebrae predispose to premature degeneration of the lumbosacral disc and a genetic predisposition has been proposed (Morgan 1999; Damur-Djuric *et al.* 2006; Gong *et al.* 2020; Gluding *et al.* 2021). The clinical consequence of cervicothoracic and thoracolumbar transitional vertebrae in dogs needs to be explored.

#### 1.5.4 Spinal bifida occulta

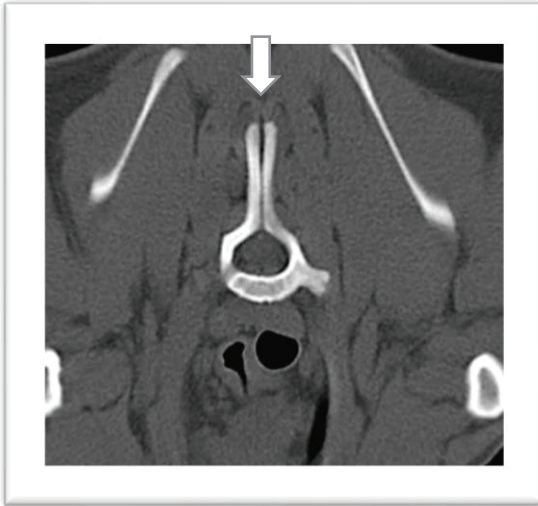


Figure 11. Computed tomography image of the thoracic vertebral column of a pug (transverse view). Spinal bifida occulta with cleft formation of the spinous process of the 1<sup>st</sup> thoracic vertebra (white arrow). Illustration by the author.

Spina bifida refers to a group of developmental defects characterized by a failure of fusion of the vertebral arches. This can, but does not need to, include protrusion of the spinal cord. Spina bifida occulta refers to the closed and mild form of spinal dysraphism (Wilson *et al.* 1979). In dogs, clinically relevant spina bifida has been reported in the lumbar spinal cord and English bulldogs are potentially predisposed (Wilson *et al.* 1979). In humans, spina bifida usually involves the transitional levels of the spine, most commonly at the lumbosacral transition (Mellado *et al.* 2011).

#### 1.5.5 Block vertebrae

Block vertebrae develops as a consequence of failure of segmentation during embryogenesis and result from partial or complete fusion of two or more vertebrae (Westworth & Sturges 2010). Block vertebrae are commonly not associated with neurological deficits in dogs (Westworth & Sturges 2010).

## 1.6 Central nervous system inflammation

Inflammation is part of the body's normal healing process (White & Mantovani 2013). The body continuously responds to tissue damage and abnormal load, where inflammation and fibrosis is part of the normal physiological repair process. For balanced tissue repair, and regeneration, the level of immune activation after injury needs to be optimized (Raziyeva *et al.* 2021). Hence, the distinction between physiology and pathology is defined by matters of degree, enough repair can heal and restore tissue integrity and function, whereas excessive repair may lead to tissue dysfunction (White & Mantovani 2013).

In the CNS, resident innate immune cells, including microglia, CNS macrophages and astrocytes, exhibit neuroinflammatory potential following activation (Perry & Teeling 2013; Wohleb & Godbout 2013). In the face of inflammatory or pathological insults, resident immune cells constitute the first line of defence against pathogens. Resident immune cells also regulate the adaptive arms of immune responses by producing cytokines that potentiate neuroinflammation (Wohleb & Godbout 2013). Cytokines are small, cell-signalling, protein molecules that enable communication between immune cells. Cytokines help direct immune responses by recruitment of peripheral immune cells to sites of inflammation (Wohleb & Godbout 2013). Dysregulation of these responses contributes to many CNS disorders (Yin *et al.* 2017).

Inflammatory disease affecting the CNS in dogs can be due to infectious or immune-mediated causes. Today, immune-mediated disease processes are regarded as more common, compared to infectious conditions, causes of inflammatory CNS disease in dogs (Tipold 1995; Muñana 1996; Tipold & Stein 2010; Barber *et al.* 2012; Hoon-Hanks *et al.* 2018; Gonçalves *et al.* 2021; Elbert *et al.* 2022). The inflammatory infiltrate in the CNS of dogs with NME is commonly described as 'lymphohistiocytic', expressing the different cell-types involved in the inflammation (Park *et al.* 2012). Lymphocytes and plasmacells are components of the adaptive immune system recruited to the CNS from the peripheral circulation (Weller *et al.* 1996). A histiocyte may be explained as a tissue macrophage (digest pathogens) or a dendritic cell (antigen presenting cell). Consequently, the term 'histiocyte', embraces cells of both the monocyte macrophage and the dendritic cell system (Cline 1994). Inflammation of the spinal cord parenchyma (myelitis) and the surrounding meninges (meningomyelitis)

has mostly been reported in combination with inflammatory brain disease (encephalitis) (Tipold 1995; Griffin *et al.* 2008; Tipold & Stein 2010; Cornelis *et al.* 2019) (figure 12).

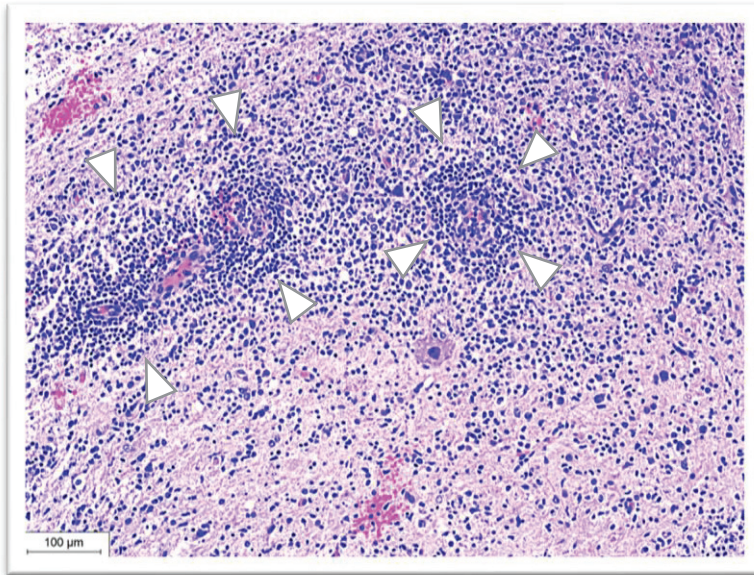


Figure 12. Lymphohistiocytic inflammation as indicated by massive perivascular cuffs (white arrowheads) and infiltration of predominantly lymphocytes and plasmacells into the spinal cord at the level of the second lumbar vertebra in a 2.5 year old male pug, presenting with neurological deficits indicating a focal thoracolumbar myelopathy (this pug is not included in the material for this thesis). Hematoxylin and eosin (H&E); Photo credit Veronica Janson.

## 1.7 Myelopathies in dogs

The word myelopathy is composed of the Greek word *myelon* for spinal cord and *pathos* for disease or injury (<https://en.wiktionary.org/wiki/myelon>). Myelopathies in dogs are common and cause motor (paresis) and sensory (ataxia) dysfunction (de Lahunta *et al.* 2021c). Depending on the location of the myelopathy along the spine (the functional segment involved), affected dogs will present with



involvement of the pelvic limbs or of all four limbs (de Lahunta *et al.* 2021e). Myelopathies have numerous different aetiologies, all characterised by their differences in clinical characteristics (Cardy *et al.* 2015). Patients with myelopathies therefore constitute a heterogeneous group, all presenting with unique individual combinations of age of onset, clinical course, time of progression, symmetry or asymmetry of neurological deficits and presence or absence of pain.

## 1.8 Myelopathy in pugs

Shortly following the publication on constrictive myelopathy (CM) by Fisher in early 2013 (Fisher *et al.* 2013), several other reports describing pugs with signs of a thoracolumbar myelopathy of similar onset, progression and absence of signs of pain appeared in the literature (Flegel *et al.* 2013; Bismuth *et al.* 2014; Full 2014; Mauler *et al.* 2014). In these reports, the common clinical phenotype was described to be caused by different disorders including subarachnoid diverticulae (SAD) (Flegel *et al.* 2013; Bismuth *et al.* 2014; Mauler *et al.* 2014) and intervertebral disc disease (IVDD) (Flegel *et al.* 2013; Bismuth *et al.* 2014; Full 2014) associated or not with presence of CVMs (Flegel *et al.* 2013; Full 2014).

Pugs with this characteristic, clinical phenotype were called ‘wobbly-pugs’ and were often seen in clinical practice in Sweden and elsewhere. Swedish insurance data (AGRIA insurance data statistics: 2006–2011), data from the orthopaedic foundation of America (<https://ofa.org/chic-programs/browse-by-breed/?breed=PG>) and the number of clicks on ‘wobbly pugs’ on the internet, also suggested that ‘wobbly’ pugs were common and that they presented worldwide.

The breed specific appearance and familiar clustering of pugs presenting with signs of a thoracolumbar myelopathy, further suggested that genetic factors influenced the development of this condition. Although a genetic investigation had been undertaken (Bagheri *et al.* 2015), no genes associated with the disorder had been identified.

In addition to pug dogs presenting with a myelopathy involving the T3-L3 spinal cord segment, occasional pugs presented with a myelopathy of the cervical area. Dogs with a cervical myelopathy showed ataxia, characterized by a dysmetric gait with hypoflexion and overreaching,

commonly more pronounced in the thoracic limbs and tetraparesis, suggesting involvement the C1-C5 spinal cord segment (Foss & Berry 2009; Rohdin *et al.* 2014). Sometimes the neurological signs of a cervical myelopathy in pugs would go unrecognized by the owners. Instead the owners' complaint consisted of wearing of the nails and the skin on the dorsal aspect of the paws of the thoracic limbs (Rohdin *et al.* 2014).

The work of this thesis has mainly focused on the pug presenting with a thoracolumbar (T3-L3) myelopathy. Consequently, the main traveller on this journey is the pug presenting with progressive ataxia, paraparesis and incontinence- 'the wobbly pug'.

## 1.9 Medical terminology for the wobbly pug

The characteristic clinical phenotype of pugs with a thoracolumbar myelopathy, the ‘wobbly’ pugs, has been given many different names. In this thesis, the term pug dog myelopathy (PDM) will be used to describe the clinical entity (figure 13).



Figure 13. The ‘working-name’ of the clinical disease that pugs with progressive long-lasting thoracolumbar myelopathy present with has changed during the work of this thesis. The photo of the pug is used with permission from Shutterstock. Illustration by the author.



## 2. Aims of the thesis

The overall aims of the thesis were to investigate the prevalence of PDM, to characterize the disorder clinically and pathologically and to explore underlying aetiologies through diagnostic imaging-, pathological-, biomarker studies and genetic investigation.

The specific aims were to:

- Define the prevalence of gait abnormalities in a cohort of Swedish pugs.
- Investigate associated conditions of abnormal gait, including other health disorders prevalent in the breed.
- Determine the clinical importance of congenital vertebral malformations for the development of PDM.
- Identify the clinical and neuropathological characteristics of PDM
- Analyse a potential contribution of an autoimmune aetiology to the development of PDM.
- Investigate the molecular genetic background for PDM.



### 3. Materials and methods

This section includes a brief description of materials and methods used in the separate papers included in the thesis. A more detailed description is provided in the respective papers.



Painting by James Joseph Tissot (c.1870).

Used with permission from Bridgeman Images.

### 3.1 Questionnaire (paper I)

The prevalence of gait abnormalities in a cohort of Swedish pugs was determined using an owner-based questionnaire targeting signs of gait abnormality and video footage showing the dogs' gait. The survey also included questions regarding the general health of the pug. In case the dog was no longer alive, the owners were asked to provide information regarding the reason/cause of death. An online version of the questionnaire was distributed in 2015 and a paper based version of the questionnaire in 2016 to all owners of a Swedish pug of 1, 5 or 8 years of age registered in the Swedish Kennel Club. The paper-based version included one additional question regarding the preferred sitting position of the pug. Besides that one single question, the versions were identical. The contact details to the pug owners were obtained from the Swedish Kennel Club.

### 3.2 Dogs (paper II-V)

The studies included in this thesis were approved by the Local Ethical Committee in Uppsala and in Stockholm, Sweden. Informed owner consent was obtained from all owners.

Client owned dogs, with and without neurological deficits, were prospectively recruited at the University Animal Hospital (UDS) at the Swedish University of Agricultural Sciences (SLU), Uppsala and at Anicura Albano Small Animal Hospital, Danderyd, Sweden (paper II, III, IV and V). Pugs with neurological deficits were retrospectively recruited at the Norwegian University of Life Sciences, Norway (paper II). Pugs without neurological deficits were retrospectively recruited from the Royal Veterinary College (RVC) at the University of London, United Kingdom (paper II). Additionally, the genetic study (paper V) included phenotyped dogs that had donated blood to the biobank at SLU, Uppsala, Sweden.

This thesis included 167 dogs; 79 pugs with PDM, 67 control pugs, 17 dogs of breeds other than pugs being investigated for various neurological diseases (neuro controls) and four pugs previously diagnosed with NME (NME pugs). The number of pugs with PDM and pugs without neurological deficits (control pugs) included in the different studies (papers II-V) are summarized in figure 14.



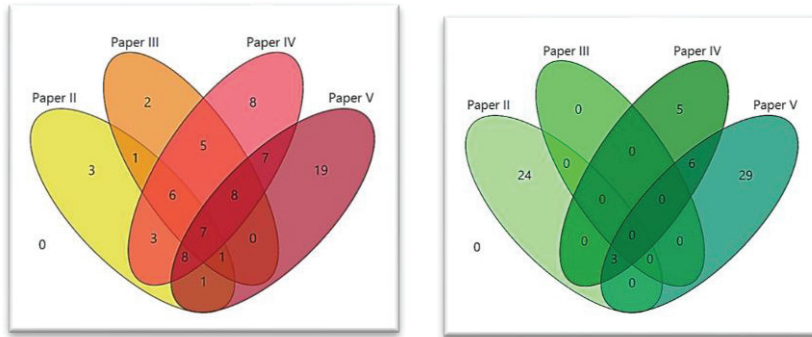


Figure 14. Venn diagram showing numbers of PDM pugs (n = 79) (left) and control pugs (n = 67) (right) included in paper II, III, IV and V

### 3.2.1 Criteria for inclusion of dogs (paper I-V)

#### Paper I

The study population consisted of pugs registered in the Swedish Kennel Club that were 1, 5 or 8 years of age year 2015 and 2016 and whose owners accepted to participate in the questionnaire based study.

#### Paper II –V

Affected pugs were included if they presented with a history of one month or more of a gait abnormality described by the owner as incoordination or weakness affecting both pelvic limbs. To be included, a neurological examination also had to confirm pelvic limb ataxia and paraparesis suggestive of a myelopathy localized to the thoracolumbar spinal cord. Onset of clinical signs of PDM was determined from the owners' first recognition of incoordination or weakness affecting the pelvic limbs.

Control pugs (paper II, IV and V) were included if they presented without signs of ataxia, paresis or incontinence. The clinical phenotype was verified either by physical examination (conducted by a board certified neurologist for paper II, IV and V or by a board certified surgeon for paper II), or by having the owner confirming that the pug had not shown any signs of incoordination or weakness affecting the pelvic limbs, had not shown worn nails or affectation to the skin on the dorsum aspect of the paws of the pelvic limbs and had been fully continent (paper V). Control

pugs had to be at least 6 years old to be included in the biomarker study (paper IV) and 8 years old to be included in the genetic study (paper V).

### 3.3 Methods of examination

#### 3.3.1 Gait analysis (paper I)

The investigators used a questionnaire, targeting immediate and indirect signs of abnormal gait. A normal gait was defined as coordinated walking or trotting without signs of pacing (Hildebrand 1968) with no visible or audible lameness or paresis and with no scuffing of the nails and/or skin on the dorsum of the paws.

Pugs assessed by their owners to have an abnormal gait and pugs reported by their owners as having a normal gait, but where an unsound gait was indicated later in the questionnaire (by their owners' response to questions about wearing of nails and/or paws) were assessed by the authors as having an abnormal gait.

In addition to being asked to complete the questionnaire, the owners of all pugs were encouraged to send video footage of their pug. Two authors (board certified neurologists) evaluated all videos independently and classified the dogs as having normal or abnormal gait. Results from the two neurologists were compared and in cases of disagreement the videos were examined again in normal and slow motion before arriving at a final, joint decision.

#### 3.3.2 Physical and neurological examination

The physical and neurological examinations of all affected pugs included in paper II-V were performed by a board certified neurologist. The neurological examination was performed as previously described (de Lahunta *et al.* 2021d).

#### 3.3.3 Presence of congenital vertebral malformations (paper II)

Advanced imaging was carried out before or immediately after death in order to associate any potential focal spinal cord pathology (FSCP) with the

presence of vertebral column lesions, as well as to guide the histopathologic investigation of the spinal cord. The focal spinal cord pathology was confirmed by findings at either MRI (n=15), pathology and MRI (n=14) or pathology (n=1). Focal spinal cord pathology was defined as any focal compressive spinal cord lesion and/or focal intramedullary T2W hyperintensity on MRI, confirmed in sagittal and transversal plane, and/or as an extensive focal loss of nervous tissue on histopathology.

Computed tomography (CT) of the pugs examined in Sweden was performed under general anaesthesia or immediately post-mortem with a dual slice CT scanner (Siemens, Somatom); 2 mm slice thickness (n=32) or a 64 slice CT scanner (Siemens, Somatom) 2 mm slice thickness (n=1). The CT exams performed in the UK and Norway were performed under sedation or anaesthesia using a 16 slice helical CT scanner (PQ 500, GE healthcare) 2 mm slice thickness (n=3) and a 128 slice CT scanner (Siemens, Somatom) 0.625 mm slice thickness (n=21) respectively.

After completion of the axial CT study, sagittal and dorsal reconstructions were made. The UK studies were reconstructed using a soft tissue algorithm while the pugs examined in Sweden and Norway were reconstructed in a soft tissue and a bone algorithm with medium and high frequency reconstruction algorithms. A standard image archiving and communication system software (Osirix foundation, V.5.5.2) was used to evaluate the images.

For each CT study, the number of thoracic and lumbar vertebrae was recorded and each vertebra was assessed for the presence of hemivertebra and associated kyphosis (Gutierrez-Quintana *et al.* 2014), hypo- and aplasia of the CAPs (Penderis *et al.* 2005), transitional vertebrae (Westworth & Sturges 2010), spina bifida (Westworth & Sturges 2010) and block vertebrae (Westworth & Sturges 2010). When the hemivertebrae caused kyphosis the Cobb angle was calculated (Guevar *et al.* 2014). In addition, the presence of spondylosis or any other acquired vertebral column abnormality, e.g. IVDD, was described.

The magnetic resonance imaging (MRI) studies were acquired using either a magnet operating at 0.2 Tesla (Esaote Vet-MR Grande, Esaote, Genoa, Italy) (n=20), or at 1.5 Tesla (Siemens, Magnetom Essenza, Siemens Munich, Germany) (n=9). At a minimum, the MRI study included sagittal T1- and T2-weighted images of the entire thoracolumbar spine and transverse T1- and T2-weighted images of the area of interest.

### 3.3.4 Neuropathological characterization (paper III)

Euthanasia was performed when requested by the owners (no animals were sacrificed because of this study). This allowed inclusion of pugs with different degrees of neurological deficits. The dogs were included in the study following informed consent by the owners.

Gross pathology was performed at the Section of Pathology, SLU or at the National Veterinary Institute, Uppsala, Sweden. The entire CNS and samples from the peripheral neuromuscular system (brachial plexus, triceps muscle, sciatic nerve, cranial tibial and gastrocnemius muscle) were carefully sampled and fixed in 10% neutral buffered formalin for transport to a board-certified pathologist.

After the spinal cord had been evacuated, the vertebral column was carefully examined and cut sagittally. Midsagittal sections were performed of thoracolumbar vertebrae and intervertebral discs with macroscopically degenerative changes and/or overt intervertebral disc herniations (IVDHs) on macromorphology, and/or where compression of the spinal cord had been demonstrated on MRI. Discs adjacent to IVDHs were also included. The sections were placed in 10% neutral buffered formalin and decalcified using 20% formic acid (Kristensens solution).

Histopathological examination of the CNS and samples from the neuromuscular system were performed at the Department of Pathology, University of Veterinary Medicine, Hannover, or at the Section of Clinical and Comparative Neuropathology, Ludwig-Maximilians-Universität (LMU), Munich, Germany. After fixation, specimens of various anatomical sites of the brain, spinal cord and peripheral nerves were processed for routine histopathology, embedded in paraffin wax and sectioned at 3-4  $\mu\text{m}$ . Tissues were stained using routine hematoxylin and eosin (H&E), and examined by light microscopy.

Histopathology of the vertebral column was performed at the Section of Pathology, SLU. Decalcified tissue was embedded using paraffin wax and processed to 4-5  $\mu\text{m}$  sections. Tissues were routinely stained with H&E. The overall morphology and integrity of the intervertebral discs and adjacent vertebrae, in addition to other apparent features, were assessed and recorded.

### 3.3.5 Biomarkers in blood and cerebrospinal fluid (paper IV)

In addition to PDM and control pugs, this study also included neuro controls and NME pugs (see page 46).

Blood and CSF were collected in all PDM pugs, control pugs and neuro control dogs, at the same sampling occasion for later analysis of GFAP, anti-GFAP autoantibodies and NME risk genotype assessment. Blood and CSF from previously sampled pugs with NME were also included in the study. Blood samples from the cephalic vein were collected in 5 ml EDTA tubes (Vacuette vacuum tubes EDTA K2, Swevet, Sweden) for the genetic testing and in 5 ml serum tubes (Vacuette vacuum tubes, serum Sep Clot Activator, Swevet, Sweden) for the GFAP-analyses. Cerebrospinal fluid samples (CSF) were obtained and collected in 3.5 ml plastic tubes (Ellerman tubes, Swevet, Sweden) in association with anaesthesia or euthanasia for analysis of glial fibrillary acidic protein (GFAP) and anti-GFAP autoantibodies. In general, the CSF was collected from the lumbar area in all dogs that underwent routine diagnostic work-up and from the cerebellomedullary cistern in all the dogs that underwent euthanasia. After collection, whole blood, serum after centrifugation and CSF was immediately frozen and stored in  $-70^{\circ}\text{C}$  until analysis.

GFAP in serum and CSF and anti-GFAP autoantibodies in CSF were analyzed by ELISA, performed at SLU, as previously described (Toda *et al.* 2007; Miyake *et al.* 2013). Previously described cut-off values in dogs separating normal and abnormal concentrations were applied (Toda *et al.* 2007; Miyake *et al.* 2013). Dog leucocyte antigen (DLA) class II-associated genetic risk testing was performed by a linked STR marker test (Pedersen *et al.* 2011) at the Veterinary Genetics Laboratory (UC Davies, California, USA). The DLA class II susceptibility variants were determined and interpreted as previously described; N/N haplotype- low risk of developing NME, N/S- low risk of NME and S/S- increased risk of NME (Greer *et al.* 2010).

### 3.3.6 Genetic investigation (paper V)

Genome-wide association studies (GWAS) were performed using a mixed logistic model, a Bayesian model adapted for mapping complex traits (BayesR), and cross-population extended haplotype homozygosity (XP-EHH) to search for disease-associated selected loci. Part of the sample was

genotyped using Illumina 230 K CanineHD BeadChip (Illumina, San Diego, CA, USA) and part was whole genome sequenced at low coverage using Gencove's low-pass sequencing solution (Gencove Inc, New York, NY, US). Chromosomal positions of SNPs were based on the dog CanFam3.1 genome assembly (Hoepfner *et al.* 2014). Before merging the samples, the SNP-chip genotype data was imputed, using the low-pass sequenced sample as the reference panel.

Before imputation, SNPs not present in the reference panel were excluded, as were SNPs with a minor allele frequency (MAF) of  $<0.05$  and a call rate of  $<95\%$  to remove low confidence genotypes. The imputation was performed using Impute2 v2.3.2 (Howie *et al.* 2009), using the 86 low-pass sequenced pugs as the reference panel.

Quality control was performed using PLINK v1.9 (Chang *et al.* 2015). To remove any biases in the genotyping due to the different technologies used in the two sets of dogs, an association analysis was run between the genotyping techniques and subsequently deleted 6,229 variants with p-values  $<1e-03$ . After quality control, 2,140,239 SNPs remained for analysis.

Using the KING v2.2.7 test of relatedness (Manichaikul *et al.* 2010), duplicates were identified within and between the genotyping technique groups. The duplicates were confirmed from records of the individual registration numbers of the dogs. From these duplicate pairs, the individual with the lower call rate was excluded. After duplicates were removed, pugs with a relatedness threshold of 0.177 were identified and one pug from each related pair was excluded.

### 3.3.7 Statistics (paper I-V)

The statistical analyses were performed using a commercially available statistical software program (JMP Pro v. 11.2.0-15.2.0, Cary, NC, USA). Data was analyzed using descriptive as well as inferential statistics.

In paper I-IV, descriptive statistics of data for continuous variables were reported as means and standard deviations or medians and interquartile ranges as deemed appropriate by the variable distributions.

Differences in proportions of categorical data were tested using Chi-squared-tests, Fisher's exact test or logistic regression (paper I, II, III and

IV). A contingency table was used to explore whether there appeared to be a likely association between tested variables.

In paper I, differences in proportions concerning dog characteristic variables (age, sex, weight) and general health disorders (seizures, syncope, dyspnoea, surgery for BOAS, pigmentary keratopathy, corneal ulcers, abnormal scratching around neck/ears/head, chronic skin problems, demodicosis, PDE, fly snapping and licking in the air) and age groups (1, 5 and 8 years of age) and gait abnormality groups were tested.

In paper II, differences in proportions concerning dog characteristic variables (sex, age at clinical onset), number of thoracic and lumbar vertebrae, type of CVM, spondylosis and presence of neurological deficits (yes/no) were tested. In paper III differences in proportions concerning dog characteristic variables (clinical onset, duration of clinical signs, presence of incontinence, presence of CNS inflammation) and histopathological findings (malacia, syringomyelia/hydromyelia) were tested. In paper IV, differences in proportions concerning clinical phenotype positive anti-GFAP autoantibody concentrations and NME susceptibility haplotypes, were tested.

Linear regression analysis was performed in paper IV to test for an association between concentrations of CSF GFAP and concentrations of CSF anti- GFAP autoantibodies in affected pugs.

Differences in continuous variables, including laboratory findings in paper IV were tested using the non-parametric Wilcoxon signed rank test.

Possible associations between presence of gait abnormalities and dog characteristic variables (age, sex, weight) and presence of general health disorders (described above for paper I) were investigated using backward stepwise multivariable logistic regression analysis. Only variables with a P value of  $<0.2$  in the univariable regression analysis were included in the multivariable analysis. For paper I-IV level of statistical significance was set at  $P < 0.05$ .

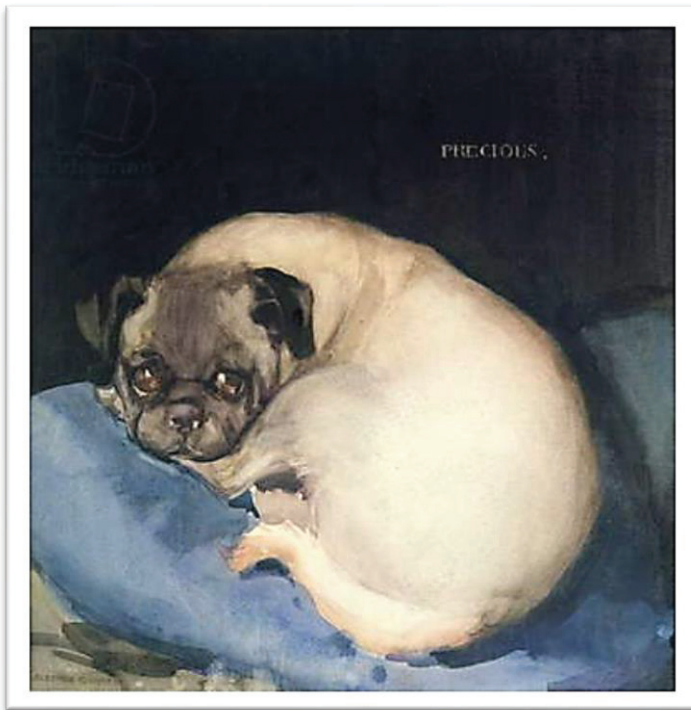
For paper V, the sum of risk alleles at the top variant per associated locus was compared between cases and controls using two-tailed Welsh Two-Sample t-test. Phenotypic variance explained by the associated loci was calculated using ANOVA, with sex and genotyping techniques as covariates.





## 4. Results

This section summarizes the results from the separate papers included in the thesis. More detailed descriptions of the results are presented in the separate papers.



Painting by Alethea Garstin (1894-1978)

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#### 4.1 Prevalence of gait abnormalities, and association between gait abnormalities and other health disorders in the pug breed (paper I)

Five hundred and fifty pug owners participated in the study. The overall response rate was 26.0%; 19.8% for the online version and 32.1% for the paper based version of the questionnaire. Gait abnormalities were reported in 30.7 % of Swedish pugs according to the owners' responses. Indeed, the single most common cause of death/euthanasia, reported by the owner, was an abnormal gait. In addition we found that, in general, young pugs presented with gait abnormalities characterized by non-progressive thoracic limb involvement, whilst older pugs presented with gait abnormalities characterized by progressive pelvic limb involvement. Thoracic limb involvement included wearing of nails and the skin on the dorsal aspect of the paws. Of the cases where the owner submitted a video of the pug showing an abnormal gait only 3/19 (15.8%) pugs presented with a gait abnormality consistent with lameness.

Of the pugs described by their owners as having an abnormal gait (n=111); thoracic limb involvement was described in 33 (34.4%), pelvic limb involvement in 42 (43.8%) and both thoracic and pelvic limb involvement in 21 (21.8%). Fifteen owners did not respond to the question regarding which limbs were affected (data not provided in the manuscript). Pug owners thus reported abnormal gait more often in the pelvic limbs than in the thoracic limbs. The gait was however described as normal by owners of pugs, 57/128 (44.5%), that also described wearing of the nails and/or the skin on the dorsum of their pug's paws (figure 15). The majority of the pugs, described by their owners as having a normal gait, despite wearing of the nails, presented with thoracic limbs involvement. When adding these dogs to the group of pugs with abnormal gait, thoracic limb involvement became more common than pelvic limb involvement.



Figure 15. Pugs presenting with wearing of the nails and of the skin on the dorsum of the paws causing bleeding and skin abrasions. Photo by the author.

The result of the questionnaire study further showed a significant association between gait abnormalities and age, and between gait abnormalities and dyspnoea.

Among a fixed set of responses to the question on presence of pain in pugs with gait abnormality the most common owner response was ‘reluctance to go for walks’ 17/30 (56.7%) and the least common response was ‘an irritable mood’ 1/30 (3.3%).

#### 4.2 The clinical importance of vertebral malformations for the development of PDM (paper II)

Fifty-seven pugs; 30 *with* and 27 *without* neurological deficits, were included in the study. Congenital vertebral malformations were present in the majority of pugs (96%) regardless of neurological status. Pugs *without* neurological deficits presented with 3.6 CVM/pug and pugs *with* neurological deficits with 3.7 CVM/pug in the thoracolumbar region. The most common location for CAP hypo- or aplasia was T10-T11 regardless of neurological status. In the majority of pugs with neurological deficits, a

CVM was found in the thoracolumbar spine immediately adjacent to the FSCP considered responsible for the neurological deficits. This suggested that part of the CVMs (13%) may have contributed to the development of FSCP.

Our study did not find any associations between presence or type of CVM in the thoracolumbar vertebral column and neurological deficits. An association was however shown between pugs with neurological deficits from a T3-L3 myelopathy and with transitional vertebra in the lumbosacral region, with lumbosacral transitional vertebra being more common in pugs with neurological deficits.

### 4.3 Neuropathological characterization of PDM (paper III)

Thirty pugs with neurological deficits from a thoracolumbar myelopathy, 20 male and 10 female pugs, underwent neuropathological characterization. All pugs remained ambulatory, with faecal incontinence affecting 80% of the pugs, by the time of euthanasia. The median age at clinical onset was 84 months (IQR 66-96 months). Mean duration of clinical signs before euthanasia was 13 months (SD 7.5)

Histopathologic examinations of affected pugs confirmed meningeal fibrosis with concomitant spinal cord parenchymal lesions were a consistent finding in the thoracolumbar spinal cord in 29/30 pugs (figure 16). The parenchymal spinal cord lesion was characterized by malacia, with the spinal cord parenchyma completely replaced by glial scar formation, in 24 pugs. Focal leptomeningeal proliferation and adhesions were in nine pugs associated with formation of a diverticula, in some creating multiple compartments of the subarachnoid space. Mild to moderate meningeal proliferations were also recognized throughout the meninges of the CNS unrelated to the FSCP in 14 pugs. In two pugs meningeal fibrosis also covered an area of grey matter parenchymal atrophy in the cranial cervical area (figure 16).

Lymphohistiocytic aggregates, indicating mild to severe, inflammation were found in the CNS related and unrelated to the parenchymal lesion in 13/30 (43%) of the examined pugs (figure 16).

Vertebral column pathology accompanied the focal parenchymal lesion and included vertebral malformations (16/17 evaluated by CT imaging) and IVDD (4/15 evaluated by histopathology). In five pugs, an IVD was mildly to moderately compressing the spinal cord at the level of the FSCP. An interesting finding was the protrusions of chondroid tissue into the adjacent endplate and the subchondral bone of some pugs of which the vertebral column was histopathologically investigated (figure 16).

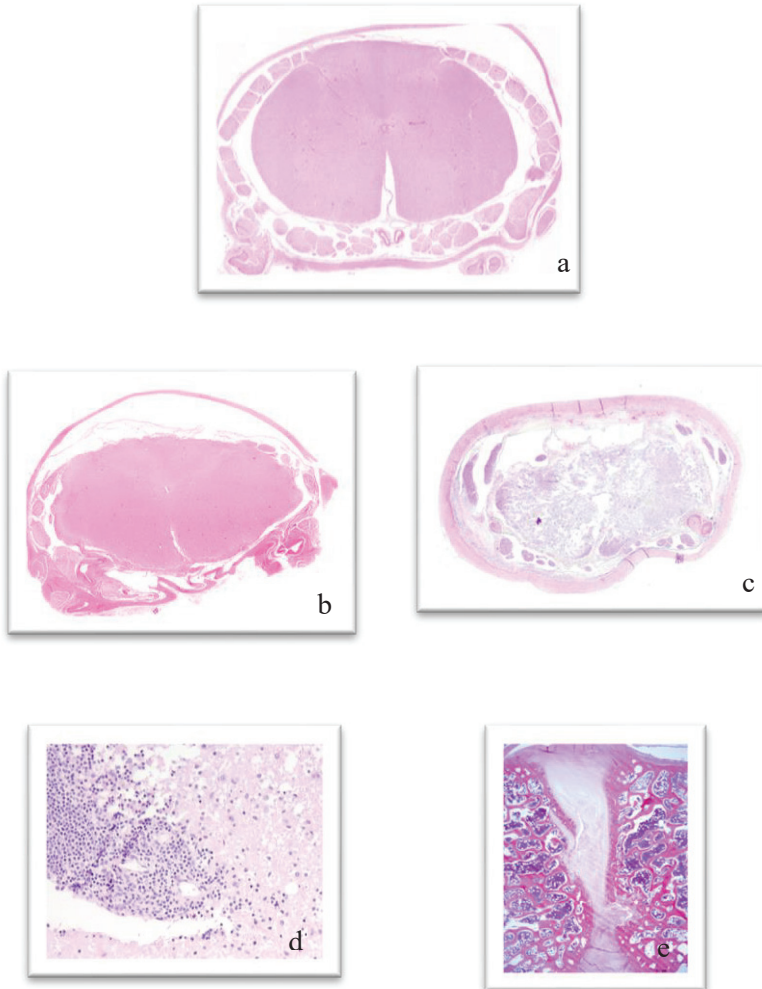


Figure 16. Histopathology of pugs with PDM. a) normal spinal cord in a pug without neurological deficits, b) moderate meningeal proliferation and moderate parenchymal atrophy, in the cervical spinal cord (C2-C3 level) of a 2 year old pug wearing nails of thoracic limb, c) extensive meningeal proliferations and severe parenchymal spinal cord destruction in the thoracolumbar spinal cord of 7 a year old pug, d) moderate lymphohistiocytic inflammation involving the spinal cord of a 9 year old pug, e) protrusions of chondroid tissue into the adjacent endplate and the subchondral vertebral bone, in the thoracic spinal cord of a 3 year old pug. Credit to Peter Wohlsein, Kaspar Matiasek and Alexandra Leijon.

#### 4.4 Investigation of a potential contribution of autoimmunity to the development of PDM (paper IV)

In total 87 dogs were included in the study; 52 affected pugs, 14 control pugs, 17 neuro controls and four NME pugs (see page 46).

Affected pugs presented with a positive CSF anti-GFAP autoantibody concentration (78.9%). This was a higher proportion compared to in the neuro controls (37.5%) ( $P$ -value 0.018) (figure 17).

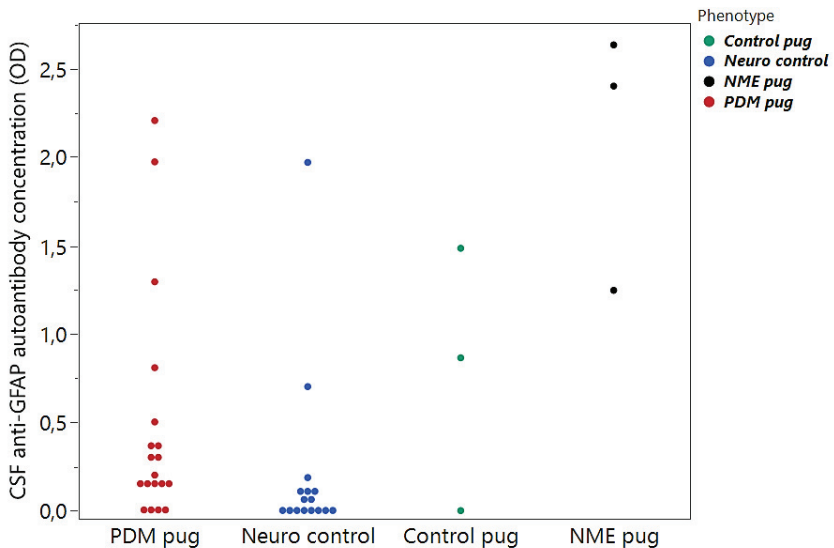


Figure 17. Anti-GFAP autoantibody concentrations in the CSF of PDM pugs ( $n = 19$ ), neuro controls ( $n=16$ ), control pugs ( $n=3$ ) and NME pugs ( $n=3$ ). CSF; cerebrospinal fluid, GFAP; glial fibrillary acidic protein, PDM; pug dog myelopathy, NME; necrotizing meningoencephalitis, OD; optic density. Cut off value 0.10 OD.

Male pugs with the DLA class II associated risk haplotype (N/S) presented with clinical signs of PDM at a younger age (70 months) than male pugs with the N/N haplotype (78 months) ( $P$ -value 0.036). The earlier onset of clinical signs associated with the N/S haplotype in affected male pugs indicate that the risk haplotype, even in a heterozygous state, may cause a more severe PDM phenotype. Our results from this study support a contribution of the immunological system, potentially autoimmunity, in the development of PDM.

## 4.5 Investigation of a genetic contribution to PDM (paper V)

Thirty-one pugs (10 cases / 21 controls), were genotyped using Illumina 230 K CanineHD BeadChip, and 86 pugs (60 cases / 26 controls) were whole genome sequenced at low coverage using Gencove's low-pass sequencing genotyping solution. After the data set had been adjusted for relatedness, the final data set consisted of 89 pugs (51 cases and 38 controls). Thirty-two of the affected pugs (62.7%) and 14 of the control pugs (36.8%) were male. The mean onset of clinical signs for PDM pugs was 78 months (SD 23) and mean age at phenotypic confirmation of control pugs being unaffected was 132 months (SD 19).

The BayesR analysis identified 19 associated loci, capturing 67 genes within the regions defined by LD  $r^2 > 0.8$  of the top variants. Of these, 34 were identified as candidate genes for the PDM phenotype, 14 with multiple functions leveraged towards the PDM phenotype. Similarly, the XP-EHH analysis identified three regions, capturing four candidate genes, all of which with potential functions in PDM.

When comparing the risk index, i.e., the sums of risk alleles from the 19 associated loci, we found a statistically significant difference between cases and controls ( $p=3.7e-12$ ), with the risk index explaining 45.6% of the PDM variance ( $p=4.3e-16$ ;) and sex 15.3% ( $p=1.1e-07$ ).

In summary, a Bayesian mixture model and XP-EHH identified genetic loci harbouring genes associated with bone homeostasis and or the formation, regulation, and differentiation of cartilage, fibrotic scar tissue and immune response, in line with PDM expression. The chronology and interaction of these processes remain unclear.

A hypothetical summary model including identified genes and their potential role in the disease progression is presented in figure 18.



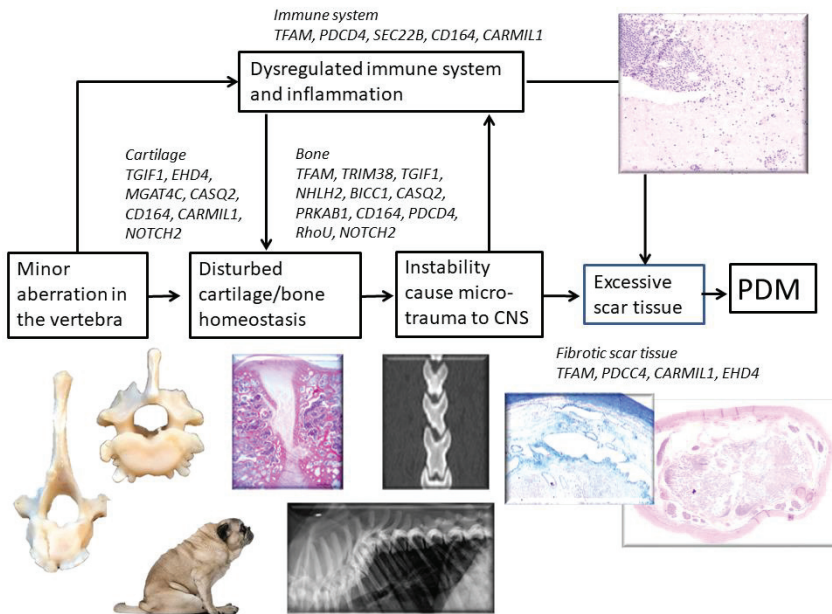


Figure 18. Hypothetical illustration of the pathophysiology including part of the identified genes and their potential involvement in the disease progression of PDM. PDM; pug dog myelopathy. Credit to Gustaf Brander. Illustration by the author.



## 5. Discussion

This section includes a discussion related to each paper included in this thesis. A more detailed discussion is provided in the respective papers.



Painting by Henry Gillard Glindoni (c.1810)

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## 5.1 Prevalence of gait abnormalities, and association between gait abnormalities and other health disorders in the pug breed (paper I)

The work in this thesis showed a high prevalence of gait abnormalities in Swedish pugs. Wearing of the nails and/or the skin on the dorsum of the paws, scuffing, is associated with neurological disorders as a consequence of proprioceptive deficits and motor dysfunction (Platt & Garosi 2012; Carr & Dycus 2016; de Lahunta *et al.* 2021c). The high prevalence of wearing of the nails and dorsum of the paws reported in the questionnaire and the fact that lameness was not a common finding in submitted videos suggested that the majority of gait abnormalities in the pugs were indeed related to neurological disorders. Although we were not able to precisely identify the prevalence of PDM, the results of the questionnaire study showed that gait abnormalities were common in pugs and that the majority were likely of neurological origin.

Two separate phenotypes were recognized in the study on gait of pugs and included younger pugs with thoracic limb involvement and older pugs with pelvic limb involvement. Younger pugs with thoracic limb involvement were in general described as having non-progressive gait abnormalities, while older pugs with pelvic limb involvement were described as having progressive gait abnormalities. In addition, a significant number of pug owners described their dogs' gait as normal, but at the same time responded that they wore down their nails and/or skin on the dorsum of their paws. Together this suggests that in general, there is a less severe phenotype involving the thoracic limbs, causing wearing of nails and skin abrasions from scuffing, and a more severe phenotype in older pugs that cause progressive ataxia and paraparesis.

In paper I we discussed the inconsistent finding of owners reporting an abnormal gait more often in the pelvic limbs while the conclusion was that gait abnormalities were actually more commonly seen in the thoracic limbs. The results needed to follow this discussion have been added in the result section of this thesis. When including the pugs, described as having a normal gait but with wearing of the nails and/or dorsal pedal skin, to the abnormal gait group, thoracic limb involvement was more common. This inconsistency may be explained by pug owners not associating wearing of the nails and/or the skin on the dorsal pedal skin of the paws with an abnormal gait.

### 5.1.1 Associated conditions of abnormal gait

Not surprisingly, age was associated with gait abnormalities. The older the pug the more likely it would be to present with an abnormal gait. Furthermore, owners reported an increase in prevalence of most of the general health disorders, included as potential comorbidities, with age of their dog.

Pugs with gait abnormalities were more likely to present with dyspnoea. Obesity is a risk factor for BOAS (Liu *et al.* 2016) and dyspnoea might be the natural consequence of a pug that is not exercising and therefore gains weight. An association between weight and gait abnormalities was however not found and gait abnormalities might therefore be related to dyspnoea in other ways. Potentially a specific phenotype predisposes the pug for both these debilitating conditions and that breed-related characteristics cause health problems not previously associated with the brachycephalic syndrome (Rohdin *et al.* 2018). It has recently been suggested that a broader definition, or term, of ‘the brachycephalic syndrome’ is warranted to incorporate the prevalence of all reported vertebral and cranial abnormalities in these types of breeds (Santifort *et al.* 2022).

### 5.1.2 Importance of vertebral malformations in the development of PDM (paper II)

A potential link between the specific clinical phenotype of PDM and the presence of particular CVMs in the pug was introduced by Fisher (Fisher *et al.* 2013). This naturally justified our study on the prevalence of CVMs in pugs with and without neurological deficits. However, an association between presence and type of CVMs in the thoracolumbar vertebral column, between pugs with and without neurological deficits, could not be confirmed.

Our results therefore did not support previous studies showing that thoracic hemivertebrae and severity of kyphosis associate with neurological disease (Moissonnier *et al.* 2011; Guevar *et al.* 2014). The difference in results was likely the effect of our study only including a few young pugs. Dogs less than a year old represent the majority of clinical cases related to hemivertebrae (Charalambous *et al.* 2014). Although hemivertebrae are

more common in other brachycephalic breeds, compared to the pug, they have been associated with neurological deficits more often in pugs (Gutierrez-Quintana *et al.* 2014; Ryan *et al.* 2017; De Decker *et al.* 2019). Thoracic hemivertebrae are of greater clinical importance in pugs as they present more often with associated kyphosis (Ryan *et al.* 2017).

The presence of hypo- and aplasia of the CAPs were common in pugs both with and without neurological deficits. The majority of dysplastic CAPs did not cause clinical signs of PDM. While each pug with PDM presented with one to four CAPs with hypo- and aplasia in the thoracolumbar vertebral column, no pug was found with more than one FSCP lesion in that specific spinal cord segment. It has been shown that in small breed dogs the intervertebral disc is capable of compensating for the functional loss of articular surfaces (Breit 2002) and instability as a consequence of hypo- and aplasia of the CAPs has yet to be proven. Hypo- and aplasia of the CAPs may therefore be an unrelated epiphenomenon in pugs with PDM (Driver *et al.* 2019).

Pugs with neurological deficits from a confirmed T3-L3 lesion presented with lumbosacral transitional vertebrae more often than pugs without neurological deficits. Although a reasonable explanation for this was not provided in the original manuscript, it could be speculated that the transitional vertebra cause disruption of the biomechanical axis, with increased motion of the column cranial to the lumbosacral area as described in people (Golubovsky *et al.* 2020).

The lack of association between multiple types of CVMs and PDM in this study demonstrated the need to look for other/additional factors for the development of PDM in pugs.

### 5.1.3 Neuropathological characteristics of PDM (paper III)

Neuropathological investigations were undertaken to provide a basic understanding of the pathophysiology of PDM (Rohdin *et al.* 2020) and as suggested in paper II, to look for factors other than CVMs for the development of PDM. The results suggested that the clinical disorder, PDM, is caused by the formation of profound meningeal fibrosis that interferes with the vascular supply to the spinal cord as well as cause obstruction of CSF flow with subsequent parenchymal spinal cord destruction. Similar findings have been reported in dogs with

experimentally induced adhesive, constrictive arachnoiditis (Mc *et al.* 1954; Hoffman *et al.* 1983). The longstanding, slowly progressive nature of PDM suggests that in many of the examined pugs, we were looking at the end-stage of the disease process. Pathologically, this was represented by severe intraparenchymal destruction, with the spinal cord parenchyma completely replaced by glial scar formation. Histopathological examinations of the CNS further revealed lymphohistiocytic infiltrations in a considerable proportion of PDM-affected pugs, similar to the type of infiltrations found in pugs with NME. Interestingly, pugs with intraparenchymal destruction and malacia presented with a longer duration of clinical signs before euthanasia and less often with lymphohistiocytic inflammation in the CNS on histopathology than pugs with a shorter duration of clinical signs before euthanasia. This may indicate that there is an early inflammatory phase and a later chronic proliferative stage in which fibrosis and adhesions become permanent (Aldrete 2004).

In addition to the finding of meningeal proliferations in the thoracolumbar spine, meningeal fibrosis was also occasionally reported in the upper cervical spine, specifically the C2-C3 area (Rohdin *et al.* 2014). The cervical meningeal fibrosis was in general milder than the thoracolumbar meningeal fibrosis, but similarly accompanied by focal parenchymal lesions. This difference in severity of the cervical versus the thoracolumbar involvement corresponded to the separate clinical phenotypes recognized already in the first study. In paper I, we described one group of pugs with milder non-progressive gait abnormalities involving the thoracic limbs, and one group with more severe, progressive gait abnormalities involving the pelvic limbs.

The predictive locations of meningeal proliferations, also described as ‘CMs’ and ‘SADs’, along the spinal cord suggest that these two sites (the C2-C3 area of the cervical spine and the thoracic spine) are predisposed to the formation of meningeal proliferations (Skeen *et al.* 2003).

Vertebral malformations may cause instability and repeated microtrauma to the spine with the build-up of meningeal proliferations. Pugs presented with vertebral column pathology (in the majority of cases a CVM) at the level of the corresponding thoracolumbar FSCP. However, CVMs are not described in the C2-C3 area of pugs. The C2-C3 disc, of normal signal intensity, has been described as bulging and to cause a flattened shape of the spinal cord over the C2-C3 area (Rohdin *et al.* 2014)

(figure 19). Future studies, to investigate in which way specific areas of the vertebral column trigger the meninges to proliferate, are certainly warranted.

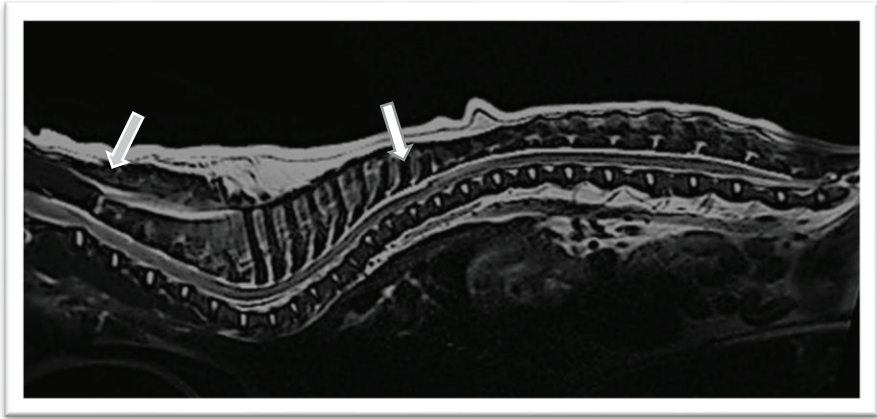


Figure 19. Magnetic resonance imaging of the spinal cord of a pug (T2W sagittal view). A subarachnoid diverticula (SAD) is found in the dorsal aspect of the thoracolumbar spinal cord at T9 (white arrow). In the cervical spine the C2-C3 disc is bulging, causing a flattened shape of the spinal cord, but without obvious SAD formation (grey arrow). Illustration by the author.

In summary, the pathological study suggested PDM to be a disorder including meningeal, parenchymal and vertebral column pathology. Pugs previously given diagnoses including ‘constrictive myelopathy’, ‘subarachnoid diverticulae’, ‘intervertebral disc disease’, ‘vertebral malformations’, as well as combinations of these (Bagheri *et al.* 2015; Ryan *et al.* 2017; Alcoverro *et al.* 2018; Alisauskaite *et al.* 2019; De Decker *et al.* 2019; Driver *et al.* 2019; Nishida *et al.* 2019; Tauro *et al.* 2019), likely represent a continuum or a spectrum of the same disorder.

#### 5.1.4 A potential autoimmune contribution to PDM (paper IV)

The somehow surprising finding of mild to severe lymphohistiocytic infiltration in the CNS in nearly half the pugs that underwent



neuropathological investigation warranted a study to further explore a potential autoimmune influence on the development of PDM.

Our results, showing that the majority of PDM affected pugs presented with anti-GFAP autoantibodies in CSF, support a contribution of the immunological system on the development of PDM. The presence of anti-GFAP autoantibodies in CSF, which is consistently present also in dogs with NME (Matsuki *et al.* 2004), has previously led to the suggestion of an autoimmune CNS process (Toda *et al.* 2007). A dysregulated immune response could interfere with the normal physiological repair process resulting in excessive fibrous tissue. In people it has been suggested that some patients are predisposed to the development of fibrous adhesions caused by various factors including autoimmune-related mechanisms (Idris *et al.* 2014; Khan *et al.* 2016).

A potential link between PDM and NME was found by showing the genetic susceptibility DLA class II haplotype, previously described in NME, to be associated with development of PDM. The earlier onset of clinical signs associated with the N/S haplotype in affected male pugs indicated that the risk haplotype (S), even in a heterozygous state, may cause a more severe PDM phenotype. Application of this knowledge in breeding needs to consider the presence of the risk haplotype in 40% of the pug breed population. Eliminating the haplotype entirely from the breed could lead to a substantial loss of genetic diversity across the genome (Greer *et al.* 2010).

### 5.1.5 The genetic background for PDM (paper V)

Owing to the breed specific occurrence of PDM, a genetic contribution to the development of the disease was suspected. Indeed, the genetic investigation of genomic DNA identified several genes with potential relevance in the development of PDM, including genes involved in bone homeostasis and formation, regulation, and differentiation of cartilage, in the development of fibrosis and in regulating inflammatory responses.

The results of this study should be interpreted in the light of the fact that it is currently unclear which factors truly drive the development of PDM and which are secondary to the disease process itself. For instance, the pathological hallmark of PDM is meningeal fibrosis. However, the meningeal fibrosis might be the appropriate, natural result of increased load, the result of exacerbated inflammation associated with a dysregulated

immune response, or a consequence of dysfunctional healing ability or formation of functional scar tissue.

A potential link to bone homeostasis and to cartilage is interesting. The loss of stability and integrity of the spine, with progression of spinal kyphosis, has been documented in a young pug and in mature pugs with thoracolumbar myelopathy (De Rycke *et al.* 2016; Wyatt *et al.* 2018). Surgical stabilisation of the spine has further proven useful for pugs presenting with clinical signs consistent with PDM (Charalambous *et al.* 2014; Mathiesen *et al.* 2018; Aikawa *et al.* 2019; Tauro *et al.* 2019; Farré Mariné *et al.* 2021; Mavrides *et al.* 2021).

An interesting and not frequently reported in the literature, finding from the pathology study were the protrusions of chondroid tissue into the adjacent vertebral endplate and the subchondral bone of some pugs (so called Schmorl's nodes) (Gaschen *et al.* 1995; Baltzer *et al.* 2012). These protrusions of chondroid tissue could potentially indicate the loss of cartilaginous integrity of the endplates. Could it be that 'the path of least resistance' for the disc material in pugs is not through the most common path seen in most cases, dorsally through the annulus fibrosis, but through the cartilaginous end-plate? Although IVDD has been reported as common in pugs the majority of affected pugs present with comparably mild IVDD (Full 2014).

Cartilage does not only provide shock absorbing properties and flexibility to the spine but it is also the main tissue providing stability in other parts of the body. According to the American, the British and the Swedish Kennel clubs, the pug should have 'thin, small, soft like black velvet ears'. The similar folded ear pinna is in specific breeds of cats associated with osteochondrodysplasia (Malik *et al.* 1999; Takanosu & Hattori 2020; Rorden *et al.* 2021).

Furthermore, loss of rigidity (chondromalacia) has been proposed, but not pathologically confirmed, to be the cause of the increased risk of severe collapse of the cartilage in the respiratory tract, particularly the larynx of pugs (Dupré & Heidenreich 2016). Future studies should aim at investigating whether our findings, with several identified genes involved in bone homeostasis, differentiation of cartilage, fibrosis development and inflammatory regulation, could suggest a more generalized disorder affecting multiple support structures of pugs' bodies.

## 5.2 Pain

When owners of pugs with a gait abnormality were asked about signs of potential pain in their dogs, their most common response was ‘reluctance to go for walks’ (paper I). Causes for reluctance to go for walks could be several, including the increased effort of walking with an abnormal gait as a consequence of a potential spinal cord disease.

Pug dog thoracolumbar myelopathy has previously been described as nonpainful (Fisher *et al.* 2013). However, the profound meningeal fibrosis interferes with the vascular supply and cause ischemia to the spinal cord. People with ischemic spinal cord lesions describe chronic pain that increase with exertion (Rice *et al.* 2004). We therefore have to consider the possibility of PDM being a painful condition and that refusing to go for walks is an expression of discomfort.

## 5.3 Limitations related to the thesis:

### 5.3.1 Specific limitations related to paper I.

Describing the prevalence, the proportion of existing cases, of a disorder in a population is challenging. The quality of the result from a questionnaire-based study is depending on the owner’s cooperation, willingness to participate and their ability to recognize the disorder. Reporting hospital prevalences may be more reliable; however these results may also be misleading as not all owners seek veterinary care for their dog’s disorder e.g. a risk for selection bias. This was shown in paper I where the majority of pug owners had not sought veterinary care for their dogs gait abnormality.

Even though the general study population was most likely representative of the Swedish pug breed (Statistics Sweden 2012) the final study population may have been biased. Owners of pugs with obvious gait abnormalities may have been more prone to respond. There may also have been another population of pug owners with specific interests in responding, e.g. breeders.

The response rate in our questionnaire study was low and in addition not all owners responded to all the questions included in the questionnaire. The low response rate might have been affected by the Swedish petition for the

right of brachycephalic breeds to breathe; launched the same year the invitation was sent and supported by a large number of Swedish veterinarians. The petition might have made pug owners more reluctant to participate in the study. A concern with a low response rate is nonresponse bias. However, a linkage between nonresponse rate and non-response biases need not induce non-response bias in questionnaire surveys (Groves 2006; Choung *et al.* 2013). The reliability of a sample, big or small, will ultimately be based on how representative of the population the chosen sample is.

### 5.3.2 General limitations paper II-V:

A general concern of this thesis was the challenge to recruit healthy control pugs for the study. It was difficult to recruit neurologically healthy pugs for CT examination and CSF sampling, but also for neurological examination and blood sampling. Sampling of CSF requires anaesthesia and therefore, naturally, brings a certain risk for the patient. Sampling in association with euthanasia provided an alternative. The difficulty in recruiting control pugs could also potentially be due to fixation of the disorder in the breed population.

To reduce the risk of including control pugs that would develop PDM later in life, we actively aimed at recruiting older control pugs. Pugs develop PDM at an average age of 7 years (Alisauskaite *et al.* 2019; Lourinho *et al.* 2020; Rohdin *et al.* 2020) and pugs with PDM in Sweden are euthanized on average 1 year after the onset of clinical signs (Rohdin *et al.* 2020). Therefore, for inclusion in paper V, control pugs had to be at least 8 years of age. Even so, one control pug, included at the age of 8.5 years in study V, presented with mild neurological deficits affecting the pelvic limbs at the age of 10 years (personal communication). Still, by including older pugs we likely reduced the risk of including pugs with a potentially more severe or aggressive phenotype (onset at a younger age).

Not all control pugs had a neurological examination performed, potentially making our results less reliable. However, based on the results of the video analyses (paper I) there was agreement between owners and specialist on how to interpret the dogs' gait, except when it came to scuffing of the paws. Dragging or scuffing (causing wear of the nails and/or the dorsal skin of the paws) was interpreted as a gait abnormality by the specialist but not always by the owners. It was therefore considered less

likely that the clinical characteristics of the PDM pugs, presenting with progressive incoordination of the pelvic limbs and weakness, would not be recognised by the owners.

Another general concern of this thesis was that the sample sizes of study II, IV and V were too small providing low statistical power and reduced chance of detecting a true effect (type II error) (Button *et al.* 2013).

In study IV, the unfortunate loss of samples stored in a freezer that broke down during work on this thesis contributed to the lack of consistency related to the blood and CSF samples.

A further limitation was the lack of an extensive and robust examination protocol determined in advance for all the upcoming studies. The examination protocol during this thesis was extended as we, with time, learned more about the disorder. It also has to be acknowledged that at the onset of the thesis, in 2014, the availability of advanced imaging, e.g. CT and MRI, in Sweden was limited.



## 6. Conclusions and future remarks

In this thesis we have shown that:

- The Swedish pug breed presented with a high prevalence of gait abnormalities. The character of the gait abnormalities suggested that the majority were related to neurological disorders.
- In general young pugs presented with non-progressive thoracic limb involvement while older pugs presented with progressive pelvic limb involvement.
- The prevalence of gait abnormalities increased with age and there was an association between gait abnormalities and dyspnoea.
- Congenital vertebral malformations were as common in pugs *without*, as in pugs *with*, neurological deficits.
- Although the majority of CVMs were incidental findings, part of all the CVMs may contribute to the development of FSCP, as CVMs often accompanied the spinal cord lesion in pugs with PDM.
- Meningeal fibrosis and associated spinal cord destruction were pathological characteristics in pugs with PDM.
- Lymphohistiocytic and plasmacytic infiltration of the CNS was commonly present in pugs with PDM.

- Pugs with PDM presented with a positive CSF anti- GFAP autoantibody concentration significantly more often than dogs of breeds other than pugs being investigated for various CNS diseases.
- The NME DLA class II risk haplotype often part of a heterozygous “N/S” genotype may be a risk factor for male pugs.
- The genetic study suggested involvement of genes implicated in bone homeostasis, the formation, regulation, and differentiation of cartilage, fibrotic scar tissue, and inflammatory responses are likely underlying PDM aetiology.



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## Implications for future research

- Design of longitudinal studies, including advanced imaging of pug spines from a younger age, to enhance the understanding of the disease process, and to identify potential risk factors for the development of PDM.
- Identify appropriate screening methods of dogs for breeding in the future to reduce the prevalence of PDM in the pug breed.
- Gene expression studies of differences in relevant tissues from pugs to explore the functions of candidate genes in PDM.
- Further investigation of a potential association between PDM and dyspnoea - are we looking at a systemic disease?



## References

- Aikawa, T., Kanazono, S., Yoshigae, Y., Sharp, N.J. & Munana, K.R. (2007). Vertebral stabilization using positively threaded profile pins and polymethylmethacrylate, with or without laminectomy, for spinal canal stenosis and vertebral instability caused by congenital thoracic vertebral anomalies. *Veterinary Surgery*, 36(5), 432-41. <https://doi.org/10.1111/j.1532-950X.2007.00289.x>
- Aikawa, T., Shimatsu, T. & Miyazaki, Y. (2019). Hemilaminectomy, Diverticular Marsupialization, and Vertebral Stabilization for Thoracolumbar Spinal Arachnoid Diverticula in Five Dogs. *J Am Anim Hosp Assoc*, 55(2), 110-116. <https://doi.org/10.5326/jaaha-ms-6762>
- Alcoverro, E., McConnell, J.F., Sanchez-Masian, D., De Risio, L., De Decker, S. & Goncalves, R. (2018). Late-onset recurrence of neurological deficits after surgery for spinal arachnoid diverticula. *The Veterinary record*, 182(13), 380. <https://doi.org/10.1136/vr.104579>
- Aldrete, J.A. (2004). Chronic adhesive arachnoiditis. *Br J Anaesth*, 93(2), 301; author reply 301-3. <https://doi.org/10.1093/bja/aeH591>
- Alisauskaite, N., Cizinauskas, S., Jeserevics, J., Rakauskas, M., Cherubini, G.B., Anttila, M. & Steffen, F. (2019). Short- and long-term outcome and magnetic resonance imaging findings after surgical treatment of thoracolumbar spinal arachnoid diverticula in 25 Pugs. *J Vet Intern Med*. <https://doi.org/10.1111/jvim.15470>
- Angevine, J.B., Jr. (1973). Chapter 7. Clinically relevant embryology of the vertebral column and spinal cord. *Clin Neurosurg*, 20, 95-113. [https://doi.org/10.1093/neurosurgery/20.cn\\_suppl\\_1.95](https://doi.org/10.1093/neurosurgery/20.cn_suppl_1.95)
- Archer, J. & Monton, S. (2011). Preferences for Infant Facial Features in Pet Dogs and Cats. *Ethology*, 117(3), 217-226. <https://doi.org/https://doi.org/10.1111/j.1439-0310.2010.01863.x>
- Atchley, T.J., Vukic, B., Vukic, M. & Walters, B.C. (2022). Review of Cerebrospinal Fluid Physiology and Dynamics: A Call for Medical Education Reform. *Neurosurgery*, 91(1). [https://journals.lww.com/neurosurgery/Fulltext/2022/07000/Review\\_of\\_Cerebrospinal\\_Fluid\\_Physiology\\_and.1.aspx](https://journals.lww.com/neurosurgery/Fulltext/2022/07000/Review_of_Cerebrospinal_Fluid_Physiology_and.1.aspx)
- Bagheri, S., Venta, P., Ballegeer, E., Lin, Y. & Patterson, J. (2015). Investigation of subarachnoid diverticulum and other spinal cord diseases in pugs. . In: *Merial-NIH National Veterinary Scholars Symposium. "Solving complex challenges at the interface of humans, animals and their environment, California, USA.*

- Bailey, C.S. (1975). An embryological approach to the clinical significance of congenital vertebral and spinal cord abnormalities. In: *Congenital Vertebral and Spinal Cord Abnormalities*. (11). 426-434.
- Bailey, C.S. & Morgan, J.P. (1992). Congenital spinal malformations. *The Veterinary Clinics of North America. Small Animal Practice*, 22(4), 985-1015.
- Baltzer, W.I., Hillebrand, L., Smith, T.J. & Stieger-Vanegas, S.M. (2012). Surgical management of a Schmorl's node in an Airedale Terrier and review of the literature. *Vet Comp Orthop Traumatol*, 25(2), 167-71. <https://doi.org/10.3415/vcot-11-06-0088>
- Barber, R.M., Porter, B.F., Li, Q., May, M., Claiborne, M.K., Allison, A.B., Howarth, E.W., Butler, A., Wei, S., Levine, J.M., Levine, G.J., Brown, D.R. & Schatzberg, S.J. (2012). Broadly reactive polymerase chain reaction for pathogen detection in canine granulomatous meningoencephalomyelitis and necrotizing meningoencephalitis. *J Vet Intern Med*, 26(4), 962-8. <https://doi.org/10.1111/j.1939-1676.2012.00954.x>
- Barber, R.M., Schatzberg, S.J., Corneveaux, J.J., Allen, A.N., Porter, B.F., Pruzin, J.J., Platt, S.R., Kent, M. & Huentelman, M.J. (2011). Identification of risk loci for necrotizing meningoencephalitis in Pug dogs. *Journal of Heredity*, 102 Suppl 1, S40-6. <https://doi.org/10.1093/jhered/esr048>
- Bergknut, N., Meij, B.P., Hagman, R., de Nies, K.S., Rutges, J.P., Smolders, L.A., Creemers, L.B., Lagerstedt, A.S., Hazewinkel, H.A. & Grinwis, G.C. (2013). Intervertebral disc disease in dogs - part 1: a new histological grading scheme for classification of intervertebral disc degeneration in dogs. *Veterinary journal (London, England : 1997)*, 195(2), 156-63. <https://doi.org/10.1016/j.tvjl.2012.05.027>
- Bismuth, C., Ferrand, F.X., Millet, M., Buttin, P., Fau, D., Cachon, T., Viguier, E., Escriou, C. & Carozzo, C. (2014). Original surgical treatment of thoracolumbar subarachnoid cysts in six chondrodystrophic dogs. *Acta ceterinaria scandinavica*, 56, 32. <https://doi.org/10.1186/1751-0147-56-32>
- Bouma, J.L. (2016). Congenital Malformations of Vertebral Articular Processes in Dogs. *The Veterinary Clinics of North America. Small Animal Practice*, 46(2), 307-26. <https://doi.org/10.1016/j.cvsm.2015.10.006>
- Breit, S. (2002). Osteological and morphometric observations on intervertebral joints in the canine pre-diaphragmatic thoracic spine (Th1-Th9). *Veterinary Journal*, 164(3), 216-23. <https://doi.org/10.1053/tvj.2002.0714>
- Brisson, B.A. (2010). Intervertebral disc disease in dogs. *Vet Clin North Am Small Anim Pract*, 40(5), 829-58. <https://doi.org/10.1016/j.cvsm.2010.06.001>
- Brocal, J., De Decker, S., José-López, R., Manzanilla, E.G., Penderis, J., Stalin, C., Bertram, S., Schoenebeck, J.J., Rusbridge, C., Fitzpatrick, N. & Gutierrez-Quintana, R. (2018). C7 vertebra homeotic transformation in

- domestic dogs - are Pug dogs breaking mammalian evolutionary constraints? *Journal of Anatomy*, 233(2), 255-265. <https://doi.org/10.1111/joa.12822>
- Brown, J., Podadera, J., Ward, M., Goldsmid, S. & Simpson, D. (2021). The presence, morphology and clinical significance of vertebral body malformations in an Australian population of French Bulldogs and Pugs. *Australian veterinary journal*, 99(9), 378-387. <https://doi.org/https://doi.org/10.1111/avj.13094>
- Button, K.S., Ioannidis, J.P.A., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S.J. & Munafò, M.R. (2013). Power failure: why small sample size undermines the reliability of neuroscience. *Nature Reviews Neuroscience*, 14(5), 365-376. <https://doi.org/10.1038/nrn3475>
- Cantile, C., Chianini, F., Arispici, M. & Fatzer, R. (2001). Necrotizing meningoencephalitis associated with cortical hippocampal hamartia in a Pekingese dog. *Veterinary Pathology*, 38(1), 119-22. <https://doi.org/10.1354/vp.38-1-119>
- Cardy, T.J., De Decker, S., Kenny, P.J. & Volk, H.A. (2015). Clinical reasoning in canine spinal disease: what combination of clinical information is useful? *The Veterinary record*, 177(7), 171. <https://doi.org/10.1136/vr.102988>
- Carr, B.J. & Dycus, D.L. (2016). Canine gait analysis. *Recovery & Rehab*, March/April, 93-100.
- Chang, C.C., Chow, C.C., Tellier, L.C.A.M., Vattikuti, S., Purcell, S.M. & Lee, J.J. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience*, 4(1), s13742-015-0047-8. <https://doi.org/10.1186/s13742-015-0047-8>
- Charalambous, M., Jeffery, N.D., Smith, P.M., Goncalves, R., Barker, A., Hayes, G., Ives, E. & Vanhaesebrouck, A.E. (2014). Surgical treatment of dorsal hemivertebrae associated with kyphosis by spinal segmental stabilisation, with or without decompression. *Veterinary journal (London, England : 1997)*, 202(2), 267-73. <https://doi.org/10.1016/j.tvjl.2014.08.011>
- Chikly, B. & Quaghebeur, J. (2013). Reassessing cerebrospinal fluid (CSF) hydrodynamics: a literature review presenting a novel hypothesis for CSF physiology. *J Bodyw Mov Ther*, 17(3), 344-54. <https://doi.org/10.1016/j.jbmt.2013.02.002>
- Choung, R.S., Locke, G.R., Schleck, C.D., Ziegenfuss, J.Y., Beebe, T.J., Zinsmeister, A.R. & Talley, N.J. (2013). A low response rate does not necessarily indicate non-response bias in gastroenterology survey research: a population-based study. *Journal of Public Health*, 21(1), 87-95. <https://doi.org/10.1007/s10389-012-0513-z>
- Clarke, B. (2008). Normal bone anatomy and physiology. *Clin J Am Soc Nephrol*, 3 Suppl 3(Suppl 3), S131-9. <https://doi.org/10.2215/cjn.04151206>
- Cline, M.J. (1994). Histiocytes and Histiocytosis. *Blood*, 84(9), 2840-2853. <https://doi.org/https://doi.org/10.1182/blood.V84.9.2840.2840>

- Cooper, J.J., Schatzberg, S.J., Vernau, K.M., Summers, B.A., Porter, B.F., Siso, S., Young, B.D. & Levine, J.M. (2014). Necrotizing meningoencephalitis in atypical dog breeds: a case series and literature review. *J Vet Intern Med*, 28(1), 198-203. <https://doi.org/10.1111/jvim.12233>
- Cordy, D.R. & Holliday, T.A. (1989). A necrotizing meningoencephalitis of pug dogs. *Veterinary Pathology*, 26(3), 191-4. <https://doi.org/10.1177/030098588902600301>
- Cornelis, I., Van Ham, L., Gielen, I., De Decker, S. & Bhatti, S.F.M. (2019). Clinical presentation, diagnostic findings, prognostic factors, treatment and outcome in dogs with meningoencephalomyelitis of unknown origin: A review. *Veterinary Journal*, 244, 37-44. <https://doi.org/10.1016/j.tvjl.2018.12.007>
- Damur-Djuric, N., Steffen, F., Hässig, M., Morgan, J.P. & Flückiger, M.A. (2006). Lumbosacral transitional vertebrae in dogs: classification, prevalence, and association with sacroiliac morphology. *Vet Radiol Ultrasound*, 47(1), 32-8. <https://doi.org/10.1111/j.1740-8261.2005.00102.x>
- De Decker, S., Packer, R.M.A., Cappello, R., Harcourt-Brown, T.R., Rohdin, C., Gomes, S.A., Bergknut, N., Shaw, T.A., Lowrie, M. & Gutierrez-Quintana, R. (2019). Comparison of signalment and computed tomography findings in French Bulldogs, Pugs, and English Bulldogs with and without clinical signs associated with thoracic hemivertebra. *J Vet Intern Med*. <https://doi.org/10.1111/jvim.15556>
- De Geer, C.M. (2018). Intervertebral Disk Nutrients and Transport Mechanisms in Relation to Disk Degeneration: A Narrative Literature Review. *J Chiropr Med*, 17(2), 97-105. <https://doi.org/10.1016/j.jcm.2017.11.006>
- de Lahunta, A., Glass, E. & Kent, M. (2021a). Cerebrospinal fluid and hydrocephalus. In: de Lahunta, A., Glass, E. & Kent, M. (eds) *de Lahunta's Veterinary Neuroanatomy and Clinical neurology*. 5th edition. Philadelphia, PA, USA: Elsevier, Saunders. 79-86.
- de Lahunta, A., Glass, E. & Kent, M. (2021b). Neuroanatomy gross description and atlas of transverse sections and magnetic resonance images. In: de Lahunta, A., Glass, E. & Kent, M. (eds) *de Lahunta's Veterinary Neuroanatomy and Clinical Neurology*. 5th edition. Philadelphia, PA, USA: Elsevier, Saunders. 25-26.
- de Lahunta, A., Glass, E. & Kent, M. (2021c). Small animal spinal cord disease. In: de Lahunta, A., E., G. & Kent, M. (eds) *de Lahunta's Veterinary Neuroanatomy and Clinical Neurology*. 5th edition. Philadelphia, PA, USA: Elsevier, Saunders. 267-273.
- de Lahunta, A., Glass, E. & Kent, M. (2021d). The neurological examination. In: de Lahunta, A., Glass, E. & Kent, M. (eds) *de Lahunta's Veterinary Neuroanatomy and Clinical Neurology*. 5th edition. Philadelphia, PA, USA: Elsevier, Saunders. 531-546.
- de Lahunta, A., Glass, E. & Kent, M. (2021e). Upper motor neuron. In: de Lahunta, A., Glass, E. & Kent, M. (eds) *de Lahunta's Veterinary*

- Neuroanatomy and Clinical Neurology*. 5th edition. Philadelphia, PA, USA: Elsevier, Saunders. 241-242.
- De Rycke, L.M., Crijns, C., Chiers, K., van Bree, H.J. & Gielen, I. (2016). Late-onset wedge-shaped thoracic vertebrae in a six-month-old pug. *The veterinary record. Case reports*, 4(1). <https://doi.org/10.1136/vetreccr-2016-000317>
- Dewey, C.W., Davies, E. & Bouma, J.L. (2016). Kyphosis and Kyphoscoliosis Associated with Congenital Malformations of the Thoracic Vertebral Bodies in Dogs. *Vet Clin North Am Small Anim Pract*, 46(2), 295-306. <https://doi.org/10.1016/j.cvsm.2015.10.009>
- Done, S.H., Drew, R.A., Robins, G.M. & Lane, J.G. (1975). Hemivertebra in the dog: clinical and pathological observations. *The Veterinary record*, 96(14), 313-7.
- Dreha-Kulaczewski, S., Joseph, A.A., Merboldt, K.-D., Ludwig, H.-C., Gärtner, J. & Frahm, J. (2015). Inspiration is the major regulator of human CSF flow. *Journal of Neuroscience*, 35(6), 2485-2491.
- Driver, C.J., Rose, J., Tauro, A., Fernandes, R. & Rusbridge, C. (2019). Magnetic resonance image findings in pug dogs with thoracolumbar myelopathy and concurrent caudal articular process dysplasia. *BMC Vet Res*, 15(1), 182. <https://doi.org/10.1186/s12917-019-1866-0>
- Dupré, G. & Heidenreich, D. (2016). Brachycephalic Syndrome. *Vet Clin North Am Small Anim Pract*, 46(4), 691-707. <https://doi.org/10.1016/j.cvsm.2016.02.002>
- Ekenstedt, K.J., Crosse, K.R. & Risselada, M. (2020). Canine Brachycephaly: Anatomy, Pathology, Genetics and Welfare. *J Comp Pathol*, 176, 109-115. <https://doi.org/10.1016/j.jcpa.2020.02.008>
- Elbert, J.A., Yau, W. & Rissi, D.R. (2022). Neuroinflammatory diseases of the central nervous system of dogs: A retrospective study of 207 cases (2008-2019). *Canadian Veterinary Journal*, 63(2), 178-186.
- Evans, H.E. & de Lahunta, A. (2013a). Spinal cord and meninges. In: Evans, H.E. & de Lahunta, A. (eds) *Miller's Anatomy of the Dog*. 4th edition. ( ). St. Louis, Missouri, USA: Elsevier, Saunders. 604-609.
- Evans, H.E. & de Lahunta, A. (2013b). Vertebral column. In: Evans, H.E. & de Lahunta, A. (eds) *Miller's Anatomy of the Dog*. 4th edition. St. Louis, Missouri, USA: Elsevier, Saunders. 113-123.
- Farré Mariné, A., De Risio, L., Mascort, J., Jiménez Peláez, M., López, M., Stabile, F. & Luján Feliu-Pascual, A. (2021). Transthoracic Vertebral Distraction and Stabilization in 10 Dogs with Congenital Thoracic Vertebral Malformations. *Vet Comp Orthop Traumatol*, 34(5), 367-374. <https://doi.org/10.1055/s-0041-1731807>
- Feng, X. & McDonald, J.M. (2011). Disorders of bone remodeling. *Annu Rev Pathol*, 6, 121-45. <https://doi.org/10.1146/annurev-pathol-011110-130203>
- Fisher, S.C., Shores, A. & Simpson, S.T. (2013). Constrictive myelopathy secondary to hypoplasia or aplasia of the thoracolumbar caudal articular

- processes in Pugs: 11 cases (1993-2009). *Journal of the American Veterinary Medical Association*, 242(2), 223-9. <https://doi.org/10.2460/javma.242.2.223>
- Flegel, T. (2017). Breed-Specific Magnetic Resonance Imaging Characteristics of Necrotizing Encephalitis in Dogs. *Front Vet Sci*, 4, 203. <https://doi.org/10.3389/fvets.2017.00203>
- Flegel, T., Henke, D., Boettcher, I.C., Aupperle, H., Oechtering, G. & Matiasek, K. (2008). Magnetic resonance imaging findings in histologically confirmed Pug dog encephalitis. *Veterinary radiology & ultrasound*, 49(5), 419-24.
- Flegel, T., Muller, M.K., Truar, K., Loffler, C. & Oechtering, G. (2013). Thoracolumbar spinal arachnoid diverticula in 5 pug dogs. *The Canadian veterinary journal*, 54(10), 969-73.
- Foss, K.D. & Berry, W.L. (2009). What Is Your Neurologic Diagnosis? *Journal of the American Veterinary Medical Association*, 234(8), 1009-1011. <https://doi.org/10.2460/javma.234.8.1009>
- Full, A., Dewey, C.J., Bouma, J.L. (2014). Prevalence and magnetic resonance imaging of intervertebral disc disease in pugs with caudal articular process dysplasia of the thoracolumbar spine. In: *ACVR Proceedings*, St Louis, MO, USA.
- Galis, F., Carrier, D.R., van Alphen, J., van der Mije, S.D., Van Dooren, T.J.M., Metz, J.A.J. & ten Broek, C.M.A. (2014). Fast running restricts evolutionary change of the vertebral column in mammals. *Proceedings of the National Academy of Sciences of the United States of America*, 111(31), 11401-11406. <https://doi.org/10.1073/pnas.1401392111>
- Gaschen, L., Lang, J. & Haeni, H. (1995). Intravertebral disc herniations (Schmorl's node) in five dogs. *Veterinary radiology & ultrasound*, 36(6), 509-516. <https://doi.org/10.1111/j.1740-8261.1995.tb00304.x>
- Giampietro, P.F., Blank, R.D., Raggio, C.L., Merchant, S., Jacobsen, F.S., Faciszewski, T., Shukla, S.K., Greenlee, A.R., Reynolds, C. & Schowalter, D.B. (2003). Congenital and idiopathic scoliosis: clinical and genetic aspects. *Clinical Medicine and Research*, 1(2), 125-36. <https://doi.org/10.3121/cm.1.2.125>
- Giampietro, P.F., Raggio, C.L., Blank, R.D., McCarty, C., Broeckel, U. & Pickart, M.A. (2013). Clinical, genetic and environmental factors associated with congenital vertebral malformations. *Mol Syndromol*, 4(1-2), 94-105. <https://doi.org/10.1159/000345329>
- Gluding, D., Stock, K.F., Tellhelm, B., Kramer, M. & Eley, N. (2021). Genetic background of lumbosacral transitional vertebrae in German shepherd dogs. *Journal of Small Animal Practice*, 62(11), 967-972. <https://doi.org/https://doi.org/10.1111/jsap.13380>
- Golubovsky, J.L., Colbrunn, R.W., Klatte, R.S., Nagle, T.F., Briskin, I.N., Chakravarthy, V.B., Gillespie, C.M., Reith, J.D., Jasty, N., Benzel, E.C. & Steinmetz, M.P. (2020). Development of a novel in vitro cadaveric model for analysis of biomechanics and surgical treatment of Bertolotti



- syndrome. *The Spine Journal*, 20(4), 638-656.  
<https://doi.org/10.1016/j.spinee.2019.10.011>
- Gonçalves, R., De Decker, S., Walmsley, G., Butterfield, S. & Maddox, T.W. (2021). Inflammatory Disease Affecting the Central Nervous System in Dogs: A Retrospective Study in England (2010-2019). *Front Vet Sci*, 8, 819945. <https://doi.org/10.3389/fvets.2021.819945>
- Gong, H., Slunsky, P., Klass, L.G. & Brunnberg, L. (2020). Prevalence of lumbosacral transitional vertebrae in dogs in Berlin. *Pol J Vet Sci*, 23(2), 261-265. <https://doi.org/10.24425/pjvs.2020.133641>
- Greer, K.A., Schatzberg, S.J., Porter, B.F., Jones, K.A., Famula, T.R. & Murphy, K.E. (2009). Heritability and transmission analysis of necrotizing meningoencephalitis in the Pug. *Research in Veterinary Science*, 86(3), 438-42. <https://doi.org/10.1016/j.rvsc.2008.10.002>
- Greer, K.A., Wong, A.K., Liu, H., Famula, T.R., Pedersen, N.C., Ruhe, A., Wallace, M. & Neff, M.W. (2010). Necrotizing meningoencephalitis of Pug dogs associates with dog leukocyte antigen class II and resembles acute variant forms of multiple sclerosis. *Tissue Antigens*, 76(2), 110-8. <https://doi.org/10.1111/j.1399-0039.2010.01484.x>
- Griffin, J.F., Levine, J.M., Levine, G.J. & Fosgate, G.T. (2008). Meningomyelitis in dogs: a retrospective review of 28 cases (1999 to 2007). *Journal of Small Animal Practice*, 49(10), 509-517. <https://doi.org/https://doi.org/10.1111/j.1748-5827.2008.00588.x>
- Groves, R.M. (2006). Nonresponse Rates and Nonresponse Bias in Household Surveys. *Public Opinion Quarterly*, 70(5), 646-675. <https://doi.org/10.1093/poq/nfl033>
- Guevar, J., Penderis, J., Faller, K., Yeaman, C., Stalin, C. & Gutierrez-Quintana, R. (2014). Computer-assisted radiographic calculation of spinal curvature in brachycephalic "screw-tailed" dog breeds with congenital thoracic vertebral malformations: reliability and clinical evaluation. *PLoS one*, 9(9), e106957. <https://doi.org/10.1371/journal.pone.0106957>
- Gutierrez-Quintana, R., Guevar, J., Stalin, C., Faller, K., Yeaman, C. & Penderis, J. (2014). A proposed radiographic classification scheme for congenital thoracic vertebral malformations in brachycephalic "screw-tailed" dog breeds. *Veterinary radiology & ultrasound*, 55(6), 585-91. <https://doi.org/10.1111/vru.12172>
- Hansen, H.J. (1952). A pathologic-anatomical study on disc degeneration in dog, with special reference to the so-called enchondrosis intervertebralis. *Acta Orthop Scand Suppl*, 11, 1-117. <https://doi.org/10.3109/ort.1952.23.suppl-11.01>
- Hensinger, R.N. (2009). Congenital scoliosis: etiology and associations. *Spine (Phila Pa 1976)*, 34(17), 1745-50. <https://doi.org/10.1097/BRS.0b013e3181abf69e>
- Higgins, R.J., Dickinson, P.J., Kube, S.A., Moore, P.F., Couto, S.S., Vernau, K.M., Sturges, B.K. & Lecouteur, R.A. (2008). Necrotizing meningoencephalitis

- in five Chihuahua dogs. *Veterinary Pathology*, 45(3), 336-46.  
<https://doi.org/10.1354/vp.45-3-336>
- Hildebrand, M. (1968). Symmetrical gaits of dogs in relation to body build. *Journal of Morphology*, 124(3), 353-60.  
<https://doi.org/10.1002/jmor.1051240308>
- Hoepfner, M.P., Lundquist, A., Pirun, M., Meadows, J.R.S., Zamani, N., Johnson, J., Sundström, G., Cook, A., FitzGerald, M.G., Swofford, R., Mauceli, E., Moghadam, B.T., Greka, A., Alföldi, J., Abouelleil, A., Aftuck, L., Bessette, D., Berlin, A., Brown, A., Gearin, G., Lui, A., Macdonald, J.P., Priest, M., Shea, T., Turner-Maier, J., Zimmer, A., Lander, E.S., di Palma, F., Lindblad-Toh, K. & Grabherr, M.G. (2014). An Improved Canine Genome and a Comprehensive Catalogue of Coding Genes and Non-Coding Transcripts. *PLOS ONE*, 9(3), e91172.  
<https://doi.org/10.1371/journal.pone.0091172>
- Hoffman, G.S., Ellsworth, C.A., Wells, E.E., Franck, W.A. & Mackie, R.W. (1983). Spinal arachnoiditis. What is the clinical spectrum? II. Arachnoiditis induced by Pantopaque/autologous blood in dogs, a possible model for human disease. *Spine*, 8(5), 541-51.
- Holm, S., Maroudas, A., Urban, J.P.G., Selstam, G. & Nachemson, A. (1981). Nutrition of the Intervertebral Disc: Solute Transport and Metabolism. *Connective Tissue Research*, 8(2), 101-119.  
<https://doi.org/10.3109/03008208109152130>
- Hoon-Hanks, L.L., McGrath, S., Tyler, K.L., Owen, C. & Stenglein, M.D. (2018). Metagenomic Investigation of Idiopathic Meningoencephalomyelitis in Dogs. *J Vet Intern Med*, 32(1), 324-330.  
<https://doi.org/https://doi.org/10.1111/jvim.14877>
- Hou, D., Kang, N., Yin, P. & Hai, Y. (2018). Abnormalities associated with congenital scoliosis in high-altitude geographic regions. *Int Orthop*, 42(3), 575-581. <https://doi.org/10.1007/s00264-018-3805-2>
- Howie, B.N., Donnelly, P. & Marchini, J. (2009). A Flexible and Accurate Genotype Imputation Method for the Next Generation of Genome-Wide Association Studies. *PLoS Genetics*, 5(6), e1000529.  
<https://doi.org/10.1371/journal.pgen.1000529>
- Idris, Z., Ghazali, F.H. & Abdullah, J.M. (2014). Fibromyalgia and arachnoiditis presented as an acute spinal disorder. *Surg Neurol Int*, 5, 151.  
<https://doi.org/10.4103/2152-7806.143364>
- Ikeda, K., Nakayama, Y. & Ishii, S. (1992). Congenital absence of lumbosacral articular process: report of three cases. *J Spinal Disord*, 5(2), 232-6.  
<https://doi.org/10.1097/00002517-199206000-00016>
- Kaplan, K.M., Spivak, J.M. & Bendo, J.A. (2005). Embryology of the spine and associated congenital abnormalities. *The Spine Journal*, 5(5), 564-576.  
<https://doi.org/https://doi.org/10.1016/j.spinee.2004.10.044>

- Khan, M.U., Devlin, J.A. & Fraser, A. (2016). Adhesive arachnoiditis in mixed connective tissue disease: a rare neurological manifestation. *BMJ Case Rep*, 2016. <https://doi.org/10.1136/bcr-2016-217418>
- Kobayashi, Y., Ochiai, K., Umemura, T., Goto, N., Ishida, T. & Itakura, C. (1994). Necrotizing meningoencephalitis in pug dogs in Japan. *Journal of comparative pathology*, 110(2), 129-36.
- Kramer, J.W., Schiffer, S.P., Sande, R.D., Rantanen, N.W. & Whitener, E.K. (1982). Characterization of heritable thoracic hemivertebra of the German shorthaired pointer. *Journal of the American Veterinary Medical Association*, 181(8), 814-5.
- Kuwabara, M., Tanaka, S. & Fujiwara, K. (1998). Magnetic resonance imaging and histopathology of encephalitis in a Pug. *The Journal of veterinary medical science*, 60(12), 1353-5.
- Lackmann, F., Forterre, F., Brunberg, L. & Loderstedt, S. (2022). Epidemiological study of congenital malformations of the vertebral column in French bulldogs, English bulldogs and pugs. *Veterinary Record*, 190(1), e509. <https://doi.org/https://doi.org/10.1002/vetr.509>
- Levine, J.M., Fosgate, G.T., Porter, B., Schatzberg, S.J. & Greer, K. (2008). Epidemiology of necrotizing meningoencephalitis in Pug dogs. *J Vet Intern Med*, 22(4), 961-8. <https://doi.org/10.1111/j.1939-1676.2008.0137.x>
- Liu, N.-C., Troconis, E.L., Kalmar, L., Price, D.J., Wright, H.E., Adams, V.J., Sargan, D.R. & Ladlow, J.F. (2017). Conformational risk factors of brachycephalic obstructive airway syndrome (BOAS) in pugs, French bulldogs, and bulldogs. *PLOS ONE*, 12(8), e0181928. <https://doi.org/10.1371/journal.pone.0181928>
- Liu, N.C., Adams, V.J., Kalmar, L., Ladlow, J.F. & Sargan, D.R. (2016). Whole-Body Barometric Plethysmography Characterizes Upper Airway Obstruction in 3 Brachycephalic Breeds of Dogs. *J Vet Intern Med*, 30(3), 853-65. <https://doi.org/10.1111/jvim.13933>
- Lloyd, R.A., Butler, J.E., Gandevia, S.C., Ball, I.K., Toson, B., Stoodley, M.A. & Bilston, L.E. (2020). Respiratory cerebrospinal fluid flow is driven by the thoracic and lumbar spinal pressures. *The Journal of Physiology*, 598(24), 5789-5805. <https://doi.org/https://doi.org/10.1113/JP279458>
- Lodato, D.L. & Hedlund, C.S. (2012). Brachycephalic airway syndrome: pathophysiology and diagnosis. *Compendium*, 34(7), E3.
- Lourinho, F., Holdsworth, A., McConnell, J.F., Gonçalves, R., Gutierrez-Quintana, R., Morales, C., Lowrie, M., Trevail, R. & Carrera, I. (2020). Clinical features and MRI characteristics of presumptive constrictive myelopathy in 27 pugs. *Vet Radiol Ultrasound*, 61(5), 545-554. <https://doi.org/10.1111/vru.12890>
- Malik, R., Allan, G.S., Howlett, C.R., Thompson, D.E., James, G., McWhirther, C. & Kendall, K. (1999). Osteochondrodysplasia in Scottish Fold cats.

- Australian veterinary journal*, 77(2), 85-92.  
<https://doi.org/https://doi.org/10.1111/j.1751-0813.1999.tb11672.x>
- Manichaikul, A., Mychaleckyj, J.C., Rich, S.S., Daly, K., Sale, M. & Chen, W.-M. (2010). Robust relationship inference in genome-wide association studies. *Bioinformatics*, 26(22), 2867-2873.  
<https://doi.org/10.1093/bioinformatics/btq559>
- Maroudas, A., Stockwell, R.A., Nachemson, A. & Urban, J. (1975). Factors involved in the nutrition of the human lumbar intervertebral disc: cellularity and diffusion of glucose in vitro. *Journal of Anatomy*, 120(Pt 1), 113-30.
- Mathiesen, C.B., de la Puerta, B., Groth, A.M., Rutherford, S. & Cappello, R. (2018). Ventral stabilization of thoracic kyphosis through bilateral intercostal thoracotomies using SOP (String of Pearls) plates contoured after a 3-dimensional print of the spine. *Veterinary Surgery*, 47(6), 843-851. <https://doi.org/10.1111/vsu.12939>
- Matsuki, N., Fujiwara, K., Tamahara, S., Uchida, K., Matsunaga, S., Nakayama, H., Doi, K., Ogawa, H. & Ono, K. (2004). Prevalence of autoantibody in cerebrospinal fluids from dogs with various CNS diseases. *Journal of Veterinary Medical Science*, 66(3), 295-7.  
<https://doi.org/10.1292/jvms.66.295>
- Matsuki, N., Takahashi, M., Yaegashi, M., Tamahara, S. & Ono, K. (2009). Serial examinations of anti-GFAP autoantibodies in cerebrospinal fluids in canine necrotizing meningoencephalitis. *The Journal of veterinary medical science*, 71(1), 99-100.
- Mauler, D.A., De Decker, S., De Risio, L., Volk, H.A., Dennis, R., Gielen, I., Van der Vekens, E., Goethals, K. & Van Ham, L. (2014). Signalment, clinical presentation, and diagnostic findings in 122 dogs with spinal arachnoid diverticula. *J Vet Intern Med*, 28(1), 175-81.  
<https://doi.org/10.1111/jvim.12241>
- Mavrides, D., Charalambous, M. & Freeman, P. (2021). Long-term follow-up of spinal segmental stabilization for surgical treatment of dorsal hemivertebrae associated with kyphosis in brachycephalic dogs. *Canadian Veterinary Journal*, 62(12), 1323-1327.
- Mc, L.R., Bailey, O.T., Schurr, P.H. & Ingraham, F.D. (1954). Myelomalacia and multiple cavitations of spinal cord secondary to adhesive arachnoiditis; an experimental study. *Archives of pathology*, 57(2), 138-46.
- McDonnell, J.J., Knowles, K.E., deLahunta, A., Bell, J.S., Lowrie, C.T. & Todhunter, R.J. (2003). Thoracolumbar spinal cord compression due to vertebral process degenerative joint disease in a family of Shiloh Shepherd dogs. *J Vet Intern Med*, 17(4), 530-7.  
<https://doi.org/10.1111/j.1939-1676.2003.tb02474.x>
- Mellado, J.M., Larrosa, R., Martín, J., Yanguas, N., Solanas, S. & Cozcolluela, M.R. (2011). MDCT of variations and anomalies of the neural arch and its processes: part 1--pedicles, pars interarticularis, laminae, and spinous

- process. *AJR Am J Roentgenol*, 197(1), W104-13. <https://doi.org/10.2214/ajr.10.5803>
- Mitchell, T.J., Knowler, S.P., van den Berg, H., Sykes, J. & Rusbridge, C. (2014). Syringomyelia: determining risk and protective factors in the conformation of the Cavalier King Charles Spaniel dog. *Canine Genetics and Epidemiology*, 1(1), 9. <https://doi.org/10.1186/2052-6687-1-9>
- Miyake, H., Inoue, A., Tanaka, M. & Matsuki, N. (2013). Serum glial fibrillary acidic protein as a specific marker for necrotizing meningoencephalitis in Pug dogs. *The Journal of veterinary medical science*, 75(11), 1543-5.
- Moissonnier, P., Gossot, P. & Scotti, S. (2011). Thoracic kyphosis associated with hemivertebra. *Veterinary Surgery*, 40(8), 1029-32. <https://doi.org/10.1111/j.1532-950X.2011.00876.x>
- Morgan, J.P. (1968). Congenital Anomalies of the Vertebral Column of the Dog: A Study of the Incidence and Significance Based on a Radiographic and Morphologic Study. *Veterinary radiology*, 9, 21-29.
- Morgan, J.P. (1999). Transitional lumbosacral vertebral anomaly in the dog: a radiographic study. *J Small Anim Pract*, 40(4), 167-72. <https://doi.org/10.1111/j.1748-5827.1999.tb03784.x>
- Mortazavi, M.M., Quadri, S.A., Khan, M.A., Gustin, A., Suriya, S.S., Hassanzadeh, T., Fahimdanesh, K.M., Adl, F.H., Fard, S.A., Taqi, M.A., Armstrong, I., Martin, B.A. & Tubbs, R.S. (2018). Subarachnoid Trabeculae: A Comprehensive Review of Their Embryology, Histology, Morphology, and Surgical Significance. *World Neurosurg*, 111, 279-290. <https://doi.org/10.1016/j.wneu.2017.12.041>
- Muñana, K.R. (1996). Encephalitis and meningitis. *Vet Clin North Am Small Anim Pract*, 26(4), 857-74.
- Nishida, H., Nakata, K., Maeda, S. & Kamishina, H. (2019). Prevalence and pattern of thoracolumbar caudal articular process anomalies and intervertebral disk herniations in pugs. *Journal of Veterinary Medical Science*, 81(6), 906-910. <https://doi.org/10.1292/jvms.18-0521>
- Nykamp, S.G., Scrivani, P.V., Kennedy, S. & Bliss, S.P. (2001). What is your diagnosis? Attenuation of the dorsal and lateral columns of contrast material and slight ventral deviation of the dorsal column at T12-T13 and T13-L1. *Journal of the American Veterinary Medical Association*, 218(9), 1417-8. <https://doi.org/10.2460/javma.2001.218.1417>
- O'Neill, D.G., Darwent, E.C., Church, D.B. & Brodbelt, D.C. (2016). Demography and health of Pugs under primary veterinary care in England. *Canine Genet Epidemiol*, 3, 5. <https://doi.org/10.1186/s40575-016-0035-z>
- O'Neill, D.G., Packer, R.M.A., Francis, P., Church, D.B., Brodbelt, D.C. & Pegram, C. (2021). French Bulldogs differ to other dogs in the UK in propensity for many common disorders: a VetCompass study. *Canine Med Genet*, 8(1), 13. <https://doi.org/10.1186/s40575-021-00112-3>
- O'Neill, D.G., Skipper, A., Packer, R.M.A., Lacey, C., Brodbelt, D.C., Church, D.B. & Pegram, C. (2022). English Bulldogs in the UK: a VetCompass

- study of their disorder predispositions and protections. *Canine Med Genet*, 9(1), 5. <https://doi.org/10.1186/s40575-022-00118-5>
- O'Neill, D.G., Sahota, J., Brodbelt, D.C., Church, D.B., Packer, R.M.A. & Pegram, C. (2022). Health of Pug dogs in the UK: disorder predispositions and protections. *Canine Medicine and Genetics*, 9(1), 4. <https://doi.org/10.1186/s40575-022-00117-6>
- Packer, R.M., Hendricks, A., Tivers, M.S. & Burn, C.C. (2015). Impact of Facial Conformation on Canine Health: Brachycephalic Obstructive Airway Syndrome. *PLoS one*, 10(10), e0137496. <https://doi.org/10.1371/journal.pone.0137496>
- Packer, R.M.A., O'Neill, D.G., Fletcher, F. & Farnworth, M.J. (2019). Great expectations, inconvenient truths, and the paradoxes of the dog-owner relationship for owners of brachycephalic dogs. *PLOS ONE*, 14(7), e0219918. <https://doi.org/10.1371/journal.pone.0219918>
- Park, E.S., Uchida, K. & Nakayama, H. (2012). Comprehensive Immunohistochemical Studies on Canine Necrotizing Meningoencephalitis (NME), Necrotizing Leukoencephalitis (NLE), and Granulomatous Meningoencephalomyelitis (GME). *Veterinary Pathology*, 49(4), 682-692. <https://doi.org/10.1177/0300985811429311>
- Parker, H.G., Dreger, D.L., Rimbault, M., Davis, B.W., Mullen, A.B., Carpintero-Ramirez, G. & Ostrander, E.A. (2017). Genomic Analyses Reveal the Influence of Geographic Origin, Migration, and Hybridization on Modern Dog Breed Development. *Cell reports*, 19(4), 697-708. <https://doi.org/10.1016/j.celrep.2017.03.079>
- Pedersen, N., Liu, H., Millon, L. & Greer, K. (2011). Dog leukocyte antigen class II-associated genetic risk testing for immune disorders of dogs: simplified approaches using Pug dog necrotizing meningoencephalitis as a model. *Journal of veterinary diagnostic investigation*, 23(1), 68-76. <https://doi.org/10.1177/104063871102300110>
- Penderis, J., Schwarz, T., McConnell, J.F., Garosi, L.S., Thomson, C.E. & Dennis, R. (2005). Dysplasia of the caudal vertebral articular facets in four dogs: results of radiographic, myelographic and magnetic resonance imaging investigations. *Veterinary Record*, 156(19), 601-5. <https://doi.org/10.1136/vr.156.19.601>
- Perry, V.H. & Teeling, J. (2013). Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Semin Immunopathol*, 35(5), 601-12. <https://doi.org/10.1007/s00281-013-0382-8>
- Platt, S. & Garosi, L. (2012). Examining the neurological emergency. In: Platt, S. & Garosi, L. (eds) *Small Animal Neurological Emergencies*. London, UK: Manson Publishing. 21.
- Raziyeva, K., Kim, Y., Zharkinbekov, Z., Kassymbek, K., Jimi, S. & Saparov, A. (2021). Immunology of Acute and Chronic Wound Healing. *Biomolecules*, 11(5). <https://doi.org/10.3390/biom11050700>

- Rice, I., Wee, M.Y. & Thomson, K. (2004). Obstetric epidurals and chronic adhesive arachnoiditis. *British journal of anaesthesia*, 92(1), 109-20.
- Rohdin, C., Jäderlund, K.H., Ljungvall, I., Lindblad-Toh, K. & Häggström, J. (2018). High prevalence of gait abnormalities in pugs. *Veterinary Record*. <https://doi.org/10.1136/vr.104510>
- Rohdin, C., Ljungvall, I., Häggström, J., Leijon, A., Lindblad-Toh, K., Matiasek, K., Rosati, M., Wohlsein, P. & Jäderlund, K.H. (2020). Thoracolumbar meningeal fibrosis in pugs. *J Vet Intern Med*, 34(2), 797-807. <https://doi.org/10.1111/jvim.15716>
- Rohdin, C., Nyman, H.T., Wohlsein, P. & Hultin Jaderlund, K. (2014). Cervical spinal intradural arachnoid cysts in related, young pugs. *The journal of small animal practice*, 55(4), 229-34. <https://doi.org/10.1111/jsap.12167>
- Rorden, C., Griswold, M.C., Moses, N., Berry, C.R., Keller, G.G., Rivas, R., Flores-Smith, H., Shaffer, L.G. & Malik, R. (2021). Radiographical Survey of Osteochondrodysplasia in Scottish Fold Cats caused by the TRPV4 gene variant. *Human Genetics*, 140(11), 1525-1534. <https://doi.org/10.1007/s00439-021-02337-5>
- Rowe, G.G. & Roche, M.B. (1953). The etiology of separate neural arch. *J Bone Joint Surg Am*, 35-a(1), 102-10.
- Rusbridge, C., Knowler, S.P., Pieterse, L. & McFadyen, A.K. (2009). Chiari-like malformation in the Griffon Bruxellois. *Journal of Small Animal Practice*, 50(8), 386-393. <https://doi.org/https://doi.org/10.1111/j.1748-5827.2009.00744.x>
- Ryan, R., Gutierrez-Quintana, R., Ter Haar, G. & De Decker, S. (2017). Prevalence of thoracic vertebral malformations in French bulldogs, Pugs and English bulldogs with and without associated neurological deficits. *Veterinary Journal*, 221, 25-29. <https://doi.org/10.1016/j.tvjl.2017.01.018>
- Safra, N., Pedersen, N.C., Wolf, Z., Johnson, E.G., Liu, H.W., Hughes, A.M., Young, A. & Bannasch, D.L. (2011). Expanded dog leukocyte antigen (DLA) single nucleotide polymorphism (SNP) genotyping reveals spurious class II associations. *The Veterinary Journal*, 189(2), 220-226. <https://doi.org/https://doi.org/10.1016/j.tvjl.2011.06.023>
- Santifort, K.M., Gutierrez-Quintana, R., Bernardini, M., Kortz, G.D., Gomes, S.A., Lowrie, M., De Decker, S., Glass, E.N. & Bergknut, N. (2022). Vertebral vascular canal dysplasia in French and English Bulldogs: Clinical, CT, and MRI characteristics and prevalence. *Vet Radiol Ultrasound*, 63(3), 281-291. <https://doi.org/10.1111/vru.13067>
- Schlensker, E. & Distl, O. (2013). Prevalence, grading and genetics of hemivertebrae in dogs. *Eur J Comp Anim Pract*, 23, 119-123.
- Schlensker, E. & Distl, O. (2016). Heritability of hemivertebrae in the French bulldog using an animal threshold model. *Veterinary Journal*, 207, 188-9. <https://doi.org/10.1016/j.tvjl.2015.10.044>
- Schrauwen, I., Barber, R.M., Schatzberg, S.J., Siniard, A.L., Corneveaux, J.J., Porter, B.F., Vernau, K.M., Keesler, R.I., Matiasek, K., Flegel, T., Miller,

- A.D., Southard, T., Mariani, C.L., Johnson, G.C. & Huentelman, M.J. (2014). Identification of novel genetic risk loci in Maltese dogs with necrotizing meningoencephalitis and evidence of a shared genetic risk across toy dog breeds. *PLoS One*, 9(11), e112755. <https://doi.org/10.1371/journal.pone.0112755>
- Shibuya, M., Matsuki, N., Fujiwara, K., Imajoh-Ohmi, S., Fukuda, H., Pham, N.T., Tamahara, S. & Ono, K. (2007). Autoantibodies against glial fibrillary acidic protein (GFAP) in cerebrospinal fluids from Pug dogs with necrotizing meningoencephalitis. *Journal of Veterinary Medical Science*, 69(3), 241-5. <https://doi.org/10.1292/jvms.69.241>
- Shim, D.M. & Oh, S.K. (2008). Congenital absence of unilateral lumbar articular process - a case report. *Asian Spine J*, 2(2), 106-8. <https://doi.org/10.4184/asj.2008.2.2.106>
- Skeen, T.M., Olby, N.J., Munana, K.R. & Sharp, N.J. (2003). Spinal arachnoid cysts in 17 dogs. *Journal of the American Animal Hospital Association*, 39(3), 271-82. <https://doi.org/10.5326/0390271>
- Skerritt, G. (2018a). Venous drainage of the spinal cord and brain. In: Skerritt, G. (ed.) *King's Applied Anatomy of the Central Nervous System of Domestic Mammals*. 2nd edition. Oxford, London, UK: Wiley, Blackwell Science. 25-31.
- Skerritt, G. (2018b). The meninges and cerebrospinal fluid. In: Skerritt, G. (ed.) *King's Applied Anatomy of the Central Nervous System of Domestic Mammals*. 2nd edition. Oxford, London, UK: Wiley, Blackwell science. 13-24.
- Skerritt, G. (2018c). Arterial supply to the central nervous system. In: Skerritt, G. (ed.) *King's Applied Anatomy of the Central Nervous System of Domestic Mammals*. Oxford, London, UK: Wiley, Blackwell science. 8-12.
- Smith, G.K. & Walter, M.C. (1988). Spinal decompressive procedures and dorsal compartment injuries: comparative biomechanical study in canine cadavers. *American Journal of Veterinary Research*, 49(2), 266-73.
- Sparrow, Duncan B., Chapman, G., Smith, Allanceson J., Mattar, Muhammad Z., Major, Joeline A., O'Reilly, Victoria C., Saga, Y., Zackai, Elaine H., Dormans, John P., Alman, Benjamin A., McGregor, L., Kageyama, R., Kusumi, K. & Dunwoodie, Sally L. (2012). A Mechanism for Gene-Environment Interaction in the Etiology of Congenital Scoliosis. *Cell*, 149(2), 295-306. <https://doi.org/https://doi.org/10.1016/j.cell.2012.02.054>
- Swainston-Goodger, W. (2013). The pug dog its history and origin. In: *The Pug Dog its History and Origin*. Vintage Dog Books.
- Takanosu, M. & Hattori, Y. (2020). Osteochondrodysplasia in Scottish Fold cross-breed cats. *Journal of Veterinary Medical Science*, 82(12), 1769-1772. <https://doi.org/10.1292/jvms.20-0299>
- Talarico, L.R. & Schatzberg, S.J. (2010). Idiopathic granulomatous and necrotising inflammatory disorders of the canine central nervous system: a review and



- future perspectives. *J Small Anim Pract*, 51(3), 138-49. <https://doi.org/10.1111/j.1748-5827.2009.00823.x>
- Tauro, A., Rose, J., Rusbridge, C. & Driver, C.J. (2019). Surgical Management of Thoracolumbar Myelopathies in Pug Dogs with Concurrent Articular Facet Dysplasia. *VCOT Open*, 02(01), e60-e72.
- Thomson, C.E. & Hahn, C. (2012). Hierarchical organisation in the nervous system. In: Thomson, C.E. & Hahn, C. (eds) *Veterinary Neuroanatomy*. Elsevier, Saunders. 35.
- Timmann, D., Konar, M., Howard, J. & Vandeveld, M. (2007). Necrotising encephalitis in a French bulldog. *J Small Anim Pract*, 48(6), 339-42. <https://doi.org/10.1111/j.1748-5827.2006.00239.x>
- Tipold, A. (1995). Diagnosis of inflammatory and infectious diseases of the central nervous system in dogs: a retrospective study. *J Vet Intern Med*, 9(5), 304-14. <https://doi.org/10.1111/j.1939-1676.1995.tb01089.x>
- Tipold, A. & Stein, V.M. (2010). Inflammatory diseases of the spine in small animals. *Vet Clin North Am Small Anim Pract*, 40(5), 871-9. <https://doi.org/10.1016/j.cvsm.2010.05.008>
- Toda, Y., Matsuki, N., Shibuya, M., Fujioka, I., Tamahara, S. & Ono, K. (2007). Glial fibrillary acidic protein (GFAP) and anti-GFAP autoantibody in canine necrotising meningoencephalitis. *The Veterinary record*, 161(8), 261-4.
- Uchida, K., Hasegawa, T., Ikeda, M., Yamaguchi, R. & Tateyama, S. (1999). Detection of an autoantibody from Pug dogs with necrotizing encephalitis (Pug dog encephalitis). *Veterinary Pathology*, 36(4), 301-7. <https://doi.org/10.1354/vp.36-4-301>
- Uchida, K., Park, E., Tsuboi, M., Chambers, J.K. & Nakayama, H. (2016). Pathological and immunological features of canine necrotising meningoencephalitis and granulomatous meningoencephalitis. *Veterinary Journal*, 213, 72-7. <https://doi.org/10.1016/j.tvjl.2016.05.002>
- Urban, J.P., Smith, S. & Fairbank, J.C. (2004). Nutrition of the intervertebral disc. *Spine (Phila Pa 1976)*, 29(23), 2700-9. <https://doi.org/10.1097/01.brs.0000146499.97948.52>
- von Praun, F., Matiasek, K., Grevel, V., Alef, M. & Flegel, T. (2006). Magnetic resonance imaging and pathologic findings associated with necrotizing encephalitis in two Yorkshire terriers. *Vet Radiol Ultrasound*, 47(3), 260-4. <https://doi.org/10.1111/j.1740-8261.2006.00137.x>
- Weller, R.O., Engelhardt, B. & Phillips, M.J. (1996). Lymphocyte targeting of the central nervous system: a review of afferent and efferent CNS-immune pathways. *Brain Pathol*, 6(3), 275-88. <https://doi.org/10.1111/j.1750-3639.1996.tb00855.x>
- Westworth, D.R. & Sturges, B.K. (2010). Congenital spinal malformations in small animals. *The Veterinary Clinics of North America. Small Animal Practice*, 40(5), 951-81. <https://doi.org/10.1016/j.cvsm.2010.05.009>

- White, D. (2013). Screening for hemivertebra in pugs. *Veterinary Record*, 173(1), 24-24. <https://doi.org/https://doi.org/10.1136/vr.f4289>
- White, E.S. & Mantovani, A.R. (2013). Inflammation, wound repair, and fibrosis: reassessing the spectrum of tissue injury and resolution. *Journal of Pathology*, 229(2), 141-4. <https://doi.org/10.1002/path.4126>
- Wilson, J.W., Kurtz, H.J., Leipold, H.W. & Lees, G.E. (1979). Spina bifida in the dog. *Veterinary Pathology*, 16(2), 165-79. <https://doi.org/10.1177/030098587901600202>
- Wohleb, E.S. & Godbout, J.P. (2013). Basic aspects of the immunology of neuroinflammation. *Mod Trends Pharmacopsychiatry*, 28, 1-19. <https://doi.org/10.1159/000343964>
- Wyatt, S., Goncalves, R., Gutierrez-Quintana, R. & De Decker, S. (2018). Outcomes of nonsurgical treatment for congenital thoracic vertebral body malformations in dogs: 13 cases (2009-2016). *Journal of the American Veterinary Medical Association*, 253(6), 768-773. <https://doi.org/10.2460/javma.253.6.768>
- Yin, J., Valin, K.L., Dixon, M.L. & Leavenworth, J.W. (2017). The Role of Microglia and Macrophages in CNS Homeostasis, Autoimmunity, and Cancer. *J Immunol Res*, 2017, 5150678. <https://doi.org/10.1155/2017/5150678>

## Popular science summary

A number of diseases can affect the dog's spinal cord. Regardless of the underlying cause the clinical consequence of spinal cord disease is a wobbly, uncoordinated, gait with loss of strength. Most spinal diseases cause unique combinations of clinical characteristics including how the disease behaves over time, symmetry of clinical signs, and the presence or absence of pain and incontinence. Dogs with spinal cord disease therefore show highly variable clinical presentations. However, unlike most dogs with spinal cord disease affecting the thoracolumbar spinal cord pugs share a common clinical presentation characterized by slowly progressive signs involving the pelvic limbs without obvious pain, often developing incontinence. There is a lack of understanding as to how common these 'wobbly pugs' are and what causes their strikingly similar clinical presentation. The term pug dog myelopathy (PDM) is used to describe the clinical entity of pugs with thoracolumbar spinal cord disease.

The research presented in this thesis has focused on the pugs with spinal cord disease by investigating their prevalence in the Swedish pug population and by exploring underlying causes for the development of PDM.

A questionnaire based study was used to investigate the prevalence of signs indicating spinal cord disease in pugs. Pug owners were asked specific questions about their dog's gait as well as questions about their dog's general health situation. In addition pug owners participating in the study were asked to submit a video of their dog's gait. Gait abnormalities were common in Swedish pugs and the character of the abnormal gait suggested an underlying spinal cord disorder. In addition, gait abnormalities of younger pugs usually affected the front limbs with

wearing of the nails and the skin of the paws from scuffing. Gait abnormalities in older pugs were instead more commonly affecting the hind limbs. While gait abnormalities of older pugs gradually worsened over time the gait abnormality in younger pugs did not. In general, older pugs presented more often with gait abnormalities. In addition, there was an association between the presence of gait abnormalities and respiratory problems. Potentially breed-related characteristics of the pug predispose it to health problems not previously linked to the brachycephalic syndrome.

Vertebral anomalies may contribute to spinal cord disease. Vertebral anomalies were however as common in pugs without signs of a wobbly, uncoordinated, weak gait, as in pugs with PDM. These results suggested that there must be other or additional factors that contribute to the development of PDM in pugs.

Neuropathological examinations showed that the clinical disease, PDM, was caused by the formation of profound meningeal fibrosis with subsequent parenchymal spinal cord destruction. The common characteristic pathological findings suggest PDM likely represent a continuum or a spectrum of the same disease process. In a significant number of examined pugs inflammatory infiltrations of the brain and the spinal cord were also found. The inflammation consisted of cells that characterize another disease known to affect the central nervous system in pugs, but also other breeds of dogs; necrotizing meningoencephalitis (NME). The results from the pathology study also indicated that inflammation may represent an early component in the development of the disease and that the extensive spinal cord destruction represented the final stage of a protracted disease process. Whether the scar tissue in the spinal cord meninges were the natural result of an increased load, the result of an abnormal immune response or as the result of an inability to form functional scar tissue, is currently unknown, but the devastating consequences of its presence was evident. The excessive fibrous tissue interfered with the vascular supply to the spinal cord as well as caused obstruction of cerebrospinal fluid (CSF) flow to the adjacent spinal cord section which atrophied over time.

The importance of inflammation for the development of PDM, were investigated by biomarkers previously used in studies of pugs with NME. Biomarker studies of blood and CSF provided further support for the immune system to be involved in the development of PDM. Molecular

genetic studies identified several genetic regions that could be associated with the clinical phenotype. Pugs with PDM differed from healthy controls in regions that harbour genes that could be linked to bone, cartilage, scar tissue, and inflammation. How these genes are important or interact for the development of PDM needs to be determined in future studies.

The thesis presents results that increase the understanding of this complex and devastating spinal cord disease in pugs.



## Populärvetenskaplig sammanfattning

Ett flertal sjukdomstillstånd kan drabba hundens ryggmärg. Oavsett bakomliggande orsak leder ryggmärgssjukdom till olika grad av förlamning och vingliga, ostadiga, rörelser. Ryggmärgstillstånd orsakar unika kombinationer av sjukdomsförlopp, symmetri av neurologiska bortfall, förekomst av smärta och inkontinens. Hundar uppvisar därför tydligt varierande sjukdomsbild vid tillstånd i ryggmärgen. Mopsar med sjukdomstillstånd i bröst-ländryggmärgen visar dock, till skillnad från andra hundar, en ovanligt likartad sjukdomsbild. Utmärkande för dessa mopsar är vinglighet och långsamt tilltagande förlamningssymtom i bakbenen, utan uppenbara smärtsymtom, ofta med inkontinens. Förkortningen PDM (pug dog myelopathy) används för att beskriva den sjukdomsbild som karakteriserar dessa vingliga mopsar.

Uppgifter saknas om hur vanliga dessa 'vingelmopsar' är och vad som orsakar deras påfallande likartade symtombild. Denna avhandling har fokuserat på mopsar med ryggmärgssjukdom genom att undersöka dess förekomst i den svenska mopspopulationen samt studera bakomliggande orsaker till PDM. En enkätstudie användes för att undersöka förekomst av rörelseavvikelser som indikerade ryggmärgssjukdom. Mopsägare ombads besvara specifika frågor kring hur deras hund rörde sig samt även frågor om hundens allmänna hälsoläge. Mopsägarna uppmanades dessutom att skicka in videofilmer på hur deras mopsar rörde sig.

Resultaten av enkätstudien visade att det var vanligt att mopsar rörde sig på ett sätt som tyder på ryggmärgssjukdom. Resultatet visade också att yngre mopsars rörelseavvikelser i regel drabbade frambenen med slitage på klor och huden på ovansidan av tassarna från att släpa i marken. Äldre mopsars rörelseavvikelser drabbade istället vanligare bakbenen. De äldre

mopsarnas rörelseavvikelser i bakbenen försämrades vanligtvis med tiden vilket inte var lika vanligt för de yngre mopsarnas rörelsebesvär.

Det var generellt vanligare med rörelseavvikelser på äldre mopsar. Dessutom sågs ett samband mellan förekomst av rörelsebesvär och andningsbesvär. Möjligen är det så att ras-relaterade egenskaper hos mopsen predisponerar den för hälsoproblem som inte tidigare kopplats till brakycefalt syndrom.

Kotförändringar har ansetts kunna bidra till ryggmärgssjukdom. Våra resultat visade dock att kotförändringar var lika vanligt förekommande på mopsar utan ostadighet och förlammningssymtom i bakkroppen som på mopsar med PDM. Följaktligen måste det finnas andra eller ytterligare orsaker som bidrar till att mopsar blir vingliga.

Vid patologisk undersökning av PDM sågs gemensamma karaktäristiska förändringar som tyder på att de vingliga mopsarna sannolikt representerar varianter av ett och samma sjukdomstillstånd. Utmärkande var en omfattande men lokalt begränsad ärrbildning, fibros, av ryggmärgshinnorna i anslutning till ett kraftigt skadat (förtvinat) ryggmärgsavsnitt. I ett betydande antal av de undersökta mopsarna sågs även inflammatoriska celler i hjärna och ryggmärg. Inflammationen bestod av celler som vanligtvis karaktäriserar en annan sjukdom som ses i det centrala nervsystemet på mops, men även andra hundraser; nekrotiserande meningoencefalit (NME). Resultaten från den patologiska undersökningen indikerade dessutom att inflammation var en tidig komponent i utvecklandet av sjukdomen och att den omfattande förtvining som påvisades i ryggmärgen representerade slutstadiet av en lång sjukdomsprocess. Huruvida fibrosen i ryggmärgshinnorna uppkommer som en naturlig följd av ökad belastning, till följd av ett onormalt immunsvaret eller till följd av en oförmåga att bilda ärrvävnad som fungerar, är i dagsläget okänt men det är uppenbart att förekomsten av fibros får förödande konsekvenser. Ärrvävnaden i ryggmärgshinnorna påverkar kärlförsörjningen och flödet av ryggmärgsvätska till det angränsade ryggmärgsavsnittet som med tiden förtvinar.

Biomarkörer som tidigare använts i studier av mopsar med NME analyserades för att undersöka betydelsen av inflammation för uppkomst av PDM. Biomarkörstudierna av blod och ryggmärgsvätska gav ytterligare stöd för att immunsystemet är inblandat i utvecklandet av de symtom som vi ser hos mopsar med PDM. Molekylärgenetiska undersökningar



identifierade ett flertal olika genetiska regioner som kunde kopplas till sjukdomsbilden för PDM. Vingliga mopsar skiljde sig från friska kontroller i regioner som innehöll gener som kunde kopplas till ben, brosk, ärrvävnad och inflammation. På vilket sätt dessa gener har betydelse eller samverkar för uppkomst av PDM behöver fastställas i framtida studier.

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# High prevalence of gait abnormalities in pugs

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The objective of this prospective study was to determine the prevalence of gait abnormalities in a cohort of Swedish pugs by using an owner-based questionnaire targeting signs of gait abnormality and video footage showing the dog's gait. This study also evaluated associated conditions of abnormal gait, including other health disorders prevalent in the breed. Five hundred and fifty (550) pugs registered in the Swedish Kennel Club, of one, five and eight years of age, in 2015 and 2016, were included in the study. Gait abnormalities were reported in 30.7 per cent of the responses. In the majority of cases, the character of the described gait indicated a neurological cause for the gait abnormality. An association was observed between abnormal gait and age, with gait abnormalities being significantly more common in older pugs ( $P=0.004$ ). An association was also found between abnormal gait and dyspnoea, with dyspnoea being significantly more common in pugs with gait abnormalities ( $P<0.0001$ ). This study demonstrated that the prevalence of gait abnormalities was high in the Swedish pug breed and increased with age. Future studies on the mechanisms behind these gait abnormalities are warranted.

## Introduction

Gait is a manner of coordinated limb movement, with the canine walk and trot described as symmetrical gaits.<sup>1</sup> Although incompletely studied, the gait of short-legged dogs, including the pug, has been described.<sup>2</sup> Abnormal gait can be the result of orthopaedic and/or neurological conditions. The pug breed is predisposed for specific orthopaedic conditions<sup>3-4</sup> and neurological problems in the breed have become increasingly recognised in the last few years.<sup>5-14</sup>

In a British study, lameness, as the result of orthopaedic problems, and spinal cord disorders, characterised by paresis and ataxia, were reported in 2.4 per cent versus 1.4 per cent of the pugs attending primary veterinary care.<sup>15</sup> The prevalence of spinal cord disorders presented from the UK<sup>15</sup> corresponds poorly to a

Swedish report,<sup>1</sup> which suggested a sevenfold increase in mortality rate for ataxia, paresis and collapse in pugs compared with other breeds. Adding the attention 'wobbly pugs' are given on the internet suggests a need to systematically determine the prevalence of gait abnormalities in the breed.

The aim of this prospective study was to investigate the prevalence of gait abnormalities in a cohort of Swedish pugs by using an owner-based questionnaire targeting signs of gait abnormality. Specialist evaluation of the gait using video footage of parts of the patient population, to compare with the owners' responses, was an additional aim. The study also evaluated associated conditions of abnormal gait, including other health disorders prevalent in the breed.

## Materials and methods

### Data collection

An invitation to participate in the study was sent by mail to all owners of pugs registered in the Swedish Kennel Club that had dogs aged one, five or eight years in 2015. The following data were obtained from the Swedish Kennel Club register: pedigree number, the dog's name and date of birth, name and address of the owner. An online standardised questionnaire<sup>16</sup> was sent to all owners who accepted the invitation. To increase the number of dogs included in the study, a second

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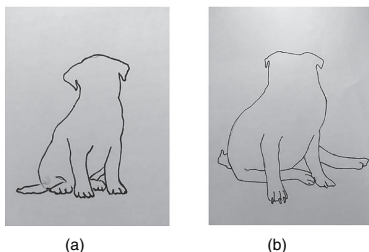
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<sup>16</sup>AGRIA insurance data. Updated dog breed statistics: 2006–2011. 2015. AGRIA Insurance Ltd, Stockholm, Sweden.

<sup>16</sup>Netigate survey platform, Stockholm, Sweden.



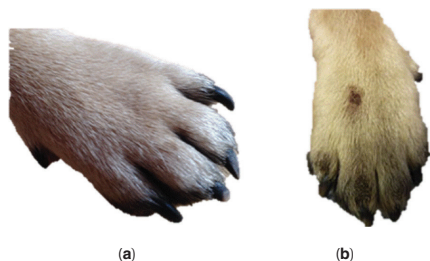
**FIG 1:** The following question was added to the paper-based questionnaire sent out in 2016 in the study of gait in pugs. Which sitting position does your pug prefer: (a) or (b)? Both options could be chosen.

paper-based questionnaire including the same questions as in the online version, but with one additional question (Fig 1), was sent by post to all owners of pugs that turned one, five or eight years of age in 2016 and were registered in the Swedish Kennel Club. The three age groups, rather than the whole pug population, were selected to optimise recognition of age-related gait abnormalities indicating distinct aetiologies, and to limit the amount of questionnaires/data.

### Questionnaire

The investigators used a questionnaire, targeting immediate signs of unsound gait, for example, lameness, ataxia/incoordination, weakness and indirect signs of gait abnormality (eg, inability to jump, abnormal wearing of the nails and/or the skin on the dorsum of the paws) (Fig 2a, b) (Table 1). The owners could either state that their dogs had a normal gait with none of the following signs: lameness, ataxia incoordination, weakness, inability to jump or raise up, abnormal wearing of the nails and/or the skin on the dorsum of the paws, or that their dogs had an abnormal gait with signs of lameness, ataxia incoordination, weakness, inability to jump or raise up, abnormal wearing of the nails and/or the skin on the dorsum of the paws. When an abnormal gait was stated, owners were asked to define the problem as acute; less than one-month duration, or chronic; more than one-month duration.

Pugs assessed by their owners to have an abnormal gait, and pugs reported by their owners as having a normal gait but which exposed an unsound gait later in the



**FIG 2:** In this questionnaire-based study of the gait in pugs, the owners were asked if their pug would wear their nails (a) and/or the skin (b) on the dorsum of their paws as shown in the picture.

questionnaire (by their owners' response to questions about wearing of nails and/or paws) were assessed by the authors as having an abnormal gait and included in group 1. Pugs that were not included in group 1 were described by the authors as having a normal gait (group 2).

Specific questions aimed to further characterise a possible gait abnormality and questions regarding the general health of the pug were asked. In case the pug was no longer alive, the owners were asked to provide information regarding reason/cause of death (Table 1).

For most of the questions, the respondent's answers were limited to a fixed set of responses: either a simple yes or no question, or multiple-choice questions where the respondents had several options to choose from. No preset options were available for certain questions and the owners were able to respond to them freely. The questionnaire did not offer preset options to actively respond 'do not know' and 'choose not to answer'; however, for every question there was a possibility for the owners not to respond or to respond to more than one alternative.

### Video footage

In addition to being asked to complete the questionnaire, the owners of all pugs were encouraged to send video footage of their pug, showing their dog walking back and forth, slowly on a leash, and also showing the dog walking from the side.<sup>iii</sup> Two board-certified veterinary neurologists (CR and KHJ) evaluated all videos independently, each on two separate occasions, and classified the dogs as having normal or abnormal gait. Results from the two raters were compared and in cases of disagreement the videos were examined again in normal and slow motion before arriving at a final, joint decision. The specialist's evaluation of the gait was then compared with the owner's responses in the questionnaire. A normal gait in the videos was defined as coordinated walking or trotting without signs of pacing,<sup>2</sup> with no visible or audible lameness or paresis and with no scuffing of the nails and/or skin on the dorsum of the paws.

### Statistical analysis

The statistical analyses were performed using a commercially available statistical software program.<sup>iv</sup> Descriptive statistics were used for dog characteristics, gait abnormalities and presence of comorbidities. Continuous variables were reported as median and IQR. If owners had made multiple choices each single reply was included in the analysis.

<sup>iii</sup><https://www.youtube.com/watch?v=raIfVZba218&feature=youtu.be>

<sup>iv</sup>JMP Pro V11.2.0, Cary, NC, USA.



**TABLE 1: Questions included in the questionnaire sent to owners of pugs one, five and eight years of age and registered in the Swedish Kennel Club. When limited or fixed sets of responses were available they are shown in brackets**

Questions related to gait	Questions related to general health
<p>Normal or abnormal gait?</p> <ul style="list-style-type: none"> <li>▶ Age when abnormal gait was first noticed (six months; one, two, three, four, five, six, seven or eight years of age)</li> <li>▶ Onset of abnormal gait (acute, insidious)</li> <li>▶ Course of abnormal gait (constant, progressing, improving)</li> <li>▶ What legs are involved in the abnormal gait? (thoracic limbs, pelvic limbs or both)</li> <li>▶ Symmetrical or asymmetrical involvement of legs</li> <li>▶ Ability to jump up and down a sofa</li> <li>▶ Signs indicating abnormal wearing of the nails or the skin on the dorsal aspect of the paws</li> <li>▶ What legs are involved in abnormal wearing of the nails and/or the skin on the dorsal aspect of paws? (thoracic limbs, pelvic limbs or both)</li> <li>▶ When did the abnormal wearing of the nails and/or the skin on the dorsal aspect of the paws start? (as a puppy, at one year of age, more than six months ago, more than one year ago)</li> <li>▶ Reluctance to walk on specific grounds (asphalt, parquet, gravel, grass)</li> <li>▶ Use of paw protection</li> <li>▶ Signs indicating pain (vocalising, reluctance to go for walks, resenting being lifted, unwilling to wear a collar, difficulty finding a resting position, avoiding specific positions, unwilling to be petted and irritable mood)</li> <li>▶ Tail carriage (double curled, tight, weak)</li> <li>▶ Incontinence (faecal, urinary)</li> <li>▶ Incontinence seen after laying down, during playing, during eating, when excited</li> <li>▶ Difficulties posturing while urinating/defecating</li> <li>▶ Describe your pug's clinical sign (owner allowed to respond freely)</li> <li>▶ Any previous traumatic event (owner allowed to respond freely)</li> <li>▶ Veterinary examination performed due to abnormal gait, wearing of the nails and/or the skin on the dorsum of the paws</li> <li>▶ Any relatives with unsound gait</li> </ul>	<p>Has your pug ever shown any of these signs or been diagnosed with any of these disorders?</p> <ul style="list-style-type: none"> <li>▶ Seizures</li> <li>▶ Syncope</li> <li>▶ Dyspnoea</li> </ul> <p>Has your dog had surgery for dyspnoea?</p> <ul style="list-style-type: none"> <li>▶ Pigmentary keratopathy</li> <li>▶ Corneal ulcers</li> <li>▶ Abnormal scratching around neck/ears/head</li> <li>▶ Chronic skin problems</li> <li>▶ Demodicosis</li> <li>▶ Pug dog encephalitis</li> <li>▶ Fly snapping</li> <li>▶ Licking the air</li> </ul> <p>Is the dog alive?</p> <p>In case the dog is no longer alive:</p> <ul style="list-style-type: none"> <li>▶ Was the dog euthanased or did it die?</li> <li>▶ Why did the dog die/why was the dog euthanased? (gait abnormality, dyspnoea, skin problems, incontinence, epilepsy, pug dog encephalitis, eye problems, other disorder)</li> </ul>

The chi-squared test or Fisher's exact test was used to test for differences in proportions concerning general health disorders in age groups and in gait abnormality groups. Differences in continuous variables between groups were tested using Wilcoxon test.

Possible associations between presence of gait abnormalities and dog characteristic (age, sex, weight) variables and presence of specific comorbidities (faecal and urinary incontinence, seizures, syncope, dyspnoea, pigmentary keratitis, corneal ulcer, abnormal scratching around neck/ears and head, chronic skin problems, demodicosis, pug dog encephalitis, fly snapping and licking the air) were investigated using backward stepwise multivariate logistic regression analysis. Only variables with a P value of <0.2 in the univariate regression analysis were included in the multivariate analysis. Level of statistical significance was set at P<0.05.

## Results

### Response rate

Of the 2374 invitations and questionnaires sent to selected pug owners, 26 per cent were returned. Five hundred and fifty owners specifically responded to the main question concerning gait (normal vs abnormal gait) (Fig 3).

In the 550 returned questionnaires, with responses concerning normal versus abnormal gait, the response rate for individual questions ranged from 220/550 (40.0 per cent) (signs of pain) to 528/550 (96.0 per cent) (any seizures).

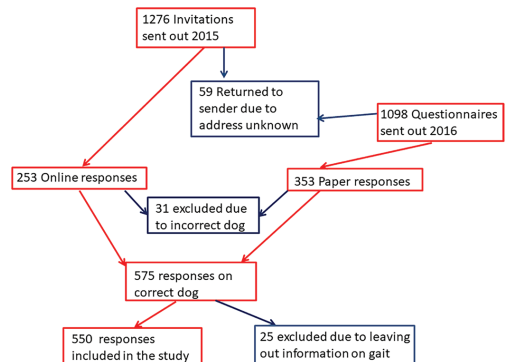
### General description of the study population

A detailed description on signalment and clinical variables in the entire cohort of pugs is presented in Table 2.

Information about sex, weight and sitting position by age groups is presented in Table 3.

### Prevalence of gait abnormality

In the responses to the questionnaire, 'a normal gait' was described by the owners in 79.6 per cent of the pugs. 'An abnormal gait for less than a month' was described in 4.4 per cent of the pugs and 'a chronic gait abnormality (>1 month duration)' in 16.0 per cent of the pugs. One hundred and twenty-eight pug owners responded that their dog wore down their nails and/or the skin on the dorsum of their paws. Fifty-seven of these 128 owners responded, in the same questionnaire, that their pug had a normal gait. Adding these 57 pugs showing indirect signs of unsound gait (wearing their nails and/or the skin on the dorsum of the paws from scuffing) resulted in a prevalence of 30.7 per cent for gait abnormalities (group 1).



**FIG 3: Flow chart over the number of responses to the questionnaires sent to owners of pugs one, five and eight years of age registered in the Swedish Kennel Club.**

**TABLE 2:** Distribution of signalment and clinical variables in 550 pugs with a normal and an abnormal gait. Pugs with an abnormal gait included all pugs perceived by their owners to have a gait abnormality, and all pugs that were reported to wear down their nails and/or the skin on the dorsum of their paws independent of their owner's perception of the gait. Note that there was a possibility for the owner to include more than one answer or provide no response to a specific question, which is the reason why the numbers in each column may not add up to the total in the adjacent column

Variable	Total number of dogs	Normal gait	Abnormal gait
Number of pugs	550	381	169
Sex			
Female	233	176	57
Male	209	136	73
Spayed female	49	33	16
Neutered male	49	28	21
Unknown sex	10	8	2
Median age (months)	58 (IQR=69)	54 (IQR=52)	61 (IQR=39)
Median bodyweight (kg)	9 (IQR=2)	8 (IQR=1)	9 (IQR=2)
Abnormal wearing of nails	128/336 (38.1%)	–	128/165 (77.6%)
Thoracic limbs	95/118 (80.5%)	–	95/118 (80.5%)
Pelvic limbs	5/118 (4.2%)	–	5/118 (4.2%)
Thoracic and pelvic limbs	18/118 (15.3%)	–	18/118 (15.3%)
Wearing nails to the extent they bleed	21/273 (7.7%)	–	21/157 (13.4%)
Abnormal wearing of skin on paws	22/335 (6.6%)	–	22/160 (13.8%)
Abnormal wearing of skin on paws to the extent it bleeds	19/150 (12.7%)	–	19/95 (20.0%)
Incontinence			
Faecal	13/357 (3.6%)	2/195 (1.0%)	11/162 (6.8%)
Urinary	23/341 (6.7%)	7/180 (3.9%)	16/161 (9.9%)
Sitting position (a)	119/329 (36.2%)	90/195 (46.2%)	29/134 (21.6%)
Sitting position (b)	210/329 (63.8%)	105/195 (53.8%)	105/134 (78.4%)
Pain	22/199 (11.1%)	1/89 (1.1%)	21/110 (19.1%)
Double curled tail	142/351 (40.5%)	88/192 (45.8%)	54/159 (34.0%)
Unable to jump up a bed or a sofa	34/186 (18.3%)	1/58 (1.7%)	30/128 (23.4%)

### Course of owner-perceived gait abnormality

The owners reported that in 63/92 (68.5 per cent) of the pugs the first signs of gait abnormality were insidious in nature, and in 36/99 (36.4 per cent) of the dogs, a progression of clinical signs was described. An insidious onset was reported in 21/32 (65.6 per cent) of the pugs with a thoracic limb involvement, and in 19/23 (82.6 per cent) of the dogs with pelvic limb involvement.

**TABLE 3:** Sex, bodyweight, preferred sitting position (Fig 1) and gait status by age group in 550 pugs. Pugs with an abnormal gait included all pugs perceived by their owners to have a gait abnormality, and all pugs that were reported to wear down their nails and/or the skin on the dorsum of their paws independent of their owner's perception of the gait. Note that there was a possibility for the owner to include more than one answer or provide no response to a specific question, which is the reason why the numbers in each column may not add up to the total in the adjacent column

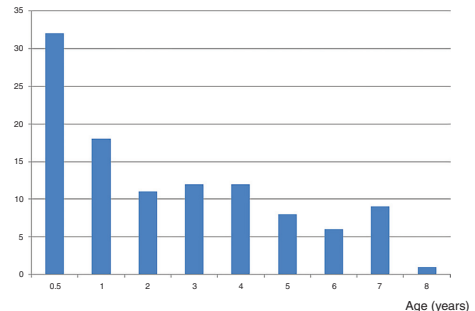
Variable	One year	Five years	Eight years
Number of pugs	168	218	164
Sex			
Female	87 (51.8%)	86 (39.4%)	60 (36.7%)
Male	69 (41.1%)	87 (39.9%)	53 (32.3%)
Spayed female	1 (0.6%)	19 (8.7%)	29 (17.7%)
Neutered male	9 (5.4%)	24 (11.0%)	16 (9.8%)
Unknown sex	2 (1.2%)	2 (0.9%)	6 (3.7%)
Bodyweight (kg)	8 (IQR=2)	9 (IQR=2)	9 (IQR=2)
Sitting position (a)	41/78 (52.6%)	51/148 (34.5%)	27/103 (26.2%)
Sitting position (b)	37/78 (47.4%)	97/148 (65.5%)	76/103 (73.8%)
Pugs with a normal gait	144/168 (85.7%)	149/218 (68.3%)	88/164 (53.7%)
Pugs with an abnormal gait	24/168 (14.3%)	69/218 (31.7%)	76/164 (46.3%)

The clinical signs were described as progressing in 6/32 (18.8 per cent) and in 13/25 (52.0 per cent) of pugs with affected thoracic versus pelvic limbs

### Characteristics of gait abnormality (group 1)

The median age when the gait abnormality started was two (2.0) years (IQR=3.5) (Fig 4). The debut of abnormal gait in the thoracic limbs was reported at a younger age (median one (1.0) year, IQR=0.5) compared with pugs with an abnormal gait affecting the pelvic limbs (median three (3.0) years, IQR=3) (P<0.001).

Number of dogs



**FIG 4:** Distribution of age of onset of gait abnormality in this questionnaire-based study in pugs. The figure includes the 109 pugs whose owners responded to the question related to onset of gait abnormality.



**TABLE 4:** Distribution of general health disorders by age in 550 pugs. Number of positive answers in relation to the total number of answers for the specific question by age group

Variable	One year	Five years	Eight years	P value
Seizures	2/163 (1.2%)	23/213 (10.8%)	22/152 (14.5%)	<0.001
Syncope	3/164 (1.8%)	28/210 (13.3%)	29/153 (19.0%)	<0.001
Dyspnoea	15/162 (9.3%)	75/211 (35.5%)	60/156 (38.5%)	<0.001
Surgery for dyspnoea	3/164 (1.8%)	12/214 (5.6%)	11/152 (7.3%)	0.054
Pigmentary keratopathy	18/154 (11.7%)	69/196 (35.2%)	56/137 (40.9%)	<0.001
Corneal ulcers	22/152 (14.5%)	79/196 (40.3%)	60/140 (42.9%)	<0.001
Abnormal scratching around neck/ears/head	5/163 (3.1%)	27/210 (12.9%)	18/147 (12.2%)	0.002
Chronic skin problems	3/157 (1.9%)	20/208 (9.6%)	12/144 (8.3%)	0.008
Demodicosis	12/158 (7.6%)	17/199 (8.5%)	12/139 (8.6%)	0.95
Pug dog encephalitis	1/158 (0.6%)	2/201 (1.0%)	0/141 (0%)	0.34
Fly snapping	1/161 (0.6%)	5/211 (2.4%)	7/148 (4.7%)	0.058
Licking in the air	17/165 (10.3%)	51/214 (23.8%)	32/154 (20.8%)	0.0021

**TABLE 5:** Distribution of general health disorders by gait abnormality in 550 pugs with a normal and an abnormal gait. Pugs with an abnormal gait included all pugs perceived by their owners to have a gait abnormality, and all pugs that were reported to wear down their nails and/or the skin on the dorsum of their paws independent of their owner's perception of the gait

Variable	Total number of dogs	Normal gait	Abnormal gait	P value
Seizures	47/528 (8.9%)	21/369 (5.7%)	24/156 (15.4%)	0.0004
Syncope	60/527 (11.4%)	29/370 (7.8%)	29/154 (18.8%)	0.0003
Dyspnoea	150/529 (28.4%)	72/367 (19.6%)	78/159 (49.1%)	<0.0001
Surgery for dyspnoea	26/530 (4.9%)	10/367 (2.7%)	15/160 (9.4%)	0.0015
Pigmentary keratopathy	143/487 (29.4%)	81/340 (23.8%)	62/145 (42.8%)	<0.0001
Corneal ulcers	161/488 (33.0%)	96/346 (27.7%)	65/141 (46.1%)	0.0001
Abnormal scratching around neck/ears/head	50/520 (9.6%)	20/364 (5.5%)	28/153 (18.3%)	<0.0001
Chronic skin problems	35/509 (6.9%)	22/359 (6.1%)	11/146 (7.5%)	0.34
Demodicosis	41/496 (8.3%)	27/347 (7.8%)	12/149 (8.1%)	0.70
Pug dog encephalitis	3/500 (0.6%)	1/350 (0.3%)	2/147 (1.4%)	0.21
Fly snapping	13/520 (2.5%)	4/364 (1.1%)	9/153 (5.9%)	0.0031
Licking in the air	100/533 (18.8%)	50/368 (13.6%)	49/162 (30.2%)	<0.0001

Among dogs with an abnormal gait (group 1), thoracic limb involvement 81/158 (51.3 per cent) was more commonly reported than pelvic limb involvement 28/158 (17.7 per cent,  $P < 0.001$ ). Of pugs with an abnormal gait, involvement of both pelvic and thoracic limbs was reported in 49/158 (31.0 per cent).

#### Abnormal wearing of nails and skin on dorsum of the paws

Wearing of the nails was more common in the thoracic limbs; thoracic limb involvement was reported in 113/118 (95.8 per cent) of the pugs wearing nails ( $P < 0.001$ ). In 56/120 (46.7 per cent) the pugs had been wearing their nails since they were a puppy, whereas 98/120 (81.7 per cent) of the pugs had been wearing their nails since one year of age. Abnormal wearing of the skin on the dorsum of the paws was most common in the thoracic limbs, and 11/17 (64.7 per cent) of the pugs who wore down the skin on the dorsum of their paws showed thoracic limb involvement. In 13/19 (68.4 per cent) the pugs had been wearing the skin on the dorsum of their paws since one year of age.

#### Prevalence of pain

Prevalence of pain in pugs with gait abnormality (group 1) is described in Table 2. The most common sign of pain

described by the owners in association with gait abnormalities was reluctance to go for walks, 17/30 (56.7 per cent). The least common signs of pain described by the owners in association with gait abnormalities were an irritable mood, 1/30 (3.3 per cent) and an unwillingness to be petted, 2/30 (6.7 per cent).

#### Prevalence of general health disorders

The prevalence of general health problems was compared between one, five and eight-year-old pugs (Table 4) and between pugs with a normal gait (group 2) and pugs with a gait abnormality (group 1) (Table 5).

A significant association was found between gait abnormality perceived by the owner and age, dyspnoea and abnormal scratching around the neck/ears and head (Table 6). A significant association was also found

**TABLE 6:** The P values and ORs and 95% CIs for dog characteristic variables and comorbidities remaining in the final multivariate logistic regression model including owner-perceived gait abnormality (no/yes) as outcome variable in 550 pugs

Variable	P value	OR (95% CI)
Age	<0.0001	1.24 (0.72 to 0.89)
Dyspnoea	0.003	2.55 (1.55 to 4.20)
Abnormal scratching around neck/ears/head	0.0008	3.83 (1.94 to 7.60)

**TABLE 7:** The P values and ORs and 95% CIs for dog characteristic variables and comorbidities remaining in the final multivariate logistic regression model including gait abnormality (no/yes) as outcome variable in 550 pugs. Pugs with an abnormal gait included all pugs perceived by their owners to have a gait abnormality, and all pugs that were reported to wear down their nails and/or the skin on the dorsum of their paws independent of their owner's perception of the gait

Variable	Pvalue	OR (95% CI)
Age	0.004	1.18 (1.09 to 1.28)
Dyspnoea	<0.0001	3.19 (2.11 to 4.85)

between abnormal gait as assessed by the authors (group 1) and age and dyspnoea (Table 7).

### Prevalence of incontinence

Of 13 pugs with faecal incontinence, three (23.1 per cent) were described by their owners as having a normal gait and eight (61.5 per cent) as having a chronic gait abnormality. Of 23 pugs with urinary incontinence, nine (39.1 per cent) were described as having a normal gait and 14 (60.9 per cent) as having a chronic gait abnormality.

### Reason for death/euthanasia

Forty-seven owners reported one or several causes for their dog's death/euthanasia: abnormal gait was recognised as the single, listed, most frequent cause of death/euthanasia of pugs in this study, 17/59 (28.8 per cent).

### Veterinary consultation

Prevalence of owners who had sought veterinary care for their pug's abnormal gait or for wearing of the nails and/or the skin on the dorsum of the paws is described in Table 8.

### Video evaluation of gait

Eighty-nine videos were sent for evaluation. Seven videos were excluded due to poor quality or other technical reasons. Twenty-three videos were not accompanied by a corresponding questionnaire, and were excluded. The remaining 59 videos included: 26 videos (44.1 per cent) of one-year-old pugs, 20 videos (33.9 per cent) of five-year-old pugs and 13 videos (22.0 per cent)

**TABLE 8:** Prevalence of pugs whose owners, of 550 pugs, had sought veterinary care for their pug's abnormal gait, for wearing of the nails or the skin on the dorsum of the paws. Pugs with an abnormal gait included all pugs perceived by their owners to have a gait abnormality, and all pugs that were reported to wear down their nails and/or the skin on the dorsum of their paws independent of their owner's perception of the gait

Variable	Number of positive answers in relation to the total number of answers for the question
Pugs perceived by their owners of having a chronic gait abnormality	36/81 (44.4%)
Pugs with an abnormal gait	48/149 (32.2%)
Pugs wearing down their nails	31/115 (27.0%)
Pugs wearing down their nails to the extent they would bleed	9/19 (47.4%)
Pugs wearing down the skin on the dorsum of the paws to the extent they would bleed	8/18 (44.4%)

of eight-year-old pugs. Forty-six of 59 (78.0 per cent) owners submitting a video recording claimed their dogs had a normal gait. Subtracting from this the number of videotaped dogs that were reported to wear their nails and/or skin on the paws (six pugs) decreased the number of pugs reported by their owners to show a normal gait from 78.0 to 67.8 per cent. The corresponding numbers for the videos analysed by the specialists were that 40 out of 59 (67.8 per cent) pugs showed a normal gait. In 16/59 (27.1 per cent) of the analysed videos, there was disagreement between owners and specialists. The specialists considered the gait as abnormal in 11 dogs, in which the owners had considered the gait as being normal. The owners assessed the gait as abnormal in five dogs, for which the authors had considered the gait as normal. In four of these five pugs, the owners described abnormal wearing of the nails. Three (3/19) pugs showed lameness as an isolated gait abnormality in the analysed videos.

### Discussion

This study showed that the prevalence of gait abnormalities in the pug breed was high and that it increased with age. Indeed, the single most common cause for death/euthanasia, reported by the owner, was an abnormal gait, which suggests gait abnormalities to be a more significant health problem than what previous, published scientific literature has suggested.<sup>15</sup>

In general, most causes of lameness are orthopaedic in origin, whereas most causes of paresis and ataxia are neurological. Wearing of the nails and/or on the dorsum of the paws, scuffing, is associated with neurological disorders as a consequence of proprioceptive deficits and motor dysfunction.<sup>16-18</sup> Although this study did not aim to differentiate orthopaedic from neurological causes for gait abnormalities, the high prevalence of wearing of nails reported in the questionnaires, and the fact that lameness was not a common finding in submitted videos, suggest that the majority of gait abnormalities in the pugs were indeed related to neurological rather than orthopaedic disorders. This is in accordance with a Swedish insurance database report, presenting a sevenfold increase in mortality rate for ataxia, paresis and collapse in the pug compared with the risk in other breeds<sup>1</sup>. Data from that same source did not show an increase in relative risk for general locomotor problems in pugs compared with the risk in other breeds. It has previously been shown that the insured dog population is similar to the general population of Swedish dogs.<sup>19</sup>

The assessment of the dog's gait was performed by the owners, likely making the results from the questionnaire less reliable. This was also suggested in the analysis of the videos, where the specialists identified more dogs with gait abnormalities than the owners did, 32.2 per cent versus 22.0 per cent. Interestingly, when adding the pugs from the videos where the owners described a normal gait but with wearing of nails and/or

skin on the dorsum of the paws, the specialists and the owners reported identical numbers of pugs with a gait abnormality.

The difficulty to evaluate locomotion, as shown in this study, has previously been described,<sup>20</sup> and the results from the video analysis could be questioned. It is reasonable to assume that mild or intermittent gait abnormalities might not have been appreciated by the owners or by the specialists; thus, the prevalence of gait abnormality in the breed found in this study could indeed have been underestimated. Ideally, the results in this study should be confirmed by objective gait studies, using kinetic and kinematic analysis, in the future.

For the video evaluation of the gait in the present study, we defined the normal gait in the pug as a coordinated walk or trot without pacing,<sup>2</sup> with no visible or audible lameness or paresis and with no wearing of the nails and/or the skin on the dorsum of the paws. Abnormal wearing of the nails and/or skin on the dorsum of the paws could not be easily identified in the submitted videos explaining why four pugs were classified as having an abnormal gait by the owners but as having a normal gait by the specialists. A relatively small proportion, 59/550 (10.7 per cent) of the responders, submitted a video footage of their dog's gait, which could have affected the result. In addition, the video analysed group of pugs were not age matched with the questionnaire-based group of pugs, and the lower proportion of submitted videos of eight-year-old pugs might have affected the result.

Pugs with a gait abnormality (group 1; 30.7 per cent of all pugs) included all pugs with an owner-reported gait abnormality (20.4 per cent) and all pugs that later in the questionnaire were reported to actually scuff, to wear down their nails and/or the skin on the dorsum of their paws. In the present study, pug owners reported abnormal gait more often in the pelvic limbs than the thoracic limbs. This finding was inconsistent with that of a gait abnormality (group 1) being more commonly reported in the thoracic limbs. Also, in almost a third of the pugs with a gait abnormality, both thoracic and pelvic limbs were affected. This inconsistency could be a result of different underlying pathology affecting the thoracic and the pelvic limbs, but could also be the consequence of owners not associating wearing of the nails and/or skin on the dorsum of the paws with abnormal gait. Furthermore, the clinical experience of the authors is that dogs with neurological gait disorders, affecting both thoracic and pelvic limbs, are easily misjudged by laymen as only affecting either or. A disorder with obvious affection of the pelvic limbs may therefore be accompanied by a subtle involvement of the thoracic limbs and vice versa. Nonetheless, neurological deficits in dogs are seen more commonly in the pelvic limbs than in the thoracic limbs in general,<sup>21</sup> and for pugs, vertebral anomalies, including hemivertebrae,<sup>8 12 14 22-25</sup> degenerative disc disease,<sup>5 26</sup> constrictive myelopathy<sup>6 23</sup>

and subarachnoid diverticula (SAD),<sup>5 7 9-11 27</sup> are found more often in the thoracolumbar area. In most of the pugs with abnormal wearing of their nails, the wearing involved the thoracic limbs and had developed already when the dog was one year old, suggesting a congenital or an early onset underlying pathology. The possibility that the wearing of nails is the result of other unrelated causes, for example, conformation, also needs to be considered. Wearing of nails and skin on the dorsum of the paws has previously been described in pugs in association with SAD<sup>13 28</sup>; in these pugs, neurological signs were more pronounced in the thoracic limbs and from a young age.<sup>13 28 29</sup>

Our results show that the prevalence of gait abnormalities in pugs is a greater health problem than what has previously been described.<sup>15</sup> Clinical data from primary care veterinary practices in the UK<sup>15</sup> included conditions that the owners had sought medical attention care for. In the present study, the majority of the owners of pugs with a gait abnormality had not sought veterinary care for their dog's gait, wearing of the nails or incontinence. Possible explanations could be that a veterinarian is only sought if the abnormality is actually appreciated by the owner as *abnormal*, or if the gait abnormality seems associated with signs suggesting suffering (pain).

This study showed an association between abnormal gait (group 1) and age, and an association between abnormal gait and dyspnoea was also confirmed. It has previously been shown that obesity is associated with the brachycephalic airway syndrome<sup>30</sup> and dyspnoea might be the natural consequence of a pug that is not exercising, and therefore gains weight. Weight was however not associated with gait abnormalities and the development of a chronic gait abnormality might therefore be related to dyspnoea and chronic airway obstruction in other, yet unknown, ways. The importance of finding an association between two common health problems in a breed, for example, dyspnoea and abnormal gait, may be questioned. However, specific breed-related characteristics may predispose the pug to health problems not previously associated with the brachycephalic syndrome.

Faecal and urinary incontinence were more common in pugs with an abnormal gait in comparison to pugs with a normal gait (Table 2). The larger number of pugs with urinary incontinence cannot be explained by neutering or spaying as these were not more frequently incontinent compared with intact pugs. A possible association between gait abnormalities and incontinence in the pug needs verification.

The majority of the pugs in this study preferred a sitting position the owners associated with dog (b) (Fig 1). The sitting position in dogs with a chronic gait abnormality was almost exclusively associated with image (b). The sitting position (b) has previously been preferred by dogs with specific orthopaedic disorders,<sup>31</sup> referred to

as a positive 'sit-test'.<sup>21</sup> Sitting with fully extended stifle joints can also be observed in dogs with spinal cord disorders.<sup>32-33</sup> However, position (b) was also reported by more than 50 per cent of owners assessing their pugs having a normal gait and also increased with age in pugs with and without gait abnormality. It needs to be verified if the preferred sitting position (b) might be an indication of a gait abnormality in pugs.

The response rate (26 per cent) of this study might have been affected by the Swedish petition for the right of brachycephalic breeds to breathe; launched the same year the invitation was sent and supported by a large number of Swedish veterinarians, it threatened many owners of brachycephalic breeds, which might have made owner population more reluctant to participate in this study. A pilot study launched before the petition reached a response rate of 35 per cent. It has, however, been shown that a low response rate does not necessarily indicate non-response bias.<sup>34</sup> Suggesting all non-responders belonged to group 2, the prevalence of gait abnormality in the entire study population would have been 7.3 per cent. Additionally, although accepting to participate in the study, many owners did not complete the survey by leaving specific questions unanswered.

Since the majority of pugs in Sweden are registered in the Swedish Kennel Club,<sup>v</sup> the general study population is most likely representative of the Swedish pug breed. The final study population might however be biased; it could be that owners of pugs suffering from obvious gait abnormalities are more prone to respond to the questionnaire. It might also be possible that there is another population of owners who have pugs with a normal gait that have specific interests in responding, for example, breeders.

In conclusion, gait abnormalities were a common finding in the pug breed with a prevalence of 30.7 per cent. Wearing of the nails and/or skin on the dorsum of the paws, predominately in the thoracic limb, was frequently found and from a comparably young age. The aim of this study was to investigate the prevalence of gait abnormalities in the pug breed with no ambition to determine their underlying pathology or prognosis. The results presented in this article may serve as a background to future, urgent studies on underlying pathology and clinical significance of gait abnormalities in the pug breed.

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**Competing interests** None declared.

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## References

- FANCHON L, GRANDJEAN D. Accuracy of asymmetry indices of ground reaction forces for diagnosis of hind limb lameness in dogs. *Am J Vet Res* 2007;68:1089-94.
- HILDEBRAND M. Symmetrical gaits of dogs in relation to body build. *J Morphol* 1968;124:353-9.
- LAFOND E, BREUR GJ, AUSTIN CC. Breed susceptibility for developmental orthopedic diseases in dogs. *J Am Anim Hosp Assoc* 2002;38:467-77.
- O'NEILL DG, MEESON R, SHERIDAN A, et al. The epidemiology of patellar luxation in dogs attending primary-care veterinary practices in England. *Canine Genet Epidemiol* 2016;3:4.
- BISMUTH C, FERRAND FX, MILLET M, et al. Original surgical treatment of thoracolumbar subarachnoid cysts in six chondrodystrophic dogs. *Acta Vet Scand* 2014;56:32.
- FISHER SC, SHORES A, SIMPSON ST. Constrictive myelopathy secondary to hypoplasia or aplasia of the thoracolumbar caudal articular processes in Pugs: 11 cases (1993-2009). *J Am Vet Med Assoc* 2013;242:223-9.
- FLEGEL T, MÜLLER MK, TRUAR K, et al. Thoracolumbar spinal arachnoid diverticula in 5 pug dogs. *Can Vet J* 2013;54:969-73.
- GUTIERREZ-QUINTANA R, GUEVARA J, STALIN C, et al. A proposed radiographic classification scheme for congenital thoracic vertebral malformations in brachycephalic "screw-tailed" dog breeds. *Vet Radiol Ultrasound* 2014;55:585-91.
- MAULIER DA, DE DECKER S, DE RISIO L, et al. Signalment, clinical presentation, and diagnostic findings in 122 dogs with spinal arachnoid diverticula. *J Vet Intern Med* 2014;28:175-81.
- MAULIER DA, DE DECKER S, DE RISIO L, et al. Spinal arachnoid diverticula: outcome in 96 medically or surgically treated dogs. *J Vet Intern Med* 2017;31:849-53.
- MEREN IL, CHAVERA JA, ALCOIT CJ, et al. Shunt tube placement for amelioration of cerebrospinal fluid flow obstruction caused by spinal cord subarachnoid fibrosis in dogs. *Vet Surg* 2011;40:1029-32.
- MOISSONNIER P, GOSSOT P, SCOTTI S. Thoracic kyphosis associated with hemivertebra. *Vet Surg* 2011;40:1029-32.
- RHODIN C, NYMAN HT, WOHLSEIN P, et al. Cervical spinal intradural arachnoid cysts in related, young pugs. *J Small Anim Pract* 2014;55:229-34.
- RYAN R, GUTIERREZ-QUINTANA R, TER HAAR G, et al. Prevalence of thoracic vertebral malformations in French bulldogs, pugs and english bulldogs with and without associated neurological deficits. *Vet J* 2017;221:25-9.
- O'NEILL DG, DARWENT EC, CHURCH DB, et al. Demography and health of pugs under primary veterinary care in England. *Canine Genet Epidemiol* 2016;3:5.
- CARR BJ, DYCUS DL. Canine gait analysis. *Recovery & Rehab* 2016;93-100.
- DELAHUNTA A, GLASS EN. Small animal spinal cord disease. In: DELAHUNTA A, GLASS EN, eds. Veterinary neuroanatomy and clinical neurology. 3rd edn. St Louis: Missouri, USA: Saunders-Elsevier, 2009:244.
- GAROSI L. Examining the neurological emergency. In: PLATT L, GAROSI L, eds. Small animal neurological emergencies. London, UK: Manson Publishing/the veterinary press, 2012:21.
- EGENVALL A, HEDHAMMAR A, BONNETT BN, et al. Survey of the swedish dog population: age, gender, breed, location and enrollment in animal insurance. *Acta Vet Scand* 1999;40:231-40.
- GILLETTE RL, ANGLE TC. Recent developments in canine locomotor analysis: a review. *Vet J* 2008;178:165-76.
- WITTE P, SCOTT H. Investigation of lameness in dogs. *In practice* 2011;33:58-66.
- BAILEY CS, MORGAN JP. Congenital spinal malformations. *Vet Clin North Am Small Anim Pract* 1992;22:985-1015.
- BOUMA JL. Congenital malformations of vertebral articular processes in dogs. *Vet Clin North Am Small Anim Pract* 2016;46:307-26.
- JEFFERY ND, SMITH PM, TALBOT CE. Imaging findings and surgical treatment of hemivertebrae in three dogs. *J Am Vet Med Assoc* 2007;230:532-6.
- WESTWORTH DR, STURGES BK. Congenital spinal malformations in small animals. *Vet Clin North Am Small Anim Pract* 2010;40:951-81.
- CHEN AV, BAGLEY RS, WEST CL, et al. Fecal incontinence and spinal cord abnormalities in seven dogs. *J Am Vet Med Assoc* 2005;227:1945-51.
- OXLEY W, PINK J. Amelioration of caudal thoracic syringomyelia following surgical management of an adjacent arachnoid cyst. *J Small Anim Pract* 2012;53:67-72.
- FOSS KD, BERRY WL. What is your neurologic diagnosis? Spinal arachnoid cysts. *J Am Vet Med Assoc* 2009;234:1009-11.
- RYLANDER H, LIPSITZ D, BERRY WL, et al. Retrospective analysis of spinal arachnoid cysts in 14 dogs. *J Vet Intern Med* 2002;16:690-6.
- LIU NC, ADAMS VI, KALMAR L, et al. Whole-body barometric plethysmography characterizes upper airway obstruction in 3 brachycephalic breeds of dogs. *J Vet Intern Med* 2016;30:853-65.
- MUIR P. Advances in the canine cranial cruciate ligament. Iowa, USA: Wiley-Blackwell, 2010.
- DEWEY CW. Myelopathies: disorders of the spinal cord in a practical guide to canine and feline neurology. In: DEWEY CW, ed. A practical guide to canine and feline neurology. Iowa, USA: Iowa state press, 2003:284.
- THOMSON CE, HAHN CN. Hierarchical organisation in the nervous system. In: THOMSON CE, HAHN CN, eds. Veterinary neuroanatomy. 1st edn. Edinburgh: Elsevier-Saunders, 2012.

<sup>v</sup>Statistics Sweden 2012.





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**34** CHOUNG RS, LOCKE GR, SCHLECK CD, *et al.* A low response rate does not necessarily indicate non-response bias in gastroenterology survey research: a population-based study. *J Public Health* 2013;21:87–95.











## Original Article

## Presence of thoracic and lumbar vertebral malformations in pugs with and without chronic neurological deficits

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## ABSTRACT

Congenital vertebral malformations (CVMs) are common in brachycephalic dogs such as the pug, and are often considered incidental findings. However, specific CVMs have been suggested to be associated with neurological deficits in pugs. The objective of this study was to investigate the clinical importance of CVMs in the pug by comparing computed tomography studies of the thoracolumbar spine from pugs without neurological deficits with those from pugs with a confirmed T3–L3 spinal cord lesion and neurological deficits consistent with a chronic T3–L3 myelopathy.

A total of 57 pugs were recruited into the study from Sweden ( $n = 33$ ), United Kingdom ( $n = 21$ ) and Norway ( $n = 3$ ); 30 with neurological deficits and 27 without. Focal T3–L3 pathology was confirmed in all pugs with neurological deficits by magnetic resonance imaging ( $n = 29$ ) and/or pathology ( $n = 15$ ). Computed tomography studies of the thoracolumbar spine from pugs with and without neurological deficits were compared to investigate possible associations between presentation of neurological deficits consistent with chronic T3–L3 pathology and signalment variables, presence of CVMs and type of CVMs. Congenital vertebral malformations were as common in pugs with, as in pugs without, neurological deficits. Regardless of neurological status, the majority of pugs (96%) presented with one or more CVM. An association between presence, or type of CVM in the T1–L3 vertebral column, and neurological deficits consistent with T3–L3 pathology could not be confirmed.

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## Introduction

Congenital vertebral malformations (CVMs) are common in brachycephalic dogs such as the pug, and include hemivertebrae, spina bifida, block vertebrae, transitional vertebrae and hypoplasia or aplasia of the caudal articular processes (CAPs) (Fisher et al., 2013; Gutierrez-Quintana et al., 2014; Bouma, 2016; Ryan et al., 2017; Bertram et al., 2018). Vertebral malformations in dogs are often considered incidental findings (Morgan, 1968; Guevar et al., 2014), but have been described clinically important in the pug (Done et al., 1975; Bailey and Morgan, 1992; Aikawa et al., 2007; Fisher et al., 2013; Charalambous et al., 2014; Fallor et al., 2014; Bouma, 2016; Mathiesen et al., 2018). A specific type of CVM,

hemivertebra, has been suggested to be associated with neurological deficits more often in pugs compared to other brachycephalic breeds like French and English bulldogs (Ryan et al., 2017).

Neurological signs related to CVMs are considered the consequence of vertebral canal stenosis and/or instability resulting in a focal myelopathy (Gutierrez-Quintana et al., 2014) during adolescence or adulthood (Aikawa et al., 2007; Westworth and Sturges, 2010; Moissonnier et al., 2011). Commonly, but not exclusively, CVMs are found in the thoracic area (Dewey et al., 2016; Ryan et al., 2017). Hence neurological dysfunction caused by CVMs most often present as a T3–L3 myelopathy; spastic and/or upper motor neuron (UMN) paraparesis and pelvic limb proprioceptive deficits (de Lahunta and Glass, 2009; Dewey et al., 2016). Anatomically, the neurological deficits from a T3–L3 myelopathy could theoretically correspond to any CVM in the T1–L3 area of the vertebral column (Evans, 1993). Gait abnormalities, with the majority indicating neurological causes, have been shown

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common in the pug (Rohdin et al., 2018), but the importance of CVMs in the development of these is not fully elucidated.

Published studies on CVMs in pugs consist of case reports, each describing a single type of CVM, lacking an objective and comprehensive overview of their clinical importance. The aim of this study was to investigate the clinical importance of CVMs in the pug by comparing computed tomography (CT) studies of the thoracolumbar spine from pugs without neurological deficits with those from pugs consistent with neurological deficits from a confirmed T3–L3 spinal cord lesion.

## Materials and methods

Dogs were eligible for participation in the study provided the owner had given informed consent and ethical approvals required for the study been obtained (Animal Ethics Committee of Sweden, Uppsala djurförsöksetiska nämnd; Approval Number C202/2014; Approval date 30 January 2015). Information retrieved from the medical records included neurological status, sex, age at onset of neurological signs and age at examination. Part of the included pugs were involved in another study (Ryan et al., 2017).

Thirty pugs with and 27 pugs without neurological deficits were included in the study. Of the 30 pugs (20 males and 10 females; median age at onset of clinical signs 88 months; IQR 67–96 months) with neurological deficits, 27 were included in Sweden and three in Norway. Of the 27 (13 males and 14 females; median age at examination 82 months; IQR 52–114) without neurological deficits, 21 were included in the UK and six in Sweden. A CT scan of the entire thoracic and lumbar vertebral column was performed in 56 pugs. In one pug without neurological deficits the study was limited to the thoracic and first three lumbar vertebrae.

### Pugs with neurological deficits

Client-owned pugs with neurological deficits were admitted for neurological evaluation at Anicura Albano Animal Hospital, Danderyd, at the University Animal Hospital, Uppsala, Sweden and at the Norwegian University of Life Sciences, Oslo, Norway, between 2014–2017. Inclusion criteria were signs of a chronic (>1 month's duration) spastic and/or UMN paraparesis and pelvic limb proprioceptive deficits, with focal T3–L3 spinal cord pathology (FSCP) confirmed by magnetic resonance imaging (MRI) and/or pathology. The pugs were recruited consecutively. No pugs were excluded due to lack of confirmed FSCP. Signs of other significant organ related or systemic disease excluded participation.

Focal spinal cord pathology was defined as any focal compressive spinal cord lesion and/or focal intramedullary T2W hyperintensity on MRI, confirmed in sagittal and transversal planes, or as an extensive focal loss of nervous tissue on histopathology. An external board certified diagnostic radiologist (VetImaging of New York, USA and Section for Radiology, Oslo, Norway) reviewed the MRI studies. Histopathological evaluation of the entire central nervous system was performed by a board certified pathologist (Institute for Pathology, Tierärztliche Hochschule Hannover and Section of Clinical and Comparative Neuropathology, Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-University Munich). All pugs with neurological deficits had a neurological examination performed by a board-certified neurologist prior to imaging.

### Pugs without neurological deficits

Pugs without neurological deficits were retrospectively selected from the case records (all part of a brachycephalic obstructive airway study) at the Royal Veterinary College University of London, UK, and prospectively from Anicura Albano Animal Hospital, Sweden.

In order to confirm a normal neurological status, included dogs, with no historical documented neurological abnormalities, had a neurologic examination performed prior to CT examination, those from the UK by a board certified surgeon and those from Sweden by a board certified neurologist.

### Computed tomography

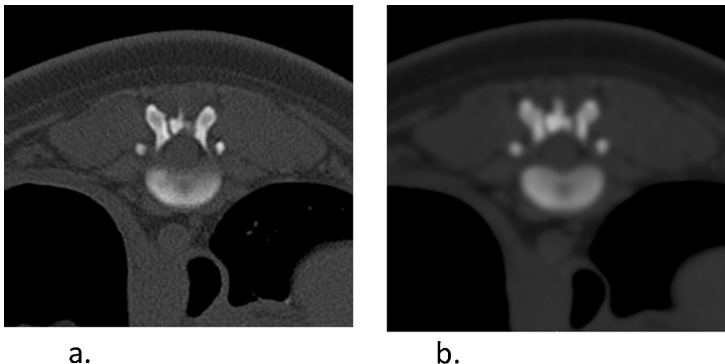
Computed tomography (CT) of the thoracolumbar vertebral column of pugs examined in Sweden was performed under general anaesthesia ( $n=23$ ) or immediately post-mortem ( $n=13$ ) with a dual slice CT scanner (Siemens, Somatom); 2 mm slice thickness ( $n=32$ ), or a 64 slice CT scanner (Siemens, Somatom); 2 mm slice thickness ( $n=1$ ). Computed tomography of pugs examined in the UK ( $n=21$ ) and Norway ( $n=3$ ) were performed under sedation or general anaesthesia with a 16 slice helical CT scanner (PQ 500, GE healthcare); 2 mm slice thickness, or a 128 slice CT scanner (Siemens, Somatom); 0.625 mm slice thickness.

After completion of the axial CT study, sagittal, dorsal and three-dimensional (3D) reconstructions were made. The studies of pugs examined in the UK were reconstructed with a soft tissue algorithm while the CT studies of pugs examined in Sweden and Norway were reconstructed with a soft tissue and a bone algorithm using medium and high frequency reconstruction algorithms. A standard image archiving and communication system software (Osirix foundation, V5.5.2) was used to evaluate all images.

All CT examinations were independently reviewed by two board certified diagnostic radiologists; one of the authors and an external reviewer (VetImaging of New York, USA). The observers were not entirely blinded to the neurological status of all dogs. Results from the two radiologists were compared, and in cases of disagreement, images were re-evaluated before the final decision was taken by one of the observers radiologists and authors. The interobserver agreement was determined for the evaluation of the CVMs. Due to differences in resolution between studies (Fig. 1), as a result of type of acquisition, the CAPs cranial to the 8th thoracic vertebrae were not evaluated.

For each CT study, the number of thoracic and lumbar vertebrae was recorded and each vertebra was assessed for the presence of hemivertebrae, associated kyphosis, block vertebrae, spina bifida, transitional vertebrae and hypoplasia or aplasia of the CAPs. When the hemivertebrae caused kyphosis the Cobb angle was calculated. Presence of spondylolysis and any other acquired vertebral column abnormality, e.g. intervertebral disc disease (IVDD) was described. In addition to comparing the CT variables between all pugs with and without neurological deficits, the CT variables were also compared between two contrasting groups of pugs <12 months ( $n=3$ ) and  $\geq 90$  months ( $n=25$ ) of age with neurological deficits, to pugs without neurological deficits.

Hemivertebra was defined as any defect in vertebral body formation as outlined by Gutierrez-Quintana (Gutierrez-Quintana et al., 2014). The Cobb angle was determined as described by Guevar (Guevar et al., 2014). Block vertebrae was defined as failure of vertebral segmentation with absence of the intervertebral disc space between two adjacent vertebral bodies (Westworth and Sturges, 2010) and spina bifida as incomplete closure of the vertebral arches resulting in a cleft through the dorsal spinous process (Westworth and Sturges, 2010). Transitional vertebrae



**Fig. 1.** Computed tomography images of the same pug at the identical level of the vertebral column reconstructed with a bone algorithm (a) and with a soft tissue algorithm using a bone window (b).

were defined as a vertebrae, localised at the junction between two divisions of the vertebral column (e.g. thoracolumbar or lumbosacral), which shows either unilateral or bilateral characteristics of adjacent divisions (Westworth and Sturges, 2010). Finally, hypoplasia or aplasia of the CAPs was defined as underdevelopment or absence of normally shaped caudal vertebral articular processes (Penderis et al., 2005).

#### Statistical analysis

The statistical analyses were performed using a commercially available statistical software program (JMP Pro v.11.2.0). Continuous variables are presented as medians and interquartile range (IQR). Associations between signalment (sex, age at clinical onset); number of thoracic and lumbar vertebrae; CVMs (hemivertebrae, block vertebrae, spina bifida, transitional vertebrae and hypoplasia or aplasia of the CAP); spondylolysis and presence of neurological deficits (yes/no) were investigated using the chi-square, Fishers exact test or logistic regression. Level of statistical significance was set at  $P < 0.05$ .

## Results

All pugs were ambulatory with moderate to severe UMN paraparesis and pelvic limb proprioceptive deficits. The FSCP was confirmed by findings at either MRI ( $n = 15$ ), MRI and pathology ( $n = 14$ ), or pathology ( $n = 1$ ). Magnetic resonance imaging (MRI), high field 1.5 T ( $n = 9$ ) and low field 0.2 T ( $n = 21$ ), was performed as part of the routine diagnostic workup of a T3–L3 myelopathy, or when the owner declined further workup immediately post-mortem ( $n = 9$ ).

Number of thoracic and lumbar vertebrae and presence of CVMs, in the pugs with and without neurological deficits are presented in Table 1.

#### Vertebral malformations in pugs with neurological deficits

All dogs ( $n = 30/30$ ) had one or more CVM. In total, 112 CVMs were found on CT examination of the 30 pugs with neurological deficits, with 3.7 malformations/pug with neurological deficits.

The most common location of hemivertebrae was T7 ( $n = 6$ ), with CAP hypoplasia or aplasia T10–T11 ( $n = 22$ ), T11–12 ( $n = 18$ ),

T12–13 ( $n = 16$ ), and with spina bifida T1 ( $n = 9$ ). The mean Cobb angle of the pugs with kyphosis ( $n = 6$ ) was  $41^\circ$  ( $35^\circ$ ,  $36^\circ$ ,  $38^\circ$ ,  $44^\circ$ ,  $45^\circ$  and  $50^\circ$ ).

Eight of 22 pugs with the CAP adjacent to the localisation of the FSCP presented with bilateral aplasia. In three pugs, a CVM immediately adjacent to the FSCP was not found (Table 2), one which presented with an adjacent intervertebral disk disease (IVDD) and one with a FSCP at T11–12 and a transitional thoracolumbar vertebrae. In five of the seven (71%) pugs presenting with a hemivertebrae, it was located adjacent to the FSCP. Median age at clinical onset of pugs with FSCP adjacent to a hemivertebrae ( $n = 5$ ) was 48 months (IQR 6–68) with a mean Cobb angle of  $42^\circ$ .

#### Vertebral malformations in pugs without neurological deficits

Ninety-three percent (25/27) had one or more CVM. In total, 97 CVMs were found on CT examination of the 27 pugs without neurological deficits, with 3.6 malformations/pug without neurological deficits.

The most common location of hemivertebrae was T9 ( $n = 4$ ), with CAP hypoplasia or aplasia T10–T11 ( $n = 17$ ), and with spina bifida T1 ( $n = 7$ ). The mean Cobb angle of the pugs with kyphosis ( $n = 3$ ) was  $48^\circ$  ( $30^\circ$ ,  $40^\circ$ , and  $75^\circ$ ; Fig. 2), the median age at examination of these pugs was 49 months (IQR 47–70).

#### Association between neurological deficits and CVMs

No significant association was found between the presence of neurological deficits and sex, age of onset of clinical signs, number of thoracic and lumbar vertebrae, presence of hemivertebrae, kyphosis associated with hemivertebrae, spina bifida, transitional thoracolumbar vertebrae, hypoplasia or aplasia of CAPs or number of vertebral malformations/dog. Pugs with neurological deficits presented with lumbosacral transitional vertebrae more often than pugs without neurological deficits ( $P = 0.041$ ).

**Table 1**

Number of thoracic and lumbar vertebrae, and presence of congenital vertebral malformations in pugs with and without neurological deficits consistent with a T3–L3 myelopathy.

Variable	Pugs without neurological deficits	Pugs with neurological deficits	$P^a$
Number of thoracic vertebrae			
12	3/27 (11.1%)	4/30 (13.3%)	0.80
13	24/27 (88.9%)	26/30 (86.7%)	
Number of lumbar vertebrae			
6	6/26 (23.1%)	9/30 (30.0%)	0.40
7	20/26 (76.9%)	21/30 (70.0%)	
Number of pugs with CVM	25/27 (92.6%)	30/30 (100%)	0.080
Number of CVM/pug	3.6 97/27	3.7 111/30	0.94
Hemivertebrae	8/27 (29.6%)	7/30 (23.3%)	0.590
Number of hemivertebrae/pug with hemivertebrae	2.3 18/8	2.3 16/7	0.96
Kyphosis associated with hemivertebrae	3/8 (37.5%)	6/7 (85.7%)	0.35
Block vertebrae	0/27 (0%)	0/30 (0%)	–
Spina bifida	7/27 (25.9%)	9/30 (30.0%)	0.73
Transitional vertebrae			
Thoracolumbar	9/27 (33.3%)	17/30 (56.7%)	0.076
Lumbosacral	6/27 (22.2%) 3/26 (11.5%)	12/30 (40.0%) 10/30 (33.3%)	0.15 0.041
Hypoplasia or aplasia of the CAPs	24/27 (88.9%)	27/30 (90.0%)	0.89
Number of hypoplasia or aplasia of the CAPs/pug with hypoplasia or aplasia of the CAPs	2.6 62/24	2.4 65/27	0.59

CVM, congenital vertebral malformation; CAP, caudal articular processes.

<sup>a</sup>  $P = 0.05$ .

**Table 2**

Localisation of focal myelopathy and thoracolumbar congenital vertebral malformations between the first thoracic and third lumbar vertebrae in individual pugs with T3–L3 pathology. In addition, any acquired vertebral column abnormality in the same region is presented. Any focal spinal cord pathologies and vertebral column abnormalities, anatomically adjacent to each other are written in bold. Focal spinal cord pathology was defined as any focal compressive spinal cord lesion, and/or intramedullary T2W hyperintensity on magnetic resonance imaging (MRI), confirmed in sagittal and transversal planes (dog numbers 1–29), or as an extensive focal loss of nervous tissue on histopathology (dog number 30) without further characterisation.

Dog number	Age at onset of clinical signs (months)	Focal myelopathy	Hemivertebrae	Kyphosis	Cobb angle	Spina bifida	Transitional vertebrae	CAP	Acquired vertebral column abnormalities
1	48	<b>T6-7</b>	<b>T6-8</b>	Yes	38°	T1	No	T10-11 T11-12 T12-13	S T3-4 S T4-5 <b>S T6-7</b> S T7-8 S T8-9 <b>IVDD T6-7</b> S T5-6 IVDD T10-11
2	72	<b>T10-11</b>	No	No		No	Trans TL	<b>T10-11</b>	
3	102	<b>T11-13</b>	No	No		No	Trans TL	<b>T12-13</b> T13-L1	IVDD T10-11
4	108	<b>T10-11</b>	No	No		No	Trans TL	<b>T10-11</b> T11-12 T12-13	No
5	72	<b>T7</b>	<b>T5-7</b>	Yes	44°	T1	No	T10-11 T11-12 T12-13 T13-L1	<b>S T4-5</b> <b>S T6-7</b> <b>S T7-8</b>
6	96	<b>T11-12</b>	No	No		No	No	T9-10 T10-11 <b>T11-12</b>	No
7	54	T7-8 <sup>a</sup>	No	No		No	No	T10-11 T11-12 T12-13	No
8	93	<b>T11-12</b>	No	No		T1	No	T10-11 <b>T11-12</b>	No
9	80	<b>T12-13</b>	No	No		No	No	T10-11 T11-12 <b>T12-13</b>	<b>IVDD T12-13</b> S T7-8 S T8-9
10	78	<b>T10-11</b>	No	No		No	No	<b>T10-11</b>	S T8-9
11	66	<b>T12-L1</b>	T7-9	Yes	36°	No	No	T9-10 T10-11 T11-12 <b>T12-L1</b>	S T4-5 S T5-6 S T7-8 S T8-9 S T9-10
12	90	<b>T12-13</b>	No	No		T1	No	T10-11 T11-12 <b>T12-13</b>	S T2-3 S T10-11
13	90	<b>T12-13</b>	No	No		No	Trans TL	T10-11 T11-12 <b>T12-13</b>	No
14	96	<b>T10</b>	No	No		T1	Trans TL	<b>T9-10</b> <b>T10-11</b> T11-12 T12-13	S T5-6
15	90	<b>T10-12</b>	T7-8	No		No	No	<b>T12-13</b>	S T6-7 S T7-8
16	101	<b>T11-12</b>	No	No		No	No	<b>T10-11</b>	S T6-7 S T7-8 S T8-9
17	3	<b>T11-12</b>	No	No		No	Trans TL	<b>T11-12</b>	No
18	96	<b>T12-13</b>	No	No		T1	Trans TL	T10-11 T11-12 <b>T12-13</b>	S T4-5 S T12-13 <b>IVDD T12-13</b>
19	79	<b>T9-12</b>	No	No		No	No	<b>T10-11</b> <b>T11-12</b>	No
20	97	<b>T12-13</b>	No	No		T1	No	No	<b>IVDD T12-13</b>
21	88	<b>T11-12</b>	No	No		No	No	T9-10 T10-11 <b>T11-12</b>	<b>IVDD T11-12</b> S T4-5 S T8-9 <b>S T11-12</b>
22	3	<b>T8</b>	<b>T8</b>	Yes	50°	No	Trans TL	T10-11 T12-13	S T3-4 S T4-5 S T5-6
23	91	<b>T10-11</b>	No	No		No	No	<b>T10-11</b>	No
24	84	<b>T11-12</b>	No	No		No	No	<b>T11-12</b>	S T9-10 S T10-11
25	68	<b>T7-T13</b>	No	No		T1	<b>Trans TL</b>	No	No
26	87	<b>T12-13</b>	No	No		T1	Trans TL	T10-11 <b>T12-13</b> T13-L1	S T8-9

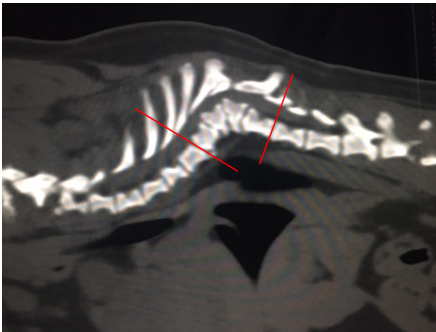


**Table 2** (Continued)

Dog number	Age at onset of clinical signs (months)	Focal myelopathy	Hemivertebrae	Kyphosis	Cobb angle	Spina bifida	Transitional vertebrae	CAP	Acquired vertebral column abnormalities
27	65	<b>T7-T10</b>	<b>T7-8</b>	Yes	45°	No	Trans TL	<b>T10-11</b> T11-12 T12-13	<b>S T7-8</b>
28	120	T11-12	No	No		No	Trans TL	No	S T4-5 S T5-6 No
29	10	<b>T6-7</b>	<b>T6-7</b>	Yes	35°	No	No	T10-11 T11-12 T12-13 T13-L1	No
30	96	<b>T12-13</b>	No	No		No	No	<b>T12-13</b>	S T9-10 S T11-12

T, thoracic vertebrae; CAP, caudal articular process hypoplasia or aplasia; Trans TL, transitional thoracolumbar vertebrae; IVDD, intervertebral disc disease; S, vertebral spondylolysis.

<sup>a</sup> In retrospect, the CAPs of pug 7 was evaluated at T7-8, showing no signs of hypoplasia or aplasia at that location on the CT examination.



**Fig. 2.** Lateral computed tomography image of a pug, without neurological deficits, with a hemivertebra and associated kyphosis, at the thoracic vertebrae T6-T7. The reference lines for the calculation of the Cobb angle are shown in red.

Two of the three pugs <12 months of age that had neurological deficits presented with hemivertebra and associated kyphosis. The group of pugs <12 months of age was however considered too small to warrant statistical analysis. When the same variables as in Table 1 were applied to the group of pugs ≥ 90 months of age, no significant association was shown between pugs ≥ 90 months of age with neurological deficits and pugs without neurological deficits.

There was no association between presence of spondylolysis and neurological deficits ( $P=0.38$ ) but with presence of spondylolysis and hemivertebrae ( $P=0.0079$ ) and thoracolumbar transitional vertebrae ( $P=0.045$ ; Table 3).

#### Interobserver agreement between certified diagnostic radiologists

There was complete agreement between the observers in describing number of thoracic vertebrae, appreciating kyphosis in association with hemivertebrae and assessing presence of spina bifida. For number of lumbar vertebrae, presence of hemivertebrae and transitional vertebrae the observers agreed in 55/56 (98.2%), 56/57 (98.2%), 55/57 (96.5%) of the cases respectively. Interobserver agreement between the observers on presence of CAP hypoplasia or aplasia, was found in 46/57 (80.7%) of the CT-studies. The interobserver agreement for studies reconstructed in both soft tissue and bone algorithm was 91.7% (33/36), and for studies reconstructed just in soft tissue algorithm 66.7% (14/21).

## Discussion

This study showed that CVMs were as common in pugs with, as in pugs without, neurological deficits. Indeed, the prevalence of CVMs in pugs was high regardless of presence of neurological signs. Where previous literature on CVMs in pugs refers to case reports, each describing a single type of CVM, this study, with a reasonable number of dogs, is the first to provide an objective and comprehensive overview of the clinical importance of CVMs in pugs. The demonstrated lack of association between multiple types of CVMs and chronic T3-L3 myelopathies is of great importance, as it shows the urgent need to look for other/additional factors for the development of FSCP in pugs.

An association between either presence or type of T1-L3 CVM and neurological deficits consistent with FSCP in pugs could not be found. However, an association was found between neurological deficits and presence of lumbosacral transitional vertebrae (Table 1). Pugs with neurological deficits included in this study all presented with thoracolumbar FSCP, hence, the spinal malformations occurring in the lumbosacral area were not likely of any relevance for their spinal cord lesions.

In the majority of pugs with neurological signs (90%), a CVM was found in the thoracolumbar spine immediately adjacent to the FSCP considered responsible for the neurological deficits (Table 2). This suggests that part of all the CVMs found (13%, 27/208) may have contributed to the development of FSCP resulting in neurological deficits. However, the study also included many CVMs without any adjacent pathology as well as pugs with FSCP without any neighbouring CVM, suggesting that additional factors, possibly breed-specific, contribute to the development of T3-L3 pathology in pugs.

**Table 3**

Presence of spondylolysis and congenital vertebral malformations between the first thoracic and third lumbar vertebrae, in pugs with and without neurological deficits indicating a T3-L3 myelopathy.

Variable	Pugs without spondylolysis (n = 18)	Pugs with spondylolysis (n = 39)	$P^a$
Hemivertebrae (n = 15)	1	14	0.0079
Spina bifida (n = 16)	5	11	0.97
Transitional vertebrae (n = 26)	11	15	0.11
Thoracolumbar (n = 18)	9	9	0.045
CAP (n = 51)	16	35	0.92
Pugs with neurological deficits (n = 30)	11	19	0.38
Pugs without neurological deficits (n = 27)	7	20	0.38

CAP, caudal articular process hypoplasia or aplasia.

<sup>a</sup>  $P=0.05$ .

Pugs with CVMs may not present with neurological deficits until they are older (Fisher et al., 2013; Charalambous et al., 2014). Therefore, we cannot exclude the possibility that some pugs, included in the group without neurological deficits, would eventually develop neurological signs relating to CVMs later in life.

In a previous study, presence of hemivertebrae was less common in pugs than in two other brachycephalic breeds, the French and English bulldog. However, hemivertebrae in pugs were associated with neurological deficits more often than in these other breeds (Ryan et al., 2017). A significant difference in the kyphotic Cobb angle has been shown between dogs with and without neurological deficits (Moissonnier et al., 2011; Guevar et al., 2014). In the present study, there was no significant difference in prevalence of hemivertebrae in pugs with or without neurological deficits, or in the mean Cobb angle of the kyphosis in pugs with or without neurological deficits. Indeed, the greatest Cobb angle (75°) was found in a pug without neurological deficits (Fig. 2). In two pugs with neurological deficits- dog number 11 and 15 (Table 2), - the hemivertebrae were not adjacent to the focal pathology. They both showed mild radiological abnormalities, one without associated kyphosis, and the second with a Cobb angle lower (36°) than the mean for both affected and unaffected pugs. Hence, previous studies suggesting the severity of kyphosis to be a key factor in the development of neurological signs in dogs with hemivertebrae was not unequivocally supported by the present study (Moissonnier et al., 2011; Guevar et al., 2014). This study only included three pugs <12 months of age with neurological deficits. Two of these pugs presented with hemivertebrae and associated kyphosis, suggesting kyphosis should be considered a risk factor for FSCP in young pugs. Statistical analysis was however, not considered warranted due to the small number of pugs in this group. According to a previous report dogs less than a year old represent the majority of clinical cases related to hemivertebrae (Charalambous et al., 2014). Significance might therefore have been confirmed if the study had included more young pugs with neurological deficits.

Hypoplasia or aplasia of the thoracolumbar CAPs was a frequent finding in pugs regardless of presence of neurological deficits. Hypoplasia or aplasia of the CAPs was also the single most common CVM found adjacent to FSCP (Table 2). In this study, hypoplasia or aplasia of the CAPs were not evaluated cranial to the 8th thoracic vertebrae due to the differences in spatial resolution between studies reconstructed in a soft tissue algorithm compared to those studies that were reconstructed in a bone algorithm (Fig. 1). However, a predictive anatomic distribution of hypoplasia or aplasia of the CAPs has been shown in the pug, with the majority of underdeveloped CAPs found between T10-T13 (Bertram et al., 2018). Therefore it is unlikely that not evaluating the CAPs cranial to T8 have influenced the result. Although hypoplastic or aplastic CAPs are frequently seen as an incidental finding (Morgan, 1968; Fisher et al., 2013), hypoplastic or aplastic thoracolumbar CAPs have been described to cause fibrous constrictive myelopathy in pugs (Fisher et al., 2013).

Spondylosis, which may develop as a consequence of chronic vertebral instability, was found associated with presence of hemivertebrae in this study (Table 3). Vertebral instability has been suggested to play an important role in the development of neurological deficits in association with CVMs (Moissonnier et al., 2011; Fisher et al., 2013; Faller et al., 2014; Mathiesen et al., 2018). An association between the presence of spondylosis and neurological deficits, suggesting more severe vertebral instability in the spines of pugs with neurological deficits, could not be found. However, to properly evaluate dynamic lesions, myelograms or CT myelograms are required, but were not performed in the present study.

There was greater interobserver agreement for the presence of CAPs hypoplasia or aplasia in pugs with neurological deficits

compared to pugs without neurological deficits. This may be a consequence of most CT-exams of pugs without neurological deficits being reconstructed only in a soft tissue algorithm resulting in less distinctively outlined borders of the CAPs (Fig. 1). Also, the distinction between a 'normal' and a hypoplastic CAP is to some extent subjective.

Pugs being recruited, evaluated and CT examined, with a skewed distribution of dogs with and without neurological deficits, at three different clinics is a limitation of the study. However, it was challenging to identify Swedish pug owners willing to sedate or anaesthetize their dog for a CT examination if the dog lacked signs of neurological deficits.

In the majority of cases, neurological status was unavailable to the diagnostic observers. Completely blinded reviews had been ideal; however, some of the pug's CT-examinations were being evaluated as part of their diagnostic workup requiring limited clinical information to be provided. Although the number of pugs in the study population was limited, with groups of specific CVMs even smaller, it has the advantage of looking at a specific breed. Other potential limitations were the small population of pugs younger than one year, the selection of only chronic cases, and the restricted examination of pugs without neurological deficits, including CT and not MRI. Despite the limitations, the recruitment of pugs from different clinics made it possible to compare CT-studies of the thoracolumbar spine of pugs with obvious neurological deficits with that of pugs with no historical documented neurological abnormalities and without deficits detected upon neurological examination.

## Conclusions

Vertebral malformations were common in pugs regardless of presence of neurological deficits. No association was found between T1-L3 CVMs and chronic neurological deficits in pugs with T3-L3 pathology. In the majority of pugs, a CVM was however found in the thoracolumbar spine adjacent to the FSCP indicating that certain CVMs may have clinical impact. Although statistically significant, the association between lumbosacral transitional vertebrae and T3-L3 pathology was unlikely of clinical relevance. The lack of association between multiple types of CVMs and chronic thoracolumbar myelopathies in a larger group of pugs should stimulate emphasizes the need to look for other or additional causes for T3-L3 pathology in this breed.

## Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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## References

- Aikawa, T., Kanazono, S., Yoshigae, Y., Sharp, N.J., Munana, K.R., 2007. Vertebral stabilization using positively threaded profile pins and polymethylmethacrylate, with or without laminectomy, for spinal canal stenosis and vertebral instability caused by congenital thoracic vertebral anomalies. *Veterinary Surgery* 36, 432–441.

- Bailey, C.S., Morgan, J.P., 1992. Congenital spinal malformations. *Veterinary Clinics of North America Small Animal Practice* 22, 985–1015.
- Bertram, S., Ter Haar, G., De Decker, S., 2018. Caudal articular process dysplasia of thoracic vertebrae in neurologically normal French bulldogs, English bulldogs, and Pugs: prevalence and characteristics. *Veterinary Radiology and Ultrasound* 59, 396–404.
- Bouma, J.L., 2016. Congenital malformations of vertebral articular processes in dogs. *Veterinary Clinics of North America Small Animal Practice* 46, 307–326.
- Charalambous, M., Jeffery, N.D., Smith, P.M., Goncalves, R., Barker, A., Hayes, G., Ives, E., Vanhaesebrouck, A.E., 2014. Surgical treatment of dorsal hemivertebrae associated with kyphosis by spinal segmental stabilisation, with or without decompression. *The Veterinary Journal* 202, 267–273.
- de Lahunta, A., Glass, E., 2009. Small animal spinal cord disease, *Veterinary Neuroanatomy and Clinical Neurology*, Third ed. Saunders Elsevier, St. Louis, Missouri, USA, pp. 243–244.
- Dewey, C.W., Davies, E., Bouma, J.L., 2016. Kyphosis and kyphoscoliosis associated with congenital malformations of the thoracic vertebral bodies in dogs. *Veterinary Clinics of North America Small Animal Practice* 46, 295–306.
- Done, S.H., Drew, R.A., Robins, G.M., Lane, J.G., 1975. Hemivertebra in the dog: clinical and pathological observations. *Veterinary Record* 96, 313–317.
- Evans, H.E., 1993. Spinal cord and meninges, *Miller's Anatomy of the Dog*, Third Ed. WB Saunders, Toronto, Canada, pp. 942–943.
- Faller, K., Penderis, J., Stalin, C., Guevar, J., Yeamans, C., Gutierrez-Quintana, R., 2014. The effect of kyphoscoliosis on intervertebral disc degeneration in dogs. *The Veterinary Journal* 200, 449–451.
- Fisher, S.C., Shores, A., Simpson, S.T., 2013. Constrictive myelopathy secondary to hypoplasia or aplasia of the thoracolumbar caudal articular processes in Pugs: 11 cases (1993–2009). *Journal of the American Veterinary Medical Association* 242, 223–229.
- Guevar, J., Penderis, J., Faller, K., Yeamans, C., Stalin, C., Gutierrez-Quintana, R., 2014. Computer-assisted radiographic calculation of spinal curvature in brachycephalic 'screw-tailed' dog breeds with congenital thoracic vertebral malformations: reliability and clinical evaluation. *PLoS One* 9, e106957.
- Gutierrez-Quintana, R., Guevar, J., Stalin, C., Faller, K., Yeamans, C., Penderis, J., 2014. A proposed radiographic classification scheme for congenital thoracic vertebral malformations in brachycephalic 'screw-tailed' dog breeds. *Veterinary Radiology and Ultrasound* 55, 585–591.
- Mathiesen, C.B., de la Puerta, B., Groth, A.M., Rutherford, S., Cappello, R., 2018. Ventral stabilization of thoracic kyphosis through bilateral intercostal thoracotomies using SOP (String of Pearls) plates contoured after a 3-dimensional print of the spine. *Veterinary Surgery* 47, 843–851.
- Moissonnier, P., Gossot, P., Scotti, S., 2011. Thoracic kyphosis associated with hemivertebra. *Veterinary Surgery* 40, 1029–1032.
- Morgan, J.P., 1968. Congenital anomalies of the vertebral column of the dog: a study of the incidence and significance based on a radiographic and morphologic study. *Veterinary Radiology* 9, 21–29.
- Penderis, J., Schwarz, T., McConnell, J.F., Garosi, L.S., Thomson, C.E., Dennis, R., 2005. Dysplasia of the caudal vertebral articular facets in four dogs: results of radiographic, myelographic and magnetic resonance imaging investigations. *Veterinary Record* 156, 601–605.
- Rohdin, C., Jäderlund, K.H., Ljungvall, I., Lindblad-Toh, K., Häggström, J., 2018. High prevalence of gait abnormalities in pugs. *Veterinary Record* 182, 1–9.
- Ryan, R., Gutierrez-Quintana, R., Ter Haar, G., De Decker, S., 2017. Prevalence of thoracic vertebral malformations in French bulldogs, Pugs and English bulldogs with and without associated neurological deficits. *The Veterinary Journal* 221, 25–29.
- Westworth, D.R., Sturges, B.K., 2010. Congenital spinal malformations in small animals. *Veterinary Clinics of North America Small Animal Practice* 40, 951–981.









## STANDARD ARTICLE

## Thoracolumbar meningeal fibrosis in pugs

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## Abstract

**Background:** Thoracolumbar myelopathies associated with spinal cord and vertebral column lesions, with a similar clinical phenotype, but different underlying etiologies, occur in pugs.

**Objectives:** To further characterize the clinical and neuropathological characteristics of pugs with longstanding thoracolumbar myelopathy.

**Animals:** Thirty client-owned pure-bred pugs with a history of more than a month of ataxia and paresis of the pelvic limbs, suggesting a myelopathy localized to the thoracolumbar spinal cord, were included in the study.

**Methods:** Prospective clinicopathological study. Included pugs underwent a complete neurological examination and gross and histopathologic postmortem studies with focus on the spinal cord. Computed tomography (n = 18), magnetic resonance imaging (n = 17), and cerebrospinal fluid analysis (n = 27) were performed before or immediately after death.

**Results:** Twenty male and 10 female pugs had a median age at clinical onset of 84 months (interquartile range, 66–96). Affected pugs presented with a progressive clinical course and 80% were incontinent. There was circumferential meningeal fibrosis with concomitant focal, malacic, destruction of the neuroparenchyma in the thoracolumbar spinal cord in 24/30 pugs. Vertebral lesions accompanied the focal spinal cord lesion, and there was lympho-histiocytic inflammation associated or not to the parenchymal lesion in 43% of the pugs.

**Conclusions and Clinical Importance:** Meningeal fibrosis with associated focal spinal cord destruction and neighboring vertebral column lesions were common findings in pugs with long-standing thoracolumbar myelopathy.

## KEYWORDS

ataxia, meninges, spinal cord

**Abbreviations:** CAP, caudal articulate process; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; CVM, congenital vertebral malformation; GFAP, glial fibrillary acidic protein; IVDH, intervertebral disc herniation; MRI, magnetic resonance imaging; SAD, subarachnoid diverticula.

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## 1 | INTRODUCTION

Myelopathy involving the thoracolumbar spinal cord is well recognized in dogs and is clinically characterized by ataxia, paresis, or paralysis of the pelvic limbs, whereas the thoracic limbs are spared.<sup>1</sup> The clinical presentation in dogs with thoracolumbar myelopathy is highly variable regarding clinical onset, course, and presence of spinal pain, depending partly on the underlying etiology.<sup>2</sup> In pugs however, thoracolumbar myelopathy with uniform clinical phenotype and clinical course, but of different etiologies, including spinal cord as well as vertebral column lesions, is described.<sup>3-27</sup> Spinal cord lesions, including subarachnoid diverticula (SAD) and constrictive myelopathy, have been given attention in pugs as separate diagnoses underlying myelopathy.<sup>11,12</sup> The histopathological features of the affected spinal cord tissues in pugs with myelopathy have been sparsely studied, mostly limited to meningeal material removed at surgery. In that respect, both SAD and constrictive myelopathy are associated with proliferation and thickening of the meninges.<sup>3,5,11,12,17</sup> Vertebral column lesions, such as congenital vertebral malformations (CVMs), including hemivertebra and hypo- or aplasia of the caudal articular processes (CAPs),<sup>4,11,21,27</sup> and acquired intervertebral disc herniation (IVDH),<sup>8,13</sup> are inconsistently described in pugs presenting as thoracolumbar myelopathy.

The high prevalence of neurological gait abnormalities in pugs<sup>6,28</sup> and the growing evidence of thoracolumbar myelopathy with a uniform clinical presentation in the breed, albeit of different etiologies, indicate that further investigations and characterization are warranted in this group of dogs. Objectives of this prospective study were to further characterize the clinical condition of pugs with longstanding thoracolumbar myelopathy, and through postmortem studies describe their neuropathological characteristics.

## 2 | MATERIALS AND METHODS

The study was approved by the local Ethical Committee (Animal Ethics Committee of Sweden [Uppsala Djurförsöksetiska Nämnd] C202/2014b). Client-owned pure-bred Swedish pugs were included in the study between 2011 and 2018 at Albano Animal Hospital, Danderyd, and at the University Animal Teaching Hospital, Uppsala.

### 2.1 | Inclusion criteria

Pugs were included in the study provided that they fulfilled the following criteria: a history of more than 1 month of a bilateral pelvic limb gait abnormality described by the owner as incoordination and weakness (onset of clinical signs was determined from the owner's first recognition of incoordination and weakness in the pelvic limbs), and a neurological examination confirming pelvic limb ataxia and paraparesis suggestive of a myelopathy localized to the thoracolumbar spinal cord. For the dog to be included, the owners also agreed to donate their dog for postmortem studies after naturally occurring death or euthanasia.

### 2.2 | Exclusion criteria

Dogs were excluded if histopathology of the thoracolumbar spinal cord was not performed at the time the pug died or was euthanized.

### 2.3 | Study population

Signalment and clinical variables were included in the data set. A thorough case history, including onset and course of clinical signs, presence of incontinence (fecal/urinary), signs indicating the dog was in pain, and result of any given treatment, was obtained at the time of inclusion. All pugs underwent a complete neurological examination performed the same day they were euthanized, by a board-certified neurologist (C. Rohdin). After euthanasia, the included pugs underwent a full postmortem examination with focus on the spinal cord and vertebral column. If practically possible, the included pugs underwent advanced diagnostic imaging, including magnetic resonance imaging (MRI) and computed tomography (CT), as well as cerebrospinal fluid (CSF) sampling and analysis, and repeat neurological examinations (by C. Rohdin). Advanced imaging was carried out before or immediately after death in order to associate any potential spinal cord lesion with the presence of vertebral column lesions, as well as to guide the histopathologic investigation of the spinal cord. Some of the recruited dogs were also included in another study investigating the presence of CVMs in pugs with and without neurological deficits.<sup>18</sup>

### 2.4 | Imaging studies

Computed tomography was performed with 2 mm slice thickness, using a dual slice CT scanner (Siemens, Somatom Spirit, Siemens Munich, Germany), or a 64 slice CT scanner (Siemens, Somatom Definition, Siemens Munich, Germany). The studies were reconstructed with a soft tissue and a bone algorithm using medium and high-frequency reconstruction algorithms. For CT studies, the examination included the entire thoracic and lumbar vertebral column and the presence of vertebral malformations, such as hemivertebra, associated kyphosis, transitional vertebrae, and hypo- or aplasia of the CAPs was evaluated.

The MRI studies were acquired using either a magnet operating at 0.2 Tesla (Esaote Vet-MR Grande, Esaote, Genoa, Italy), or at 1.5 Tesla (Siemens, Magnetom Essenza, Siemens Munich, Germany). At a minimum, the MRI study included sagittal T1- and T2-weighted images of the entire thoracolumbar spine and transverse T1- and T2-weighted images of the area of interest. Intervertebral discs were described as compressing (mildly, moderately, severely), or not compressing the spinal cord.

All CT and MRI studies were submitted to and evaluated by a single board-certified imager at a diagnostic imaging company (VetImaging of New York, New York).



## 2.5 | Cerebrospinal fluid analysis

The CSF samples were taken at the University Animal Teaching Hospital, Uppsala, Sweden, or at Albano Animal Hospital, Danderyd, Sweden, and analyzed within 30 minutes of collection. The analyses included total leucocyte- and erythrocyte cell counts, and differential leucocyte counts, and measurement of protein concentration (turbidimetry). Protein concentration was considered to be increased if  $>0.25$  g/L at the cerebellomedullary cistern, or  $>0.40$  g/L at the lumbar cistern.<sup>29</sup> Cerebrospinal fluid was interpreted to have pleocytosis if there were  $>5$  nucleated cells/ $\mu\text{L}$ , and pleocytosis was classified by the primary cell population if 1 cell type comprised  $>50\%$ .<sup>29</sup>

## 2.6 | Pathology

Gross pathology was performed at the Section of Pathology, Swedish University of Agricultural Sciences (site 1) or at the National Veterinary Institute, Uppsala, Sweden (site 2).

The entire central nervous system (CNS) were carefully sampled and fixed in 10% neutral buffered formalin for transport to a board-certified pathologist. After the spinal cord had been evacuated, the vertebral column was carefully examined and cut sagittally. Midsagittal sections were performed of thoracolumbar vertebrae and intervertebral discs with macroscopically degenerative changes, and overt disc herniations on macromorphology, or where compression of the spinal cord had been demonstrated on MRI. Discs adjacent to disc herniations were also included. The sections were placed in 10% neutral buffered formalin and decalcified using 20% formic acid (Kristensens solution).

Histopathological examination of the CNS was performed at site 2, at the Department of Pathology, University of Veterinary Medicine, Hannover (site 3), or at the Section of Clinical and Comparative Neuropathology, Ludwig-Maximilians-Universität, Munich (site 4), Germany. After fixation, specimens of various anatomical sites of the brain and spinal cord were processed for routine histopathology, embedded in paraffin wax and sectioned at 3–4  $\mu\text{m}$ . Tissues were stained using routine hematoxylin and eosin (HE), and examined by light microscopy.

Histopathology of the vertebral column was performed at site 1. Decalcified tissue was embedded using paraffin wax and processed to 4–5  $\mu\text{m}$  sections. Tissues were routinely stained with HE. The overall morphology and integrity of the intervertebral discs and adjacent vertebrae, in addition to other apparent features, were assessed and recorded.

## 2.7 | Statistics

Descriptive statistical analyses were performed using a commercially available statistical software program (JMP Pro v. 11.2.0, Cary, North Carolina). Continuous variables are presented as means and SDs and as medians and interquartile range (IQR). Associations between clinical variables and pathological findings were investigated using the Chi-square, Fishers exact test. Level of statistical significance was set at  $P < .05$ .

## 3 | RESULTS

### 3.1 | Study population characteristics

Thirty pugs fulfilled the inclusion criteria with pathology being performed by the time the pug was euthanized. Information about dog signalment, clinical variables, CSF findings, and whether or not the dog was receiving medical treatment is presented in Table 1. No pug had undergone neurosurgical intervention.

All 30 pug owners described an insidious, progressive course of their pug's pelvic limb gait abnormality. Mean duration of clinical signs before veterinary consultation was 7.1 months (SD, 6.9). None of the owners perceived their dogs to be in pain, but a reluctance to go for walks was reported by the owners of 13 pugs.

At the physical examination, all 30 pugs presented with moderate to severe pelvic limb muscle atrophy. Spinal pain was not recognized in any of the pugs upon examination. In 13 pugs, a repeated neurological examination had been performed. In 3 of them, the pelvic limb flexor reflexes gradually became weaker, with loss of muscle tone in the pelvic limbs, at reexaminations performed within 9.5, 11, and 12 months after the initial examination. These 3 pugs were examined neurologically on 7, 4, and 3 occasions respectively. In 2 out of these 3 pugs, with a normal perineal reflex upon initial examination, loss of the perineal reflex and anal tone developed over time.

All 30 pugs were still ambulatory at the day of euthanasia. Twenty-eight pugs were euthanized because of their gait abnormality or because of their associated incontinence. Other causes for euthanasia ( $n = 2$ ) were pyometra ( $n = 1$ ) and osteosarcoma of the mandibula ( $n = 1$ ). The pug with pyometra had a neurological examination performed, confirming pelvic limb ataxia and paraparesis, 1 month before acute onset of clinical signs of the pyometra. The pug with osteosarcoma had a neurological examination, confirming pelvic limb ataxia and paraparesis, and a CT of the entire thoracolumbar vertebral column, with no signs of vertebral involvement, performed 2 months before euthanasia.

### 3.2 | Imaging studies

Fifteen pugs underwent both CT and MRI imaging. All CT studies ( $n = 18$ ) were performed immediately after death. Magnetic resonance imaging was performed under general anesthesia ( $n = 7$ ) or immediately after death ( $n = 10$ ). Mean time between MRI and euthanasia in the 7 pugs examined under anesthesia was 6.3 months (SD, 4.2 months). In the 18 pugs examined by CT, 64 CVMs were identified, mean 3.6 (SD, 2.0), in the thoracolumbar vertebral column. In CT-examined pugs with histopathologically confirmed focal spinal cord lesions ( $n = 17$ ), at least 1 CVM presented immediately adjacent to the focal lesion in 16 pugs (94%) (Figure 1). Four hemivertebra and 12 hypo- or aplasia of the CAPs were found accompanying the focal lesion. In addition, 3 pugs presented transitional thoracolumbar vertebrae adjacent to the focal spinal cord lesion. In the remaining pug examined by CT, a herniated intervertebral disc, diagnosed by MRI, was located immediately adjacent to the focal spinal cord lesion. In all but 1 pug ( $n = 16$ ), examined by MRI

**TABLE 1** Distribution of signalment, clinical variables, cerebrospinal fluid, and histopathological findings of lympho-histiocytic inflammation in the central nervous system of the 30 pugs of the present study. Data on signalment and incontinence is compared to pugs in the general Swedish pug population<sup>a</sup>

Variable	The 30 pugs of the present study	The general Swedish pug population <sup>a</sup>
Number of pugs	30	550
Sex		
Male	18 (60.0%)	38.7%
Male (neutered)	2 (6.7%)	9.1%
Female	10 (33.3%)	43.1%
Female (spayed)	0 (0)	9.1%
Median weight (kg)	9.0 (IQR, 8.4-10.0)	9 (IQR, 8-10)
Coat color		
Black	5 (16.7%)	24.6%
Fawn	25 (83.3%)	71.0%
Median age (months) at clinical onset	84 (IQR, 66-96)	-
Range of age at clinical onset (months)	7-124	
Incontinence	24 (80.0%)	
Fecal	24 (80.0%)	3.6%
Urinary	7 (23.3%)	6.7%
Presented with pelvic limb gait abnormality before the onset of incontinence	23 (95.8%)	
Urinary incontinence developed before fecal incontinence	1 (4.2%)	
Abnormal CSF	19/27 (70.4%)	-
Mononuclear pleocytosis	14/27 (51.9%)	
Increase in total protein	17/27 (63.0%)	
Mean duration of clinical signs (months) before euthanasia:		-
In all Pugs	12.7 (SD, 7.9)	
In pugs with lympho-histiocytic inflammation in the CNS on histopathology <sup>b</sup>	9.8 (SD, 7.5)	
In pugs (n = 2) with lympho-histiocytic leptomeningitis	2.8 (SD, 1.8)	
In pugs (n = 28) without confirmed lympho-histiocytic leptomeningitis	13.6 (SD, 7.7)	
Pugs receiving ongoing medical treatment	7/30 (23.3%)	
Corticosteroids	4/7	
NSAIDs	2/7	
Corticosteroids + cyclosporine	1/7	
Mean duration of clinical signs (months) before euthanasia		
In pugs receiving treatment	11.9 (SD, 5.8)	
In pugs not receiving treatment	12.9 (SD, 8.5)	

Abbreviations: CSF, cerebrospinal fluid; IQR, interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs.

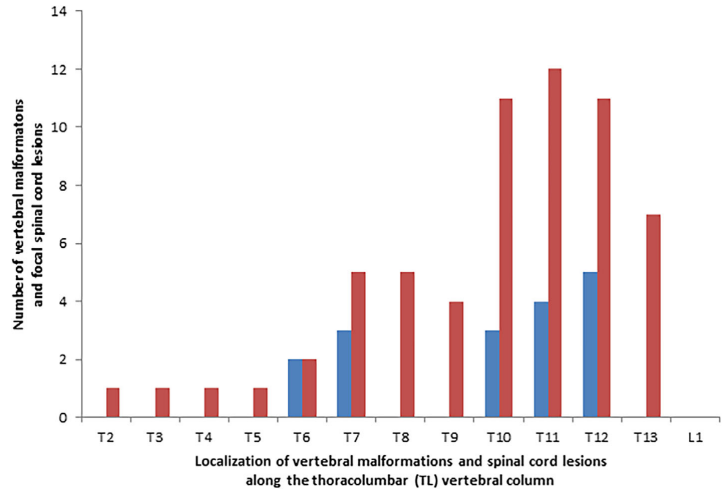
<sup>a</sup>From Reference 28.

<sup>b</sup>Four out of the 13 pugs were receiving treatment with an anti-inflammatory agent at the time of sampling.

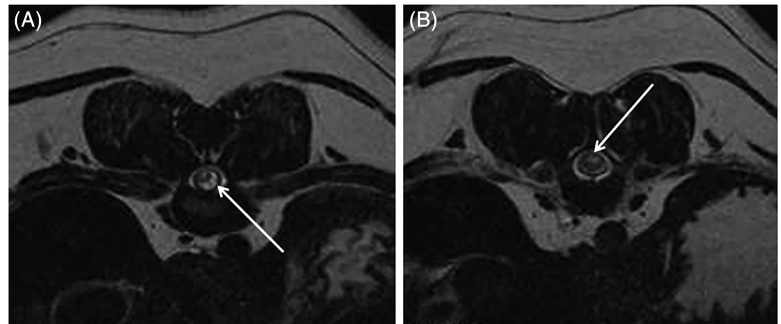
(n = 17), the spinal cord lesion presented with a focal intramedullary T2W hyperintensity. The 17th pug presented with a more extensive spinal cord T2W hyperintensity (T10-L1). A SAD, located immediately cranially or caudally to the focal intramedullary T2W hyperintensity,

was diagnosed by MRI in 9/16 (56%) pugs (Figure 2). An IVDH, compressing the spinal cord at the intramedullary T2W hyperintensity, was diagnosed by MRI in 5 pugs. The disc compression was considered mild in 2 and moderate in 3 pugs.

**FIGURE 1** Distribution of the focal thoracolumbar spinal cord lesions and their adjacent vertebral malformations, diagnosed by computed tomography, in 17 pugs with long-standing thoracolumbar myelopathy. Vertebral malformations included hemivertebra, hypo- or aplasia of the caudal articular process, and thoracolumbar transitional vertebrae. Blue bars represent focal spinal cord lesions. Red bars represent vertebral malformations



**FIGURE 2** Transversal T2W spinal cord magnetic resonance images at the level of T10-T11 in a pug. A ventrolateral subarachnoid diverticula (SAD) (A) is located immediately cranial to the intramedullary T2W hyperintensity (B). White arrow points at SAD in (A), at T2W hyperintensity in (B)



### 3.3 | Analysis of CSF

Cerebrospinal fluid was sampled from the cisterna magna ( $n = 26$ ) or from the lumbar area ( $n = 1$ ) when the dogs were under general anesthesia ( $n = 1$ ) or in association with euthanasia ( $n = 26$ ). The median number of mononuclear cells in the CSF was  $6/\mu\text{L}$  (IQR, 2-18) (range, 0-217); of protein  $0.3 \text{ g/L}$  (IQR, 0.25-0.42) (range, 0.14-0.56); and of erythrocytes  $5/\mu\text{L}$  (IQR, 0-16) (range, 0-445) (Table 1). The CSF of the pug with pyometra was unremarkable. The CSF of the pug with osteosarcoma showed an elevated total protein concentration with normal cell count.

### 3.4 | Pathology

Gross pathology was performed at site 1 ( $n = 29$ ), and at site 2 ( $n = 1$ ).

Histopathology of the CNS was performed at site 3 ( $n = 19$ ), at site 4 ( $n = 10$ ), and at site 2 ( $n = 1$ ).

Fifteen dogs had intervertebral discs evaluated histopathologically (site 1).

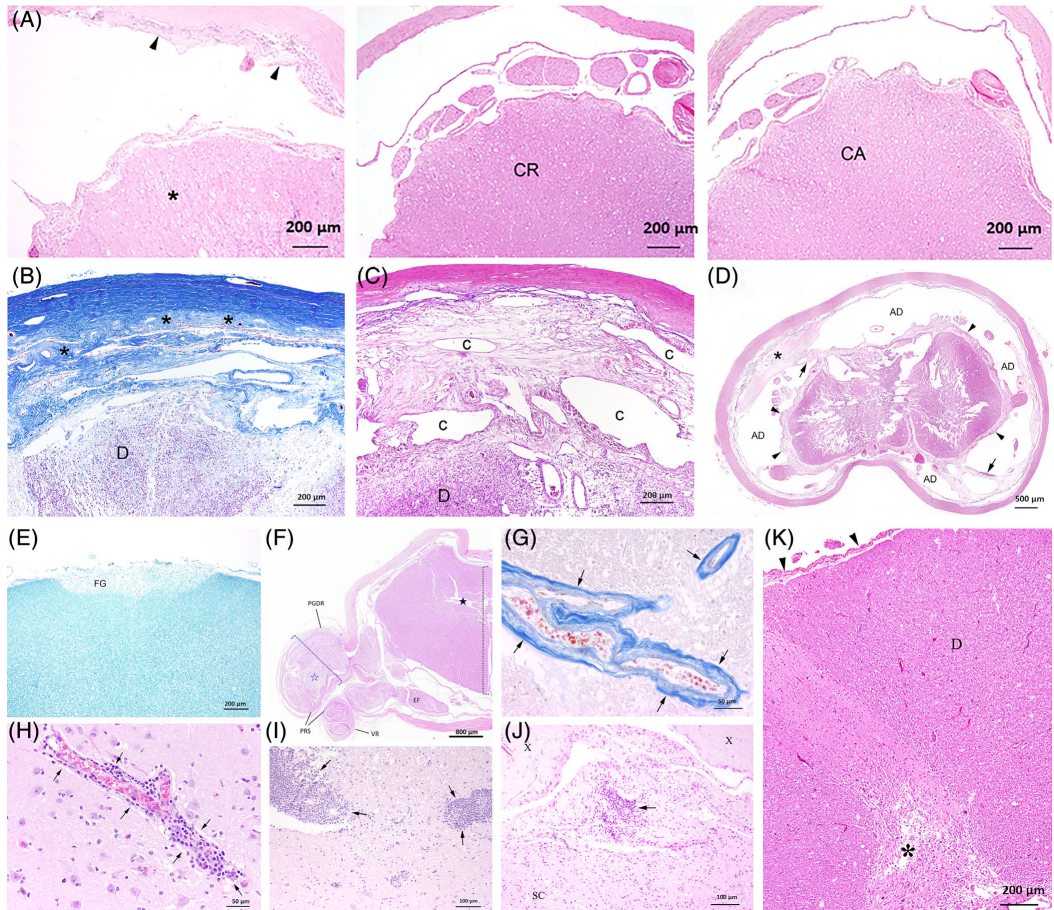
### 3.4.1 | Macroscopic findings

Vertebral column pathology included hemivertebra ( $n = 6$ ), with ( $n = 4$ ) or without ( $n = 2$ ) kyphosis, and herniating intervertebral discs ( $n = 15$ ). The morphology of the CAPs could not be assessed, as they were destroyed by the processing procedure. There was occasional focal spinal cord atrophy of the thoracolumbar spinal cord.

### 3.4.2 | Histopathology of the CNS

Lesions were confirmed in the CNS of all examined pugs including meninges, spinal cord, and nerve roots. Meningeal fibrosis and concomitant spinal cord parenchymal lesions were a consistent finding in 29/30 pugs.

Meningeal fibrosis was confirmed in the thoracolumbar spinal cord of 29 pugs. Focal, circumferential, meningeal fibrosis, limited in length along the spinal cord, in many cases involving only a few millimeters (Figure 3A), was present in 28 pugs—27 in the T5-L1 region



**FIGURE 3** Histopathologic findings in the spinal cord from pugs affected by thoracolumbar myelopathy, showing: A, focal spinal cord lesion with segmental subdural fibrosis and adjacent slices without the fibrosis only a few millimeter cranially (CR) and caudally (CA) from the damaged area along the spinal cord; HE staining. B, Severe meningeal fibrosis with adhesion of lepto- and pachymeninges (asterisks). "D" indicates dorsal tract; Azan staining. C, Formation of diverticula with multiple compartments "C" of the subarachnoid space. "D" indicates dorsal tract; HE staining. D, Focal malacia with almost total destruction of the spinal cord, leptomeningeal (arrowheads) and subdural (asterisk) fibrosis with focal adhesions (arrow) and numerous arachnoidal diverticula (AD). HE staining. E, Myelin loss in the fasciculus gracilis (FG), of the dorsal funiculus, at the level of the cervical spinal cord; Luxol fast blue staining. F, Compression radiculopathy at T7. Note the enlarged transsectional diameter (blue dotted bracket) of preganglionic dorsal root (PGDR) in relation to the maximal dorsoventral diameter of the respective spinal cord segment (black dotted bracket). The most affected dorsal root further shows endoneurial oedema (blue-lined asterisk). Both extradural parts of PGDR and ventral root (VR) present with thickening of the periradicular sheath (PRS), whereas intradural ventral rootlets are affected by a focal endoneurial fibrosis (EF), HE staining. G, Perivascular fibrosis (arrows) at the level of L3, spinal cord; Azan staining. H, Mild perivascular lympho-histiocytic inflammation (arrows) in the cerebrum; HE staining. I, Moderate lympho-histiocytic inflammation (arrows) in the spinal cord; HE staining. J, Moderate lympho-histiocytic leptomeningitis (arrows) adjacent to a thoracic, malacic, focal spinal cord (SC) lesion. "X" represent pachymeninges; HE staining. K, Lack of leptomeningeal fibrosis (arrowheads) adjacent to a focal malacic cervical spinal cord lesion (\*) in the dorsal horn. "D" represents dorsal tract; HE staining

and 1 at L5/L6. The focal meningeal proliferation was profound in 27 pugs (Figure 3B), and mild in 1 pug. In 1 other pug, the meningeal fibrosis extended the length of the spinal cord between T3 and T13.

The meningeal fibrosis involved the pachy- and leptomeninges in 13 (n = 13); pachymeninges in 3 (n = 3); leptomeninges in 3 (n = 3); and involved the meninges without distinction in 9 (n = 9) dogs. Focal

leptomeningeal proliferation and adhesions were in 9 pugs associated with formation of a diverticula, in some creating multiple compartments of the subarachnoid space (Figure 3C).

Mild to moderate meningeal proliferation was also recognized throughout the CNS unrelated to the focal spinal cord pathology in 14 pugs.

A single, thoracolumbar, focal spinal cord lesion was repeatedly found accompanying the meningeal fibrosis in 28 pugs. In 1 other pug, the lesion was more extensive, presenting between T10 and L1. The parenchymal spinal cord lesions were characterized by malacia ( $n = 24$ ), by syringomyelia ( $n = 4$ ), or by hydromyelia ( $n = 1$ ). The level of these focal lesions corresponded to the intramedullary T2W hyperintensity on MRI. The focal malacic lesions within the spinal cord were delineated by microgliosis, astrogliosis, gemistocytes, and astrocytosis, forming astroglial scars (Figure 3D). The malacic lesions, with almost total destruction of the parenchyma, were clearly demarcated from adjacent spinal cord cranially and caudally (Figure 3A). Clinical variables and histopathological findings between pugs with focal parenchymal spinal cord pathology characterized by malacia and the group of pugs with syringo/hydromyelia are presented in Table 2.

Wallerian-like degeneration could be traced further cranial from the malacic spinal cord lesions, mainly through the fasciculus gracilis in the cervical spinal cord (Figure 3E). Compared to the, in general, severe focal thoracolumbar spinal cord lesions, these neurodegenerative lesions were mild.

Nerve root entrapment and vascular encasement was found with meningeal vessels and nerve roots embedded in the circumferential meningeal fibrosis. Loss of nerve fiber density, seen involving both dorsal and ventral nerve roots (Figure 3F), hypertrophy of the dentate ligaments, and intradural tethering was found including the thoracolumbar but also the cervical and lumbosacral spine. In addition, perivascular fibrosis with collagen deposits were found throughout the CNS (Figure 3G), involving areas with and without meningeal proliferation.

Varying degrees of lympho-histiocytic CNS inflammation, including encephalitis ( $n = 9$ ) (Figure 3H), myelitis ( $n = 8$ ) (Figure 3I), and pachymeningitis with plaque-like mineralizations ( $n = 2$ ), were found in 13 pugs. Mild to moderate lympho-histiocytic leptomeningitis was found adjacent

to the focal spinal cord destruction in 2 pugs (Figure 3J). The pug euthanized because of pyometra had perivascular cuffs of lympho-histiocytic inflammatory cells in the brain. Evidence of lympho-histiocytic infiltration of the CNS was not confirmed in the pug with osteosarcoma.

Severe gray matter destruction was recognized in the cranial cervical spine in 3 pugs. One of these pugs had had surgery to remove a SAD in the dorsal C2-C3 spinal cord area 4 years before presenting with signs of a T3-L3 myelopathy. These lesions displayed the same features as the lesions in the thoracolumbar spinal cord, with ( $n = 2$ ) or without ( $n = 1$ ) (Figure 3K) associated meningeal fibrosis. There was no evidence of fibrocartilaginous material in the vasculature supplying the affected cervical or the thoracolumbar spinal cord tissue.

Meningeal fibrosis with a concomitant spinal cord lesion was absent in 1 pug. At 10 months before euthanasia, this pug was diagnosed by MRI (1.5 Tesla) with a focal intramedullary T2W hyperintensity and a probable SAD at T6/7. Histopathology displayed neurodegenerative features with mild-severe dilated myelin sheaths, swollen axons, and myelinophagia in the cervical, thoracic, and lumbar spinal cord, however, no focal lesions was identified.

### 3.4.3 | Histopathology of the vertebral column

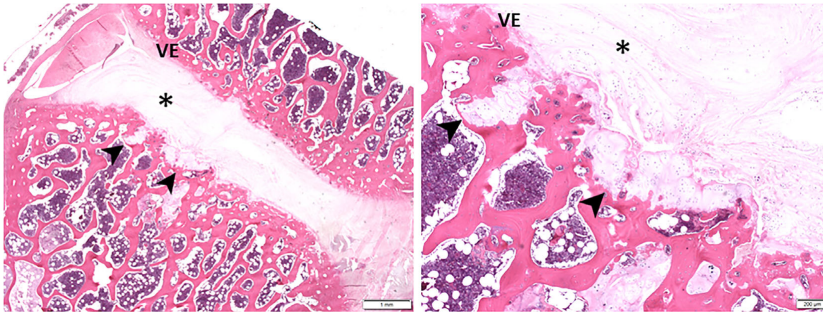
Fifteen out of 30 pugs had a total of 39 thoracolumbar discs evaluated histopathologically. Herniating intervertebral discs were found at the identical site as the focal lesion in 4 out of 15 pugs. On MRI, these 4 herniations were mildly moderately compressing the spinal cord. In 1 pug with evidence of moderate spinal cord compression from a disc diagnosed on MRI, IVDH could not be confirmed by histopathology 8 months later.

In 6 of the 15 examined pugs, protrusions of chondroid tissue, presumably originating from the nucleus pulposus with chondroid metaplasia, into the adjacent endplate and subsequently into the subchondral bone, so called Schmorl's nodes, were found (Figure 4). Schmorl's nodes were found in vertebrae adjacent to, but also unrelated to, the focal lesion in the spinal cord.

**TABLE 2** Distribution of clinical variables and histopathological findings of lympho-histiocytic inflammation in the central nervous system of 29 pugs with spinal cord lesions confirmed by histopathology. Level of statistical significance was set at  $P < .05$

Variable	Spinal cord lesion represented by malacia ( $n = 24$ )	Spinal cord lesion represented by syringomyelia/hydromyelia ( $n = 5$ )	P value
Median age (months) at clinical onset	84 (IQR, 67.5-96.0)	68 (IQR, 54.5-108.5)	.97
Mean duration of clinical signs (months) before euthanasia	13.9 (SD, 7.8)	7.1 (SD, 7.4)	.06
Incontinence			
Fecal	20/24 (83.3%)	2/5 (40.0%)	.27
Urinary	7/24 (29.2%)	0/5	.08
Pugs with lympho-histiocytic inflammation in the CNS on histopathology	8/24 (33.3%)	4/5 (80.0%)	.05

Abbreviations: CNS, central nervous system; IQR, interquartile range.



**FIGURE 4** Protrusion of chondroid tissue of the nucleus pulposus into the adjacent endplate, a so-called Schmorl's node (arrowheads), in a pug. VE, vertebral endplate; \*, intervertebral disc; HE staining

## 4 | DISCUSSION

Apart from sharing a uniform clinical presentation and course of the disease, pugs in this study also exhibited similar neuropathological features, including thoracolumbar meningeal fibrosis and concomitant spinal cord destruction. Although the homogenous clinical and pathological findings could represent a true disease, the additional finding of meningeal fibrosis unrelated to the thoracolumbar spinal cord lesion more likely suggest a predisposition, possibly breed related, to meningeal proliferation. The profound meningeal fibrosis could interfere with vascular supply to the spinal cord as well as cause obstruction of CSF flow with subsequent parenchymal spinal cord destruction.

Although the finding of concomitant CNS inflammation and neighboring vertebral column pathology might be unrelated findings, their high, and for CNS inflammation unexpected, prevalence suggest they are part of the underlying etiology.

Histopathological evidence of ongoing mild to severe lymphohistiocytic CNS inflammation, presenting distant to or at the focal spinal cord lesion, was found in 43% of the examined pugs. In addition, 52% pugs presented with a cell composition in the CSF indicating, in general mild, intrathecal mononuclear inflammation. Although an infectious etiology was considered unlikely because of the stereotype and longstanding history, this cannot be entirely excluded. On the other hand, glial fibrillary acidic protein (GFAP), the hallmark intermediate filament protein in astrocytes,<sup>30</sup> has the potential to induce an immune-mediated reaction in both dogs and humans.<sup>31–34</sup> Leakage of GFAP has been shown in clinically normal pugs and was suggested to be the result of a breed-specific astrocyte fragility.<sup>32,35</sup> Astrocyte activation, in response to a spinal cord insult from, for example, an IVDH, and/or by appropriate upregulation of GFAP,<sup>36,37</sup> may have triggered an inflammatory, possibly autoimmune, response in the CNS contributing to the lesions seen in the pugs of this study.<sup>38</sup>

The spinal cord lesions from the pugs with thoracolumbar myelopathy in this study shared many features with the spinal cord of laboratory dogs, used in research about adhesive, also called constrictive, arachnoiditis.<sup>39,40</sup> Adhesive arachnoiditis, a rare chronic myelopathy in people, is characterized by meningeal fibrosis that has been described

to cause disruption of the vascular supply and the development of arachnoid diverticula, syringomyelia and myelomalacia.<sup>41–46</sup> Familiar adhesive arachnoiditis, with a suggested genetic influence, has been described.<sup>47–49</sup> In the present study, active inflammation involving the arachnoidea at the focal spinal cord lesion was found in only 2 of the affected pugs. These 2 pugs were euthanized early after the onset of clinical signs (1.5 and 4 months), which brings to question if a focal arachnoiditis was no longer present in the other affected pugs by the time they were euthanized.<sup>3,50</sup> This was, however, not unequivocally supported by the overlap in range of duration of clinical signs before euthanasia in pugs *without* (1.5–30 months) and those *with* inflammatory infiltration of the arachnoidea. Mild inflammatory meningeal changes have previously been described in pugs with SAD.<sup>3,5,51</sup>

Pugs with intraparenchymal malacia presented with a longer duration of clinical signs before euthanasia and less often with lymphohistiocytic inflammation in the CNS on histopathology compared to pugs with syringo/hydromyelia. Although not statistically significant, this may indicate that there is an early inflammatory phase and a later chronic proliferative state in which fibrosis and adhesions become permanent in pugs with a longstanding thoracolumbar myelopathy, as suggested in human literature related to adhesive arachnoiditis.<sup>52</sup>

Vertebral column lesions, including CVMs and IVDHs, accompanied the focal meningeal fibrosis and spinal cord lesions in pugs diagnosed by CT. Unfortunately, all pugs in the present study were not examined by advanced imaging, and some CVMs, in particular articular process dysplasias, are difficult to document without CT.<sup>3</sup> Although a previous study, including pugs of similar age as in the present study, could not confirm an association between CVMs and neurological signs, it has been suggested that some CVMs in pugs are indeed clinically significant.<sup>10,11,18</sup> Vertebral malformations, congenital or potentially acquired,<sup>53</sup> may inflict direct spinal cord trauma by chronic micromotion as a consequence of vertebral canal stenosis or instability, or indirect trauma by negatively interfering with its vascular supply and thereby limiting its resistance to injury.<sup>4,7,11,14,54–56</sup> Spinal cord dynamic compression occurs in pugs with SAD and repetitive spinal cord injury could be a potential cause of thoracolumbar SADS.<sup>25</sup> Future biomechanical studies are required to confirm a potential

association between a greater risk to develop neurological signs in pugs and the nature and spatial distribution of their CAP malformations.<sup>7,10</sup>

An interesting finding was the prolapse of the nucleus pulposus through the cartilaginous endplate into the body of the vertebral body, consistent with a Schmorl's node. Schmorl's nodes are uncommonly described endplate abnormality in canines.<sup>57-59</sup> There are different hypotheses for the pathogenetic background of the Schmorl's nodes, including increased axial load and disc degeneration.<sup>60</sup> An association with ischemic necrosis, caused by interruption of the vascular supply to the vertebral endplate, has also been proposed.<sup>60,61</sup> In contrast to some other vertebral column changes found in this study, the presence of Schmorl's nodes was not correlated to spinal cord lesions.

Histopathology showed intradural tethering and hypertrophy of the dentate ligaments in pugs of this study. The spinal cord, meninges, and nerve roots move within the vertebral canal upon posturing of the head and spine.<sup>62,63</sup> Respiration, especially forced, also has an impact on spinal cord movement.<sup>64</sup> Intradural tethering and/or hypertrophy of the dentate ligament may be the consequence of increased spinal cord movement within the vertebral canal. Spinal cord movement above and below a segment of the cord where meningeal fibrosis holds the spinal cord fixed, could sustain, and potentially also contribute to progression of the injury by constant repetitive traction.<sup>39</sup>

Although all pugs included in this study presented with the similar, predictive, clinical phenotype, thoracolumbar meningeal fibrosis and parenchymal spinal cord pathology were not confirmed in 1 dog, which indeed had a T2W hyperintensity in the thoracolumbar spinal cord on MRI. As the extent of the parenchymal spinal cord pathology in general was limited in length along the spinal cord, it might therefore easily have been missed on trimming procedures.

The majority of pugs presented with incontinence, and fecal incontinence was more likely to develop before urinary incontinence. Reports on fecal and urinary incontinence in dogs, including pugs, with upper motor neuron spinal cord lesions have been sparse and mostly associated with SAD.<sup>9,16,20</sup> The character of the perineal reflex of a few affected pugs changed over time and became weaker leaving the sphincter open. This suggests that the neurological dysfunction responsible for incontinence in affected pugs could be more complex than previously proposed,<sup>20</sup> involving also lower motor neurons. In addition, repeated neurological examinations showed that, with time, the spinal reflexes in the pelvic limbs of some pugs became weaker with loss of muscle tone. The recognition of decreased spinal cord reflexes, including the perineal reflex, could be related to the observed nerve root pathology and needs further investigation.

Spinal pain was not described by any of the owners nor recognized upon examination in any of the pugs. However, reluctance to go for walks was described by more than a third of the owners. Although vascular compromise to the spinal cord in dogs, except initially, is described as non-painful,<sup>65,66</sup> people with ischemic spinal cord lesions describe chronic, intense pain that increase with exertion.<sup>67</sup> It needs to be questioned whether the decreased activity in some pugs with thoracolumbar myelopathy was because of the inability to walk

normally, because of any concomitant breed related dyspnea, or, in fact, a reflection of pain.<sup>28,68</sup>

We acknowledged that this descriptive study, having more than 1 pathologist examining the pugs, was limited by the lack of possibility for objective comparison and statistical analysis. However, the pathologists independently confirmed the major pathological findings of this study, that is, meningeal fibrosis, and concomitant spinal cord lesions, and also the presence of lympho-histiocytic CNS inflammation. Furthermore, each of them provided valuable input related to additional pathology and potential pathogenesis of pugs with longstanding thoracolumbar meningeal fibrosis. Owner attitude and financial limitations early in the project might have resulted in exclusion of some dogs undergoing the same diagnostic protocol and may have limited the opportunity to evaluate the importance of neighboring vertebral lesions.

## 5 | CONCLUSIONS

Pugs with a longstanding thoracolumbar myelopathy presented with a progressive clinical course and a high prevalence of fecal incontinence. The pathology, characterized by meningeal fibrosis with associated malacic spinal cord lesions, was accompanied by neighboring vertebral column lesions. In addition, lympho-histiocytic CNS inflammation was recognized in the CNS related and unrelated to the focal spinal cord lesion. Increased knowledge of the clinical and pathological characteristics is important for our understanding and treatment of this debilitating condition in pugs. Future studies should focus on the mechanisms behind the development of meningeal fibrosis in pugs.

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### CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

### OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

### INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Animal Ethics Committee of Sweden (Uppsala djurförsöksetiska nämnd) C202/2014.

### HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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## REFERENCES

- de Lahunta A, Glass E. Small animal spinal cord disease. In: de Lahunta A, Glass E, eds. *Veterinary Neuroanatomy and Clinical Neurology*. 3rd ed. St. Louis, MO: Elsevier; 2009:243-244.
- Cardy TJ, De Decker S, Kenny PJ, Volk HA. Clinical reasoning in canine spinal disease: what combination of clinical information is useful? *Vet Rec*. 2015;177:171.
- Alisauskaitė N, Cizinauskas S, Jeserevics J, et al. Short- and long-term outcome and magnetic resonance imaging findings after surgical treatment of thoracolumbar spinal arachnoid diverticula in 25 Pugs. *J Vet Intern Med*. 2019;33:1376-1383.
- Aikawa T, Kanazono S, Yoshigae Y, Sharp NJ, Munana KR. Vertebral stabilization using positively threaded profile pins and polymethylmethacrylate, with or without laminectomy, for spinal canal stenosis and vertebral instability caused by congenital thoracic vertebral anomalies. *Vet Surg*. 2007;36:432-441.
- Alcoverro E, McConnell JF, Sanchez-Masian D, De Risio L, De Decker S, Goncalves R. Late-onset recurrence of neurological deficits after surgery for spinal arachnoid diverticula. *Vet Rec*. 2018;182:380.
- Bagheri S, Venta P, Ballegeer E, Lin Y, Patterson J. Investigation of subarachnoid diverticulum and other spinal cord diseases in pugs. Paper presented at: Meril-NIH National Veterinary Scholars Symposium: Solving complex challenges at the interface of humans, animals and their environment; 2015; California, USA.
- Bertram S, Ter Haar G, De Decker S. Caudal articular process dysplasia of thoracic vertebrae in neurologically normal French bulldogs, English bulldogs, and Pugs: prevalence and characteristics. *Vet Radiol Ultrasound*. 2018;59:396-404.
- Bismuth C, Ferrand FX, Millet M, et al. Original surgical treatment of thoracolumbar subarachnoid cysts in six chondrodystrophic dogs. *Acta Vet Scand*. 2014;56:32.
- Chen AV, Bagley RS, West CL, Gavin PR, Tucker RL. Fecal incontinence and spinal cord abnormalities in seven dogs. *J Am Vet Med Assoc*. 2005;227:1945-1951.
- Driver CJ, Rose J, Tauro A, Fernandes R, Rusbridge C. Magnetic resonance image findings in pug dogs with thoracolumbar myelopathy and concurrent caudal articular process dysplasia. *BMC Vet Res*. 2019; 15:182.
- Fisher SC, Shores A, Simpson ST. Constrictive myelopathy secondary to hypoplasia or aplasia of the thoracolumbar caudal articular processes in Pugs: 11 cases (1993-2009). *J Am Vet Med Assoc*. 2013;242: 223-229.
- Flegel T, Muller MK, Truar K, Löffler C, Oechtering G. Thoracolumbar spinal arachnoid diverticula in 5 pug dogs. *Can Vet J*. 2013;54:969-973.
- Full A, Dewey CJ, Bouma JL. Prevalence and magnetic resonance imaging of intervertebral disc disease in pugs with caudal articular process dysplasia of the thoracolumbar spine. Paper presented at: ACVR Proceedings; 2014; St Louis, MO.
- Gutierrez-Quintana R, Guevar J, Stalin C, Faller K, Yeaman C, Penderis J. A proposed radiographic classification scheme for congenital thoracic vertebral malformations in brachycephalic "screw-tailed" dog breeds. *Vet Radiol Ultrasound*. 2014;55:585-591.
- Mathiesen CB, de la Puerta B, Groth AM, Rutherford S, Cappello R. Ventral stabilization of thoracic kyphosis through bilateral intercostal thoracotomies using SOP (String of Pearls) plates contoured after a 3-dimensional print of the spine. *Vet Surg*. 2018;47: 843-851.
- Mauler DA, De Decker S, De Risio L, et al. Signalment, clinical presentation, and diagnostic findings in 122 dogs with spinal arachnoid diverticula. *J Vet Intern Med*. 2014;28:175-181.
- Oxley W, Pink J. Amelioration of caudal thoracic syringohydromyelia following surgical management of an adjacent arachnoid cyst. *J Small Anim Pract*. 2012;53:67-72.
- Rohdin C, Haggström J, Ljungvall I, et al. Presence of thoracic and lumbar vertebral malformations in pugs with and without chronic neurological deficits. *Vet J*. 2018;241:24-30.
- Ryan R, Gutierrez-Quintana R, Ter Haar G, De Decker S. Prevalence of thoracic vertebral malformations in French bulldogs, Pugs and English bulldogs with and without associated neurological deficits. *Vet J*. 2017;221:25-29.
- Skeen TM, Olby NJ, Munana KR, Sharp NJ. Spinal arachnoid cysts in 17 dogs. *J Am Anim Hosp Assoc*. 2003;39:271-282.
- Westworth DR, Sturges BK. Congenital spinal malformations in small animals. *Vet Clin North Am Small Anim Pract*. 2010;40:951-981.
- Wyatt S, Goncalves R, Gutierrez-Quintana R, De Decker S. Outcomes of nonsurgical treatment for congenital thoracic vertebral body malformations in dogs: 13 cases (2009-2016). *J Am Vet Med Assoc*. 2018; 253:768-773.
- Longo S, Gomes SA, Lowrie M. Previously unreported magnetic resonance findings of subarachnoid fibrosis leading to constrictive myelopathy in a pug. *J Small Anim Pract*. 2019;60:324.
- Meren IL, Chavera JA, Alcott CJ, Barker AK, Jeffery ND. Shunt tube placement for amelioration of cerebrospinal fluid flow obstruction caused by spinal cord subarachnoid fibrosis in dogs. *Vet Surg*. 2017; 46:289-296.
- Aikawa T, Shimatsu T, Miyazaki Y. Hemilaminectomy, diverticular marsupialization, and vertebral stabilization for thoracolumbar spinal arachnoid diverticula in five dogs. *J Am Anim Hosp Assoc*. 2019;55: 110-116.
- Rusbridge C, Stringer F, Knowler SP. Clinical application of diagnostic imaging of Chiari-like malformation and syringomyelia. *Front Vet Sci*. 2018;5:280.
- De Decker S, Packer RMA, Cappello R, et al. Comparison of signalment and computed tomography findings in French Bulldogs, Pugs, and English Bulldogs with and without clinical signs associated with thoracic hemivertebra. *J Vet Intern Med*. 2019;33:2151-2159.
- Rohdin C, Jaderlund KH, Ljungvall I, Lindblad-Toh K, Haggstrom J. High prevalence of gait abnormalities in pugs. *Vet Rec*. 2018;182:167.
- Platt S, Olby NJ. Clinical pathology. *BSAVA Canine and Feline Neurology*. 3rd ed. UK: British Small Animal Veterinary Association; 2004:46-52.
- Hol EM, Pekny M. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. *Curr Opin Cell Biol*. 2015;32:121-130.
- Flanagan EP, Hinson SR, Lennon VA, et al. Glial fibrillary acidic protein immunoglobulin G as biomarker of autoimmune astrocytopathy: analysis of 102 patients. *Ann Neurol*. 2017;81:298-309.
- Shibuya M, Matsuki N, Fujiwara K, et al. Autoantibodies against glial fibrillary acidic protein (GFAP) in cerebrospinal fluids from Pug dogs with necrotizing meningoencephalitis. *J Vet Med Sci*. 2007;69:241-245.
- Uchida K, Park E, Tsuboi M, Chambers JK, Nakayama H. Pathological and immunological features of canine necrotizing meningoencephalitis and granulomatous meningoencephalitis. *Vet J*. 2016;213:72-77.
- Zarkali A, Cousins O, Athauda D, Moses S, Moran N, Harikrishnan S. Glial fibrillary acidic protein antibody-positive meningoencephalomyelitis. *Prac Neurol*. 2018;18:315-319.
- Toda Y, Matsuki N, Shibuya M, Fujioka I, Tamahara S, Ono K. Glial fibrillary acidic protein (GFAP) and anti-GFAP autoantibody in canine necrotizing meningoencephalitis. *Vet Rec*. 2007;161:261-264.
- Baronio D, Martinez D, Fiori CZ, et al. Altered aquaporins in the brains of mice submitted to intermittent hypoxia model of sleep apnea. *Respir Physiol Neurobiol*. 2013;185:217-221.
- Hendricks JC, Kline LR, Kovalski RJ, O'Brien JA, Morrison AR, Pack AI. The English bulldog: a natural model of sleep-disordered breathing. *J Appl Physiol*. 1987;63:1344-1350.
- Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. *Glia*. 2005;50:427-434.



39. Hoffman GS, Ellsworth CA, Wells EE, Franck WA, Mackie RW. Spinal arachnoiditis. What is the clinical spectrum? II. Arachnoiditis induced by Pantopaque/autologous blood in dogs, a possible model for human disease. *Spine*. 1983;8:541-551.
40. Mc LR, Bailey OT, Schurr PH, Ingraham FD. Myelomalacia and multiple cavitations of spinal cord secondary to adhesive arachnoiditis; an experimental study. *Arch Pathol*. 1954;57:138-146.
41. Shah J, Patkar D, Parmar H, Prasad S, Varma R. Arachnoiditis associated with arachnoid cyst formation and cord tethering following myelography: magnetic resonance features. *Australas Radiol*. 2001;45:236-239.
42. Basaran R, Kaksi M, Efendioglu M, Onoz M, Balkuv E, Kaner T. Spinal arachnoid cyst associated with arachnoiditis following subarachnoid haemorrhage in adult patients: a case report and literature review. *Br J Neurosurg*. 2015;29:285-289.
43. Morisako H, Takami T, Yamagata T, Chokyu I, Tsuyuguchi N, Ohata K. Focal adhesive arachnoiditis of the spinal cord: imaging diagnosis and surgical resolution. *J Craniovertebr Junction Spine*. 2010;1:100-106.
44. Barsoum AH, Cannillo KL. Thoracic constrictive arachnoiditis after Pantopaque myelography: report of two cases. *Neurosurgery*. 1980;6:314-316.
45. Shaw MD, Russell JA, Grossart KW. The changing pattern of spinal arachnoiditis. *J Neurol Neurosurg Psychiatry*. 1978;41:97-107.
46. Khan MU, Devlin JA, Fraser A. Adhesive arachnoiditis in mixed connective tissue disease: a rare neurological manifestation. *BMJ Case Rep*. 2016;2016:1-7.
47. Nagai M, Sakuma R, Aoki M, Abe K, Itoyama Y. Familial spinal arachnoiditis with secondary syringomyelia: clinical studies and MRI findings. *J Neurol Sci*. 2000;177:60-64.
48. Duke RJ, Hashimoto SA. Familial spinal arachnoiditis. A new entity. *Arch Neurol*. 1974;30:300-303.
49. Pasoglou V, Janin N, Tebache M, Tegos TJ, Born JD, Collignon L. Familial adhesive arachnoiditis associated with syringomyelia. *Am J Neuroradiol*. 2014;35:1232-1236.
50. Gnirs K, Ruel Y, Blot S, et al. Spinal subarachnoid cysts in 13 dogs. *Vet Radiol Ultrasound*. 2003;44:402-408.
51. Rohdin C, Nyman HT, Wohlsein P, Hultin Jaderlund K. Cervical spinal intradural arachnoid cysts in related, young pugs. *J Small Anim Pract*. 2014;55:229-234.
52. Aldrete JA. Chronic adhesive arachnoiditis. *Br J Anaesth*. 2004;93:301-303.
53. De Rycke LM, Crijns C, Chiers K, van Bree HJ, Gielen I. Late-onset wedge-shaped thoracic vertebrae in a six-month-old pug. *Vet Rec Case Rep*. 2016;4:1-7.
54. Penderis J, Schwarz T, McConnell JF, Garosi LS, Thomson CE, Dennis R. Dysplasia of the caudal vertebral articular facets in four dogs: results of radiographic, myelographic and magnetic resonance imaging investigations. *Vet Rec*. 2005;156:601-605.
55. Dewey CW, Davies E, Bouma JL. Kyphosis and Kyphoscoliosis associated with congenital malformations of the thoracic vertebral bodies in dogs. *Vet Clin North Am Small Anim Pract*. 2016;46:295-306.
56. Moissonnier P, Gosso P, Scotti S. Thoracic kyphosis associated with hemivertebra. *Vet Surg*. 2011;40:1029-1032.
57. Bergknut N, Meij BP, Hagman R, et al. Intervertebral disc disease in dogs - part 1: a new histological grading scheme for classification of intervertebral disc degeneration in dogs. *Vet J*. 2013;195:156-163.
58. Gaschen L, Lang J, Haeni H. Intravertebral disc herniation (Schmorl's node) in five dogs. *Vet Radiol Ultrasound*. 1995;36:509-516.
59. Baltzer WI, Hillebrand L, Smith TJ, Stieger-Vanegas SM. Surgical management of a Schmorl's node in an Airedale Terrier and review of the literature. *Vet Comp Orthop Traumatol*. 2012;25:167-171.
60. Kyere KA, Than KD, Wang AC, et al. Schmorl's nodes. *Eur Spine J*. 2012;21:2115-2121.
61. Peng B, Wu W, Hou S, Shang W, Wang X, Yang Y. The pathogenesis of Schmorl's nodes. *Bone Joint Surg*. 2003;85:879-882.
62. Reid JD. Effects of flexion-extension movements of the head and spine upon the spinal cord and nerve roots. *J Neurol Neurosurg Psychiatry*. 1960;23:214-221.
63. Kitahara Y, Iida H, Tachibana S. Effect of spinal cord stretching due to head flexion on intramedullary pressure. *Neurol Med Chir (Tokyo)*. 1995;35:285-288.
64. Winklhofer S, Schoth F, Stolzmann P, et al. Spinal cord motion: influence of respiration and cardiac cycle. *RoFo*. 2014;186:1016-1021.
65. Cauzinille L. Fibrocartilaginous embolism in dogs. *Vet Clin North Am Small Anim Pract*. 2000;30:155-167.
66. Cauzinille L, Kornegay JN. Fibrocartilaginous embolism of the spinal cord in dogs: review of 36 histologically confirmed cases and retrospective study of 26 suspected cases. *J Vet Intern Med*. 1996;10:241-245.
67. Rice I, Wee MY, Thomson K. Obstetric epidurals and chronic adhesive arachnoiditis. *Br J Anaesth*. 2004;92:109-120.
68. Walsh K. Chronic pain management in dogs and cats. *In Pract*. 2016;38:155-165.

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Pugs commonly present with signs of spinal cord disease, pug dog myelopathy (PDM). In this thesis the prevalence of signs of spinal cord disease and underlying mechanisms for the development of PDM were studied. Clinical and pathological characterization was performed of PDM and advanced imaging, biomarker studies of blood and cerebrospinal fluid and molecular genetic investigations were undertaken. Findings in this thesis provide data that contribute to the understanding of this complex and devastating disorder in the pug breed.

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