Characterisation of Chiari-like Malformation and secondary Syringomyelia in Selected Toy Dog Breeds using Magnetic Resonance Imaging

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Declaration of originality

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Summary

Chiari-like Malformation (CM) and secondary Syringomyelia (SM) is a complex, debilitating abnormality which compromises the normal cerebrospinal fluid movement of the central nervous system culminating in the development of fluid-containing cavities within the spinal cord and associated with behavioural signs of pain and neurological deficits. The prevalence of asymptomatic CM dogs suggest that cerebellar indentation and impaction may be normal anatomical variations and unsuitable as a definition of CM. Magnetic Resonance Imaging (MRI) remains the definitive means of diagnosing CM/SM and a morphometric technique of quantifying CM and SM on mid-sagittal MRI has been successfully applied and validated in previous studies to a cohort of Griffon Bruxellois (GB) dogs with and without CM and a mixed breed GB family crossed with a mesaticephalic breed (Australian Terrier). Using a refined technique which took account of recent research findings, morphometries using a triangulation of circles, lines and angles were used to 'map' MRIs of the whole brain and cervical region in order to quantify the severity of the CM and SM phenotype in the Cavalier King Charles (CKCS). A further morphometric analysis was undertaken to explore brachycephaly and miniaturization as risk factors for CM and SM by comparing their impact in the CKCS, Affenpinscher and Chihuahua breeds. The collective framework of lines and angles generated a unique 'signature' for the dog, characterised by "concertina" type flexures demonstrating the combined nature of segregated traits towards the severity in the phenotype. Compared to controls, CKCS with CM pain are characterised by increased brachycephaly and airorhynchy, while significant traits for SM in the three dog breeds included those reported for the GB, suggesting a common aetiology. The characterisation of the CM phenotype provides the possibility of a diagnostic tool for veterinarians and means to assist breeders with mate selection to reduce symptomatic prevalence of CM/SM.

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Abbreviations

bFEE	cardiac-gated cine balanced fast field echo
BMPs	bone morphogenetic proteins
BVA	British Veterinary Association
BOAS	brachycephalic obstructive airway syndrome
CNS	central nervous system
CKCS	Cavalier King Charles spaniels
CM	Chiari-like malformation (canine)
CM-I	Chiari type 1 malformation (human)
COI	Coefficient of Inbreeding
CSF	Cerebrospinal fluid
Cx43	Connexin43also known as Gap junction alpha-1 protein
BDNF	derived neural growth factor
DICOM	Digital Imaging and Communications in Medicine
EBV	Estimated Breeding value
F1	first generation offspring
F2	second generation offspring
FCI	The Fédération Cynologique Internationale
FGF	Fibroblast growth factor
GB	Griffon Bruxellois
GWAS	Genome-wide Association study
KC	The Kennel Club
MRI	Magnetic resonance imaging
MSX2	Homeobox gene required for proper craniofacial morphogenesis
P1	first parental cross
QTL	Quantitative Trait Locus
Runx2	Runt Related Transcription Factor 2 (protein coding gene)
SM	Syringomyelia
SPARC	Secreted Protein Acidic and Rich in Cysteine/osteonectin/BM-40)
T1W	T1-weighted
T1W	T2-weighted
TWIST	Basic helix-loop-helix transcription factors involved in cell lineage and differentiation
TGFα /β	transforming growth factor α or β
WNTs	signaling pathway first identified in wingless type drosophila

Table of Contents

Declaration of originality	i
Summary	ii
Acknowledgement	iii
Abbreviations	iv
Table of Contents	v
Figures	X
Chapter 1	x
Chapter 2	xi
Chapter 3	xi
Chapter 4	xii
Chapter 5	xii
Chapter 6	xii
Tables	xiv
Chapter 1	xiv
Chapter 3	xiv
Chapter 4	xiv
Chapter 5	xiv
CHAPTER 1	1
Introduction	
Man's Best Friend	1
Welfare concerns	
Chiari-like Malformation	
Nomenclature	
Definitions of Chiari-like Malformation	
Prevalence and Incidence of CM	
Clinical signs of symptomatic CM	
Treatment	
Syringomyelia secondary to CM	11
Definition	11
Human Syringomyelia classification	

Diagnosis of SM	13
Prevalence	13
Clinical signs	14
Treatment	15
Grading and evaluation of canine SM	15
Pathogenesis	17
Morphometric measurements associated with pathogenesis	
Studies of CM not in association with SM	20
Brachycephaly and miniaturization risk factors for secondary syringomyelia	21
Embryology	23
Introduction	23
Craniofacial Morphogenesis/Development relevant to canine CM/ SM	23
Signal transduction	24
Early Development the Central Nervous System (CNS)	24
Development the Central Nervous System (CNS)	25
Development of the Skull and Craniocervical Junction	
Vertebrate Skull	34
Genetic basis for CM/SM	43
Introduction	43
Human Genetic studies	43
Canine Genetic Studies	44
Cavalier pedigree SM database	45
Griffon Bruxellois pedigree database	47
Informal Breeding Guidelines and their Effectiveness	48
Estimated Breeding Values (EBVs)	53
Genetic studies investigating SM in the CKCS	54
Genetic studies investigating CM in the Griffon Bruxellois	56
Thesis Overview and Aims	57
Testable hypothesis	59
Research Aim	59
Specific Research Aims	59
CHAPTER 2	60
Materials and Methods	60
Introduction	60
Materials	61

	61
Inclusion and exclusion criteria	62
Assessment of CM/SM status	62
Study samples	63
Control dogs over 5 years	64
Methodology	64
Validation of significant morphometric traits in a mixed breed Griffon Bruxellois family	65
Characterisation of CM in the Cavalier King Charles Spaniel	66
Comparison of CM in different breeds	69
Standardisation of morphometric traits using 'best fit' circle and angles	70
Data Analysis	70
Morphometric mapping and 'morphing' technique	70
Statistical analysis	71
Reliability Analysis	72
Ethics Statement	72
Validation of the morphometric measurements in previous Griffon Bruxellois (GB) studie	
study and mixed breeding	s using a family 73
study and mixed breeding	s using a family 73 73
study and mixed breeding Introduction Study Cohort	s using a family 73 73 74
study and mixed breeding Introduction Study Cohort Results	s using a family 73 73 74
study and mixed breeding Introduction Study Cohort Results Study group CM/SM status and family relationships	s using a family 73 73 74 75 75
study and mixed breeding Introduction Study Cohort Results Study group CM/SM status and family relationships Magnetic Resonance image morphometric measurements	s using a family 73 73 74 74 75 75
study and mixed breeding Introduction Study Cohort Results Study group CM/SM status and family relationships Magnetic Resonance image morphometric measurements Syringomyelia	s using a family 73 7374 75 757678
study and mixed breeding Introduction Study Cohort Results Study group CM/SM status and family relationships Magnetic Resonance image morphometric measurements Syringomyelia Discussion	s using a family 73 73 74 75 75 76 76 78 79
study and mixed breeding Introduction Study Cohort Results Study group CM/SM status and family relationships Magnetic Resonance image morphometric measurements Syringomyelia Discussion CHAPTER 4	s using a family 73 73 74 74 75 75 76 76 78 79 79
study and mixed breeding Introduction Study Cohort Results Study group CM/SM status and family relationships Magnetic Resonance image morphometric measurements Syringomyelia Discussion CHAPTER 4 Use of morphometric mapping to characterise symptomatic Chiari-like malformatic	s using a family 73 73 74 75 75 76 76 78 79 79
study and mixed breeding Introduction Study Cohort Results Study group CM/SM status and family relationships Magnetic Resonance image morphometric measurements Syringomyelia Discussion CHAPTER 4 Use of morphometric mapping to characterise symptomatic Chiari-like malformat syringomyelia and associated brachycephaly in the Cavalier King Charles Spaniel	s using a family 73 73 74 74 75 75 76 76 78 79 79
study and mixed breeding Introduction Study Cohort Results Study group CM/SM status and family relationships Magnetic Resonance image morphometric measurements Syringomyelia Discussion CHAPTER 4 Use of morphometric mapping to characterise symptomatic Chiari-like malformat syringomyelia and associated brachycephaly in the Cavalier King Charles Spaniel Introduction	s using a family 73 73 74 75 75 75 76 76 78 79

Kesuits	84
Hindbrain study	84
Whole brain study	85
Statistical analysis using Discriminant Analysis	86
Overview of group findings	87
Discussion	90
Conformation similarities with Griffon Bruxellois	90
Conformation similarities with Griffon Bruxellois Brachycephaly and Airorhynchy	90 91
Conformation similarities with Griffon Bruxellois Brachycephaly and Airorhynchy Craniocervical junction conformation impact on CSF flow dynamics	90 91 91

Craniometric analysis of the hindbrain and craniocervical junction of Chihuahua, Affenpinscher and Cavalier King Charles Spaniel dogs with and without syringomyelia secondary to Chiari-like Malformation

Introduction	93
Study Cohort	93
Results	94
Individual Breeds	95
Morphometric analysis	98
Discussion	99
Breed characteristics for SM	100
Reduced occipital crest and supraoccipital bone	102
Changes in angulations which impact on the caudal cranial fossa	102
Summary	103

General Discussion	104
Natural variation and clinical significance of CM	105
Is the characterisation of CM using morphometric mapping useful?	109
Application of morphometric mapping	109
Multifactorial basis of CM/SM	111
Artificial Selection for CM and risk of SM	118
Conclusion	122
Limitations of the study	123
Future Studies	125

REFERENCES	127	
Appendix		
Tables	144	
U-tube link to Movie Morph here (https://youtu.be/TcPdH9Gcnqo)		
Legends for Morph Movies	146	
Publications	147	

Figures

Chapter 1

- Fig 1.1 Mid-sagittal T2-weighted image of brain and cervical spinal cord of CKCS with CM and SM.
- Fig 1.2 Examples of magnetic resonance imaging features of CM and SM syndrome in Griffon Bruxellois.
- Fig.1.3 Measurements made from skull radiographs illustrated in a normal GB and a GB with CM/SM.
- Fig 1.4 Treatment guidelines provided by Consultant veterinary neurologist C Rusbridge.
- Fig 1.5 Phenotype form used for the collection of DNA.
- Fig 1.6 Three representative sagittal T2-weighted MRI of the head and cranial cervical region of a dog with CM.
- Fig 1.7 Mesaticephalic German Shepherd skull compared with a miniaturised skull of the Chihuahua and the brachycephalic short base skull of the Cavalier King Charles Spaniel.
- Fig 1.8 Schematic illustration of the cellular morphogenetic process that occurs in gastulation and primary neurulation called 'Convergence Extension'.
- Fig 1.9 An illustration of convergent extension during the process of neural induction.
- Fig 1.10 Diagram of three closure points in the mouse embryo.
- Fig 1.11 Diagramatic section through the emergent spinal cord with the development of the sulcus limitans and ganglia.
- Fig 1.12 Schematic diagram of the differentiation of vesicles in the developing brain.
- Fig 1.13 Schematic developing vertebrate brain indicating the 5 divisions of the brain and 3 flexures.
- Fig 1.14 Ventricular system of the dog brain.
- Fig 1.15 Diagrammatic transverse section through the developing medulla oblongata and fourth ventricle.
- Fig 1.16 Schematic diagram of the skull components and their origins through vertebrate evolution.
- Fig 1.17 Schematic illustration of the primordial cartilages of the chondrocranium.
- Fig 1.18 Diagram of the bones that comprise the skull adapted from Miller's Anatomy of the Dog by H Evans and A de Lahunta.

- Fig 1.19 Anatomical variations in sutures associated with Inca bones.
- Fig 1.20 Seven dog breeds illustrating variation in skull morphology of both caudal and lateral aspects (not to scale).
- Fig 1.21 Pedigree of dog V showing relationship with founder bitch G and 'saturation' of suspected carriers in his first three generations.
- Fig 1.22 Key features which have ensured a successful DNA collection.
- Fig 1.23 Family tree of 32 related Griffon Bruxellois dogs.
- Fig 1.24 Comparasion of percentage dogs with and without SM in the Netherlands and other countries 2004-2009.
- Fig 1.25 Percentage of each offspring from various parental combinations for 372 CKCS between SM affected (all ages) and unaffected dogs over two and a half years of age.
- Fig 1.26 Example of mode of inheritance for SM given in Veterinary Journal 183 (2010).
- Fig 1.27 Table conversion from 2006 informal guidelines to 2012 BVA/KC guidelines (SM only).
- Fig 1.28 Three generation CKCS breed family tree with grandparents and offspring of an SM unaffected male with two SM affected mates.

Chapter 2

- Fig 2.1 Midline sagittal T1-weighted MRI of hindbrain and craniocervical region of a three year old female Griffon Bruxellois with the framework of measured lines and angles.
- Fig 2.2 Midline sagittal T1-weighted MRI of brain and craniocervical region of a female Griffon Bruxellois backcross.
- Fig 2.3 Twenty four measurements used to map the hindbrain and craniocervical junction on T1w mid-sagittal MRIs of a CKCS without SM.
- Fig 2.4 Additional morphometric measurements taken of the T2w mid-sagittal brain MRI of a CKCS with CM pain.
- Fig 2.5 T1W sagittal MRI of a five year old Chihuahua without SM with a framework of 19 measurements (11 lines and 8 angles) with three ratios used to 'map' the hindbrain and craniocervical junction.

Chapter 3

Fig 3.1 Study cohort GB family tree (n=27) including a mixed cross to an Australian terrier and subsequent backcross.

- Fig 3.2 Boxplot distribution of significantly associated traits for CM and SM BVA/KC grades (n=26).
- Fig 3.3 Key morphometric measurements made on the MRIs of dogs in three generation pedigree of the F2 Backcross.
- Fig 3.4 Head conformation and associated angle FAC in six relatives of Bitch H with and without SM.

Chapter 4

- Fig 4.1 Canonical Discriminate functions of CKCS with and without CM pain and SM.
- Fig 4.2 Descriptive boxplots of key significant traits for the 3 study cohorts; Control, CM pain and SM.
- Fig 4.3 Four mid-sagittal T2w whole brain MRI exemplars of cohorts Control, CM pain and two conformation cases of SM.

Chapter 5

- Fig 5.1 Scatterplots for canonical Discriminant Functions Analysis for 1) Breed 2) SM status in total group cohort.
- Fig 5.2 Scatterplots for canonical Discriminant Analysis for three breeds, Chihuahua, Affenpinscher and CKCS.
- Fig 5.3 Descriptive boxplots of variables associated with SM status significant for both Chihuahua and CKCS cohorts.
- Fig 5.4 Descriptive boxplots of significant variables associated with SM status in the Affenpinscher, Chihuahua and CKCS.
- Fig 5.5 Four pairs of TW1 mid- sagittal MRI of exemplar Chihuahua, Affenpinscher and CKCS with and without SM and their morphometric overlays.
- Fig 5.6 Schematic framework of selected CM traits (red lines) and 'normal' traits (blue lines) to illustrate underlying unifying pathophysiological processes.

Chapter 6

- Fig 6.1 Schematic framework of selected CM traits (red lines) and 'normal' traits (blue lines) to illustrate underlying unifying pathophysiological processes.
- Fig 6.2 Diagrammatic illustrations to show the hypothetical effects of compensatory changes in skull dimensions and brain shape when there is craniosynostosis or shortening of certain skull bones.
- Fig 6.3 Comparison of two radiographs of Australian terriers with and without CM

- Fig 6.4 Superimposed 'signatures' of four exemplar CKCS dogs aligned at fixed point * (Ch.4).
- Fig 6.5 Morphometric mapping on six sagittal MRI exemplars of whole brains with measurements of three 'best fit circles' comparing CKCS (with and without CM and SM) and other breeds (without CM or SM).
- Fig 6.6 Superimposed 'signatures' of six dogs in Fig 6.5.
- Fig 6.7 Sagittal MRI of Chihuahua with and without SM.
- Fig 6.8 Mid-sagittal MRI of CKCS with SM (A) and without SM (B) from a different research project to illustrate differences in shape and position of the cerebrum and brain stem.
- Fig 6.9 'Natural' distortion of the ventricular system of the dog with bilateral reduction of the skull base and supra-occipital bone with compensatory increase in cerebrum that might occur during brachycephaly and CM.
- Fig 6.10 Eyes of Australian terriers with and without CM.
- Fig 6.11 Radiographs of three generation of mixed breed GB family illustrating changes in the caudal skull.
- Fig 6.12 Facial features of three generation pedigree of F2 Backcross and F1 and F2 body conformation.
- Fig 6.13 Three dimensional image of brain and cervical spinal cord of F2 backcross siblings.

Tables

Chapter 1

- Table 1.1Definition of human Chiari malformation.
- Table 1.2Clinical signs of 39 CKCS with CM.
- Table 1.3List of symptoms and their frequency in human CM type 1.
- Table 1.4
 Classification of Syringomyelia in humans.
- Table 1.5
 Grading criteria for SM according to Cerda-Gonzalez and Olby.
- Table 1.6
 Main derivatives of neural crest cells (selected).
- Table 1.7No of dogs grouped according to their head conformation with separate
interparietal bones in a population of 127.
- Table 1.8Informal Breeding Guidelines 2006.

Chapter 3

- Table 3.1Summary of CM and SM status in family study group.
- Table 3.2
 Descriptive Statistics for five traits in Mixed, Pure and control GB Groups.
- Table 3.3Morphometric measurements for three generation the F2 backcross pedigree.

Chapter 4

- Table 4.1Study groups with numbers and ages of CKCS in the three study cohorts.
- Table 4.2Significant traits identified in four analyses of the hindbrain study using ANOVA
(1) and independent sample t-tests (2-4).
- Table 4.3Significant traits identified in four analyses: 1. Total group using ANOVA, 2-4
paired cohorts using independent sample t-tests.
- Table 4.4
 Canonical Discriminant Function Coefficients

Chapter 5

Table 5.1Study cohort.

 Table 5.4
 Significant variables for SM status identified in ANOVA after Tukey correction.

Chapter 1

Introduction

"Ever since the initial *post-mortem* examination description by Chiari in 1891 of the group of malformations that bears his name, it seems there has always been more questions on this subject than answers." **WS Jr Ball,** 1995.

Man's Best Friend

Any canine condition which manifests itself as pain can affect the quality of life not only of the individual dog, but also that of the owners [1,2]. The relationship between dog and man is deep rooted, going back to prehistoric times [3,4] and the strength of bond between the two cannot be overstated. It can be just as distressing for an owner to know their dog is in pain or discomfort as to be suffering pain themselves. It is particularly poignant then that the developmental abnormality known as Chiari-like Malformation (CM) is found in both man and in the dog. CM is analogous to Chiari type I malformation (CM-I) in humans, a condition which affects ~1 in 1280 people. Common symptoms in human CM patients include severe headache and neck pain, vertigo, muscle weakness, impaired fine motor skills and chronic fatigue [5,6]. None of these symptoms are unique to the CM and it is easy to see that the clinical signs of the condition in the dog can be easily overlooked. Behavioural signs of pain are therefore equally important as neurological indicators [7] in any diagnosis of canine CM. The malformation itself can be painful but it is commonly known to be associated with the spinal cord disease Syringomyelia (SM). In simplest terms, CM is the conformational changes in the skull and brain that is thought to disrupt the flow of cerebrospinal fluid (CSF) and results in the formation of a fluid filled cavity or syrinx (pleura syringes) in the spinal cord and impair its function [8]. SM can be progressively painful and manifests itself with neurological deficits. In the Cavalier King Charles spaniels (CKCS), the initial clinical sign for veterinary consultation was persistent scratching at one side of the neck or shoulder area. In 1997, Rusbidge demonstrated a link between phantom scratching and the condition SM [9].

Welfare concerns

The welfare concerns of SM secondary to CM were highlighted by the Companion Animal Welfare Council [10] in 2006 and featured in the BBC television documentary 'Pedigree Dogs Exposed' which was aired worldwide in 2008. The fact that many disabling inherited diseases in dogs were a consequence of human selection for perceived desirable traits precipitated three inquiries into dog breeding and competitive dog showing in the UK. This has led to the formation of a new independent Advisory Council on the Welfare Issues of Dog Breeding sponsored by the Dogs Trust, People's Dispensary for Sick Animals (PDSA) and the Royal Society for Prevention of Cruelty to Animals

(RSPCA) [11,12]. All three enquiries reported the need to develop evidence-based breeding strategies that address the issues of conformation and inherited diseases. The Bateson report stated there was a "need to avoid selection for extreme morphologies that can damage the health and welfare of the dog" (8.3 p45) and a "need for breed-specific advice and guidance on how to breed away from particular problems" (5.12 p 27)[2].

Dogs can communicate their emotions in a variety of ways and it is important to appreciate these when dealing with dog welfare and disease. Behavioural signs such as withdrawal, yelping, aggression, sensitivity, abnormal posture can all indicate 'neuropathic pain' i.e. pain associated with abnormal somatosensory processing in the central or peripheral nervous system. Although a clinical examination will identify some of these signs of pain, others maybe intermittent and/or depend on accurate reporting by the owner based on observations and intimate knowledge of their pet. Dogs give their total loyalty and friendship to their adopted pack leader, and it is important to this partnership that their trust is not betrayed.



The dog, as Man's best friend, has been modified through selective pedigree breeding into a large variety of form and function. As a result it is now totally dependent on man to safeguard the species welfare and health. It is important that the close relationship we have with our pets not to betray their trust. Humans and dogs each have a unique role to play towards understanding the causes and most effective treatments for the inherited developmental condition CM and SM.

Photo curtesy of Charlotte Mackaness

Chiari-like Malformation

Nomenclature

In veterinary medicine different terms have been used to describe the complexities of the skull deformities that result in syringomyelia. These have included Arnold Chiari malformation, Chiari malformation as with the human analogue, occipital hypoplasia (OH) based on the reduced development of the supraoccipital bone [13] and caudal occipital malformation syndrome (COMS) [14]. Such names have resulted in misperception and in 2006 an international veterinary working group, invited by the UK Cavalier Club, were briefed to agree a name in order to dispel confusion [15]. There was general resistance to the label Chiari malformation because it is not veterinary practice to use eponymous terms to describe disease. However such practice has not been without precedence, especially when the pathophysiology/anatomy is so complex that there is no simple alternative such as Parkinson's disease in human medicine. It was noted that Chiari Malformation, commonly used in in human medical scientific publications, no longer reflected the original description of the disease but any condition characterised by reduced posterior fossa volume and caudal descent of the hindbrain. Since dogs and cats do not have cerebellar tonsils and the hindbrain is more commonly used as an embryological term not in general use. Other names were rejected at the time because there was insufficient evidence for the pathogenesis of the condition or because they could lead to confusion; for example, COMS was sometimes used indiscriminately to include syringomyelia and 'caudal occipital' refers to the supraoccipital bone and is anatomically incorrect [14]. Similarly occipital hypoplasia, OH, was rejected because it did not reflect the complexity of the disorder. A compromise was reached by agreeing the canine term Chiari-like Malformation [15,16]. It was noted that an acronym was especially important to dog breeders and owners and the term Chiari-like Malformation and Syringomyelia is often abbreviated to a simple CM/SM. However some veterinarians use the alternative acronym CLM to distinguish the term from the human condition but in the view of the author this continues to lead to confusion.

Definitions of Chiari-like Malformation

Despite an agreement on nomenclature, the definition of CM has evolved over decades reflecting an increased understanding of the pathogenesis of secondary SM. Figure 1 provides an illustration of midsagittal T2-weighted image of brain of CKCS with CM and SM highlighting some of the anatomical components involved in the disorder. Canine CM is often considered a naturally occurring model for Chiari type 1 malformation (CM-I) in humans [14,17–21]. Notwithstanding, there is no doubt the veterinary profession draws substantially on knowledge and experience provided by the human medical profession with respect to CM/SM. Comparing the conditions in both the human and in the dog has mutual advantages and this has been strengthened over the years with combined expertise and shared information.



Fig. 1.1 Mid-sagittal T2-weighted image of brain and cervical spinal cord of CKCS with CM and SM.

White text = brain parenchyma, yellow text = bones, aqua text associated with cerebrospinal fluid flow

Human Chiari Malformation

In humans Chiari Malformation refers to a spectrum of disorders involving displacement of the cerebellum. In 1896 Chiari described four hindbrain abnormalities or 'Types' 1-1V with increasing severity of neurological deficits. These are listed on the left column in Table 1.1. Over the century, with a better understanding of the pathophysiology, these original definitions of CM have been modified and two additional types have now been included – Type CM-0 and CM- 1.5 and these definitions are summarised on the right side of Table 1.1 [16].

CM-I has been extensively investigated [5,22–28]. Initially it was described a developmental condition characterised by the displacement of the cerebellar tonsils into or through the foramen magnum [29] and association with herniation through the foramen magnum. However the current definitions of human CM-I recognises a range of abnormalities which include hindbrain descent and overcrowding of the caudal fossa which compromises CSF circulation [26,29–32]. Batzdorf suggests a division of CM that is useful for surgical treatment based on subtypes: basilar invagination, low insertion of torcula (confluence of sinuses), shallow posterior fossa and space occupying mass (arachnoid cyst) [33]. The primary distinction between CM-0 and CM-I is the presence or absence of tonsillar herniation but there is accumulating data that relate to clinical and radiological similarities between the two. Familial clustering of these disorders suggest there is a common underlying genetic basis [34] and supported by an investigation of occipital hypoplasia in the CKCS [35].

Chiari Malformation	Original discription by Chiari (Batzdorf 2001)	Modern description
Туре 0		Overcrowding of the posterior fossa with abnormal brainstem anatomy (posterior pontine tilt, downward displacement of the medulla, low-lying obex) but with normally placed cerebellar tonsils (Markunas <i>et al</i> , 2012
Type 1.5		Cerebellar and brainstem herniation through the foramen magnum. Similar to type II malformation but not associated with spinal dysraphism (Tubbs <i>et al</i> , 2004)
Туре І	Elongation of the cerebellar tonsils and medial part of the inferior cerebellar lobes into cone-like projections, which accompany the medulla into the spinal canal	Volumetrically small posterior cranial fossa with hindbrain overcrowding (Milhorat <i>et al,</i> 1999) The classic radiographic description is tonsillar herniation of at least 3mm below the foramen magnum
Туре II	Displacement of portions of the vermis and also the pons and medulla into the spinal canal and elongation of the fourth ventricle into the spinal canal	Downward displacement of the cerebellar vermis, brainstem and fourth ventricle, associated with a myelomingocoele (Geerdink <i>et al</i> , 2012)
Type III	Displacement of almost the entire hydrocephalic cerebellum into a cervical spinal bifida	Hindbrain herniation into a high cervical or occipital encephallocoele (Castillo <i>et a,l</i> 2012)
Type IV	Hypoplasia in the region of the cerebellum without displacement of the portions thereof into the spinal canl	Obsolete term describing cerebellar hypoplasia unrelated to the other Chiari malformations

Table 1.1	Definitions	of human	Chiari n	nalformatio	on in t	the last	century.
							•

Source 'Syringomyelia a disorder of CSF circulation' Editors Flint and Rusbridge. p304).

Canine Chiari –like malformation

There is less phenotypic variation in canine CM than with humans but the extent of differences between breeds is not known. The conformation of the craniocervical junction varies with respect to the size of the dog's head and with its the ligaments [36] and associated with different breeds. However, with CM, the size of cerebellar volume in the CKCS was larger than other small breeds and Labradors and posed a greater risk for overcrowding of the caudal cranial fossa and therefore SM [37]. This feature in particular makes CM in dogs dissimilar from the analogous human condition, Chiari type I malformation, although there are some similarities to Chiari type 0 malformation [34]. The term craniovertebral or craniocervical junction refers to the bony structures surrounding the medulla oblongata, the cervicomedullary junction and the upper cervical spinal cord and is constructed of the occipital bones forming the foramen magnum, the atlas and the axis. Mechanically the craniocervical junction consists of a central pivot (basioccipital bone, dens and axis) and two rings (foramen magnum and atlas).

Dr C Dewey and Dr D.Marino *et al.* in the USA, consider CM to be part of wider spectrum of craniocervical junction abnormalities (CJA) [38]. The group define Chiari-like malformation as a condition of the craniocervical junction in which there is a mismatch of the structures of the caudal

cranial fossa causing the cerebellum to herniate into the foramen magnum. This herniation can lead to fluid accumulation in the spinal cord, also known as syringomyelia [39].

In 2011, the British Veterinary Association (BVA)/Kennel Club (KC), as part of the CM/SM health screening, published the following grading for CM using the cerebellum as a measure of overcrowding of the caudal fossa.

- Grade 0, no CM the cerebellum has a rounded shape with a signal consistent with cerebrospinal fluid (CSF) between the caudal cerebellar vermis and the foramen magnum.
- **Grade 1 CM** the cerebellum does not have a rounded shape, i.e. there is indentation by the supraoccipital bone, but there is a signal consistent with CSF between the caudal vermis and the foramen magnum.
- Grade 2 CM the cerebellar vermis is impacted into or herniated through the foramen magnum.

There are no breeding guidelines provided for CM as they are for SM in the BVA/KC Health screening scheme. This is because there is no current scientific evidence to justify this however the measurement system provides the means to accumulate sufficient data towards a potential estimated breeding value for the condition.

One generally accepted definition of canine CM is the disparity in volume between the caudal cranial fossa and its contents so that the cerebellum and brainstem are herniated into or through the foramen magnum [40,41]. However, many veterinarians regard the herniation as the key characteristic feature of CM as in the analogous form of human CM type1 [42,43].

CM in the Griffon Bruxellois

Most of the early research into CM/SM has been based on the CKCS because of the popularity of the breed in the late 90s early 2000s, the association of the 'scratching cavalier' as a classical sign of SM and the proactive support of the Cavalier Club and pet owners. However SM had been diagnosed in other breeds and in 2005 an Australian Griffon Bruxellois breeder, Lee Pieterse requested the help of Dr Rusbridge to investigate CM/SM in her dogs using radiographs with the possibility of predicting SM. Consequently, with the support of funding from SM DNA Research and the Charity 'For The Love of Ollie', an investigation of 56 Griffon Bruxellois was devised which described a simple radiographic technique as a possible screening tool [44]. In the selected study group, 61 % had CM, 47 % had SM \geq 2mm wide (9% SM only, 38% CM/SM) and 18% had CCD or SM \leq 2mm wide. All dogs with CCD or SM had ventriculomegaly with 94% (34 of 36) having moderate or severe ventricular dilatation. Four dogs had clinical signs of CM/SM; all had an asymptomatic CM/SM affected dam. The results indicated that in this breed, CM is characterised by a shortening of the basicranium and supraoccipital bone with a compensatory lengthening of the cranial vault, especially the parietal bone. The radiographic study

demonstrated that one measurement ratio could be used to predict Chiari-like malformation (sensitivity of 87 per cent and specificity of 78 per cent) and that there were significant interaction factors between sex and syringomyelia for two measurement ratios. Figs.1.2 and 1.3. from the published paper illustrates the differences between a normal GB and one with CM/SM [44].





Key (a) Normal (mid sagittal T2W brain and cervical spinal cord). (b) CM (mid sagittal T2W brain and cervical spinal cord). There is coning of the cerebellar vermis into the foramen magnum and moderate ventriculomegaly. (c) CM/SM (sagittal mid-line T2W brain and cervical spinal cord). Note the fluid-filled cavity within the spinal cord. Low signal within high signal fl uid is because of fluid movement (fluid flow-void). (d) CM with SM less than 2 mm (sagittal mid-line T2W brain and cervical spinal cord with insert of transverse T2W spinal cord at level of dens). Immediately dorsal to the dens, there is a small area of high signal within the spinal cord. In this dog, there is also severe dilatation of the lateral ventricles. (e) SM and no CM with moderate ventriculomegaly (mid sagittal T2W brain and cervical spinal cord). (f) Central canal dilation and no CM (sagittal mid-line T2W brain and cervical spinal cord with insert of transverse T2W spinal cord at level of Mid-signal mid-line T2W brain and cervical spinal cord. In this dog, there is also severe dilatation of the lateral ventricles. (e) SM and no CM with moderate ventriculomegaly (mid sagittal T2W brain and cervical spinal cord). (f) Central canal dilation and no CM (sagittal mid-line T2W brain and cervical spinal cord with insert of transverse T2W spinal cord at level of mid-C2).

Fig.1.3 Measurements made from skull radiographs illustrated in a normal GB and a GB with CM/SM



Key: Skull radiographs from two Griffon Bruxellois dogs. A normal dog is on the left and a dog with Chiari-like malformation (CM)/syringomyelia (SM) is on the right. Top row, digital images of the radiographs were black and white inverted and windowed so that areas of greatest bone density were black. Middle row, a line was drawn from the nasion (point 1) following the basioccipital bone and through the dorsal occipitoatlantal joint. An ovoid shape (dotted ovoid) was drawn that was bisected by the line and incorporated the tympanic bullae for its ventral border. A dotted line extends from this, following the outline of the supra occipital bone, and terminating dorsally at the area of the lambdoid suture. The bisection of tympanic bullae ovoid with solid line was designated point 2, and the area of the lambdoid suture was designated point 4. Bottom row, the following measurements were then made: A, rostral nasal bone (point 1) to bisection of tympanic bullae ovoid with solid line (point 2); B, point 2 to perpendicular point on the dorsal cranium (point 3); C, area of the lambdoid suture (point 4) to perpendicular point on the solid line (point 5) and D, point 2 to 5.

In Georgia University USA, the presence of CM was subjectively assessed in a study of 84 GB by recording the presence of cerebellar deviation and cerebellar herniation separately but also graded CM using a version of the current British Veterinary Association (BVA) grading scheme [45]. Rather confusingly perhaps for breeders, the group did not use the same numerals as the BVA scheme;

- **CM 1** with a normal shaped cerebellum.
- **CM 2** has an indented cerebellum but signal consistent with cerebrospinal fluid exists between the caudal cerebellar vermis and the foramen magnum.
- CM 3 exhibits a cerebellum impacted or herniated into the foramen magnum.

They found that CM was present in 65% of the dogs, 39 dogs (47%) had cerebellar deviation, 55 dogs (65%) had cerebellar herniation, and 22 dogs (26%) had both. Pain for CM/SM was assessed in this study and reported no phantom scratching in any of the dogs and no other cranial cervical junction abnormalities (e.g., dorsal angulation of the dens, atlanto-axial subluxation) were identified.

Prevalence and Incidence of CM

CM has been reported in many Toy breeds particularly the CKCS, King Charles spaniels, Griffon Bruxellois, Affenpinschers, Chihuahuas, Pomeranians, Maltese, Boston and Yorkshire terriers [38,43,46–48]and brachycephalic cats [49]. CM is considered ubiquitous in the CKCS [50–52]. The Griffon Bruxellois (known as the Brussels Griffon in the USA) has a high prevalence of the disorder with a conservative estimate of 65% having CM and 42- 52% having syringomyelia [44,45,53]. There has been no in depth studies of other breeds as yet.

Any information on the prevalence of CM is clearly dependent on how it is defined and this is usually in the context of SM. Accurate phenotyping is pivotal to facilitate genetic studies into this disorder. Harcourt-Brown *et al* while investigated the prevalence of CM in clinically unaffected dogs [43] concluding that the anatomical variations in 199 dogs analysed found 44% dogs had cerebellar indentation and 22% dogs with cerebellar impaction and these represented normal variations and therefore unsuitable as definitions of CM.

The incidence of symptomatic CM is not known at the present time since signs of pain are difficult to recognise by owners or their veterinarians to measure objectively. Furthermore neuropathic pain associated with SM in dogs may also be due to CM and difficult to differentiate between the two morbidities. Breed Club Health Censuses do give owners the opportunity to volunteer information about the health status of their dogs but the results are subject to the robustness of the questions asked and how comprehensive they are. The Cavalier Club Census 2013 reported that 11.2% (327 owners) of their dogs had been diagnosed with CM and 7.4% (217 owners) with Syringomyelia.

Clinical signs of symptomatic CM

Although the underlying aetiology for CM remains unclear, clinical signs of CM are considered to be associated with neuropathic pain i.e. when nerve fibers themselves may be damaged or dysfunctional.

Table 1.2 is derived from a study of neurological signs in 39 CKCS with CM [17]. The group investigated the deformity of the cerebellum in the median plane on MRI with respect to the supraoccipital bone and the foramen magnum and degree of ventriculomegaly. It provides a simple measure of the frequency of clinical signs. The conclusion reached by the group was that there was no correlation between these clinical signs and the severity of the herniation, SM or hydrocephalus

	Clinical Signs	Number (% affected)
	Facial nerve deficits	9 (41)
	Seizures	7 (32)
	Vestibular syndrome	7 (32)
	Multifocal central nervous signs	6 (27)
Cranial group	Ataxia	5 (23)
	Menace deficit	4 (18)
	Proprioceptive deficits	4 (18)
	Head tremor/nodding	3 (14)
	Temporal muscle atrophy	2 (9)
	Spinal hyperaesthesia	16 (94)
Spinal group	Proprioceptive deficits	8 (47)
	Persistent scratching of the shoulder and/or neck	3 (18)

Table 1.2 Clinical signs of 39 CKCS with CM

The trigeminal neuralgia or 5th cranial nerve is thought to be implicated in human CM-1 but not always. Associated neuropathic pain is perhaps through compression of the nerve root or spinal nucleus and indeed some dogs perform face or head rubbing. Oro *et al* have investigated the clinical signs in human CM-I (defined as having a cerebellar herniation 3mm or more through the foramen magnum) by means of a patient questionnaire. Table 1.3, taken from the publication gives the frequency of 265 patients that reported a wide range of symptoms but predominated by head ache (98%) predominated. It lists the presenting symptoms for over 50% of the patients [54]. Such clinical signs can only manifested by behaviour in dogs and any intermittent nature of any such signs increases the difficulty in recognition by the owner or veterinarian.

Table 1.3 List of symptoms and their frequency greater than 50% in human CM-I.

Presenting Symptom	% frequency
headache	98
dizziness	84
difficulty sleeping	72
weakness of an upper extremity	69
neck pain	67
numbness/tingling in upper extremity	62
fatique	59
nausea	58
shortness of breath	57
blurred vision	57
tinnitus	56
difficulty swallowing	54
weakness of a lower extremity	52

The classic Chiari headache is generally considered to be intense pain in the occipital region (the back of the head) that is triggered by straining (Valsalva maneuver) such as coughing, sneezing, laughing and physical activity, and usually does not last very long [16]. In dogs CM associated pain may be manifested as a yelp when being picked up, or other rapid changes in position.

A questionnaire-based behavioural analysis of 122 CKCS with neuropathic pain due to CM and SM identified a reduced quality of life for dogs that were affected. Behaviour that was positive correlation with neuropathic pain included withdrawal and reluctance to interact or participate in certain forms of exercise or aggression[7]. They may adopt unusual sleeping positions and prefer sternal decumbency [40].

Treatment

A comprehensive investigation of treatment for CM is considered out with the scope of this thesis. The therapeutic approach to the treatment of affected dogs is currently with analgesics, some of which are not licensed for dogs. Despite a range of drugs for neuropathic pain, dosages and side effects need constant management [55]. Although successful in palliating pain, dogs unresponsive to medications are either euthanized or condemned to a life of persistent, intractable pain. Cranial cervical decompression surgery is offered as a treatment to re-establish of CSF flow through the foramen magnum in order resolve the size of the syrinx rather than CM pain. The concurrent existence of occipital hypoplasia and occipital dysplasia [35] suggests that the larger keyhole foramen magnum associated with occipital dysplasia may offer less obstruction to CSF flow and may affect the severity of CM and SM clinical signs and age of onset of SM but the evidence is speculative [55]. Reducing CSF production with proton pump inhibitors or diuretics can have long term side effects since they have system effects and are more important for management of SM [56].

Syringomyelia secondary to CM

Definition

SM is a generic term characterised by one or more fluid filled cavities called syringes (singular syrinx) within the spinal cord. These can develop as a complication to trauma, meningitis, arachnoiditis or tumours [57] which impinge the subarachnoid space and disrupt cerebrospinal fluid (CSF) flow. The name syringomyelia proposed in 1824 by Olivier d'Angers [16]. Further descriptive terms proposed by Shuppel in 1865 were as follows:

Hydromyelia - a dilation of the central canal;

Syringomyelia - a cavity separate from central canal;

Syringohydromyelia - a combination of above.

However there has been some controversy over the distinction between hydromyelia lined by ependyma of the central canal and syringomyelia lined by glial cells within the white matter and the use of the terms 'syringohydromyelia' and syringomyelia [16,58]. Histologically the distinction between hydromyelia and syringomyelia is difficult. This is also true when the conditions are investigated by radiological and clinical means. The use of the term 'syringohydromyelia' has fallen out of usage as the additional 'hydro' is superfluous.

Human Syringomyelia classification

Human SM and hydromyelia were traditionally classified into communicating and noncommunicating types [32] indicating the presence of a connection between the syrinx and the fourth ventricle. Communicating SM was associated with hydrocephalus and complex hindbrain malformations. It is uncommon in humans and may have a different pathogenesis and management [33].Table 1.4 is provided for reference.

Classification of Syringomyelia (Milhorat. Neurosurgery Focus, 2000)				
1	communicating syringomyelia			
	central canal dilations			
		1) communicating hydrocephalus		
		2) complex hindbrain malformations		
		3) Dandy–Walker cyst		
1	noncommunicating syringomyelia			
11	central canal/paracentral syringes			
		1) Chiari malformations		
		2) basilar invagination		
		3) spinal arachnoiditis (posttraumatic,		
		4) extramedullary compressions		
		5) tethered cord		
		6) acquired tonsillar herniation		
		primary parenchymal cavitations		
		1) spinal cord trauma		
		2) ischemia/infarction		
		3) intramedullary hemorrhage		
111	atrophic cavitations			
	(syringomyelia ex vacuo)			
1V	neoplastic cavitations			

Table 1.4 Classification of Syringomyelia in humans

Text in red is comparable to canine conditions

Diagnosis of SM

Diagnosis of SM can only be definitively confirmed by magnetic resonance imaging (MRI) or post mortem [20,41,59,60]. However radiographs [20,44], computed tomography (CT) [60,61] ultrasound [20] thermal imaging [62] and head conformation [63] have been used to indicate CM and likelihood of SM. A syrinx can extend the whole length of the spinal cord [64] but is commonly (76% of cases) located in the cranial region (C1-C4) of the cervical spinal cord. It is possible that ' reduced-cost screening' which is limited to the cervical region of the spinal cord may miss a syrinx in the lower region [64][65].

Prevalence

The earliest probable case of a SM in the dog, deduced retrospectively from the clinical history and radiographs, was a dog presented to the Royal Veterinary College in 1987. However it was a decade later, coinciding with better diagnostic capability of magnetic resonance imaging (MRI), that abnormalities of the occipital bones and cavitation in the spinal cord were first described [66]. It was Rusbridge in 1997 who first described what was then called Syringohydromyelia, and its association with 'persistent scratching' in Cavaliers [9]. Further cases were reported in Australia [21]. More recently the widespread use of advanced neuroimaging techniques has led to the diagnosis of SM in several breeds of toy breed dogs.

As with CM, accurate estimates for SM prevalence are compounded by the difficulties of obtaining a definitive diagnosis. In addition, as not all animals with SM are symptomatic, it is difficult to obtain reliable data on disease incidence. The frequency of asymptomatic SM in humans has been reported as being 51.2% [67] [68]. A study of 555 CKCS declared asymptomatic by their owners revealed SM to be in 25% of dogs aged one year rising to 70% in dogs aged 6 years [50]. The overall prevalence in the tested population was 46% [69]. In populations of Griffon Bruxellois studied previously, 42- 52% of dogs have SM but this is not always in association with a classical CM [44,45,53]).

In the UK, electronically stored patient health records from primary-care practice are emerging as a useful source of epidemiological data in companion animals. It uses large volumes of health data from UK primary-care practices participating in the VetCompass animal health surveillance. A retrospective study of the prevalence of CM/SM included all dogs within the VetCompass Programme (September 1, 2009–June 13, 2014) [70]. Overall, the period prevalence of symptomatic CM/SM was 0.05 per cent for all breeds but in the same period the prevalence in the Cavalier King Charles Spaniel (CKCS) was 1.6 per cent i.e. relatively much higher. The overall low figure in this survey was a reflection of the data collection which only included dogs that were sent to referral by the vet and confirmed by MRI. Other breeds at increased odds included the King Charles Spaniel, Affenpinscher, Chihuahua and Pomeranian. Insured dogs had 4.6 times the odds of having a diagnosis of CM/SM compared with uninsured dogs

because it was more likely to be referred or receive a MRI investigation. Pain was the most commonly associated clinical sign (67 dogs, 72 per cent). They report that, despite its low overall period prevalence, the high proportion of affected dogs identified with chronic pain suggests a significant welfare issue [71].

A questionnaire-based prevalence study of symptomatic SM was undertaken with all 240 CKCS registered in the Danish Kennel Club in 2001. Dogs were 6 years at the time of investigation so as to take account of late onset of the condition. The group estimated a prevalence of symptomatic SM at 15.4% in the population and estimated heritability of symptomatic SM as 0.81. The group concluded that prevalence of symptomatic SM was high and that genetics have a high impact on clinical disease expression [69].

The UK Kennel Club Annual Health Report 2015 listed the results of the CM/SM testing scheme by breed [72]. Affenpinscher, Boston terrier, CKCS, Chihuahua (both coat types), Griffon Bruxellois, Papillon and Pomeranian are represented and additionally one French Bulldog and a Pekinese without SM. Only 3 dogs had no CM – two Griffon Bruxellois and a Chihuahua. The CKCS breed owners, have been subsidized by the Kennel Club Charitable Trust by waiving the scheme fees for dogs screened before 2013 and, of a total 44 dogs screened over 5 years, eleven CKC were without SM , twelve with SM and six with a central canal dilation.

Clinical signs

The clinical signs of SM in dogs are variable and, since the conditions are usually accompanied with CM, behavioural signs of neuropathic pain such as vocalisation, unwillingness to exercise and being withdrawn may be hard to differentiate between the two morbidities. Some signs when severe can be particularly distressing for the owner as well as the affected dog. They include sensitivity to touch, particularly around the neck and shoulders, face rubbing, 'air scratching'- a reflex scratching motion (dysaesthesia) towards the neck but not making contact with the skin – which makes walking on a leash particularly stressful. It is an example of neuropathic pain whereby the nerve fibers themselves are damaged and dysfunctional [16,73,74]. Damage to the spinal cord can be manifested as cervicothoracic scoliosis, thoracic and pelvic limb ataxia and thoracic limb paresis [47,75,76]. SM pain has been positively correlated with the width and symmetry of the syrinx on the vertical axis and association with the dorsal horn rather than the length [77].

A questionnaire --based behaviour analysis of Cavalier King Charles spaniels with neuropathic pain due to Chiari-like malformation and syringomyelia [7] found that, in 122 dogs, the severity of neuropathic pain was positively associated with certain fear-associated behaviour and with decreased owner-perceived quality of life.

Treatment

An in-depth investigation of the treatment of SM is not considered part of the scope of this thesis but an algorithm (Fig 1.4) is provided courtesy of Dr C Rusbridge.





Essentially there are two forms of treatment of SM; i) medical, focusing on reducing inflammation and CSF production and the management of neuropathic pain, ii) surgical, craniocervical decompression is similar to that performed in human patients to alleviate clinical signs. Following surgery 80% of dogs improved [78] but long term improvement is less successful [55]. There are several different opinions in the veterinary profession as to the best approach taken to treat the multifocal condition of CM/SM which varies with their personal expertise. Most importantly is including differential diagnosis such as dermatological diseases and other spinal diseases such as intervertebral disc disease.

Grading and evaluation of canine SM

In the USA, Cerda-Gonzalez and Olby have graded the severity of SM as a percentage of the spinal cord affected and direct comparisons between studies with UK and USA should take account of this (Table 1.5). This scheme is similar to the Rusbridge 2004 phenotype evaluation which was used in setting up a worldwide DNA collection for CKCS in conjunction with Mitral Valve Disease and Epilepsy [79]. In addition to stereotypical clinical signs of SM and any MRI results, the phenotype form

required pedigree with a view to genotyping, linkage analysis and positional gene cloning planned at the time (Fig. 1.5).

Table 1.5 Grading criteria for SM according to Cerda-Gonzalez and Olby

Grading criteria for SM (USA)					
Grade	% of spinal cord				
0	none				
1	<33%				
2	33-60%				
3	>60%				

Fig. 1.5 Phenotype form used for the collection of DNA in UK.

Syringomyelia - Mitral	Valve dise	ease - Epilepsy			
Phenotype Form Send to: Dr Clare Rusbridge- Confidential Fax: (011-44) 208 786-0525 or email: <u>neuro.vet@btinternet.com</u>					
Pedigree Name:					
Date of birth: Case No		<u>Owner's nar</u>	me		
Color B B/T R T <u>Gender</u> M MN	F FN	Vet name/practice (prac	ctice stamp)		
Sire's pedigree name					
Dam's pedigree name					
Date of Sampling -		Referring Clinician			
Syringomyelia (please tick appropriate	e box)				
No clinical signs 🗆	Aae	of onset (if appropriate) –	yrsmths		
Shoulder scratching 🗆 🛛 Neck pain 🗆	Scoliosis 🗆	Peivic limb ataxia 🗆	Thoracic limb weakness 🗆		
Mas MRI carried out Yes □ No	□ Wass	urgery carried out ? Yes	D No D Dateyrsmth:		
Occipital hypoplasia 🛛 Yes 🗆	No 🗆				
Cerebellar herniation Yes 🗆 Syringomyelia Yes 🗆 No 🗆	No 🗆		<1/3 diameter spinal cord		
Area of spinal cord affe	cted	D'	1/3-2/3 diameter spinal cord > 2/3 diameter spinal cord		
Medullary kinking Yes 🗆			sts notes attached		
Details of any affected relatives	NO LI	Enterleidig.			
			-		
vlitral Valve Disease (please fill in <i>i</i> tic	к appropriate	e box)			
Grade of murmur /6 Age of last heart	clearance	_yrsmthsAgem	nurmur first diagnosedyrsmths		
Grade of heart disease Are diseased		the Examined by DBoar	d Cart, Cardialogict ⊡Caparal Practitionar		
⊐Normal (0) no murmur					
□Mild (1) Evidence of heart disease (i.e □Moderate (2) Ac 1 n/ue mild counts and (or	. murmur) but no avidance of lo ft	o clinical signs (heart normal atrial on largement on wrav/or	size on x-ray/ scan and no pulmonary oedema; an: no pulmonary podomo		
□ Moderate (2) As r pras minor cough and r or □ Severe (3) As 2 but has needed or still re	quires frusemid	e for pulmonary oedema (hea	an, no pannonary occena. In failure). Has or had clinical signs of coughing		
breathlessness at exercise, so Verv severe (4) On multiple drugs to control o	ime exercise int linical signs of h	tolerance eart failure: unable to exemis			
			1950.0		
Heart medication currently receiving □Cardiologists notes / ultrasound scan resul	ts attached				
Details of any affected relatives					
Drimony Epiloney[]	Enicodic Folli	na 🗆 Compulsius	dicardar □ (a.a. fly catching)		
≤nmary ⊏priepsy⊡ Age diagnosed above –	Episodic Falli Med	ication received -	alsoraer 🗆 (e.g. ny-catorning)		
Other (please specify) -					
applicable: Age at death vrs mth	Cause of death	n an	e previous phenotype form yrs inth:		

SAVE THIS FORM FOR FUTURE HEALTH UPDATES

The British Veterinary Association define canine SM as a fluid-filled cavity that includes, or is distinct from, the central canal of the spinal cord and is graded according to its maximum internal diameter in a transverse plane.

- Grade 0, normal.
- Grade 1, central canal dilation which has an internal diameter of less than 2mm.
- **Grade 2**, SM (central canal dilation which has an internal diameter of 2mm or greater), separate syrinx, or pre-syrinx with or without central canal dilation. This grade is deemed 'affected'.

The late onset nature of the condition is taken into account by qualifying the grade with a letter indicating the age group at the time of scanning as follows: a = more than five years of age; b = three to five years of age; c = one to three years of age. The grade is not valid without the qualifying letter [80]. A **pre-syrinx** is defined as spinal cord oedema, and may be a transitional state prior to development of SM. It has the appearance of high signal intensity on T2-weighted (T2W) images consistent with marked increased fluid content within the spinal cord substance but not of free fluid. On T1 weighted images (T1W), the spinal cord is either normal or has a slightly hypointense signal.

Pathogenesis

Early theories of the pathogenesis of SM in humans were postulated from the belief that the CSF entered into the spinal cord via the fourth ventricle into the central canal or the spinal subarachnoid space via the perivascular spaces and that the syrinx fluid originates from the subarachnoid CSF. These hydrodynamic theories proposed by Chiari and Ollivier D' Angers were based on SM as being a developmental disorder [58]. A blockage such as cerebellar herniation in CM-I [81,82] or another obstruction at the foramen magnum as the driving force for syrinx formation could act a piston in conjunction with the cardiac cycle [83]. However, the subarachnoid CSF origin theory was not based on direct evidences and assumed that the perivascular spaces were the channels used to transfer CSF from the subarachnoid space into the cord [84] and does not explain why the syrinx is at a higher pressure than the subarachnoid space.

Recent articles in 2000s proposed that the syrinx fluid derived from the extracellular fluid from the spinal cord microcirculation, not from the CSF in the subarachnoid space or the fourth ventricles [18,85]. Greitz proposed an intramedullary pulse pressure theory which suggested the extra-cellular fluid within the syrinx originates from the high pressure system in the spinal cord microvasculature. High pressure CSF in the spinal cord generated during systole from an obstruction of the subarachnoid space such as a cerebellar herniation and a syrinx forms as a result of the differential low pressure caudal to the obstruction [85].

These studies did not show new clinical evidences but provided novel insights into the pathogenesis of syringomyelia. The idea that the syrinx fluid originates from the extracellular fluid may explain the pathophysiology of syrinx formation in adhesive spinal arachnoiditis but it is still difficult to explain effectively the mechanism in CM-I.

With the advent of modern visualization technologies pioneered in human medicine, phase contrast cine MRI has been used to investigate the movement of CSF flow at the level of the foramen magnum in the dog [19]. In a study using steady-state acquisition cine MRI, Driver *et al.* [86] investigated the relationship of the cerebellar pulsation to the presence and severity of SM using three groups of small breed dogs with and without CM but not SM and CKCS with CM/SM. The results showed that the CKCS with CM/SM has a significantly greater cerebellar pulsation during the cardiac cycle than control dogs or CKCS with CM only.

Although MRI studies demonstrated blockade and alternated CSF dynamics at the foramen magnum, they are unable to show direct communication of the syrinx with the CSF spaces. Recent imaging studies suggest that the extracellular fluid accumulation may play an important role and it is suggested that reduced compliance of the posterior spinal veins associated with the decreased compliance of the spinal subarachnoid space might result in disturbed absorption of the extracellular fluid through the intramedullary venous channels and formation of syringomyelia [87].

Morphometric measurements associated with pathogenesis

In a decade following the International Working Group [15] considerable research has been undertaken worldwide to understand the relationship/s between CM and SM in an attempt to elucidate the pathogenesis of SM in the dog. Attention has focused on secondary SM rather than CM.

Morphometric measurements include the use of either a) volume, or b) linear dimensions and angles to study the following anatomical features:

- 1. Cranial caudal fossa [51,60,88,89]. It was concluded that_deduced caudal fossa with SM dogs.
- 2. Cerebellum
 - a. volume [37] It was concluded that increased volume with CM/SM CKCS.
 - b. linear (Lu *et al.*, 2003; Rusbridge & Knowler, 2006 Cerda-Gonzalez, Olby & Griffith, 2015c;) It was concluded that cerebellum abnormal in dogs with CM/SM.
 - c. deformity [17] It was concluded that there was no correlation between severity of herniation and neurological signs.
- **3.** Disparity of brain parenchyma with the caudal cranial fossa [88,91,93,94]. It was concluded that there was a mismatch or rearrangement of neural parenchyma with SM dogs.

- **4. Association with craniocervical junction** [38,90,95–97]. It was concluded that CJA did not predict development or worsening clinical signs or SM but may contribute to them.
- 5. Progression over time/age of onset. In order to assess the progression of the disease such measurements have either been repeated with the same dogs over time or age matched studies [56,64,98] It was concluded that the age of onset affects severity, affected young dogs have more severe symptoms. Syringes may enlarge over a period of time.
- 6. Influence of head positioning [99] It was concluded that there was no difference in volumetric measurement with head position but there was a significant difference between the cerebellar herniation and CSF space between the cerebellum and brainstem which was larger in the flexed position. CKCS differ in their morphology in the craniocervical junction from other breeds, these differences do not account for why some CKCS develop SM and others do not.
- 7. Association with cranial blood supply
 - a. **venous volume** [100] It was concluded that there was reduced sinus volume in CKCS with CM/SM compared to dogs with CM only.
 - b. **jugular foramen** [44,101]. It was concluded that a reduced skull base with narrowing of the jugular foramina can increase cranial venous pressure and impair CSF absorption.

8. Association with ventricular circulation

- a. **CSF flow**[19]. It was concluded that the differences in CSF velocity at C2-C3 and foramen magnum associated with SM.
- b. **cerebellar pulsation** [86]. It was concluded that abnormal cerebellar pulsation could lead to a mismatch in the timing of arterial and CSF pulse waves predisposing to SM.

9. Association with pain

- **a. Syrinx dimensions**: [77,102]. It was concluded that maximum syrinx width was the strongest predictor of pain, scratching behaviour and scoliosis in dogs with syringomyelia. Both pain and syrinx size were positively correlated with syrinxes located in the dorsal half of the spinal cord.
- b. Craniocervical abnormalities: Olby and Cerda-Gonzalez *et al* investigating the impact of SM in the CKCS in association with atlantooccipital overlapping (AOO) [73], atlantoaxial bands [95], medullary elevation and morphometric idex [90] failed to identify any link with pain [98].

Studies of CM not in association with SM

Natural anatomical variation in breeds with respect to CM has been quantified in two studies.

Studies in the USA, Dewey and Marino define CM (referred to as CLM) as an abnormally shaped supraoccipital bone which results in rostrally directed compression of the caudal aspect of the cerebellum [38]. They proposed that CM was part of a wider spectrum of craniocervical junction abnormalities (CJA) without reference to SM. In a morphometric study of 216 CKCS and 58 non CKCS dogs, they evaluated compression index values associated with the presence of CM and other craniocervical junction abnormalities using both MRI and Computed tomography (CT). They defined CJA as a general term for CM, atlantooccipital subluxation, atlantoaxial instability, occipitoatlantoaxial malformation, atlanto occipital overlapping (AOO), and dens abnormalities that all occur in the craniocervical region of small-breed dogs. They describe these common CJAs as analogous to Chiari type I malformation in humans called as basilar invagination. This anomaly is a rostrally displaced C1 dorsal arch that can cause cerebellar compression and AOO and is similar condition in dogs. They define CM 'to denote disorders causing neural tissue constriction at the cervicomedullary junction'. Dogs were assessed for cerebellar compression (CC), medullary kinking (ventral spinal cord compression at the C1–C2 articulation), and **dorsal compression** (dorsal spinal cord compression at the C1–C2 articulation). A compression index was calculated for each of these 3 locations in each dog. No reference was made to cerebellar herniation and no account was taken of the presence or absence of SM.

All 274 dogs had CC; medullary kinking was identified in 187 (68.2%) and dorsal compression was identified in 104 (38.0%). Atlanto-occipital overlapping was identified in 76 (27.7%) dogs. Breed of dog (CKCS vs non-CKCS) and value of cerebellar compression index were the only significant predictors of atlanto-occipital overlapping. The CKCSs had an almost 5-fold decrease in risk of atlanto-occipital overlapping nearly doubled for every 10% increase in CC index [38].

In the UK, Harcourt-Brown *et al.* investigated the prevalence of CM in 199 clinically unaffected dogs [43]. Using angular and linear morphometric analysis which took account of the degree of brachycephaly (cranial index) and head position, they investigated evidence of cerebellar indentation and impaction into or through the foramen magnum. They found that in 185 non-Cavalier King Charles Spaniel (CKCS) dogs, indentation occurred in 44% (95% CI, 47-51%) and impaction was identified in 22% (95% CI, 16-28%). Since no asymptomatic, non-CKCS dogs showed herniation they concluded that the high prevalence of cerebellar indentation and impaction may be normal anatomical variations and therefore unsuitable as definitions of CM.

Further attempts to provide standardised morphometric measurements on MRI for traits associated with SM have been provided by Cerda-Gonzalez *et al* with respect to the atlas position and atlantooccipital

overlapping [73]. Four standardised measurements which assessed the proximity of the atlas to the foramen magnum on T1-weighted mid sagittal MRI on 271 dogs <15kg were found to be reliable. Brachycephalic dogs had more cranially and dorsally positioned atlas bones compared to other skull types and decreased bodyweight. This study provides evidence that miniaturization is a risk factor for cranio-cervical abnormalities associated with SM.

Brachycephaly and miniaturization risk factors for secondary syringomyelia

It is not surprising that any incongruities between the capacity of the skull and brain size is considered a disorder associated with brachycephaly and miniaturization. However, the extent to which these two risk factors contribute to the morphological abnormalities associated with CM and secondary SM has not been fully investigated. Toy breed dogs that have been reported with SM-affected secondary to CM include Cavalier King Charles spaniels (CKCS), King Charles spaniels, Griffon Bruxellois, Affenpinschers, Yorkshire terriers, French bulldogs, Havanese, Chihuahuas, Pomeranians, Boston terriers, Maltese terriers, Yorkshire terrier, Papillons, miniature Dachshunds, Shih Tzu, Bichon Frisé, Staffordshire bull terrier, and several cross breeds [14,44,48,53] Although some of these breeds are recognised in the dog world as brachycephalic because of their obviously shortened craniofacial bones but there is considerable variation in skull shape within breeds as is body weight. Mesaticephalic Poodles were used in a control group in one study investigating skull shape of the CKCS with and without SM [103] but a Poodle was one of three case studies with CM/SM [48]. The influence of body weight has been shown to a consideration in any investigation where there are breed variations in neural parenchyma associated with CM/SM [104]. Body size in small dogs compared to larger types has been shown to be correlated to a single genetic variant *IGF*. This single-nucleotide polymorphism haplotype is common to all small breeds and nearly absent from giant breeds, suggesting that the same causal sequence variant is a major contributor to body size in all small dogs [105]. In the dog, brain to body size ratio (encephalisation) has decreased with domestication compared to farm animals [106]. Additionally, as the adult bodyweight decreases the brain size becomes relatively larger [107]. The dog genome project showed that the tight selection by breeders has resulted in more diversity between breeds than within breeds [108] and there is some evidence that a simple genetic architecture underlies morphological variation in dogs[109].

Fig. 1.7 illustrates the morphological diversity of *Canis familiaris* in four dogs. A and B are over 100 years old skulls exhibited in the Huntarian Natural History Museum, Glasgow University.

Dog A, labelled 'lap dog' considered to be a Chihuahua, B is a Bulldog. Note the angle of the hard palate and nasal bones? relative to the cranial base in dogs A, B and C a CKCS skull. This dorsal retroflection of the jaw, known as airorhynchy, is a feature of the brachycephalic breeds [108] and absent in the mesaticephalic skull of German Shepherd (D). Certain skull characteristics, such as the
angle between the hard palate and the neurocranium, appear static during wolf development, whereas in domestic dogs the angle differs substantially from that of wolves throughout development. This has led to the conclusion that dog skull shape is neomorphic by some investigators [110].



Fig. 1.7 Comparison of four canine skulls to illustrate brachycephalic

A Lapdog (miniaturised Toy breed) B. Bulldog(brachycephalic large breed) C. Cavalier King Charles Spaniel (brachycephalic Toy Breed). D. German Shepherd (Mesaticephalic large breed). Note the dorsal retroflection of the muzzle (airorhynchy) in A-C and the hypogenesis of the occipital bones in C, characteristic of CM, compared to A,B and D.

(A same scale as B, skulls courtesy of Huntarian Museum, Glasgow University; C and D not the same scale, skulls courtsey of Dr C Rusbridge).

The genetics involved in brachycephaly is complex and considered in more detail later in this chapter. Relative to the grey wolf, different forms of brachycephalic groups have been identified in dogs but even these have evolved considerably in the last decade. Fifty years ago the CKCS was not considered a brachycephalic dog but it is now [103]. Defective growth of the basioccipital and basisphenoid bones results in shortening of the basicranial axis. This affects the growth patterns of the normal brain and results in compensatory changes in the cranial vault [111]. In the rostral to caudal shortening in the brachycephalic dog, the skull becomes rounded dorsally and rostrally and the dog has a comparatively broad head. Consequently the normal Griffon Bruxellois skull is wider than it is long, in comparison to the German Shepherd which is often twice as long as it is wide [112]. However why do some brachycephalic dogs have SM and others do not? If there is a strong association between CM/SM and brachycephaly, then one would expect to see cases in the larger brachycephalic breeds such as Boxers and English Bulldogs for which there are no known cases to the author.

There is some confusion with the use of the term 'brachycephaly' which stems from the Greek roots meaning 'short' and 'head' and first coined by a Swiss human anthropologist A. Retzius who classified ancient human skull found in Europe. The cephalic or cranial index is the maximum width of the skull

multiplied by 100 divided by the maximum length of the cranium and provides a standard for fetal head shape. However in the dog world, brachycephaly is synonymous with foreshortening of the facial bones rather than the cranium. In dogs the length of the skull includes the muzzle and termed craniofacial index [113]. Schmidt *et al* investigating the skull type of the CKCS describes the characteristics of brachycephaly as having a shortened face, rostrally elevated palate, an arched zygomatic apophysis, forward facing eyes, maxillary hypogenesis, aberrant conchal growth and dorsally rotated teeth [114]. The vast majority of welfare concerns for the brachycephalic breeds are concerned with respiratory disorders, particularly BOAS (brachycephalic obstructive airway syndrome) not CM/SM.

Embryology

Introduction

CM/SM is considered a developmental disorder - i.e. an aberrant event occurring during embryology that impacts on later growth. An understanding of the normal development of the brain and skull, together with the cervical junction, is fundamental to identifying any deviations associated with CM and secondary SM that may arise. The mammalian brain and skull develop concurrently, in a coordinated manner and normal development of the embryo is dependent on the three factors:

- 1. Genotype: genetic programme inherited from parents and its successful execution.
- 2. Epigenetic: environmental factors which influence genetic determination.
- 3. Physical: forces which act through space-occupying cavities and organs, muscle action, hydraulic pressures, etc.

An irregularity in one or more of these aspects may contribute to the range of anomalies associated with CM/SM.

Craniofacial Morphogenesis/Development relevant to canine CM/ SM

There exists enormous diversity in cranial morphology with respect to skeletal size and proportion between wild and domestic canids [115], indeed in the entire canid family it is greater than in any other mammalian species. Such diversity originates from the set of DNA instructions in the developing zygote that determines the phenotype. Translating these instructions through cell differentiation and morphogenesis is performed an orderly manner. This is achieved by transcription factors that regulate the identity and patterning of embryonic tissues which ensure that genes are turned on and off at critical times so that cell activity, migratory patterns and metabolic states are highly ordered [116,117]. An understanding of these integrated mechanisms has been revealed by studies in model vertebrate organisms such as the zebrafish, frog, chicken and mouse [118–121]. Transcription factors that regulate the expression of the cells of embryonic tissues are underpinned by intracellular communication in a process known as signal transduction [116,117,122,123]. In any multifactorial condition such as

CM/SM, which involves the development of the brain and spinal cord, it is therefore reasonable that irregular transcription processes may be involved.

Signal transduction

Genes encode the cytoplasmic signalling pathways that regulate the behaviour of surrounding cells by producing positive and negative intercellular signalling molecules. The genetically conservative **homeobox genes** (a large family of similar genes that direct the formation of many body structures during early embryonic development) switch on cascades of other genes using transcription factors. Part of the homeobox tool kit is the HOX and PAX genes that encode nuclear transcription factors for rostrocaudal and dorsoventral patterning and cranio facial development and are highly conserved in vertebrate morphology[124–126]. Sox genes are relevant to CM/SM in that subgroups of the genes are involved with central nervous system neurogenesis, oligodendrocyte development, chondrogenesis, and neural crest cell development [127–129]. Examples of regulatory growth factors include brain derived neural growth factor (BDNF) and transforming growth factor TGF α and TGF β which are derived from a variety of local organs. Organizing centers, called signalling centers or nodes, are created which regulate the behaviour of the surrounding cells by producing a variety of extracellular matrix proteins, cell adhesion molecules and cytoplasmic signalling pathway components [130,131]

Early Development the Central Nervous System (CNS)

Neurulation

The earliest research into neurulation was undertaken by Professor of Anatomy, Wilhem His in 1874 and centered on physical analogues [132]. However, with the advent of electron microscopy and the revelations of subcellular structures such as cytoskeletal elements and intracellular vesicles, it became possible to choreograph the changes in cell shape changes in the developing neurula [133–135]. Current research into neurulation now focuses on both the physical mechanisms and molecular changes i.e. epigenetics. An excellent review of how molecular and physical mechanisms are integrated during neurulation has been provided by Vijayraghaven and Davidson [136].

The invaginated neuroepithelium (epithelial cell sheet) forms the neural tube and canal during gastrulation in a process called primary neurulation [137]. In contrast, secondary neurulation, which occurs at the caudal level, is not achieved by invagination but involves the mesenchymal cells undergoing epithelialization and neural lumen formation within a solid cylindrical mass of cells (tubulogenesis). This creates the neural tube at the lowest portion of the spinal cord called the tail bud [138,139].

The formation of the neural plate is described as **neural induction** whereby the dorsal midline ectoderm is differentiated into the neuroepithelium [140]. Neural induction occurs when the action of bone

morphogenetic proteins (BMPs) is prevented by antagonists (chordin, noggin and follistatin) released from the primitive node, and allows the ectoderm to form neuroectoderm [121,141]. The nodal family of proteins, a subset of the transforming growth factor beta (TGFβ) superfamily, is responsible for mesoderm induction, patterning of the nervous system, and determination of dorsal- ventral axis in vertebrate embryos. FGFs (Fibroblast growth factors) and WNTs (signaling pathway first identified in wingless type *drosophila*) are involved in maintaining the mesodermal state, and BMPs are involved in process is called 'patterning' the mesoderm [142,143]. This establishes genetically programmed subsets of cells in proper relation to each other and to surrounding tissues [117,141].

Development the Central Nervous System (CNS)

Role of the Notochord

The mesoderm cells of the vertebrate gastrula which migrate along the midline defining the embryo axis give rise to the notochord [117]. The notochord plays a key role as an embryonic regulator controlling the patterning and proliferation of a wide variety of organs. It is the source of the 'Hedgehog' signalling pathway. Mammals have three Hedgehog homologues, Desert (DHH), Indian (IHH), and Sonic (SHH), latter named after a computer game common in appearance [144]. It is the Sonic hedgehog which initiates the next stage in neural development known as 'neural induction'[123,145,146]. Studies in the Zebrafish and *Xenopus* have shown that the notochord coordinates the development of all three body axes [147,148]. Although the notochord plays an inductive role in neurulation, the actual process of 'bending' of the neural plate to form a tube is due to structural changes within the cells of the neuroepithelial cells. The lengthening and narrowing of this field of cells, called **convergent extension** and illustrated in Fig 1.8. Structural changes within the cells of the neuroepithelial cells is a well-conserved signalling pathway that underlies this and other morphogenetic processes (adapted from Keller et al [134,149].

Fig 1.8 Schematic illustration of the cellular morphogenetic process that occurs in both gastrulation and primary neurulation called 'Convergence Extension'.



Following adhesion and fusion, the neural fold apices remodel to create two continuous epithelial layers: the surface ectoderm on the outside and the inner neural tube [135,150]. The HOX genes are responsible for organising neural folding and the changes in shape are illustrated in Fig. 1.9. After adhesion and fusion, the neural fold apices remodel to create two continuous epithelial layers: the surface ectoderm on the outside and the inner neural tube [135,150]. Apoptotic processes (cell death) are important during neural tube closure which is not merely the proliferation of cells meeting in the midline [136,151]. Computational models of neurulation have been used to test the theories of force generation involved in cell and tissue morphogenesis [136].

Fig 1.9 An illustration of convergent extension during the process of neural induction



Sequence of shape change: Neural induction under the influence of the

notochord a) neural plate bends at a median hinge

point (MHP and is elevated to forma neural fold.

b) paired dorsolateral hinge points (DLHPs) at the lateral sides of the folds and predominantly at the future brain levels)

c) midline apposition and fusion of the edges with remodelling to separate neuroectoderm of the neural tube and surface ectoderm

Adhesion, fusion and remodelling of the midline (c)

Rostro-caudal sequence of neurulation

Closure of the neural tube is not like a zipper action starting at one end but from three or more points depending on the species. In the mouse, the first neural tube closure is initiated at the hindbrain/cervical boundary (6 somite stage) and spreads both rostrally and caudally from this site [135,150]. This is illustrated in Fig 1.10 below. The second closure event occurs around the forebrain/midbrain boundary and a third closure initiates independently at the rostral extremity of the forebrain and the spread of the closure spreads caudally to fuse with rostral closure (closure 2) at the anterior neuropore [151]. A partial failure in closure 1 (craniorachischisis) in the lumbar or lumbosacral region results in Spina Bifida. Failures at other sites (exencephaly) are fatal.

Fig 1.10 Diagram of three closure points in the mouse embryo



The cranial end of the notochord is the stomodeum where an ectodermal evagination forms the hypophyseal (Rathke's) pouch which gives rise to the anterior lobe of the pituitary [152] where it continues to play an important controlling endocrine mechanism in the adult.

Neural crest cells

An important player in the development of both the CNS and the skull are the neural crest cells generated in primary induction from the crests of the neural fold [113,117,145,153]. The neural crest is formed from ectomesenchymal tissue i.e. involving ectoderm cells and not just mesoderm which is the usual origin of mesenchyme (mesenchymal connective tissue) [154]. Mesenchymal cells have the ability to migrate easily, whereas the epithelial cells do not show much mobility [148,155]. At the neural crest, neuralizing and epidermalizing influences interact by means of WNT activation and BMP inhibition (e.g. Noggin) [145]. The outcome are cells that possess outstanding multipotency that contribute not only to the peripheral nervous system but also to the ectomesenchymal precursors of the cranial skeleton [145,156]. Translocated neural crest cells undergo cytodifferentiation upon reaching their predetermined destinations. The diversity of cell types are partly genetically determined but also specified by local environmental influences. Indeed, the influence of the neural crest cells is seen as fundamental to the origin of vertebrates in the 'New Head' theory expounded by Gans and Northcutt and others [117,153,157]. Vertebrate species exhibit different patterns of neural crest cell emigration. Mammals differ from birds in that their neural crest cells emigrate from the 'crest' of the neural fold whereas bird NCC arise after the closure of the neural tube [158].

Neural crest cells are present in the early somite embryos from the optic primordia throughout the brain. They divide as they migrate providing the major source of connective tissue components, including cartilage, bone and ligaments of the facial and oral regions. This active phenomenon has been demonstrated Japanese quail *in vitro* studies [159].

Cranial neural crest cells segments into regions adjacent to the brain. Differentiation of these cells results in the anlagen of cranial nerve ganglia [156,160]. Paraxial and splanchnic mesoderm populate the developing branchial arches and eventually give rise to the skeletal elements (membranous and endochondral skull and facial bones) connective tissue (cartilage, bone and ligaments), and musculature of the vertebrate head. The trunk neural crest cells, dorsolateral to the neural tube, condense to form ganglion of the autonomic nervous system and sensory nerves as the spinal cord. Trunk neural crest cells do not contribute to the skeletal or connective tissue which is derived from paraxial mesoderm (somites). This is summarized in the Table 1.6 below modified from the excellent review article written by F. Santagati and F. Rijli [161].

Main derivatives of neural crest cells (selected)							
		Cranial crest	Trunk crest				
	Sensory nervous system	ganglia of cranial nerves	spinal ganglia				
		enteric nervous system	part of enteric nervous system				
Nouronal colls		parasympathetic ganglia: ciliary,	parasympathetic ganglia: pelvic				
ineuronal cens	Autonomic nervous system	pterygopalatine, otic and	plexus				
		submandibula					
			Sympathetic ganglia				
Non-neuronal cells	skolotal colls	face and skull bones and visceral	none				
	SKEIELAI CEIIS	cartilages					
		meningies of prosencephalon and					
		part of the mesencephalon	none				
	Connective tissue	dental papilla					
		walls of aortic and arch derivied ar	none				
		skin; smooth muscle, dermis,	none				

Table	1.6	Main	derivatives	of neural	l crest cell	s (selected)

Development of the spinal cord

The development of the axial skeleton is concurrent with the development of the CNS. The neural tube is surrounded dorsally by ectoderm, dorsolaterally by paraxial mesoderm and neural crest cells and ventrally by the notochord. Fig 1.11 represents a schematic illustration of the proliferation of neuroblasts in the neural tube into zones that become the grey cells of the cord with a shallow groove, called the sulcus limitans. This separates the dorsal alar plate (primordium of the dorsal horn) and ventral basal plate (primordium of the ventral horn). The development of the sulcus limitans, alar and basal plates extends from the mesencephalon (midbrain) along the full length of the spinal cord [117]

Paraxial mesoderm (presomitic or somitic mesoderm) forms simultaneously with the neural tube. Paraxial mesoderm lateral to the developing brain (cephalic paraxial mesoderm) consists of seven **somitomeres,** as revealed by scanning micrographs of the neurula stage in the avian [117] and human [162] embryo. The paraxial mesoderm lateral to the notochord condenses to form somites which differentiate into sclerotome precursor for bone, myotome precursor for muscle and dermatome precursor for dermis.



Fig 1.11 Diagrammatic section through the emergent spinal cord with the development of the sulcus limitans and ganglia

The neural ectoderm or mantle layer (grey) seperates at the sulcus limitans and differentiates into the alar plate (primordium of the dorsal horn) and basal plate (primordium of the ventral horn). The central canal is lined by ependymal epithelium. Neural crest cells (green) migrate ventrally and through apoptotic processes condense to form ganglia. Ectoderm is coloured yellow and paraxial mesoderm red.

Fate Mapping

Animal models have played a pivotal role in understanding the genetic mechanisms and pathways involved in some disease processes, particularly through the investigation of mutant phenotypes in mice [120,163] and with quail-chick chimeras [158]. Modern techniques of cell marking and identification methods, known as fate mapping, are providing detailed accounts of cell migrations and tissue lineages by tracking mesenchymal populations in the embryo. The combination of cellular and molecular advances in genetics studies of vertebrate craniofacial development during the gastrula, neurula, and early organogenesis makes it possible to track the origins of specific tissues in detail. An example of this is using a transgenic mouse with a permanent neural crest cell lineage marker, Wnt1-Cre/R26R, [124,164–166]. Morriss-Kay *et al*, examined the tissue origins of the mouse cranial base using this marker, and a mesoderm lineage cell marker, Mesp1-Cre/R26R.[167]. Extensive fate mapping studies show that neural crest cells and muscle progenitor cells are more extensively mixed than previously believed during development [168]. Interactions between muscles and tendons during craniofacial development are similar to those observed in the limb, despite the distinct embryological origin of these cell types in the head [169].

The Formation of the Brain

The initial segmentation of the rostral ectodermal tube is expressed as the forebrain (prosencephalon), midbrain (mesencephalon), hindbrain (rhombencephalon) and spinal cord, with the hindbrain further segmented into rhombomeres. [117,156,170,171]. See Fig 1.12 below which has been adapted from a figure in Fundamentals of Canine Neuroanatomy and Neurophysiology (Wiley Blackwell 2015) [172]. Secondary divisions of prosencephalon and rhombencephalon form the typical five vesicle brain with cavities in the cranial tube that become ventricles in the mature brain [113,156]; The alar and basal plates of the prosencephalon form the telencephalon and diencephalon, respectively.

Fig 1.12 Schematic diagram of the differentiation of vesicles in the developing brain.



Three primary flexures appear in this rostral part of the neural tube. Two of these convex dorsally; the **cephalic flexure** at midbrain level (10 somite stage) and the **cervical flexure** at the junction of the hindbrain and the spinal cord (20 somite stage). The intervening **pontine flexure** concaves dorsally (32 somite stage). This is illustrated in Fig 1.13.





There are two organising centers involved in inductive signaling in head organisation, the frontal brain (prosencephalon) directing the nose, eyes and rostral skull base and the caudal skull base influenced by the rhombic brain (rhombencephalon). The existence of two skull formation centers is substantiated by cyclopic and otocephalic malformation patterns [173].

The development of the brain and sense organs in the neurulating embryo results in the differential disappearance of the mesodermal cranial somites. Rhombomeres, the eight distinct segments of the neural tube, are distal to the cephalic flexure and particularly sensitive to Hox genes. They are programmed to form only one part of the hindbrain in a very precise manner and each part develops its own set of ganglia and nerves. The cranial base is formed from the segmented paraxial mesoderm (somitomeres) [174].

Development of the cerebellum

The cerebellum starts as a proliferation of the metencephalic alar plate and bilateral dorsal outgrowths called rhombic lips expand medially through the roof plate of the fourth ventricle fusing in the midline. As the pontine flexure deepens the enlarged metencephalon becomes folded against the dorsal laminar of the medulla and developing choroid plexus [117] Neurepithelial cells in the rhombic lips develop along several lines but unique to the cerebellum is the densely populated external germinal layer that makes up the cortex and incorporates the Purkinje neurons. In the dog the greatest development of this layer is seven days postnatally and attributed to being able to stand and walk in a coordinated manner. It is perhaps pertinent to note this later growth of the cerebellum in the context of its deformation in CM.

Development of the Ventricular System

The development of the ventricular system is in dynamic harmony with all the evolving arrangements of differentiating tissues in the cephalic region. The initial shape of the five ventricles (telencephalon, diencephalon, mesencephalon, metacephalon and myelencephalon) is determined by the primary and secondary brain flexures [113,117]. The disproportionate expansion of the cerebral hemispheres alters the configuration of the lateral ventricles which narrow and become c-shaped. These flexures also create specific communicating channels; the narrow interventricular foramina (Monroe) which connect to the lateral ventricle, the cerebral aquaduct (Sylvius) from third to fourth ventricle and the median aperture (Magendie) from fourth to the subarachnoid space via the cisterna magna of primates but not in dogs and cats See Fig 1.14 below. The left and right lateral apertures open to the subarachnoid space via the quadrigeminal cistern.[175].

The ependymal cells lining the ventricles, continuous with that of the central canal of spinal cord, contain the CSF. It is thought the hydrostatic tension within the ventricles acts like a scaffold for the developing parenchyma and that the pontine flexure is a result of buckling from forces.[16,176]. Recent

research has demonstrated that there is a linear relationship between CSF volume and body weight in dogs



Fig. 1.14 Ventricular system of the dog brain

Red arrows movement of filtrate from the venous system into the ventricles at the choroid plexus; Aqua arrows movement of CSF through the ventricles to the subarachnoid space.

Choroid plexus

The choroid plexus develops as outgrowths from the medial wall of each telencephalon but continue as paired plexus in the roof of the diencephalon, through the interventricular foramen of the third ventricle into the fourth ventricle. Here they lie each side of the median line until turn laterally at the caudal end of the cerebellar peduncle to enter the lateral aperture [113,117,175]. The ependymal cells that line the vesicles become modified with microvilli and are sealed together to prevent water soluble molecules entering the CSF (tanycytes) and become choroid epithelial cells [177]. This single layer of cells adheres to the highly vascularised pia mater to form the choroid plexus which provides an extensive increased surface area for metabolite exchange forms a blood/CSF interface. The combined tissue, called the tela choroidea, produces CSF by secretion and ultrafiltation [178]. The largest subarachnoid space (cisterna) is the where the caudal surface of the cerebellum meets the dorsal surface of the medulla oblongata known as the cisterna cerebellomedullaris or cisterna magna. The arrangement is illustrated in the Fig 1.15 below [172]. The choroid plexus plays a major part in regulating neuroparenchymal homeostasis. CSF leaves the ventricular system via small lateral apertures of the fourth ventricle.



Fig. 1.15 Diagrammatic transverse section through the developing medulla oblongata and fourth ventricle.

Diagram adapted from those in Fundamentals of Canine Neuroanatomy and Neurophysiology by E. Uemura.

As the pontine flexure develops, the cells of the metencephalon and caudal myelencephalon migrate ventrally and laterally to form the roof of the fourth ventricle (medullary velum) and this highly vascular pia mater invaginates as the choroid plexus.

Development of the Skull and Craniocervical Junction

Introduction

The primary function of the skull is protection of the brain and sense organs but the facial region, consisting of 36 bones, accommodates feeding, respiration and communication. Despite its complexity, primary segmentation can be shown to underpin it's development and structure [165]. The box-like cranium of the dog is shaped for the muscle attachments, particularly of the jaw, with the development of the sagittal crest and occiput [113]. The number and complex shapes of the facial bones increase the surface area supporting respiratory and olfactory functions and the jaw for implantation of teeth [113]. Human intervention through dog breeding has selected anatomical variants of the canine skull and neck for a huge range of uses including hunting techniques, physical strength and agility for protection or merely for appearance. This is particularly true of features that are anthropomorphic such as large, forwardly directed eyes and snub noses.

Development of bone

Both mesoderm and neural crest cells contribute to undifferentiated mesenchyme that develop into the cartilage and bone in the head [118,166,179]. Ossification is an inductive process under the influence of adjacent tissues which act on the preosteogenic cells. There are two signalling pathways with different growth patterns **i**) **endochondral ossification** which has a hyaline precursor. The growth

pattern is three dimensional, slowly expanding from deep seated centre within a hyaline cartilage anlagen ii) **intramembranous ossification** which occurs in sheet-like osteogenic membranes but the final osseous tissue is identical to that of endochondral ossification. Both result in the formation of a matrix of osteoblasts and its calcification into bone. Sometimes both methods can participate in forming a single bone, effacing different origins [131,165].

Bone is a complex organ which retains a dynamic nature of plasticity. The cells communicate with each other through many signalling processes that necessitate locally generated growth factors and effector cytokines (non-antibody proteins released by one cell population on contact with specific antigen which act as intercellular mediators). Superimposed on some of these processes can be hormonal regulation [180].

During embryonic development, osteoblasts originate from local mesenchyme. In response to specific stimuli, these precursor cells commit to oseteogenic lineage and differentiate into mature osteoblasts. The distinct stages in this lineage and its genetic and epigenetic control have been extensively researched during the last 20 years using *in vitro* culture cells. The genetic and transcriptional control of bone formation has been reviewed by Javed *et al* [181].

The formation of bone by osteoblasts and the reabsorption of bone by osteoclasts is 'coupled' but remodelling is controlled by osteoblasts and influenced by mechanical stress via muscle attachment, nutrition such as vitamin D, calcitonin, parahormone and sex hormones.

Genetic and Transcriptional Control of Bone Formation

The cellular and molecular events leading to cartilage and bone formation have been extensively investigated using cell culture of developing embryonic bone of normal and genetically modified chick and mouse embryos [181,182]. Members of the bone morphogenetic protein (BMP) family induce bone formation at genetically determined sites called 'ossification centre' and, in controlling where skeletal precursors cleave or segment, these produce separate skeletal elements connected by joints [183,184]. Key factors regulating the gene expression series that bring about induction, proliferation, and maturation of oesteoblasts are immensely complex and interact osteoclasts in bone remodelling in a continuous process. Review: [181].

Vertebrate Skull

The dog skull, as other vertebrates, is a product of its evolutionary origins [185,186]. In her review of the mammalian skull, Morriss-Kay [187] provides an evolutionary context as a means of understanding its complex structure. She describes four components:

i. cartilaginous **neurocranium** a trough like skull base which has a primitive cartilaginous forerunner called the **chondrocranium** supporting the brain and sensory organs

- ii. cartilaginous **viscerocranium** (splanchnocranium or orognathofacial complex). These are modified supports for the gill arches of early aquatic vertebrates associated with feeding but also part of the senses. In later evolutionary vertebrates these features contributes to the jaws, hyroid and inner ear bones [113,186];
- iii. dermal dermatocranium or cranial vault (calvaria), the membrane formed bones which encases the telencephalon and the nose which originated from the bony head armour without a cartilaginous precursor
- iv. sclerotomal occipital region.

Fig.1.16 below, adapted from Morriss-Kay [187] is a schematic diagram of the skull components and their origins through vertebrate evolution. It is interesting to note the occipital region which is formed from sclerotome somites in a similar manner to the formation of vertebrae. Incorporation of the occipital vertebrae into the skull is supported by the annexation of the upper part of the spinal cord into the brain, together with the first 2 spinal nerves as cranial nerves X1 and X11 [188].

Fig. 1.16 Schematic diagram of the skull components and their origins through vertebrate evolution.



Dentition is derived phylogenetically from ectodermal placoid scales with ectoderm forming dental lamina and neural crest cells dental papilla [189] An evolutionary change in the dermal skull roof of both birds and mammals was the loss of lateral walls of the neurocranium thus enabling the brain to expand.

Chondrocranium

This is made up of the parachordal and trabecular cartilages which are neural crest in origin plus the branchial cartilages from paraxial mesoderm [113,167,189,190]. In an illustrated comparative study of the trabeculae cranii in the dogfish, trout, frog and salamander, de Beer demonstrated that the rostral development of the enlarged brain moved the mouth to lie ventrally and that these cartilaginous rods were modified branchial arches [191] and illustrated below (Fig. 1.17)





The parachordal and trabecular cartilages enlarge and fuse with olfactory, optic and otic capsules to form the trough-like chondrocranium which ossifies endochondrally ventral to the brain and combines later with the membrane formed bone of the desmocranium. The notochord remains the signalling centre for parachordal cartilages. McBratney-Owen *et al.* investigated the tissue origins of the mouse cranial base using both *Wnt1-Cre/R26R*, and a mesoderm lineage cell marker, *Mesp1-Cre/R26R*. They showed that neural crest cells contribute to all of the cartilages that form the ethmoid, presphenoid, and basisphenoid bones with the exception of the hypochiasmatic cartilages. The basioccipital bone and non-squamous parts of the temporal bones are mesoderm derived [167]. In the mouse, the prechordal-chordal boundary is between the basisphenoid and basioccipital bones [152]. In the dog Beagle foetus, several ossification centres arise in the cartilage of the chrondrocranium which mark the formation of the occipital and sphenoid complexes and the vormer and ethmoid bones [113]. Premature fusion of the basisphenoid and presphenoid (intersphenoidal synchondrosis) shortens the basicranial axis. However this allows the lower jaw to continue to grow unopposed rostrally and arch dorsally in brachycephalic dogs, as typical of such breeds as the bulldog and Griffon Bruxellois.

Fig. 1.18 below is a diagram adapted from Miller's Anatomy of a Dog [113]. Notice that that initial ossification of the supraoccipital and alisphenoid is 41 mm and the basioccipital is the last of the

occipitals to ossify at 57mm but the first for the cranial base (clivus). The median elements of the basicranium- the basiccipital and basisphenoid and paired ossifications for the presphenoids and ethmoid bones- fuse with their lateral component wing (obitosphenoids and alisphenoids to form the trough-like sphenoidal complex. However, fusion between these two sphenoids is never complete in the dog [113]. The Pituitary Gland develops directly above the hypophyseal cartilage (basisphenoid bone and spheno-occipital synchondrosis) and sits in the concavity of the sella turcica.

The cranial base is angled at the level of the hypophyseal fossa where the rostral prechordal and caudal chordal parts meet. In the early embryo this angle is very obtuse but by the time of ossification it should have flattened. Consequently inadequacy of cartilage growth will result in a short cranial base with increased angulation [192]. In humans, the clivus (basioccipital–supraocciput angle) is a useful parameter to differentiate various causes of foetal ventriculomegaly and in particular Chiari type II malformation [193].





Diagramatic dog skull which provides the sequence of initial ossification by means of numerals which indicate foetal size in mm. Early foetal ossification (under 50mm) is highlighted by a green dot. Shaded bones with dotted outline are formed in cartilage.

Calvaria

The bones of the calvaria initiate as osteogenic foci which condense in the mesenchyme between the brain and lateral surface ectoderm. These develop as paired frontal and parietal centers, an unpaired interparietal bone and superficial areas around the sense organs (lacrimal and nasal, temporal, tympanic ring). The latter fuses with the supraoccipital bone on day 45 of gestation and usually loses its identity. In the developing canine foetus, each bone of the calvaria shows a central ossification centre trabecular network that spreads to cover the brain to form roofing bones [113]. Studies using a transgenic mouse with a permanent neural crest cell marker [166,167,194] have revealed that frontal and squamosal bones are neural crest derived, in contrast to the parietal and interparietal bones which are mesodermal origin. However the unossified sutural membrane between the parietal is also of neural crest origin and can therefore function as an organising centre because they represent tissue interfaces [195].

Growth of the calvarial bones is a combination of 1) sutural growth, 2) surface apposition and resorption (remodelling) and 3) centrifugal displacement of the expanding brain [131]. When intracranial pressures become excessive, as in hydrocephalus, both plates of the calvaria becomes thinned and grossly expanded. Conversely, any reduced functional matrix force of the developing brain results in small calvaria e.g. micocephalics. Normal forces acting on the calvaria, such as muscles that act on the superstructure of the cranium, add to the dimensions, but not the intracranial capacity[131].

Interparietal bone

On the 45th day of gestation an unpaired, median, interparietal bone appears superficial to the paired parietal bones and to the supraoccipital. Usually this fuses with the dorsorostral border of the supraoccipital as the sagittal crest, but in some dogs it remains a separate entity, the *os interparietale* (Table 1.7). Although it is more obvious inside the cranium, an unfused interparietal bone can be observed in the adult dog [113].

Table 1.7Number of dog foetuses, grouped according to their head conformation, with unfusedinterparietal bones

Breed type	No of dogs examined	no of dogs with separate interparietal		
brachycephalic	33	17		
mesaticephalic	30	9		
dolichocephalic	64	0		
total dogs	127	26		

However, in the Beagle foetus that Evans studied there was always a separate interparietal bone for a short period which fused indistinguishably with the squamous part of the supraoccipital. A study of neonatal CKCS [196] found that the lambdoid suture appeared more advanced in closure in the CKCS when compared to control (beagle) dogs. Premature closure of the lambdoid suture is associated with

reduction in the posterior fossa volume in human CM 1[197]. The interparietal bone begins to fuse before birth and closure is completed between the second and fourth year. Tubbs *et al* [29] reported a 16% incidence in 50 adult skulls over 30 years.

The interparietal bone, in human *Os Incae*, is formed in a persistent mendosal suture that runs horizontally from the medial portions of the lambdoidal sutures. This suture is a normal variant in the human skull and is well-known in anatomy and radiology textbooks. It is called the Inca bone because it was first described in skulls of Inca tribe in Peru in the belief that it was an ethnic characteristic of this ancient civilization [198]. Since then numerous anatomists have reported a separate interparietal bone in adult skulls of various ethnic groups and it has been suggested that it might be inherited. In Japan, access to 1207 dehydrated Japanese skulls of which 752 were foetuses enabled Matsumura *et al.* to study the development of the interparietal part of the supraoccipital (called occipital squama) in foetal skulls. They concluded that inter-parietal bones may occur due to failure of fusion between primary and secondary centres of ossification of occipital bone [199]. The preinterparietal bones, which are developed from the additional 4th pair of interparietal ossification centres, are clearly differentiated from other anomalies in the lambda region by the shape of their territory and by their location. (See Fig 1.19 below)





(A) multiple 'extra' intra sutural bones (called worminan bones) in sagittal suture (B) multiple worminan bones in sagittal and lambdoidal sutures (C) Interparietal bone bounded by sagittal and Mendosal sutures. The superior median fissure divides the interparietal bone into left and right halves. N.B Group reported 11 children with craniosynostosis in the presence of an interparietal bone[197].

Occipital bones

As previously stated, the four occipital bones that surround the foramen magnum do not have the same origins in the mammal vertebrate skull including the dog [157] The basioccipital is part of the chondrocranium and it is the first to ossify in the clivus (skull base). The two lateral exoccipitals are also endochondral bones and initiate as condensations of paraxial mesoderm that follow the changing contours of the developing brain [200]. Forming the dorsal border of foramen magnum is the supraoccipital. However this bone is derived from cephalic paraxial mesoderm [153,158] and its

development is influenced by the evolution of the interparietal bone, already noted, becomes incorporated with the supraoccipital in many mammalian species including man and the dog. Sometimes the bones can remain unfused in the adult dog, more apparent internally or as the external sagittal crest [113]. The nuchal crest separates the dorsal and caudal divisions of the skull and the external occipital protuberance forms a ridge or crest which is poorly developed in some dogs, e.g. Griffon Bruxellois.

The varying proportions of these four occipital bones and their shape and size of the foramen magnum is illustrated in Figs 1.20 depicting dog skulls circa 1900 from the Hunterian Museum, University of Glasgow. The variety of form is a feature of breeding selection that has occurred over thousands of years and still continues.

Fig. 1.20 Seven dog breeds illustrating variation in skull morphology of both caudal and lateral aspects (not to scale).



(Skulls courtesy of Hunterian Museum, Glasgow, photography by John T Knowler)

Occipital dysplasia (Dorsal notch)

The dorsal notch of the supraoccipital is considered a form of incomplete ossification of the bone and can be referred as occipital dysplasia [201]. Four of the seven breeds represented in the 100 year old skulls in Fig 1.20 possess this anatomical phenomenon. In a study of dog skulls from the Roman era, it has been suggested that the incidence of the notch in their dog population was a result of inbreeding [202]. Bagley *et* al identified occipital dysplasia in two dogs concurrently with syringomyelia and tetraparesis and paraparesis and suggested there may be an association between them all [66] However Rusbridge *et al* established a coexistence of occipital dysplasia and hypoplasia with SM in CKCS and demonstrated that it was not possible to differentiate either condition with herniation of the cerebellum. Post mortem findings identified a tough membrane that covered the keyhole defect and suggested that this tissue was more compliant than bone and offered less resistance to the cardiac cycle and CSF flow dynamics. It was postulated that this might also reduce painful clinical signs associated with CM and resulted in late onset clinical signs [35]. Driver *et al* showed that the height of the foramen magnum in CKCS increases with time [56] i.e. undergone dynamic change and this finding has also been confirmed by the author in the GB breed (unpublished). It may also be that atrophied bone increases the size of the foramen magnum.

Craniocervical junction

The assembly of the four occipital bones and the first two cervical vertebrae make up the craniocervical junction. Mechanically this consists of a central pivot (basioccipital bone, dens and axis) and two rings (foramen magnum and atlas). The embryology mirrors the functionality with the central pillar originating from the axial portion of the occipital and first two cervical sclerotomes, whereas the ring structures come from the lateral portion of the first two cervical sclerotomes [203]. Developmental anomalies of the axial portion may result in anomalies of the dens pivot and the basiocciput. Furthermore, disturbances in the development of the lateral portion may result in abnormalities of the occipital condyles, atlas arch and lateral masses of the atlas and axis [203]. In other words a developmental anomaly resulting in a Chiari malformation may also be associated with abnormalities of the atlas, axis and dens. In the dog, the most important craniocervical junction abnormality associated with CM is atlanto-occipital overlapping, which has been reported as similar to basilar invagination in humans [38,51]. Both conditions are characterised by increased proximity of the cranial cervical spine to the base of the skull [204]. The defining characteristic of human basilar condition is the invagination of the odontoid process of the axis through or towards the foramen magnum, often with compression of the neural tissue by the dens [204]. Other less common canine craniocervical junction anomalies include atlantoaxial subluxation [95,205] and dorsal angulation of the dens [206]. Occipital dysplasia (i.e. widened foramen magnum) also may be seen [35,66]; however this is probably an acquired condition due to overcrowding of the caudal cranial fossa, mechanical pressure from the cerebellum and supraoccipital bone resorption [56].

A fibrous band caudal to the foramen magnum that compressed the cord and subarachnoid space has been shown to be associated with CM/SM in CKCS [95]. It is also seen in other breeds where resection of the tissue in a Pomeranian patient to resolve the compression also resolved the associated pain [97]. Similar atlantoaxial banding is found in humans [207].

Skull Growth

Skull growth results from a combination of i) bone remodeling (deposition and resorption) which is directional and unlike interstitial growth which is multidirectional expansion ii) apposition of bone at sutures and synchondroses (endochondral bone junction sites) iii) transposition-displacement of enlarged and remodeled bones [131]. Prenatally growth is uneven, reflecting and accommodating the developing brain. The rostral and caudal sections (divided by the hypophyseal fossa or sella turcica) increase their length at different rates and at different times with the sphenoid and basioccipital bones developing more slowly. Expansion of the cranial base occurs by primary growth of the cartilage and by expansion at the synchondroses i.e. at the suture lines [166].

Sutures

Sutures are sites of cellular proliferation and fibre formation which creates immovable bone joints (synathroses) which can absorb stresses. Their size, type and location are genetically determined and closure occurs in a defined pattern in relation to the brain [166]. The sutures of the calvaria are different from those of the facial skeleton reflecting slightly different mechanisms of intramembranous osteogenesis [131]. In humans the anterior cranial fossa involves the displacement of spheno-frontal and spheno-ethoidal sutures.

Early closure or fusion of sutures called craniosynostosis, usually before the age of seven years in humans, results from the expression of those genes encoding fibroblast growth factor receptor (FGFRs) and the transcription factors TWIST and MSX2. The spheno-occipital synchondrosis makes a significant contribution to growth post-natally [208]. A recent study found that the spheno-occipital synchondrosis was closed in 80% of dogs at 4 months old and that this suture seemed to ossify earlier in CKCS compared to other brachycephalic dogs, which in turn ossified earlier than mesaticephalic dogs [114].

Genetic basis for CM/SM

Introduction

The dog genome project has provided data and the resources for the unique characteristics of breeds which enable investigations for the genetic basis of complex traits. There are 2.5 million single Griffon Bruxellois nucleotide polymorphisms (SNPs) available for use in linkage analysis in order to locate mutations [209,210]. Molecular markers were used to study genetic relationships in a diverse collection of 85 domestic dog breeds [211]. Differences among breeds accounted for approximately 30% of genetic variation: see reviews [212,213]. Studies of the evolutionary history of the dog have shown that dog breeds define distinct genetic units which are divided into at least 4 hierarchical groupings [212]. The genomes of CKCS and Griffon Bruxellois are clustered with those of mostly modern breeds including other spaniels, gundogs, hounds and terriers. Founder events and stringent breeding practices, including line breeding, have made purebred dogs a closed genetic pool [214]. Closely related breeds are more likely to share ancestral chromosomes and hence carry the same disease allele/s. The higher inbreeding in isolated populations also has the effect of leading to larger physical regions of genetic identity, shared on chromosomal segments, which are involved in disease expression. The relatively low genetic heterozygosity of purebreds make them particularly attractive for use in genetic mapping but should be used with caution [215].

Since CM is considered a congenital abnormality and has variable success in surgical or medical treatment, knowledge of the genetics raises the possibility of prevention in the first instance. Genetics also has the potential to improve the treatment of affected dogs, such as the use of targeted gene therapeutics. Thus studies of CM/SM could not only be part of patient care but also guide treatment approaches. Furthermore, in dog breeding, understanding the inheritance of CM/SM creates the real opportunity of reducing incidence of the conditions, if not eradicating it. Accurate and consistent phenotyping is crucial for successful genotyping and the differences in reported genetic findings are perhaps due to differences in the parameters that are used in the phenotyping and in analysis. Nomenclature may also lead to confusion since it implies similarity when perhaps there are differences in the phenotype. For example, in the human CM-I analogue, the phenotype is described by some as a hindbrain overcrowding and underdeveloped posterior cranial fossa [216] whereas others see the phenotype as an abnormality of cranio-cervical junction [217] thus generating different conclusions. This may also be the case in the dog phenotype.

Human Genetic studies

The genetic basis for the human analogue Chiari type 1 malformation (CM-I) has been elucidated by three lines of inquiry i) familial aggregation ii) twin studies and iii) association with other genetic conditions. Studies of human families affected with CM-I with and without SM revealed the frequency

of transmission to be less than expected on the basis of pure Mendelian inheritance, suggesting autosomal dominant inheritance with incomplete penetrance [218,219]. The multifactorial nature of CM/SM is underlined by a variety of investigations worldwide. A research group in Spain performed a case-control association study of 303 tag single nucleotide polymorphisms (SNP) across 58 candidate genes involved in early paraxial mesoderm development in a sample of 415 CMI patients and 524 sexmatched controls [220]. The genes selected were involved in the signalling gradients that occur during segmental patterning of the occipital somites (i.e. FGF8, Wnt, or retinoic acid pathways and from bone morphogenetic proteins or BMP, Notch, Cdx and Hox pathways). Others include placental angiogenesis, sclerotome development or CMI-associated syndromes. Single-marker analysis identified nominal associations with 18 SNPs in 14 genes. Further analysis identified a risk haplotype for classical CM-I in two of the 14 genes (ALDH1A2 and CDX1) suggesting that common variants in genes involved in somatogenesis and foetal vascular development may confer susceptibility to CM-I.

CM-I has been associated with several known genetic syndromes. Chondrodysplastic disorders (achondroplasia and thanatophoric dwarfism [221–223] and Crouzon syndrome [224,225] are just two such examples. However gene identification studies are hindered by the complexity of inheritance patterns and aetiologies of CM-I and SM. As mentioned in the previous section, premature fusion of bones that limit growth of the skull (synchondrosis) and resultant impact on growth of the developing brain e.g. variety of syndromes reported in human CM-I for which the genetic basis has been explored [226].

In a more specific study, measured correlations between ten cranial morphologies delineating the caudal fossa were coupled with high-density SNP genome screen of 23 families with 71 CM affected individuals. This investigation revealed significant evidence for linkage to regions on two chromosomes, 9 and 15. The latter region contains a biologically plausible gene for CMI, namely fibrillin-1, which is a major gene in Marfan syndrome and has been linked to Shprintzen–Goldberg syndrome, of which CMI is a distinguishing characteristic [31].

A comprehensive summary of genetic diseases and syndromes that are associated with CM-I are provided in the book 'Syringomyelia a disorder of CSF circulation', p74-6 [16].

Canine Genetic Studies

Since SM was first identified in the CKCS [9], a primary aim of those researching the disorder has been to find the genetic basis for the condition because finding the gene/s responsible for CM/SM would improve understanding of the underlying molecular and cellular pathogenic mechanisms and thereby enhance diagnosis, prognosis and clinical management of the conditions. Moreover, it may be considered possible to develop a genetic test which would identify carriers and thereby devise breeding strategies to reduce or eliminate this devastating condition in the CKCS and other affected breeds. The aim of the genetic studies involves two aspects

- 1. identifying the phenotypic variations that occur in the disorder
- 2. collecting suitable DNA of these phenotypic variations

Since CM is present in variable degrees of expression in nearly 100% of the CKCS dogs, genetic studies in this single breed are not feasible but remained a possibility for SM. CM has a lower incidence frequency in other Toy Breeds such as the Griffon Bruxellois, King Charles spaniel, Affenpinschers, etc., making it possible to investigate the genetics of both CM and SM using these breeds.

Cavalier pedigree SM database

In 1999, a worldwide pedigree collection of MRI-confirmed SM dogs and their unaffected relatives was initiated by C Rusbridge and S P Knowler. The data was collated as a single family tree using a human genealogy computer programme (Sierra On-line Inc Bellevue, WA 98007) which had the advantage of viewing multiple relationships simultaneously compared to those that only provided a single line of descendants. A preliminary investigation, using pedigree information from 45 dogs with SM which had been supplemented with published information from the breed club and spanning 20 generations, revealed a high incidence of SM in the lines of 4 key dogs. Further detailed pedigree analysis revealed the degree of inbreeding with the same sire appearing several times in a single pedigree [13]. This study was followed up with one of 120 dogs diagnosed with SM and a family tree of more than 5,500 CKCSs spanning a maximum of 24 generations. This investigation showed 6 of 8 great grandparents of all affected dogs could be traced back to 2 female ancestors so that all 8 were descended from one or the other or both (Fig 1.21). The disease appeared to be more severe and to have an earlier onset with increased inbreeding, especially when breeding was from affected dogs. Selection for coat colour is believed to have influenced the development of both SM and Idiopathic Epilepsy [227]. Further pressures on the gene pool had resulted from breeding guidelines to reduce the incidence of mitral valve disease. The incidence of syringomyelia was very high in certain families and lines which had been extensively inbred and indicated that the inheritance is more likely to be of variable penetrance or oligogenic than simple [227].

The pedigree database creates a unique ID of each dog and their relatives and, in collaboration with the Cavalier Club, this was used to collate a DNA collection scheme called 'DNA for Healthy Cavaliers [79]. For inclusion in the DNA collection all the study dogs must have a microchip or tattoo which linked it to its pedigree validated with a Kennel Club registration. Microchipping dogs was not made mandatory in the UK until April 2016 [228] but in the Netherlands microchipping and KC registration is required as a 6 week old puppy and the registration certificate provides a three generation pedigree. Furthermore, a key feature of this particular database is that the MRI results have been verified by one person (Clare Rusbridge) and therefore provided consistency. Phenotypic information was delivered

by the owner-completed checkboxes of clinical signs and incorporated into a consent form for the data and DNA to be used for scientific purposes see Fig 1.5.

Fig 1.21 Pedigree of dog V showing relationship with founder bitch G and 'saturation' of suspected carriers in his first three generations. (taken from Rusbridge and Knowler, 2004).



DNA collection

Inherited diseases commonly emerge within pedigree dog populations, often due to use of repeatedly bred carrier sire(s) within a small gene pool and the likelihood of involving recessive genes. However, there are many factors that are intrinsically difficult about collecting DNA and collating pedigree information from a large canine population. The keys to a successful DNA collection program include (1) the need to establish and maintain support from the pedigree breed clubs and pet owners; (2) committed individual(s) who can devote the considerable amount of time and energy to coordinating sample collection and communicating with breeders and clubs; and (3) providing means by which genotypic and phenotypic information can be easily collected and stored. The experiences in setting up a worldwide DNA collection are summarised in Fig 1.22 and published in the Journal of Heredity [79].



Fig. 1.22 Key features which have ensured a successful DNA collection.

Project supported by ¹ UK DNA Archive for Companion Animal (*www.liv.ac.uk/animalDNAarchive*) and ² Boehringer Ingelheim.

The CKCS database is still in use by the author SPK and currently stands at around 13,000 dogs which can be imported into a dedicated Microsoft Access TM database which allows detailed phenotypic information and analysis of the pedigrees and inheritance. Accurate family records make linkage analysis possible in the collaborative genetic studies.

Griffon Bruxellois pedigree database

A worldwide pedigree database of Griffon Bruxellois and DNA collection was set up in 2007 in a similar manner to the CKCS database but it is much smaller and currently stands at 1563 dogs. The GB breed have dogs without CM thereby enabling the genetic basis of this condition to be studied. Data collection has been a crucial part of understanding the complexities of CM and SM and it has been used both in the radiograph study of GB [44] and the genetic studies [94,229]. Fig. 1.23 is an example of a family tree used in these investigations. The core of the family tree consists of five foundation animals (numbered 1 to 5) consisting of two normal dogs, one normal bitch and two bitches with asymptomatic Chiari-like malformation (CM)/syringomyelia (SM). The two asymptomatic CM/SM bitches had seven descendants with CM/SM, and four of these had clinical signs. Three first-generation descendants were free of CM, although they did have a CCD or an SM less than 2 mm wide. Two of these dogs (half-siblings) were mated, and two of four offspring had asymptomatic CM. One branch of the family tree

was the product of a mating between normal bitches 1 and dog 2, and all animals were free of CM. However, three of the descendants had SM or CCD without the bony defect



Fig. 1.23 Family tree of 32 related Griffon Bruxellois dogs.

What is noteworthy in this family is the differences between the branch from crossing bitch 1 and stud 2 without CM producing all offspring without CM, compared to stud 4 with no CM or SM when crossed with bitches 3 and 5 with CM/SM which resulted in high number of affected offspring. In the half-brother /sister mating (bitch 3), CM skips a generation. Both these findings indicate involvement of a recessive trait for CM.

Informal Breeding Guidelines and their Effectiveness

Following on from the two papers on inheritance [13,227], an International Working Party, at the request of the Cavalier Club, recommended informal breeding guidelines [15]. These were based on those initiated by C. Rusbridge in 2004 and shown to be effective in the Netherlands. They were similar to the guidelines for mitral valve disease that were already in place and which used an age cut off at 2.5 years. It had been decided to allow for mildly affected dogs to be used in breeding avoiding reducing the genepool. The aim of these recommendations was to reduce the incidence of early onset symptomatic syringomyelia (SM). Table 1.7

Grade	Age (years)	Syringomyelia	Comment	Breed to	
А		Absent or less than			
	Over 2.5	2mm central canal			
		dilatation in the C2-		A, C, D	
		C4 region only			
С	Under 2.5	Absent	Rescan after 2.5 years	А	
D	Over 2.5	Present	Asymptomatic	А	
E	Under 2.5	Present	Asymptomatic	Do not breed	
F	Any	Present	Symptomatic	Do not breed	

Table 1.7 Informal Breeding Guidelines 2006

Note Grade B from the previous guidelines of Clare Rusbridge have been removed because of difficulty in accurately interpreting a mild CM. Previously A * - now A, Previously B – now C.

These recommendations were a forerunner to the existing BVA/KC breeding guidelines detailed previously.

- That both the sire and the dam of a proposed mating are screened (any unscreened dog should be assumed to be "D")
- 2) Offspring of any mating should also be MRI screened before breeding.
- 3) Any dog screened before 2.5 years old has a second screen when older,
- 4) That dogs are screened from 6 months of age
- 5) That if a limited ("mini") MRI screen is performed in order to reduce financial costs for the breeder.
 - a) the minimum area covered is from approximately the level of the thalamus / corpus callosum to cervical vertebrae 5 (C5)
 - b) Both TW1 and TW2 = sagittal images are obtained in addition to TW1 and /or TW2 transverse images through the upper cervical spinal cord.
 - c) An assessment is also made for presence/absence of ear disease and ventricular enlargement.
 - d) That interpretation of images is made by Diplomate level radiologists, neurologists and, in special circumstances, by orthopaedic surgeons with recognised expertise in this area.

The majority of the dogs screened for SM were owned by a small number of breeders from Netherlands and comprised parents and offspring that had undergone an MRI prior to breeding. Fig 1.24 provides the percentage numbers of affected dogs that were compared to unaffected in the first two years (2004-6) and last two years (2007-2009) and are compared to dogs elswhere in the world which were not using

the breeding guidelines to the same extent. It illustrates an improvement in incidence in the Dutch dogs (unpublished data).



Fig 1.24 Percentage comparasion of dogs with and without SM in the Netherlands and other countries 2004-2009.

The Netherlands had an apparent higher prevalence of SM which was considerably reduced through adoption of the informal breeding guidelines.

A concern that SM asymptomatic dogs produce symptomatic offspring prompted an investigation that looked at the effectiveness of the breeding guidelines in both the CKCS and GB [53]. The effectiveness of these breeding guidelines was investigated using 643 dogs, 550 Cavalier King Charles spaniels (CKCS) and 93 Griffon Bruxellois (GB), which had either one (454 dogs) or both parents (189 dogs) with MRI-determined SM status. Offspring without SM were more common when the parents were both clear of SM (SM-free; CKCS 70 per cent, GB 73 per cent). Conversely, offspring with SM were more likely when both parents had SM (SM-affected; CKCS 92 per cent, GB 100 per cent). A mating of one SM-free parent with an SM-affected parent was risky for SM affectedness with 77 per cent of CKCS and 46 per cent of GB offspring being SM-affected (Fig 1.25). In a total of 372 cavaliers 153(36%) offspring were unaffected and 239 (64%) were affected, 129 of which resulted from breeding affected dogs with a mate of unknown status.



Fig. 1.25 Percentage of each offspring from various parental combinations for 372 CKCS between SM affected (all ages) and unaffected dogs over two and a half years of age.

(U= unknown SM status. Grades A*,A, C,D, E of informal breeding guidelines).

Investigating the heritability and mode of inheritance of SM in the CKCS breed

In 2009 the pedigree database was given to Dr S Blott and Dr T Lewis at the Animal Health Trust to investigate the heritability of SM [230]. The investigation comprised a mixed model analysis of 384 CKCS, with MRI confirmed SM, in conjunction with the Kennel Club pedigree records of all dogs registered from the mid-1980s to September 2007. It revealed a moderately high estimate of heritability of SM ($h^2 = 0.37 \pm 0.15$ standard error) when analysed as a binary trait. More recently, the heritability of symptomatic SM in dogs >6 years of age was calculated as high as 0.81 in the CKCS study in Denmark of all registered dogs in the Danish Kennel Club in 2001. This investigation included a long term follow up of asymptomatic dogs and the findings suggested that 93% (14/15 cases) remained asymptomatic if no clinical signs had developed by age 6 years [69]

Lewis *et al* indicated that the condition segregated within families and this indicated genes at more than one locus influencing SM. Fig.1. 26 below is the example used in the paper to indicate that the inheritance rules out autosomal dominant mode of inheritance for a single gene because affected offspring have resulted from parents of seemingly clear SM status. However the example given may have reached a premature conclusion because it did not take account of the late onset nature of SM and that the parent/s may or may not be clear of SM.

Fig 1.26 Example of mode of inheritance for SM given in Veterinary Journal 183 (2010).



SM0b = no SM over 3 years but less than 5 years, SM2c = affected SM under 3 years

The late onset nature of SM was highlighted by an investigation into the prevalence of SM by Parker and colleagues [50]. This investigated a population of 555 CKCS declared by their owners to be showing no clinical signs of syringomyelia that underwent MRI SM screening prior to breeding. Data were analysed by logistic regression to determine the effects of sex and age on the prevalence of syringomyelia. Only increased age was found to have a significant effect. The prevalence of syringomyelia was 25 per cent in dogs aged 12 months, increasing to a peak of 70 per cent in dogs aged 72 months or more.

As a result of evidence from these three investigations [50,53,230], it was recommended that all breeding dogs from breeds susceptible to SM be MRI screened and that the SM status at five years old is established. It was recommended that all results should be submitted to a central database that can be used by dog breeders to better enable mate selection based on estimated breeding values [231].

An official British Veterinary Association / Kennel Club Canine Health screening scheme for CM/SM was initiated in 2011 in the UK [232]. Following the recommended protocol the MR images are assessed by two scrutineers and graded for severity for both Chiari-like malformation and syringomyelia. Results are submitted to a central database, intended to generate Estimated Breeding Values (EBV) and entered into the UK Kennel Club Mate Select Computer program [233]. The following Table 1.27 provides the suggested breeding guidelines for SM and its relationship with the informal guidelines.

Age	SM Grade	2006	Normal		CCD		SM				
		equivalent	0a	0b	0c	1a	1b	1c	2a	2b	2c
			*A === CDD	A sector	C	•A with CDD	A with CCD	ŧ	D	D	E
		*A									
over 5 yrs	0a	88 CCD									
		A									
3-5yrs	0b	B46(2)D									
		С									
1•3 yrs	0c										g
		*A									bree
over 5 yrs	1a	with 000									Į
		A									1 op
3-5yrs	1b	with COD									-
1-3 yrs	1c	#									
over 5 yrs	2a	D									
3•5yrs	2b	D									
1•3 yrs	2c	E	do not breed								
Dog clinical sig SM a	with ns CM&/or ny age	F	do not breed								

Fig. 1.27 Table conversion from 2006 informal guidelines to 2012 BVA/KC guidelines (SM only)

CCD=central canal dilatation Grade depends on age at which dog was scanned. a= over 5 years, b= 1-3 years, c= less than 3 years, Green = breed, Pink = do not breed. *A = dog over 5 years no SM.

dogs with CCD aged less than 2.5 years (were often attributed a D grade).

CM – Chiari malformation, SM – syringomyelia, CCD – central canal dilatation.

Estimated Breeding Values (EBVs)

Complex polygenic conditions such as CMSM are a result of both genetic and environmental influencing factors. An EBV is the estimate of genetic risk for each animal in the pedigree from a variety of phenotypic traits. It is a more accurate metric for breeding selection than an individual dog's test score alone [230,234] and perhaps best illustrated by an actual case study. In the Fig 1.28 below, stud dog A without SM (3 years old and with a dam clear of SM over 5 years) was mated to two bitches with SM but only one has a parent clear of SM over 5 years and produced offspring that are clear of SM at 3 years of age.

Knowledge of the MRI status of previous generations is helpful to breeders but EBV gives the risk value that includes affected siblings as well. EBVs are available for all animals in the pedigree used in the analyses described above, even if they have no phenotype available. However an EBV can only be calculated for a breed if enough individual dogs across the breed have been scored [235].

Fig 1.28 Three generation CKCS family tree with grandparents and offspring of an SM unaffected male with two SM affected mates.



Large square shape indicates male, large circle indicates female. Black colour indicates SM affected, colourless indicates SM unaffected. Small square indicates dog of unknown status. Letters indicate CM/SM grade according to current BVA/KC breeding guidelines.

In 2008 Dr Sarah Blott and Dr Tom Lewis at the Animal Health Trust were funded by the Kennel Club to establish EBVs for the CKCS breed. DNA swabs were collected in the possibility of genomic breeding values (http://www.thecavalierclub.co.uk/health/ebv/genetic study.htm). Unfortunately the MRI phenotypic information from different MRI centres was inconsistent and KC registration and microchipped dogs were not mandatory so some results could not be verified. This prompted the Cavalier Club to request the BVA and KC to provide an official scheme with an agreed protocol and the consensus of two or more interpreters in a similar manner to the Hip dysplasia scheme. However the KC policy to publish the results of dogs that are screened under the scheme was not popular with high ranking Cavalier Club members. As a result, a breeder led EBV scheme was set up in Belgium called 'Cavaliers for Life' with additional funding from the Belgium Government and the specialist knowledge from the University of Leuven, which had pioneered EBVs in cattle, was enlisted to develop the specifically designed computer programme in a standardized manner, paying special attention to the fact that individual data remain invisible to the outside world http://www.cavaliersforlife.eu/. This had the huge advantage to Show breeders that the individual data is anonymous. Since Breed Clubs are run for the benefit of Show breeders they were able to influence members to support their scheme, at the same time failing to support the official BVA/KC scheme.

Genetic studies investigating SM in the CKCS

A primary goal, since the recognition of canine CM/SM as a naturally occurring model for human CM-I and SM, has been to identify the genetic factors leading to the development of these conditions in both humans and dogs. The specific steps identified by human genetic collaborators in Canada, Dr G Rouleau and Z Kibar at the Sainte Justine Research Centre in 2006 were;

1. Genetic mapping of the CM and SM gene(s) by linkage disequilibrium analysis in the CKCS breed and in other related breeds; these rely on known markers (SNPs).

- 2. Identification of candidate genes for CM and SM using the positional candidate gene approach.
- 3. Molecular characterization of the gene(s) mutated in CM and associated SM.

A small grant from Cavalier Health Foundation (#104) was awarded in 2005 with the purpose of archiving CKCS DNA. This was in collaboration with Dr Guy Rouleau at Director of CHU Sainte Justine Research Centre in Montreal (formally at McGill University) and initially Dr Berge Minassian at the Children's Hospital Toronto and Dr Diane Shelton UC San Diego. Over 1,500 DNA samples worldwide were collected (not all the dogs had MRI confirmed status) with sponsorship from the UK DNA Archive, Boehringer Ingelheim UK, Utrecht University, the Cavalier Club UK and TDDS Laboratories. The collection known as 'DNA for Healthy Cavaliers' has been supported by many veterinarians and dedicated breeders and dog owners in Europe (especially the Netherlands), USA, Canada, Australia, South Africa and Scandinavia.

An initial CKCS whole genome scan was completed in 2005 with 173 CKCS dogs distributed over 34 dog pedigrees. Selection was based on SM-affected status and familial relationship in the CKCS database and the study was led by Dr Zoha Kibar at Sainte Justine Research Centre, Montreal Canada. Genetic analysis was undertaken by Dr Marie-Pierre Dube at University of Montreal and six genomic regions with LOD (logarithm of the odds) scores above 1 that could harbour the CM/SM gene(s) identified [236]. A grant from the American Kennel Club Health Foundation (#954) with matching funds provided by ACKCSC and Cavalier Club of the USA and additional DNA samples provided by North Carolina State University and Guelph University allowed the genetic research to continue. A cohort of 198 CKCS dogs, distributed over 39 pedigrees, was genotyped with 57 additional markers that had been selected from the suggested candidate regions. Linkage analysis of these data gave persistent LOD scores above 1 in 5 candidate regions, with the highest LOD score of 3.03 being on locus 1. Ten new microsatellite markers saturating the SM locus 1 were then genotyped in a cohort of 363 CKCS distributed over 49 pedigrees. The highest LOD score of 3.07 persisted and multiple markers in the region gave suggestive LOD scores either above 1 or above 2.

Locus 1 was further investigated by genotyping 108 SNPs on 223 affected and 62 unaffected CKCS. Each of these dogs were confirmed clear of SM at 6 years of age at the time of MRI scan to allow for the late onset nature of SM. This investigation identified 10 Single nucleotide polymorphisms (SNPs) haplotype spanning 1.3 Mb which was then reconstructed in the whole cohort and found in 10% of unaffected dogs while present in only 0.2% of affected dogs. This suggested a protective effect against SM. The results also confirmed linkage of CM/SM to Locus 1 [237]. Fine mapping of this candidate region with haplotype sharing analysis with other genetically related affected breeds is currently underway.

Genetic studies investigating CM in the Griffon Bruxellois

CKCS and GB breeds are genetically related with common ancestors from the Smousje (an Affenpinscher-like dog), Pug and ruby King Charles spaniel [44]. Incomplete penetrance and variable transmission of CM indicate a complex polygenic mode of inheritance[13,44,227], which makes identification of the predisposing genetic factors using traditional genetic cloning approaches difficult. A more powerful approach to investigate the apparent genetic complexity of CM is quantitative trait loci (QTL) analysis. This aims at identifying genes or QTLs that determine the disease even though each gene contributes only a small fraction and the approach has been used successfully in complex diseases like hip dysplasia [238].

Robust phenotyping and novel morphometric measurements

Accurate phenotyping is essential in order to identify the underlying genetic basis of a disorder. The QTL study in the GB used a total of 14 measurements taken from T1-weighted sagittal MRI images of 50 CM affected dogs and 24 unaffected dogs [94]. These comprised 8 lines and 5 angles associated with the dorsum of the spheno-occipital synchondrosis. Earlier unpublished pilot studies had suggested that the height of the rostral and caudal cranial fossa may be an important variable. However accurate assessment of this is hampered if the MRI does not include the rostral skull. To overcome this problem the author used a circle extending from the cranial base line and encompassing the caudal aspect of the occipital lobe. As this circle occupied the space between the base of the skull and the dorsum of the occipital lobe, the diameter (F-diameter) could be used as a reflection of the height of the rostral cranial cavity. The center of the circle (F) was used as a vertex and the occipital circle could also be used as an assessment of the size of the caudal cranial fossa by measuring the distance and angulation of the point where the circle bisected to the dorsal cerebellum. The latter overcame the problem of inconsistently identifiable caudal cranium bony landmarks which had been a problem in previous studies [91].

Six traits were found to be significantly associated to CM in this breed and identified two loci on CFA2 and CFA14 strongly associated to cranial fossa height and height of the rostral part of the caudal cranial fossa. Linear and mixed regression analyses identified associated single nucleotide polymorphisms (SNPs) on five *Canis Familiaris* Autosomes (CFAs): CFA2, CFA9, CFA12, CFA14 and CFA24. A reconstructed haplotype of 0.53 Mb on CFA2 was strongly associated to the height of the cranial fossa (diameter F). In addition, a haplotype of 2.5 Mb on CFA14 was associated to both the height of the rostral part of the caudal cranial fossa and the height of the brain and also significantly associated to CM. The CFA2 QTL harbours the Sall-1 gene which is an excellent candidate since its orthologue in humans is mutated in Townes-Brocks syndrome which has previously been associated to CM-I [229].

Thesis Overview and Aims

The first chapter, Introduction, delivers the context for the dissertation and the concepts that underpin it. It provides a synopsis of the nomenclature, prevalence, clinical signs and treatment of both CM and SM. It highlights the existing research and focuses on the different morphological measurements used to analyse these conditions so that they can be carried forward. Risk factors such as brachycephaly and miniaturization are explored with respect to understanding their association with CM/SM.

Since CM is considered to be a congenital condition, the introduction includes an embryology section with particular emphasis on the developmental anatomy of the brain and skull in order to provide a context for any aberrations in the etiology of CM/SM. The hereditary nature of CM/SM is expanded in a section on genetics to include the work already undertaken relating to breeding guidelines and how the sample data was collected. Acquiring DNA samples to provide the means to undertake a genetic analysis of a multifactorial, late onset disorder can be really challenging and its contribution to research should not be underestimated.

Chapter Two sets out the materials and methodology used in the thesis. The material unit of analysis in the project investigation is the sagittal Magnetic Resonance Image (MRI) of the hindbrain and craniocervical junction of the dog. Details of the quality and nature of the image samples are provided and the means by which they were assessed. The morphometric traits that had already been successfully applied to the genetic studies in the GB are presented and the subsequent conceptual steps to carry forward the analysis. In order to validate the original findings in the most effective manner, five of the most significant traits for CM were tracked in a mixed GB breed family but additional traits to accommodate the whole brain were investigated in the CKCS in order to tease out symptomatic CM and SM. Finally, in order to explore the risk factors brachycephaly and miniaturization, selected traits were investigated in the CKCS, brachycephalic Affenpinscher and miniature Chihuahua. The chapter provides a broad overview of the methodology used to analyse the data including the making of morph movie clips as part of the results for which links are provided. The chapter finishes with an ethics statement.

The results section of this thesis present the findings of three published papers in the scientific journal PLOS ONE. An introduction for each chapter provides details of the specific study cohorts for each investigation and a pertinent discussion of the results puts the findings in context with other published material and includes a summary.
Chapter 3 Inheritance of Chiari-Like Malformation: Can a Mixed Breeding Reduce the Risk of Syringomyelia? [239].

Chapter 4 Use of Morphometric Mapping to Characterise Symptomatic Chiari-Like Malformation, Secondary Syringomyelia and Associated Brachycephaly in the Cavalier King Charles Spaniel [239].

Chapter 5 Craniometric Analysis of the Hindbrain and Craniocervical Junction of Chihuahua, Affenpinscher and Cavalier King Charles Spaniel Dogs With and Without Syringomyelia Secondary to Chiari-Like Malformation [240].

The thesis is brought together in the discussion, Chapter 6. This offers an overview of the essential elements that characterise CM, critically examining its robustness and usefulness with additional observations made by the author. Emphasising the multifactorial nature of the condition, the embryology and genetic information provided in the introduction are employed to suggest an underlying etiology of CM and secondary SM. Head conformation which formed part of the study of the inheritance of CM in Chapter 3 is encompassed the role of key significant CM traits, identified in the thesis, in breeding selection to reduce the risk of painful CM and SM.

Finally, although the research into CM/SM over the last decade has provided a wealth of information, there is little evidence that the incidence of the disorder has been reduced. This is best achieved if this knowledge is put to a practical use by veterinarians and breeders. These aims are reflected in the 'future steps'. An appendix section provides supplementary data and the author's list of publications.



Photo courtesy of Henny van den Berg

Testable hypothesis

The clinical consequences of Chiari-like Malformation (CM) and Syringomyelia (SM) are due to changes in brain and spinal cord conformation and these can be quantified on sagittal Magnetic Resonances images to clearly define the phenotype and severity of the disorder.

Research Aim

The aim of this study was to characterise the phenotype and severity of CM/SM in selected toy breed dogs so that it can be used for diagnosis and facilitate genetic studies which are crucial to understanding the condition and reducing disease incidence through breeding selection. This dissertation builds on ten years of research already undertaken by the author investigating CM/SM in the Cavalier King Charles Spaniel (CKCS) and Griffon Bruxellois (GB) Toy dog breeds (see publication list in the Appendix).

Specific Research Aims

- 1. Validate the morphometric measurements in previous Griffon Bruxellois studies using a family study and mixed breeding.
- 2. Characterise the phenotype associated with the severity of the CM and SM in the Cavalier King Charles Spaniel.
- 3. Compare phenotypic for CM in the Griffon Bruxellois and Cavalier King Charles Spaniel with Chihuahua and Affenpinscher breeds to explore the relationship between brachycephaly and miniaturization and CM/SM.

Chapter 2

Materials and Methods

Introduction

The morphometric analysis of Chiari-like malformation (CM) and syringomyelia (SM) in the Griffon Bruxellois (GB) had previously established a framework of lines on sagittal magnetic resonance DICOM images [94]. The use of the unique occipital circle measurement, with its 'f-diameter', not only allows proportional evaluations to be completed but a means of standardizing the morphometries, especially when used in conjunction with ratios. Since the circumference of the circle reflects the shape of the neural parenchyma rostral to the cerebellum, it displays its relationship and juxtaposition within the cranium, highlighting any conformation anomalies associated with the severity of both CM and SM. Thus the occipital circle provides an ideal foundation with which to investigate CM/SM in other breeds.

A series of 14 measurements, comparing the images of GB dogs with and without CM/SM, had identified eight significant variables for CM, three of which were unique to CM and five of which were significant for SM. Furthermore, when the 14 morphometric traits were subjected to Quantitative Trait Loci (QTL) analysis, six traits were found to be associated to CM [229]. One of the traits; the height of the cranial fossa (f-diameter) was strongly associated to a haplotype on CFA2 which harbours the Sall-1 gene which is associated with CM-I in Townes-Brocks syndrome.

Since a T1-weighted sagittal MRI is part of the protocol for the diagnosis of CM/SM by veterinarians and also used for screening prior to breeding, it is, therefore, widely available for study. Such common usage makes this image an ideal choice to include in the characterisation of the complex conditions CM/SM. Having already established which significant traits were the most helpful to use in the previous studies it, account should also be taken of recent findings from other research groups. In view of these factors, the initial morphometries have been enhanced and modified accordingly in order to meet the specific aims under investigation. This chapter provides information about the overarching materials and methods that are applied in the studies and the conceptional processes involved in achieving the thesis aims. Details of study cohorts for any particular study are provided in the results section because they are pertinent to the statistical evaluation in the chapter.

Materials

This dissertation uses Digital Imaging and Communications in Medicine (DICOM) T1-weighted (T1W) or T2-weighted (T2W) midsagittal images of the brain and upper cervical spinal cord. The quality of the MR image (MRI) is dependent on both the strength of the MRI machine and the skills of the operator, neither of which are always under the control of the researchers. The size of the magnetic coil determines the area that can be imaged. According to the size of the dog, this area can be limited to a scan that would not include the whole skull if the area of interest was the cervical region. MRI scanners come in different magnet field strengths measured in teslas or "T", and those in the study ranged from 0.2 to 3 Tesla. When viewed at high magnification, j-peg images derived from the DICOM of 0.2 T scanners appear pixilated and it can be difficult to make out smaller anatomical details that can be seen T3 machines.

An **MRI sequence** is a number of radiofrequency pulses and gradients that result in a set of images with a particular appearance. Each sequence has a number of parameters, and multiple sequences are grouped together into an MRI protocol. They include features such as the time to echo (TE) and the time to repetition (TR) but the simplest method used to divide them is according to the dominant influence on the appearance of tissues. This has resulted in the division of all sequences into proton density (PD) weighted, T1 weighted, T2 weighted, diffusion weighted, flow sensitive and 'miscellaneous'. There are also a number of 'optional add-ons', such as fat or fluid attenuation, or contrast enhanced that are available to specialists.

In simple terms, the signal in MR images is high or low (bright or dark), depending on the pulse sequence used, and the type of tissue in the image region of interest. Tissues with increased water appear dark on T1-weighted image as does low proton density such as bone whereas fat appears bright in comparison. Increased water appears bright in T2-weighted image but bone is less contrasted. Since the bones in the skull are an area of interest, T1-weighted images were selected for the study, as these were the most diagnostically useful images from the low field MRI studies. The exception was images from a 3 Tesla system as the signal-to-noise ratio was suboptimal for standard T1-weighted images and so T1-weighted fluid attenuated inversion recovery (T1W FLAIR) images were used. The images were viewed using an eFILM DICOM viewer by specialist interpreting the status.

MR scanners used in the investigation

Where possible the same machine was used in any one study but this was not always possible. In the Netherlands, Stone Lion Veterinary Hospital and Veterinary Teaching Hospital of University of Helsinki used a 0.2T MR scanner (Esaote S.p.A Genova, Italy) where the dogs were positioned in sternal recumbency. Fitzpatrick Referrals used a 1.5T scanner (Siemens Symphony Mastro Class, Enlargen, Germany) and positioned the dogs in dorsal recumbency.

Inclusion and exclusion criteria

A minimal inclusion criterion was T1-weighted or T2-weighted sagittal and transverse images of the brain and cranial cervical spinal cord although for many dogs the imaging protocol was more comprehensive. Any images of the dog's head in the flexed position or misaligned in the coil were excluded from the study.

Criteria for images inclusion

- i. Inter-thalamic adhesion to as far caudally in the neck as possible but at least to C4/C5 intervertebral disc space.
- ii. A maximum slice thickness 4mm with head and neck aligned in the sagittal plane.
- iii. The central nervous system from the cisterna magna to the C4/C5 intervertebral disc space must be continuously visible in a single sagittal image.

Only dogs with Medical records or completed phenotype forms with written consent of owner participated in the study. Mandatory details of date of birth, age at MRI scan and compulsory microchip were required and attributed to the DICOM images. Pedigree and KC registration details were requested but not mandatory for some studies.

Assessment of CM/SM status

Although other veterinary surgeons have collaborated in the research studies, consistency of phenotypic information was ensured by having every assessment of CM and SM status made by one veterinary neurologist Dr C Rusbridge or the scrutineers of the official BVA/KC CM/SM Health scheme [80] of which she is one of three. The protocol and grading scheme are detailed in the Table below. Additional grading by a ECVN neurologist using the same criteria as the BVA/KC were i) GB in study 1 in the Netherlands were graded by Netherlands KC grading scheme at Utrecht University using a system adopted from the BVA/KC scheme and protocols (the mixed breeds were graded by the BVA scheme). ii) Chihuahua dogs in Finland

The British Veterinary Association/Kennel Club Chiari Malformation and Syringomyelia Health Scheme grading of Chiari-like malformation and syringomyelia according to the British Veterinary Association / Kennel Club Chiari-like malformation and Syringomyelia Health Scheme is as follows:

Chiari-like malformation

Grade 0 - No Chiari malformation

- Grade 1 Cerebellum indented (not rounded)
- Grade 2 Cerebellum impacted into, or herniated through, the foramen magnum.

Syringomyelia is defined as a fluid-filled cavity that includes or is distinct from the central canal of the spinal:

Grade 0 - Normal (no central canal dilation, no presyrinx, no syrinx)

Grade 1 - central canal dilation or a separate syrinx, which has an internal diameter of less than 2mm or a pre-syrinx alone.

Grade 2 - syringomyelia (central canal dilation which has an internal diameter of 2mm or greater, a separate syrinx, or pre-syrinx with central canal dilation). In this instance the syrinx is measured by the maximum internal diameter in a transverse plane.

The syringomyelia grade is qualified with a letter indicating the age group at the time of scanning as follows: a = more than five years of age; b = three to five years of age; c = one to three years of age.

Pre-syrinx is defined as spinal cord oedema, and may be a transitional state prior to development of syringomyelia. Pre-syrinx has the appearance of high signal intensity on T2W images consistent with marked increased fluid content within the spinal cord substance but not of free fluid. On T1W images the spinal cord is either normal or has a slightly hypointense signal [32].

Study samples

The majority of CM/SM cases and controls have been sourced from Dr C Rusbridge who has been collecting data from different centres worldwide for over fifteen years either as patients or sent to her for diagnostic evaluation. The acquisition of CM/SM information is thus not restricted to cases from one particular location or within a limited timescale. Owners are requested to provide a pedigree which is checked against the Registration certificate to be included on the CKCS and GB databases, owned by the author and Dr C. Rusbridge, which can be imported into the dedicated Microsoft Access [™] database. The CKCS database comprising nearly 13,000 has approximately 80% linked together in a pedigree family tree and 90% of the 1,570 GBs are linked in a single tree. DNA samples have been provided for over 1,000 CKCS, approximately 78% have known MRI status and 288 GB have provided DNA samples with known MRI status. Recruitment and fundraising involved engaging and supporting breeders and pet owners, not only on an individual level but also via public media. Using Dr Rusbridge's website <u>http://www.veterinary-neurologist.co.uk/</u> News Blog <u>http://clarerusbridge-news.blogspot.co.uk/</u> which the author set up and maintains together with a closed Face Book group 'CMSM Research' <u>https://www.facebook.com/CMSMresearch.</u>

Study dogs are divided into groups according to their BVA grading for CM and SM. Since all the CKCS have CM, these dogs were divided into groups according to their SM status only and, where applicable, if they were symptomatic or not. SM can be a progressive condition [50,98] but not always. In a study

of CKCS, 43% of the dogs did not show progression of the disease [53]. Therefore, in any investigation, asymptomatic dogs confirmed clear of SM at 5 years or older were selected when possible or younger dogs were age matched. Dogs screened for CM/SM prior to breeding did not have a neurological examination but all symptomatic dogs underwent a neurological examination. Clinical signs [40] included MRI to the cranial lumbar region as standard but more caudal if the dog had any clinical signs of lumbar pain.

Control dogs over 5 years

An important criterion for the characterisation of the CM and SM is obtaining suitable numbers of controls that are free of CM and SM. Syringomyelia may be late onset condition so ideally dogs five years and older that are free of CM and or SM would be the best controls. However asymptomatic dogs do not undergo a MRI unless being screened for breeding purposes or undergoing tests for other conditions. The expense of an MRI is prohibitive and consequently the author has been involved personally in both fundraising and recruitment over the last decade to enable breeders and pet owners willing to screen their dogs at no cost to themselves in order to help the breed. Syringomyelia DNA Research was set up in 2006 as a dedicated account for voluntary donations which have funded over 75 dogs in UK, Canada and Australia have been funded by private donations. A dedicated fundraising scheme called 'Rupert's Fund' was set up in 2011 specifically to identify dogs over five years of age free of late onset SM.

Methodology

In an initial GB study in 2014, a framework of 14 measurements were made on T1-weighted sagittal MRI on the hindbrain and craniocervical region and illustrated in Fig 1.1 below [94]. Since the DICOM images were obtained from a variety of sources and variable quality it used landmarks of the most easily identified features. First the image was "windowed" to create improved contrast and highlight bony landmarks (upper image Fig 1.1 with an inset magnification of the area of the optic nerve and canal). The lower image demonstrates the same without the windowing effect. These morphometric measurements were subsequently used in quantitative trait loci analysis [229].



Fig. 2.1: Midline sagittal T1-weighted MRI of hindbrain and craniocervical region of a three year old female GB with the framework of measured lines and angles

Key: All line measurements start from one of 9 points:

(a) dorsum of spheno-occipital synchondrosis

(b) basion of basioccipital bone

(c) rostral edge of the dorsal lamina of the atlas

(d) junction between the supraoccipital bone and the occipital crest

(e) most dorsal point of intersection of the cerebellum with the occipital lobe circle

(f) center of occipital lobe circle placed on the cranial baseline (HAI) and extending to encompass

the occipital lobes. The centre of the circle is F

(g) point at which the optic nerve deviates into the canal (inset)

(h) most caudal point of the olfactory bulb

(i) intersection point with the extended HA baseline

5 angles measured are (1) abc (2) caf, (3) aid, (4) agh and (5) afg.

Validation of significant morphometric traits in a mixed breed Griffon Bruxellois family

A simplified framework of measured lines and angles is used to validate the conformational features associated with CM in the mixed breed family (Fig 2.2.) The grid consists of the three lines ae, bc and f-diameter and two angles fac and agd (called aid in previous study). These had the greatest significance associated with CM, four which were also significant traits for SM, and had actually been linked to particular chromosomes.

Fig. 2.2. Midline sagittal T1-weighted MRI of brain and craniocervical region of a female GB backcross



Key:

(a) dorsum of spheno-occipital synchondrosis

(b) basion of basioccipital bone

(c) rostral edge of the dorsal lamina of the atlas

(d) junction between the supraoccipital bone and the occipital crest

(e) most dorsal point of intersection of the cerebellum with the occipital lobe circle

(f) centre of occipital lobe circle placed on the extended cranial baseline (AB) (G) intersection point with the extended AB baseline and DC.

The five traits measured in the study are lines ae, bc and f-diameter (blue) and angles FAC (yellow) and AGD (red).

A comparison of these traits in a family would allow them to be tracked in each generation in order to validate their association with CM and SM and identify any segregation to suggest mode of inheritance.

Characterisation of CM in the Cavalier King Charles Spaniel

It is hypothesized that the clinical consequences of CM and SM that result from changes in brain and spinal cord conformation in the GB are the same as those in the CKCS and that segregated traits are additive to the severity of the condition. Since CM is ubiquitous in the breed it is possible to distinguish between those that are symptomatic with CM but do not have SM. Additional measurements to assess brachycephaly might clarify any association with symptomatic CM and SM

CKCS Hindbrain Study

Fig 2.3 illustrates the 24 measurements taken (13 lines and 11 angles) and used to construct a 'grid' that generated a craniocervical 'map' of each sagittal image in the study. Five lines and three angles that were significant for CM in the Griffon Bruxellois are marked with a * [94].

The measurements used in the original GB investigation were augmented to include:

- i. The position of the odontoid process (dens) relative to the atlas since this was thought to impact on the degree of cranial cervical stenosis, angling of the medulla and/or obstruction of CSF channels.
- ii. Additional triangulation of angles arising from the basicranium to landmarks in the cranial caudal fossa to reflect any overcrowding of the cerebellum and medulla oblongata.

Fig. 2.3 Twenty-four measurements used to map the hindbrain and craniocervical junction on T1w mid-sagittal MRIs of a CKCS without SM.



Key

(a) dorsum of spheno-occipital synchondrosis

(b) basion of basioccipital bone

(c) rostral edge of the dorsal lamina of the atlas

(d) junction between the supraoccipital bone and the occipital crest

(e) most dorsal point of intersection of the cerebellum with the occipital lobe circle

(f) centre of occipital lobe circle placed on the baseline at the level of the basioccipital bone (ab) and extending to encompass the occipital lobes. Diameter of circle = f-diameter

(g) point at which the optic nerve deviates into the optic canal

(h) rostral edge of supra-occipital bone

(*i*) intersection point with ventrally extended line dc with the caudally extended ab baseline (forms angle 3 dib)

(j) most rostral aspect of the dens of the axis bone

(k) extended line from point b along the best fit line of the ventral medulla oblongata to where it changes angle to the spinal cord.

(l) rostral extension of baseline abi (hence becoming baseline labi)

11 angles measured are (1) lae, (2) fac, (3) dib, (4) fae (5) aeb (6) abd (7) bdi (8) ebd (9) jcb (10) afg (11) dbk.

* significant for CM in the Griffon Bruxellois [94]

CKCS Whole Brain Study

The minimum inclusion criteria was imaging of the entire brain parenchyma and cervical region. In order to ensure consistency with the hindbrain study, 32 dogs that had both T1W and T2W were included (i.e. overlapped), together with 11 'hindbrain' measurements. These comprised: angles 2,3,7,9 and 10, lines ab, bc, ac, ai, and bk and the diameter of 'best fit' occipital circle with centre at f (f-diameter). All these and the additional measurements i- iii listed below were recorded by author SPK using the DICOM reading software package Mimics[®].

Additional measurements illustrated in Fig 2.3 included: -

- i. Forebrain circle (diameter m) and distance/line mf
- ii. Olfactory circle (diameter n), the distances/line nf and angles mfn and nfa
- iii. Total brain area and its ellipticity (E). Ellipticity is defined as a mathematical relationship between of the largest radius to the smallest radius in the ellipse and measures how ovoid the shape is. Both E and brain area were calculated automatically by the Mimics® software programme.

A further 6 measurements were recorded by collaborator Ms C. Cross viewed in eFILM [™] 18.

- iv. two measurements that represented flattening of the rostral forebrain
- v. olfactory bulb size (length and width)
- vi. the angulation of the olfactory bulb with the hard palate

Fig. 2.4 Additional morphometric measurements taken of the T2w mid-sagittal brain MRI of a CKCS with CM pain.



Key : For identity of points a-l see Fig.2.3. Three 'best fit' circles (coloured aqua) and an ellipse (coloured red) that follow the outline shape of the neural parenchyma as closely as possible.

- top angle angulation between the frontal and parietal lobes
- bottom angle -angulation between the dorsal OB and the frontal lobe
- *OB angle angulation between the OB and hard palate*
- *mfn and nfg*.

Comparison of CM in different breeds

In this study T1W midsagittal brain and upper cervical spinal cord images were compared using 19 measurements taken (11 lines and 8 angles) and used to construct the now familial 'grid framework' to explore the caudal fossa and craniocervical junction and record differences in the juxtaposition of hindbrain, spinal cord and skull in dogs with and without SM. The CKCS, shown to have similar traits to the GB, is compared to the miniature Chihuahua and the brachycephalic Affenpinscher which is quite similar to the Griffon Bruxellois in conformation (Fig 2.5).

Measurements used in the original GB investigation [94] (marked *) were augmented to include:

- i. The position of the odontoid process (dens) relative to the atlas since this was hypothesised to impact on the degree of craniocervical stenosis, angling of the medulla and/or obstruction of CSF channels
- ii. Additional triangulation of angles arising from the basicranium to landmarks in the caudal fossa to reflect any overcrowding of the cerebellum and medulla oblongata.

Fig. 2.5 T1W sagittal MRI of a five year old Chihuahua without SM with a framework of 19 measurements (11 lines and 8 angles) with three ratios used to 'map' the hindbrain and craniocervical junction.



Key

- (a) dorsum of spheno-occipital synchondrosis
- (b) basion of basioccipital bone
- (c) rostral edge of the dorsal lamina of the atlas

(d) junction between the supraoccipital bone and the occipital crest

(e) most dorsal point of intersection of the cerebellum with the occipital lobe circle

(f) centre of' best fit' occipital lobe circle placed on the cranial baseline (abi) and extending to encompass the occipital lobes. The centre of the circle is f and its diameter (f-diameter) indicates the maximun height of the caudal calvaria dorsal to the spheno-occipital synchondrosis (g) point at which the optic nerve deviates into the optic canal (*i*) intersection point of the extended cranial baseline (*ab*) caudally with extended line *dc* ventrally to form angle 3. This indicates the relative positions of the supra and basioccipital bones to the atlas. (*j*) most rostral aspect of the dens of the axis bone.

(k) extended line from point b along the best fit line of the ventral medulla oblongata to where it changes angle to the spinal cord (degree of medullary kinking).

8 angles measured are (1) afg, (2) fac, (3) dib, (4) jcb (5) aeb (6) ebd (7) bdi (8) dbk * trait used in previous GB study [94].

Standardisation of morphometric traits using 'best fit' circle and angles.

The 'best fit' circle is used as a means of standardizing morphometric traits between dogs of various sizes and breeds. The diameter of the circle provides a linear value and the radius can be used as a proportional distance for other measurements such as the relative position of the other anatomical features such as the cerebellum to the basioccipital bone. This feature has been identified as a statistically significant feature in the GB foundation study. It is especially important for images for which the forebrain is unavailable as a means of reference for the neural parenchyma. The size of the circle is governed by the shape of the occipital lobes within the caudal cranial fossa and in addition to the distance between the cranial height and the skull base. Angles associated with the 'best fit circle' have been used to provide comparative values for the position of anatomical features relative to one another.

Data Analysis

The sagittal MR images were anonymised and measurements were taken by author S P Knowler, initially blinded to SM status, using a DICOM reading software package Mimics® 14.12 – 16.0 Materialise (15 3001 Leuven Belgium). This programme allows viewing of DICOM images and has a windowing facility to control the image contrast hence enabling consistent identification of bony landmarks and, in addition, precise tools to measure the juxtaposition of the cerebrum and cerebellum and generate three-dimensional 'masks' of the brain. The measurements were collated with Microsoft Excel 2007 (http://office.microsoft.com/en-us/excel/).

Adobe Photoshop (<u>http://www.adobe.com</u>) was used to resize and aligned the framework of angles and lines and while keeping the ratios constant. It provides the ability to manipulate images while ensuring that the integrity of the original image is retained. Quantitative comparisons are possible with reorientated or resized and the use overlays thus provide a powerful visual tool.

Morphometric mapping and 'morphing' technique

Multiple measurements were taken which used both bone and tissue landmarks to explore the brain and craniocervical junction. Because the lines and angles are interrelated, any deviations from the 'normal' juxtaposition of hindbrain, spinal cord and skull could enhance understanding of the pathogenesis of CM and SM. To take account of differences in natural anatomical variations in the CKCS, particularly

size of head, two trait ratios were included in the analysis. These were related to the height of the cranial fossa to the representative distance across the foramen magnum (f-diameter/line bc) and the height of the supraoccipital bone (f-diameter/line cd). Advanced statistical analysis has the potential to ascertain those traits best suited to identify the abnormalities associated with CM/SM.

The morphometries, as with the GB study, are underpinned by the 'best fit' occipital circle f. As previously stated, this circle follows the contour the occipital lobes dorsal to the cerebellum and extends caudally and ventrally to the level of the skull base (i.e. basioccipital bone) as closely as possible. The diameter of the circle provides a linear value of the approximate height of the caudal cranial fossa parenchyma, while the circle radius provides a proportional distance to assess the juxtaposition of anatomical features such as the inter-thalamic adhesion, sella turcia, etc. Moreover, the circle provides an important means of standardizing the morphometric traits between various sizes of dog.

JPEGs of the images generated by Mimics[®] were imported into Photoshop[®]. By applying the 'maintain fixed ratio' tool of the software, the images were resized so that the occipital circle was the same size in all exemplar images and the skull baseline ' labi ' was rotated to the horizontal plane and aligned to facilitate comparison of skull angulations. These images were used to make a photo morphing movie using Abrosoft Fantamorph[®] 5.4.5 software (<u>http://www.abrosoft.com)</u>.

Statistical analysis

Statistical analysis and interpretation was supervised by Statistical Consultant Dr Angus McFadyen (akm-stats). IBM SPSS for Windows[®] v20-22 was used to calculate measurement reliability (Intraclass Correlation Coefficient (ICC) model) and to analyze variables in the study cohort

Analysis of Variance (ANOVA), with a Bonferroni correction for multiple testing was used to analyze the traits for total cohorts. The independent sample t-test with Levene's test for equality of variance was used for differences between paired subgroup combinations. Descriptive statistics (box plots) were generated for selected significant variables. Correlations were tested with Pearson r correlation test. Any significance between groups was tested using Linear or Multiple Regression models. P-values were considered significant: < 0.05 for ANOVA and with Bonferroni correction, < 0.017 for the t-test. Since segregated traits associated with CM and SM have been shown to be additive to the severity, Linear Discriminant Function Analysis (DA) were applied to the data in order to examine the relationships between the significant variables in more depth. DA takes account of any correlations between variables and how reliable these are for predicting the group to which each dog had been assigned. This method is therefore helpful to identify the most important phenotypic trait variables that distinguish between each group. In such an analysis, the selected traits are evaluated by using cross-validation to avoid data bias and to confirm the prediction model. Although DA makes more assumptions regarding the data and hence is perhaps less flexible than Logistic Regression, it has the advantage of providing a pictorial

representation of the classifications of more than two groups. It has been shown via simulations that, when the normality assumptions are not too drastically violated, there is very little difference in the results between DA and Logistic Regression and both are equally robust techniques for classification models.

Reliability Analysis

Measurement reliability was assessed for inter and intra observer reliability using intra-class correlation [ICC] in SPSS; A mixed model with 10 dogs was used to assess intra measurer reliability of 2 lines, 2 angles and 1 circle repeated with a time interval of a year by author S.P. Knowler. ICC values less than 0.5 are indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.90 indicate excellent reliability. Intra-rater reliability was found to be ICC value 0.86 for author S P Knowler and indicated a relatively high consistency maintained between the previous GB study [94] and the CKCS study (different breed).

Ten dogs were also measured independently by collaborators Ms C Cross and Dr C Rusbridge to assess inter-observer reliability in the CKCS whole brain study. After these measurements were obtained, Ms C Cross then made an independent second measurement of 10 cases to ensure high intra-observer reliability: All exceeded 0.96 and 95% confidence intervals were considered narrow.

Ethics Statement.

All studies were retrospective study was based the analysis of MRI (DICOM) obtained for diagnostic purposes or for pre-breeding health screening for CM/SM. Full written consent was obtained from all owners and actual dogs remained anonymous (reference NASAP-2015-001-SVM).

Ethics approval for the mixed breeding project was not sort because the dogs were family pets that lived with owner and breeder Henny van den Berg. All breeding, both mixed cross and pedigree, complied with the rules and authorization of the Raad van Beheer op Kynologisch Gebied. This is the principal cynological organization in the Netherlands which oversees pedigree dog health and welfare and regulates breeding registrations, including inspections of breeding premises, microchipping and DNA profiling.

Chapter 3

Validation of the morphometric measurements in previous Griffon Bruxellois (GB) studies using a family study and mixed breeding

Introduction

Morphometric analysis of 155 DICOM T1W mid sagittal images of the brain and cervical region of GB dogs identified six significant traits for CM characterised by an apparent shortening of the entire cranial base and possibly by increased proximity of the atlas to the occiput. As a compensatory change, there appeared to be an increased height of the rostral cranial cavity with lengthening of the dorsal cranial vault. The considerable reorganization of the brain parenchyma included ventral deviation of the olfactory bulbs and rostral invagination of the cerebellum under the occipital lobes. A unique opportunity to validate these findings was provided by an accidental mating between the brachycephalic Griffon and a mesaticephalic Australian terrier. Through MRI screening of the parents, first generation offspring (F1) and a subsequent backcross to the pure GB, it was possible to track key significant traits associated with CM and SM and compare them to those of pure breeding dogs in the GB family. Additionally, analysing the DICOMs of the extended GB family of the hybrid cross made it possible to investigate the inheritance of CM/SM.

Pedigree analysis of human familial aggregations of CM-I malformation which is similar to CM, suggest both autosomal dominance with reduced penetrance [31,218] and autosomal recessive [241], but most likely the pattern of inheritance is oligogenetic and determined by the cumulative effect of variants in various genes [23]. Canine CM does not appear to segregate in a Mendelian manner in the familial studies of CKCS and the evidence supports the multifactorial nature of inheritance [13,35,242]. It was hoped that an analysis of the key significant traits identified for CM and SM in Fig 2.2 within the context of a four-generation family would not only provide validation, but also illuminate aspects of the mode of inheritance in this complex disorder. Therefore, the specific aims of this chapter were as follows:

- 1) To investigate five significant phenotypic traits for CM in the GB, Australian terrier and their hybrid crosses.
- 2) To elucidate any patterns of inheritance by comparing significant phenotypic traits related to both CM and SM in a family of pure and mixed breeding dogs.

Study Cohort

The family (n=27) comprised three foundation bitches C,D, H and two foundation dogs E and K that were part of the previously mentioned larger 155 GB cohort [94] used in genetic studies[229]. Furthermore, foundation Dog E was the offspring of a CM/SM affected GB that formed part of separate family (n= 32) where CM was investigated using radiographs [44]. The study cohort has two key black and tan coat GB siblings; a CM affected male (dog A) involved in the outcross to the Australian terrier bitch and his unaffected sister (bitch G) whose pure breed GB male offspring was used in a backcross to the F1 hybrid. Unlike the majority of countries, in the Netherlands (the breeder's home), the black and tan coated GB is called a Griffon Belge and considered a separate breed (race) to the GB by the Federation Cynologique Internationale (FCI) [243]. However, in order to ease reading, different races of Griffon Bruxellois are referred collectively as GB in this manuscript. Two of the dogs in the extended family cohort (dogs A and H) had three matings each (i.e. comprising six matches) and the two older dogs that had previously been bred and scanned made a total of eight matches in the study.

Apart from Dog E, the entire family group were owned by co-author and breeder Henny van den Berg. Mating decisions were entirely those of Henny van den Berg and based on her assimilated knowledge of the CM and SM, the MRI status of the parents, head shape and coat colour. Selection for conformation in dog breeding is subjective and morphometric measurements were not considered by the breeder. Observations of head shape from previous matings that produced SM affected dogs prompted her selection of dogs with the longest skulls to mate with her CM affected dogs with shorter skulls. Selection was not based on head shape only; other factors were taken into consideration for example temperament and gait. All the dogs lived in the same household during the whole study period with similar vaccinations, exercise and a raw meaty bones diet, thus minimising any environmental factors that might influence multifactorial traits such as CM and SM. T1W sagittal DICOMs of the brain and cervical region were available for 26 of the 27 dogs. Dog 27 was euthanized when severe CM/SM was diagnosed in a preliminary MRI before a sagittal image was obtained. Nineteen offspring were imaged at 12 -15 months. Seven dogs were rescanned and their revised CM and SM status reported. All the dogs in the study were scanned at the same veterinary centre using a 0.2 Tesla MRI machine (Esaote Grande, Italy) [244].

Results

Study group CM/SM status and family relationships

The CM/SM status and family relationships are summarized in Fig 3.1 and in Table 3.1.

Fig 3.1 Study cohort GB family tree (n=27) including a mixed cross to an Australian terrier and subsequent backcross.



Offspring were designated the combined letters (lowercase) of their parents to facilitate understanding of the relationships. Bitch C and Dog F were both a parent and an offspring so designated a single letter as a first generation parent (P1) to ease reading. Hence ed1 is renamed 'C' and 'eh3' renamed 'F'. Only one of the 19 offspring was CM0 (F1 hybrid ab1). There were four offspring with CM-I; GB ac2 and fg3 and both second filial (F2) backcross progeny (abfg1 and abfg2). The 13 remaining offspring were CM2.

Parental	Sire			progeny number				progeny number			Dam		
	CM/SM status			СМ			litter	SM			CM/SM status		
cross	СМ	SM	code	CM0	CM1	CM2	Total (N)	SM0c	SM1c	SM2c	code	СМ	SM
AxB*	cm2	sm0b	А	1	0	3	4	3	1	0	В	cm0	sm0a
AxC	cm2	sm0b	Α	0	1	1	2	1	0	1	С	cm1	sm1b
AxH	cm2	sm0b	Α	0	0	2	2	1	0	1	Н	cm2	sm0b
ExH	cm1	sm2b	Е	0	0	3	3	2	1	0	Н	cm2	sm0b
KxH	cm2	sm1c	К	0	0	2	2	0	1	1	Н	cm2	sm0b
ExD	cm1	sm2b	Е	0	1	0	1	0	1	0	D	cm2	sm0a
FxG	cm2	sm0c	F	0	1	2	3	3	0	0	G	cm0	sm0c
ABxFG#	cm2	sm0c	FG1	0	2	0	2	2	0	0	AB4	cm2	sm0c
total (N) progeny				1	5	13	19	12	4	3			

Table 3.1 Summary of CM and SM status in family study group

The progeny could not be confirmed lifetime clear of the disease because SM can be a late onset disease and the offspring were MRI screened at one year old. According to the BVA/KC nomenclature for SM, dogs screened less than three years of age are designated by the letter c. However, in this manuscript, to facilitate easier reading, a letter accompanying the SM grade is not stated in the text unless the dog underwent a MRI aged three to five years (designated b) or greater than five years (designated a). Three progeny (ac1, ah1 and kh1) and one parent E were SM2 affected. Two dogs, C and K, were SM1a and b respectively. Dogs eh1 and kh2 were both SM1 at one year but when re-scanned at three years eh1 remained SM1b. In contrast, F1 Hybrid ab3 was SM1 at one year and SM0 when rescanned at 2.7 years. Parents B and D were SM0a.

Magnetic Resonance image morphometric measurements

Descriptive statistics for significant traits for both CM and SM are provided as Boxplots in Fig 3. 2.

Fig 3.2. Boxplot distribution of significantly associated traits for CM and SM BVA/KC grades (n=26).



Top row; 0 = CM0 (normal), 1 = CM-I (intermediate), 2 = CM2 (affected). Bottom row; 0 = SM0 (normal), 1 = SM1 (intermediate), 2 = SM2 (affected).

Table 3.2 compares the mean, maximum and minimum trait values for the six mixed breed offspring and the 12 pure-breed offspring relatives compared to previous baseline study (GB control cohort) together with the trend for CM and SM risk [94]. The means of the mixed breed group poses less risk for all traits than the other groups and the pure-breed group less risk than the control group.

		CM traits								
		f- diameter	Line bc	Line ae	angle FAC	angle AGD				
Cohor	mm	mm	mm	degrees	degrees					
	Mean	41.96	16.48	30.7	74.36	69.09				
Mixed Breed	Minimum	40.24	15.34	29.14	70.44	65.13				
Onspring ii=0	Maximum	45.31	18.69	32.67	79.4	74.35				
	Mean	42.8	14.68	30.61	74.53	79.41				
Purebred GB	Minimum	40.2	11.85	29.27	67.63	63.52				
onspring n=15	Maximum	46.11	16.84	33.02	84.31	105.7				
	Mean	42.47	13.15	31.68	71.2	81.93				
GB control	Minimum	37.8	10.68	28.09	52.83	64.49				
group n=155	Maximum	47.85	17.27	36.4	86.51	109.21				
Less risk for CM	if value is	smaller	longer	shorter	wider	smaller				

Table 3.2. Descriptive Statistics for five traits in Mixed, Pure and control GB Groups.

Table 3.3 provides individual values of the morphometric traits for the three generation F2 Backcross arranged as a pedigree so that comparisons are easier to view.

Breed/cross	P1	P1	F1	F2	F2	F1	P1	P1	
	Griffon	Griffon	Griffon	backcross	backcross	Hybrid	Griffon	Aust. T	
Dog code	F	G	fg1	abfg1	abfg2	ab4	Α	В	* Less risk
CM status	CM2	CM0	CM2	CM1	CM1	CM2	CM2	CM0	if value is
f-diameter	43	40.8	43.8	45.3	40.2	40.3	42.2	40.2	smaller
line bc	14.5	16.1	13.5	17.7	15.7	15.8	16.8	18.7	longer
line ae	30.2	30.5	31	32.7	29.1	29.7	32.1	29.3	shorter
Angle FAC	80.2	73.9	69.7	70.6	78.5	72.9	73.7	80	wider
Angle AGD	77.8	69.5	92	65.1	66.3	69.7	66.9	58.6	smaller

Table 3.3 Morphometric measurements for three generation the F2 backcross pedigree.

The degree of risk for CM is indicated by the trend in values (*final column of the Table 3.3). Both F2 backcross progeny exhibited varying degrees of intermediate CM-I, but bitch abfg2 has only one out of the five risk factors: a shorter line bc. However, dog abfg1 has three of the five risk factors; larger f-diameter, longer line ae and smaller angle FAC. The dogs with no CM (B and G) have a smaller f-diameter, longer line bc, short line ae and smaller angle AGD. Furthermore, Bitch G (CM0), when compared to sibling A with CM2, had a smaller f-diameter and line ae (less risk), similar line bc and angle FAC and angle AGD.

Fig 3.3 illustrates the traits as a framework superimposed on the MRIs.



Fig 3.3. Key morphometric measurements made on the MRIs of dogs in three generation pedigree of the F2 Backcross.

The CM0 F1 hybrid ab1 provides an additional control comparison. The lines and angles have been linked together providing a visual representation of the interrelationships between the individual traits. Sire fg1 and son abfg1 have similar values for angle FAC($\sim70^\circ$) but, different values for line bc. Similarly, dam ab4 and daughter abfg2 have similar trait values for line bc (~15.7) but different values for angle FAC.

Syringomyelia

Four of the dogs in the cohort (E, ac1, ah1 and kh1) had syringomyelia (SM2) and four dogs (K, C, eh1 and kh2) had central canal dilation less than 2mm (SM1). All these dogs are related to either Dog E with SM2 or Dog K with SM1 (Fig 3.1) with the exception of offspring ah1. Angle FAC was of special interest because it had been found to be significant for CM and not SM in the former baseline study [94]. Fig 3.4 illustrates Bitch H with 5 offspring from three different sires. This bitch (CM2 SM0a) was described by the owner/co-author as having a 'huge head' compared to the breed average and she has a large angle FAC. When mated to Dog K with small angle FAC (SM1b) the two offspring with small angle FAC (kh1 and kh2) both had SM. The offspring with larger angle FAC (kh3) did not have SM. Fig 3.4 the head shape and MRIs of Dogs K, H and kh1 are illustrated in together with further examples of Bitch H's other matches with Dogs A and E. The SM affected dogs demonstrate the lack of skull development caudal to the ear pinna (behind the ears) compared to dogs with no SM. This supports the radiographic evidence in the previous GB family study [44] and head conformation in the CKCS [63].



Fig 3.4. Head conformation and associated angle FAC in six relatives of Bitch H with and without SM.

TW1 sagittal MRI of the caudal fossa and cranial-cervical junctions with superimposed morphometric framework of lines and angles for parents and offspring enhances comparison. The differences in the size of angle FAC are reflected in the lack of skull development caudal to the ear pinna (behind the ears) for dogs with SM compared to dogs with no SM ('normal' caudal skull shaded aqua colour). The photos of the heads have been resized to allow comparison using two vertical lines (black) placed at the outer eye and the origin of the external pinna (red) a consistent distance apart.

Discussion

Chiari-like Malformation has been shown to be a risk factor for SM and this developmental malformation can also be painful and result in decreased quality of life [8,245]. In this study five conformation traits for CM were analysed in an extended family involving a mixed breed cross which took advantage of an accidental mating between a mesaticephalic breed and the brachycephalic GB inspiring the three year study. T1-weighted sagittal DICOM images were compared with a purebred outcross family with varying affectedness for CM and SM. The results generated here builds on data from four previous studies in the GB that investigates the phenotype, the risk of CM and SM and its inheritance and the successful identification of two Qualitative Trait Loci and candidate genes [44,53,94,229]. Furthermore, analysis of familial morphometric differences in the eight litters and 19

progeny, we were able to identify potential traits that might be useful for grading the severity of CM and SM.

Clarification of the CM phenotype

The framework of traits proved useful in refining the definition of CM. Both the "angle" traits relate to the displacement of the atlas bone relative to the dorsum of the supraoccipital bone and skull base. An increased angle AGD reduces the volume (space) available for CSF to flow freely. Differences in angle FAC reflect the alignment of the atlas and supraoccipital crest, the degree of 'invagination' of the cerebellum under the cerebral parenchyma and proximity to the sphenoid occipital synchondrosis. Therefore, if line bc from the basion of the basioccipital bone to the rostral edge of the dorsal lamina of the atlas (i.e. distance across the craniocervical junction) is considered in combination with the other traits, this may impart an additional degree of risk for overcrowding and result in SM or painful CM. Since all the traits exhibited continuous variation, it is feasible that angle FAC, significant for CM, may be protective for SM when it is wide and that the combined effect on the caudal fossa volume might be additive if the angles and lines are considered together rather than individually.

This study supports the hypothesis that CM in the dog is a more global skull disorder rather than a caudal skull abnormality. Furthermore, the occipital bone insufficiency associated with rostral cranium doming seen in dogs K, kh1 and ah1 Fig 3.4 (aqua arrow) has been documented in other studies [8,44,246] and determined to be risk factor for SM in a study of conformation in the CKCS [63]. Repeat MRI screening for late onset SM offers the opportunity to monitoring morphometric changes over time [56].

Patterns of inheritance for CM/SM

This study is too small to make conclusions about inheritance. However, the F2 backcross had CM-I status from CM2 parents despite the high incidence of inherited CM in the other family members (Fig 3.1). These results, together with the fact CM is known to skip a generation in an earlier familial study of 33 GB [44], suggests the involvement of recessive traits that are protective against CM and/or gene penetrance is variable involving individual traits that are additive in severity. Line bc is associated with CFA9 and CFA24 and line ae with CFA14 [229], i.e. these segregated traits may or may not be expressed in any one individual as illustrated by the variations in F1and F2 generations.

SM2 dog E and SM1 dogs C and K were mated to SM0 dogs. Of their eight offspring two were SM2 and two SM1. SM0 parents A and H when mated together produced both an SM0 and SM2 offspring. All these findings support evidence from two previous studies of inheritance in the BG [44,53].

Chiari-like Malformation

An outcross does have the potential to reduce prevalence of inherited CM and SM. In eight matches only one offspring (hybrid ab1) was CM0. However his external conformation resembled his mesaticephalic Australian terrier dam and least desirable phenotypically. The purebred GB outcross with parent G with CM0 produced one of three offspring with CM-I. Three remaining CM-I dogs were all related (E, C and ac2) suggesting they had inherited similar skull conformation. The 13 CM2 progeny all had at least one CM2 parent. However, an exception was the F2 backcross progeny. These had less severe CM-I than both their CM2 parents.

Syringomyelia

Although the offspring were too young to confirm SM clear status, the morphometric measurements used in this study provide an indicator of risk for SM. For example, any reduction in distance between point B and C represents less area for the hindbrain. An increased f-diameter (height of the rostral cranial cavity) is a significant risk factor for CM and SM [94]. This increase in height is thought to be a developmental compensation to accommodate the forebrain and occurs in response to cranial base craniosynostosis and overcrowding in other parts of the skull [112]. In a previous study of 93 Griffons [94], 67 of the dogs had CM (72%) but nine of the 26 CM free dogs had SM and were similar to Dog E in this study. Although the cerebellum is not compressed or herniated into the foramen magnum, it is invaginated rostrally under the parenchyma of the occipital lobes and/or there is generalized ventriculomegaly indicating obstructed CSF dynamics. This is possibly due to arachnoid adhesions [16], but the possibility that there may be a thoracic or lumber syrinx cannot be ruled out because only the caudal spinal cord was imaged [247].

Summary

This is study of an outcross between a normal mesaticephalic Australian terrier and GB with CM and subsequent backcross to a GB. Techniques developed in an earlier study to quantify CM and SM in GBs were used as a control and applied to the extended family group to investigate inheritance of CM and SM in pure and cross breeding. Comparing the familial inheritance of five significant traits on MR images associated with CM and SM we showed variants exhibited segregation and suggested that a protective role existed. The traits were useful to quantify CM and SM and useful to distinguish the phenotype. Furthermore such variants may be additive towards the severity of CM and SM. The definition of CM is refined as a more global cranium and craniocervical junction abnormally characterised by insufficiency of the supra and basioccipital bones with compensatory rostral cranium doming, shortening of the skull base and increased proximity of the cervical vertebrae to the occiput resulting in overcrowding of the neural parenchyma in the caudal fossa. These craniocervical traits may be useful to quantify CM and risk of SM to assist breeders with mate selection. Such a system requires validation to ensure appropriateness for all breeds at risk.

Chapter 4

Use of morphometric mapping to characterise symptomatic Chiarilike malformation, secondary syringomyelia and associated brachycephaly in the Cavalier King Charles Spaniel

Introduction

Syringomyelia has been extensively investigated in the CKCS for over a decade. CM, defined in terms of the caudal cranial fossa, is ubiquitous in the breed [20,37,51,60] and cerebral cranial volumes [93,248]. Despite this, there has never been any correlation found between the severity of CM, SM and clinical signs[73]. The question remains as to why some dogs are symptomatic and others are not when graded the same CM status.

Other studies have associated brachycephaly with CM and SM [63,114] but the exact correlation between these features has yet to be elucidated. In the previous chapter, the morphometric analysis of CM and SM in the GB and a mesaticephalic cross indicated that segregated traits were additive towards the severity of the CM phenotype and helpful in confirming the risk of brachycephaly. One trait played a protective role for GB dogs at risk for SM [249]. In the CKCS, a genome wide linkage study also identified a novel locus for SM associated with CM and a haplotype that inferred protection against SM [250]. In this chapter, the study aims is to quantify symptomatic CM, SM and associated brachycephaly in the CKCS breed by mapping T1W DICOM in the mid-sagittal plane hindbrain and craniocervical junction using refined morphometric techniques detailed in Chapter 2, Fig 2.3. These hindbrain images the widely available because they form part of the protocol for the BVA/KC scheme. However, in order to investigate the impact of brachycephaly, mid-sagittal MRIs of the entire brain are required so that rostral forebrain flattening and olfactory lobe rotation are included (Chapter 2 Fig 2.4. As outlined in more detail in the methodology section, two separate studies were completed because i) the T1W study was consistent with the previous study ii) the difficulty of obtaining sufficient numbers of control dogs and suitable MRI data with whole brains and iii) T2W DICOM are better suited for the detection of water and fluids [94]. The olfactory bulb of brachycephalic dogs is ventrally orientated [251] and it is postulated that this may be more extreme in CM.

Brachycephaly is defined as foreshortening of the facial skeleton with restricted growth of the basiccipital and basisphenoid bones manifesting as a shortening of the basicranial axis [112]. The CKCS breed description [252] indicates a greater cranial facial length than the GB, and although head conformation studies show an increased cranial index to be a risk factor for SM [63], it is not known if a more ventrally orientated olfactory bulb or rostral forebrain flattening are also risk factors.

It is hypothesised that the clinical consequences of CM and SM that result from changes in brain and spinal cord conformation in the GB are the same as those in the CKCS and that segregated traits

including brachycephaly are additive to the severity of the condition. Therefore the specific aims of this chapter were as follows:

To quantify symptomatic CM, SM and associated brachycephaly in the CKCS breed by

- Mapping the hindbrain and craniocervical junction using refined morphometric techniques previously validated in the GB [94] and mixed breed [249]. These employed T1W MRI in the mid-sagittal plane.
- Quantifying brachycephalic conformation in association with CM/SM by mapping the entire brain to include rostral forebrain flattening and olfactory lobe rotation on T2W MRI in the mid-sagittal plane.

Study Cohort

The study dogs comprised 162 CKCS. The DICOM data was identified from two sources:-

- 90 CKCS undergoing diagnostic investigation that included T1W imaging of the brain at the Stone Lion Veterinary Hospital or DICOM that has been sent to CR for the purposes of diagnostic interpretation and inclusion in the genetic study [250] and selected because they were accompanied by DNA samples suitable for genetic studies.
- 2. 72 CKCS undergoing diagnostic investigations at Fitzpatrick Referrals Ltd over a two year period.

The CKCS were divided into three groups:

Control cohort: unaffected CKCS without SM without any clinical signs and/or behavioural signs of pain reported by their owners. These comprised SM0 and SM1 dogs that were five years or over or age matched dogs.

CM Pain cohort: CKCS without SM but with clinical and/or behavioural signs of neuropathic pain for example vocalisation, unwillingness to exercise and being withdrawn. These signs had been consistent over months and other sources of pain had been eliminated. They were SM0/SM1 dogs of any age.

SM cohort: CKCS with SM with or without clinical and/or behavioural signs of pain. These comprised SM2 dogs of any age.

Table 4.1 provides the ages and numbers of dogs assigned to each group. In order to limit ambiguity, the Control cohort in the hindbrain study comprised SM0 dogs only over the age of 5 years (mean age 6.2 years, median 5.4 years). The Control cohort for the whole brain study mean was age 4.4 years (median 4.9 years).

	н	indbrain(T	W1) study	Whole brain (TW2) study					
age	'Normal'	CM pain	SM	Total	'Normal'	CM pain	SM	Total	
≥5years	31	16	18	65	7	11	7	25	
3 -4.9 years	0	4	23	27	7	6	7	20	
<3yrs	0	8	30	38	2	8	17	27	
Total	31	28	71	130	16	25	31	72	

Table 4.1. Study groups with numbers and ages of CKCS in the three study cohorts

Hindbrain Study

The study included the T1W mid-sagittal images of 130 (78 females and 52 males) CKCS. Minimum inclusion criterion was imaging of the hindbrain and cervical region. All 90 images from Stone Lion Veterinary Hospital plus 40 T1W images from Fitzpatrick Referrals Ltd. Fig 2.3 (Chapter 2) illustrates the 24 measurements taken (13 lines and 11 angles) and used to construct a 'grid' that generated a craniocervical 'map' of each sagittal image in the study.

Whole brain Study

This study of 72 CKCS (39 males and 33 females) used anonymised and randomised T2W sagittal images of whole brain Fitzpatrick Referrals Ltd. Fig 2.4 (Chapter 2). Overall, 50 measurements, comprising 24 hindbrain and 26 whole brain were made to quantify the phenotypic differences CKCS with and without SM and with CM pain.

Results

Hindbrain study

In this study cohort 61 of 130 dogs (46.9%) had insufficient imaging of the forebrain prohibiting measurements of line fg and angle 10 (afg) (called angle 5 in GB study). Statistical analysis of the total group employing ANOVA identified 14 significant variables and differentiated by the three possible paired subgroup combinations (independent t-test). Table 4.2 tabulates these results, t-test results with p-values between 0.05 and 0.017 are also reported. The five traits associated with five *Canis familiaris* Autosomes in previous GB studies – f-diameter, lines bc, ae and angles 2, 3 and 10 was tested using a Wilcoxon rank-sum test and passed the significance threshold (P<0.05). Table 4.3 details nine significant traits (in bold) identified in the 72 whole brain group with p< 0.05 for ANOVA and with Bonferroni correction, < 0.017 for the t-test. Angle 2, which was uniquely significant for CM but not SM in the GB study [94,229], was significant in the total group and in combinations involving the control cohort.

Group	1.Total cohort		2.control v SM		3.control	v CM pain	4. CM pain v SM	
	n=130		n=99[28 +71]		n=59[28+31]	n=102[31,71]	
variables	F	p-value	t	p-value	t	p-value	t	p-value
∠3* (dib)	13.53	<0.001	-5.34	<0.001			-2.65	0.011
∠5 (aeb)	13.5	0.007			3.35	0.001		
∠7 (bdi)	14.85	<0.001	4.81	<0.001			4.02	<0.001
∠9 (jcb)	14.8	<0.001			-2.23	0.03	4.59	<0.001
∠10* (afg)	3.88	0.024					2.74	0.008
∠11 (ebk)	4.3	0.016	2.82	0.006	2.26	0.027		
line f-d*	6.3	0.002	-4.04	<0.001				
line ab	4.1	0.019			2.41	0.019	-2.7	0.009
line bc*	6.7	0.002					3.68	<0.001
line ae*	4.9	0.009	-4.06	<0.001				
line ac	4.3	0.015	2.51	0.014			2.3	0.024
line ai	10.7	<0.001	4.62	<0.001			2.91	0.004
line bk	9.5	<0.001	3.16	0.002			4.35	<0.001
ratio f-d/bc	12.4	<0.001	-3.66	0.001			-4.34	<0.001

Table 4.2 Significant traits identified in four analyses of the hindbrain study using ANOVA (1) and independent sample t-tests (2-4).

*trait significant in previous GB studies F: F-test from one factor ANOVA, t: t-test statistic from independent sample t-test.

Whole brain study

 Table 4.3 Significant traits identified in four analyses in whole brain study: 1. Total group using ANOVA, 2-4 paired cohorts using independent sample t-tests.

Group	1.Total group		2.Contr	ol v SM	3.Control v CM pain		4. pain v SM	
	n=	n=72		-47	n	=41	n= 56	
variables	F	p-value	t	p-value	t	p-value	t	p-value
∠2^ (fac)	5.93	0.004	2.25	0.03	3.41	0.002		
∠3* (dib)	4.39	0.016	-2.9	0.007				
∠7 (bdi)	4.57	0.014	2.95	0.013				
∠10* (afg)	4.86	0.011	2.79	0.008			-2.55	0.014
line bk	4.1	0.021			-3.28	0.003		
line ai	5.2	0.008	3.22	0.002				
Ellipticity	7.27	0.01	3.72	0.001	2.73	0.01		
bottom	3.47	0.037					2.82	0.007
OB angle	3.69	0.03			-3.15	0.003		

*trait significant in previous GB studies, OB = olfactory bulb F: F-test from one factor ANOVA significance t: t-test statistic from independent sample t-test.

Statistical analysis using Discriminant Analysis

Discriminant Analysis is a method used in statistics to find linear combination of features that separates two or more groups which all have continuous variables. It proved useful in identifying a total of eight significant traits which best separated the Control cohorts from CM pain and SM cohorts. The DA canonical coefficients for each group (Appendix Table 4.3) are plotted in the conventional manner in Fig 4.1. Function F is the ratio of <u>between</u> group variations to <u>within</u> group variation with higher values indicating the likelihood of a group effect. In the hindbrain study, the angles 5, 7, and 9 were identified as most significant in DA. After cross-validation for the whole group, an average of 64.6% was correctly classified (SM 78.9%; 45.2 % Control; 50.0% CM pain). In the whole brain study, five traits (line bk and n-diameter, Angle 2(fac), Ellipticity and OB angle) were all identified and on average 72.5% of the group were correctly classified (93.3% Control; 68% CM Pain; 65.5% SM affected)





Hindbrain study: Average 64.6% correctly classified with SM affected highest separation of 78.9%. Function 1 increases with larger angles 5, 7 and 9 and Function 2 increased with larger angles 5 and 7 but smaller angle 9. Thus, SM cohort has smaller angles 5, 7 and 9 than other groups because the centroid is furthest on the left. The CM pain cohort on average has larger angles 7 and 9. The Control cohort has highest centroid in Function 2 hence they would, on average, they have larger angles 5 and 7 and smaller angle 9.

Whole brain study: Average 72.5% of the group was correctly classified with 93.3% of Control cohort. Function 1 the combined effect of decreases with of ellipticity and angle 2 (fac) but increases with lines bk, the n-diameter and the olfactory bulb angle. Function 2 increases with line bk, ellipticity and olfactory bulb and decreases with angle 2 and the n-diameter. It follows that the group has the centroid on the left side and has a more elliptic brain, wider angle 2 but smaller olfactory bulb angle (i.e. the olfactory bulb is not so rotated).

Overview of group findings

Control cohort; has the most ovoid (least spherical) shaped elliptical brain with a tendency towards a wider angle 2 (p = 0.004) and angle 5 (p = 0.007) with least ventral rotation of the olfactory bulb (p = 0.030) i.e. it is the least brachycephalic group compared to the others.

CM pain cohort; has a short basicranium (line ab) with a resultant compensatory increased cranial height (small angle 7) and increased brachycephaly with olfactory bulb more ventrally rotated (p = 0.003) and rostral forebrain flattening (p = 0.007) compared to Control CKCS. However, in comparison with SM dogs, the cohort has a longer line bc and a wider angle 9 increases the volume of the caudal fossa, which may lessen obstruction to CSF flow and the risk of developing SM.

SM cohort; has a tendency towards a bigger f-diameter (p = 0.002) i.e. greater compensation to rostral caudal shortening, smaller angles 7 and 9 and shorter line bk (p < 0.001) at the craniocervical junction compared to other groups. The additive effects of other traits give two phenotypic variables predisposing to SM. These are:

- 1. Reduced supra and basioccipital bone lengths with an increased proximity of both the atlas and the dens. All these anomalies reduce the volume of the caudal fossa (wide angle 3, short line bc);
- 2. More brachycephalic (smaller angles 2, 5 and 10) with compensatory cranial height (fdiameter), with the hindbrain being invaginated into the cranial fossa and the craniocervical vertebrae invaginated towards the foramen magnum.



Cavalier Photo courtesy Nicki Hughes

Fig 4.2 provides descriptive boxplots of a selection of variants used in the text which distinguish significant differences between the three groups.





Fig 4.3 Four mid-sagittal T2w whole brain MRI exemplars of cohorts Control, CM pain and two conformation cases of SM.



The occipital circle has been standardised in all the images and the baseline 'labi' aligned to facilitate comparison. Colour codes for morphometric 'signatures':

Blue = Control; **Yellow** = CM pain; **Red/Crimson** = SM affected (two cases). Green: all groups=angles 2 and 3 and lines bc and bk. Superimposed- CM pain (yellow) has most extreme range. White*=greatest parenchyma height from skull baseline and is rostral to occipital circle in CM pain and SM affected case 2, white x =caudal displacement of occipital lobe. white/blue bar drawn at most rostral point of forebrain and olfactory circles indicates angulation of forebrain flattening. The blue bar (Control dog) has been superimposed on the three other group dogs for comparison. CM pain has greatest rostral forebrain flattening; The SM case 2 has the greatest olfactory lobe deviation. Orange: brachycephaly- lines mark the position and relationship of the upper nasal bone and the hard palate. SM case 2 has the greatest brachycephaly with angulation at the nasion and the lower palatine/incisive bones. Aqua: dens. This lies closest to the basioccipital in SM case 1. The different angle of dens in CM pain dog was found to be significant for the study.

Black arrows suggest displacement resulting from craniosynostosis, **black** * indicates deviation (shortening) of occipital bone in CM pain and SM case 2.

Discussion

The study aimed to quantify symptomatic CM, SM and associated brachycephaly in the CKCS breed by mapping the hindbrain and craniocervical junction using refined morphometric techniques previously validated in the GB [94] and mixed breed [249] and to quantify brachycephalic conformation in association with CM/SM by mapping the entire brain to include rostral forebrain flattening and olfactory lobe rotation.

Variations in size and shape of the skull known to exist in CKCS breed, the late onset nature of SM, the spectrum of clinical signs for CM/SM and difficulty of identifying behavioural pain, all add to the complex nature of the condition. For these reasons, the characterisation of symptomatic CM and SM affectedness focuses on the anatomical similarities associated with these morbidities rather than the traits associated with 'normal' CKCS conformation which can be quite diverse. The use of the 'best fit 'occipital circle with the f-diameter and its ratio with the traits representing the size of the foramen magnum (line bc) and supra-occipital bone (line cd) have been key features in the characterisation. The early closure of the of the sphenoid occipital synchondrosis in the CKCS [114] makes the landmark 'point a' (Fig 4.3) a useful anchor point to superimpose the exemplar dogs' morphometric 'signatures' in order to compare them.

Angle 3 (dib) plays a major role in the morphometric analysis in the hindbrain study since it links both the supraoccipital and atlas bones with the basicranium. It also contributes to angle 7 and linked to lines ac, bc and bd. These quantify areas within the caudal fossa. The alignment of Angle 3 is independent of the dens. However the relationship between its proximity and angulation can be quantified with morphometric mapping and this angle is particularly significant with respect to SM and the Control group (Table 4. 2, p <0.001). Conversely, line bc measures the distance from the occiput to the atlas across the foramen magnum and the increased length in the CM pain cohort is an important distinction between this group and the SM cohort (Table 4.2, p <0.001).

Conformation similarities with Griffon Bruxellois

The five significant variables for SM (f-diameter, lines bc and ae and angles 3 and 10) were identified in both GB and CKCS suggesting a common aetiology. Furthermore, a smaller angle 2 (fac) which was significant for CM but not SM in the GB study [94] and a wider angle 2 was shown to have a protective role against SM in a mixed breed family [249]. In this study, angle 2 was significant in the forebrain study (control v CM pain and SM). Genetic studies [229] have confirmed that the increased f-diameter (significant locus CFA2 and the CM candidate *Sall-1* gene) and increased length of lines ae and fg (significant locus CFA14) reflect the increased height of the cranium and rostral caudal fossa. It is hoped that the current CKCS genetic studies will shed some light on the aetiology of both CM and SM [237,250].

Brachycephaly and Airorhynchy

Although the CKCS is recognised as having a brachycephalic skull [103], facial length is very varied in the breed, with the muzzle becoming fashionably shorter and more dorsally rotated (airorhynchy) in the last decade [63,103]. It is entirely credible that craniofacial conformation makes a significant contribution to CM and risk to SM as it does in the GB breed [44,45,94]. Thus, CM is not just a reduction in the cranial base and caudal fossa. The 'ellipticity' of the brain provides a quantitative value to compare the natural oval shape of the control cohort in Chapter 4 to the more global brachycephalic CM pain and two SM cases. The reduced size and rotation of the olfactory bulb, together with the clival angle (cranial base angulation between the ethmoidal plane and the clival plane [168], is associated with a shortened muzzle and increased stop and a 'face' that tilts up like a human. This is illustrated in Fig. 4.5 which has the nasal and palate bones emphasized as orange lines. It highlights the dynamic changes of the skull conformation and brain parenchyma associated with progressive brachycephaly and airorhynchy, shortening of the basicranium and supraoccipital bones and the proximity and angulation of the atlas and dens.

A recent study of suture closure and skull morphology in dogs[253] investigates the prebasial angles (angle between the hard palate and the cranial base of skull). These suggest that as the phenotype morphs from normal (Control) to CM pain and then to SM affected there is increasing airorhynchy. This is recognised as greater retroflexion of the facial skeleton on the cranial base in the most extreme case (SM case 2) which also has the greatest olfactory bulb rotation and 'stop' (nasion). The olfactory bulb links directly to the subarachnoid space via the cribriform plate of the ethmoid bone[254]. Any reduction in its size would impact on the absorption through the choroid membranes [254] and influence CSF dynamics. In humans the clivus-supraoccipital angle has been used to predict the size of foetal posterior fossa and diagnose CM-I1 malformation [193].

Craniocervical junction conformation impact on CSF flow dynamics

The traits associated with the craniocervical junction, line bc across the foramen magnum, and angles 3, 9 and 11 and lines bk and cj relating to the alignment and proximity of atlas and dens (first two cervical vertebrae) were all significant for SM. This is not surprising since the cisterna magnum which is the most common site the production of CSF [113]. Although atlanto-occipital overlapping was only found in 27.7% of dogs with SM in one study involving different breeds[38], there is no reason to exclude the possibility of an additive effect towards the severity of SM. Phase contrast MRI, or cine MRI which produce phase contrast images throughout cardiac cycle [19,86] provide a visual record of the impediment of CSF at the craniocervical junction. Basilar invagination and indeed any reduction in area associated with the arachnoid villi in the choroid plexus might inhibit CSF flow in both proliferation and function.

Summary

Morphometric mapping using a triangulation of lines, angles and circles is useful for defining SM and CM pain phenotypes. The results confirm that it is essential to consider the whole brain in the characterisation of CM which takes account of the brachycephaly and its additive effect on CM/SM. Through the standardisation of the 'best fit' circles and ellipse, it is possible to quantify differences in conformations associated with brachycephaly and the proximity of the cervical vertebrae to the skull that result in CM pain and SM. Linking the angles and lines to create a unique 'signature 'for each dog enables comparisons to be made relative to size and altered position of anatomical features. The Control cohort had the most natural, wolf-like, skull conformation in terms of ellipticity. The CM pain cohort was characterised by increased brachycephaly with greatest rostral forebrain flattening, shortest basicranium and compensatory cranial height. However, in this cohort, an increased distance between the occiput and atlas provided fewer impediments to CSF dynamics at the foramen magnum and reduced the risk for SM. The SM cohort exhibited two conformation anomalies. One phenotype variation was influenced by incongruities at the craniocervical junction and increased proximity of the dens producing a 'concertina' type flexure with medullary elevation. The other phenotypic variation was influenced by increased brachycephaly resulted in a 'concertina' type flexure similar to the CM pain cohort. However, both SM variations were characterised by an apparent reduction in caudal fossa volume which compromised the CSF dynamics in the spinal cord.

Chapter 5

Craniometric analysis of the hindbrain and craniocervical junction of Chihuahua, Affenpinscher and Cavalier King Charles Spaniel dogs with and without syringomyelia secondary to Chiari-like Malformation

Introduction

The morphometric mapping technique applied to the CKCS in the previous chapter confirmed similarities with the GB breed but the technique has yet to be applied to other Toy breeds. Two particular risk factors associated with CM/SM are miniaturization and brachycephaly [63,89,96]. The Chihuahua is the smallest known dog breed for which SM has been reported. Both British and American breed standards state that a Chihuahua must not weigh more than 2.7 kg (6 lb). An investigation of this miniature breed may therefore elucidate the pathogenesis of CM/SM. The Affenpinscher is a brachycephalic dog, often referred to as the "monkey dog". It is quite similar in appearance to the GB but typically larger and with a less stocky build. Although the Affenpinscher wirehaired head has rostrocaudal shortening similar to the GB, its muzzle is not as flattened [255] and provides an interesting brachycephalic variation for CM/SM investigation. In this chapter, the CKCS which has been investigated in depth in the previous chapter is compared to the Chihuahua and the Affenpinscher. Therefore, the specific aims of this chapter were as follows:

- 1. Characterise the phenotypic variables of the caudal fossa and craniocervical junction associated with SM (i.e. characterise the phenotypic variables of canine CM).
- 2. Elucidate any conformation similarities between the breeds that might suggest a common aetiology and assist in diagnosis.
- 3. Identify any protective conformation traits that might contribute to generating estimated breeding values in order to reduce the risk of SM through selective breeding.

Study Cohort

This investigation comprised a total of 273 T1W DICOM sequences in the mid-sagittal plane of the hindbrain and craniocervical junction of which 132 were CKCS, 42 Affenpinschers and 99 Chihuahuas (53 DICOMS obtained from the Veterinary Teaching Hospital of University of Helsinki that had participated in a low cost screening examination for CMSM with voluntary participation of owners which was part of another study.

Each breed was grouped according to its SM status. CKCS and Affenpinschers that were not graded by the BVA were evaluated by Dr C.Rusbridge (a BVA/KC CMSM Health Scheme scrutineer).
Collaborator A-M Kiviranta examined and imaged 53/99 Chihuahuas and their SM status was evaluated jointly by C. Rusbridge and collaborator T. Jokinen who were blinded to their clinical status. Table 5.1 provides details of the group composition.

		TOTAL			
		Chi	Affens	CKCS	Total number dogs
gender	male	51	16	48	115
	female	48	26	84	158
age group	<3yrs	32	11	36	79
	3-4.9yrs	34	15	32	81
	≥5yrs	33	16	64	113
SM grade	0	34	28	45	107
	1	23	6	18	47
	2	42	8	69	119
Group	Total	99	42	132	273

Table 5.1: Study cohort.

Chi = *Chihuahua*; *Affens*= *Affenpinscher*.

The craniometric analysis comprised 19 measurements (11 lines and 8 angles) to explore the caudal fossa and craniocervical junction in order to record the differences in the juxtaposition of hindbrain, spinal cord and skull in dogs with and without SM (see Fig 2.5).

Results

Total cohort

Since the three breeds differ markedly in size and head shape, an initial DA investigation of the 19 variables and three ratios was performed to determine those that best discriminated between the three breeds. It revealed that a minimum of six lines, three angles and two ratios could be used to correctly classify an average of 94.4% of the dogs when cross-validated (Fig 5.1). The f-diameter of the 'best fit' occipital circle (f-diam), Angles 3 and 4 were identified as the most significant variables that distinguished between the groups when the breeds were combined and SM status substituted as the independent variable in the multivariate analysis. However there was only a 61.9% successful prediction for correct SM classification (Fig 5.1). Scatterplots for both canonical DA are provided in Fig. 5.1: 1) Breeds 2) SM status. Table S1 lists the most significant traits used in the construction of the plots.



Fig 5.1 Scatterplots for canonical Discriminant Functions Analysis for 1) Breed 2) SM status in total group cohort.

Discriminant analysis is used to determine the minimum number of dimensions needed to describe differences between the group for 1) Breed and 2) SM. These significant variables identified are allocated a weight within each discriminant function (Appendix, Table 5.2) and the two functions plotted against each other to illustrate group seperation. The 11 traits can distinguish with 100% accuracy for CKCS, 92.8% for Chihuahuas and 90% for Affenpinschers. Separation in SM status using 3 traits yields 75.7% accuracy for SMO, 73.9% for SM2 but 0% for SM1 predictive success for each group.

Individual Breeds

Scatterplots, generated when DA was applied to each breed, identified different significant traits that were most important for each cohort (Fig 5.2). In the Chihuahua this was Angle 3 (dib) and Angle 4 (jcb) both which relate to the craniocervical junction and the proximity and alignment of the atlas and dens with the basioccipital bone (skull base). Affenpinschers appear similar to the GB in that the most useful trait was f-diameter which is increased with SM. However the Affenpinschers are unique in that it is the distance of the dens to the atlas that best separates the subgroups with 92% correctly placed for SM0. In the CKCS scatterplot, line id (distance between the occipital crest and the level of the cranial baseline abi) is plotted against ratio f-diameter/bc (the height of the caudal cranial fossa / distance of the atlas from the basioccipital across the foramen magnum). The function coefficients used in the analysis are provided in the Appendix, Table 5.3.

Since SM is a late onset condition, the status of the SM1 dogs (central canal dilatation less than 2mm) cannot be confirmed less than 5 years old. Of the total SM0 dogs for each breed, 19% (8/42) Affenpinschers, 12% (12/99) Chihuahua and 3% (4/132) CKCS were under three years old and 21% (9/42) Affenpinschers, 8% (8/99) Chihuahua and 7% (9/132) CKCS were three years to less than five

years old. The phenotypes of these relatively low numbers of SM0 dogs with unknown status were useful to compare those with SM1 and SM2 dogs of a similar age. The ambiguity of subgroup SM1 is demonstrated in Fig 5.2 with low percentage predictive scores of the original groups classified as correct (13% Chihuahuas, 16% Affenpinschers and 0% CKCS).





Chihuahua: Left to right, there is less risk of SM left to right as angle 3 decreases and angle 4 increases (atlas further caudal from the supraoccipital and basioccipital bones) Affenpinscher: Left to right, increased risk of SM with decreasing line cj and increasing f-diameter) CKCS: Left to right, increasing risk of SM with increasing line id and ratio f-diameter: ai (i.e. dogs that are more brachycephalic).

The 14 significant variables for SM identified by ANOVA analysis after Tukey correction are provided in Table 5. 2. These included the 5 traits * found in the previous GB analysis supporting the idea of a common aetiology.

		Chihuahua		Affenpinscher		CKCS	
no	variable	F	p-value	F	p-value	F	p-value
1	bi	7.294	0.001	1.692	0.197	11.516	<0.001
2	ai	6.455	0.002	1.86	0.169	10.982	<0.001
3	bk	8.966	<0.001	0.582	0.563	3.802	0.025
4	∠7(bdi)	8.819	<0.001	2.126	0.133	13.217	<0.001
5	∠3 (dib)*	9.406	<0.001	2.847	0.07	14.548	<0.001
6	f-diam:bc	8.957	<0.001	2.307	0.113	9.463	<0.001
7	cj	0.711	0.494	5.39	0.009	1.511	0.225
8	bc*	4.306	0.016	3.566	0.038	2.319	0.102
9	ae*	1.802	0.17	3.248	0.05	5.408	0.006
10	f-diam*	2.106	0.127	6.991	0.003	8.21	<0.001
11	∠4 jcb	16.77	<0.001	0.394	0.677	2.711	0.07
12	fg*	4.443	0.014	0.83	0.444	0.65	0.525
13	ac	1.696	0.189	0.497	0.612	5.22	0.007
14	f-diam:ai	8.446	<0.001	3.884	0.029	15.329	< 0.001

Table 5.2: Significant variables for SM status identified in ANOVA after Tukey correction.

* Traits identified as significant in previous GB study. L=angle. Significant p values ≤ 0.05 for SM affectedness are highlighted in bold.

Descriptive boxplots, highlighting the significant morphological differences and similarities that were identified in the univariate analysis, have been grouped into two Figs 5.3 and 5.4 to ease reading. Colour coded circles indicate which trait is significant for the breed. Since the breeds are plotted on the same axis for each trait, the differences in size between breeds is apparent: the largest CKCS (yellow), the smallest Chihuahua (blue) and the Affenpinscher (green) in between. The mean values for SM0, SM1 and SM2 have been linked with a colour coded line for each breed. However since the statistical analysis identified significance between SM0 and SM2, a thin grey line has been drawn between their mean values in order to accentuate differences between the two or disparity with SM1. A red bar has been added to the coloured line if the significance was between SM0 and SM1 (only example line bc) or SM1 and SM2 (nine examples). A double red bar has been added if there was an additional significance between SM0 and Fig 5.3 illustrates six traits that are significant in both the Chihuahua and the CKCS breeds and Fig 5.4 three traits shared by the remaining traits (Table 2) shared by the Affenpinschers or those unique to each breed.

Fig 5.3. Descriptive boxplots of variables associated with SM status significant for both Chihuahua and CKCS cohorts.



The mean values for SM0, SM1 and SM2 have been linked with a colour coded line for each breed. In addition, a thin grey line has been drawn between mean values SM0 directly to SM2. Unless indicated by red bar/s, the significance is between SM0 and SM2.

Fig 5.4. Descriptive boxplots of significant variables associated with SM status in the Affenpinscher, Chihuahua and CKCS.



The mean values for SM0, SM1 and SM2 have been linked with a colour coded line for each breed. In addition, a thin grey line has been drawn between mean values SM0 directly to SM2. Unless indicated by red bar/s, the significance is between SM0 and SM2.

Morphometric analysis

Fig 5.5 compares the morphometric grids for three exemplar breed pairs; Chihuahua, Affenpinscher and CKCS. Each pair of sagittal MR images is matched as far as possible for age and the diameter of its occipital circle (f-diameter), but opposing SM status. SM2 (affected) red coloured grid is superimposed over that of the SM0 image (unaffected blue grid), highlighting the discrepancies between the paired dogs.

All the SM affected dogs (b) have a smaller line ac, cj and angles 2 and 4 but they vary in degrees and the proximity and angulation of the atlas and dens. For example, dogs 1b and 3b have the same length line bc as their counterparts but a smaller angle 7 and medullary kinking associated with a smaller angle 8 (dbk). Despite the breeds' differences in morphometric proportions, Fig 5.5 illustrates the cumulative similarities of the shared deformities associated with SM.

Fig 5.5. Four pairs of TW1 mid- sagittal MRI of exemplar Chihuahua, Affenpinscher and CKCS with and without SM and their morphometric overlays.



The pairs of dogs have been matched as far as possible in age and size of the occipital circle (f-diameter) which is typically larger in Affenpinschers and CKCS dogs with SM. AOO= atlanto-occipital overlap. Note the reduced occipital crest in all 3 dogs with SM (a) and marked ventriculomegally in SM dogs 2b and 3b. The images in each pair have been morphed with each other to provide a dynamic illustration (morph movie- appendix 1).

Discussion

This study aimed to distinguish the phenotypic variables of the caudal skull, hindbrain and craniocervical junction in three different breeds and how these predispose the formation of one or more syringes in the spinal cord. Individual predisposing features, such as medulla oblongata elevation (kinking) [79,90] and atlanto-occipital overlapping [38,97,205], have previously been evaluated in other studies using different parameters. They show to be closely associated with SM but are not an evitable consequence i.e. having this anatomical feature is not always associated with SM and vice versa. Equally, dens abnormalities have been reported [206] but a consistent association with SM remains unclear [73]. This study differs in that it quantifies the combined influence of all predisposing features including the reduced caudal fossa size and attempts to identify protective elements. The 19 selected

morphological measurements were successful in providing a comprehensive means of analysing the proportions and juxtapositions of the hindbrain and craniocervical junction in the three breeds.

Seven significant traits identified in previous GB studies namely f-diameter, line bc, ae, fg and angles 1 (in that study angle 5) 2 and 3 were also identified in this investigation and supports the basis of a shared aetiology of CM/SM [94,229,249]. Although there were differences in breed size (Fig 2) and absolute values (Fig.4A and 4B), the trends for SM0 to SM2 status were broadly similar in the three breeds.

The relationship between the traits is demonstrated by the morphing movies S4-6. These dynamic illustrations reveal two flexures associated with SM that are not appreciated in the static DICOM images. The morph movies help illustrate how seemingly minor changes in skull and vertebral conformation can be additive towards distorting the neural parenchyma and thereby compromising the CSF flow dynamics both within the brain and the spinal cord. Furthermore, despite the dissimilar proportions in the morphometries of the three breeds, the underlying forces that are revealed in the movies appear similar but in different degrees.

Breed characteristics for SM



Photos: Chihuahua courtesy of Miryam Lowman Bodimeade (left), Affenpinscher courtesy of Jessica Gruninger, Cavalier courtesy of Nicki Hughes

Chihuahuas

Angle 3(dib) and 4(jcb) were identified as the most discriminating factors in this breed (Fig 3). An increase in Angle 3 is associated with flattening and deformation of the cerebellum whereas Angle 4 flexure represents overcrowding at the craniocervical junction and neural parenchyma (Fig 5.4). The significant traits (p<0.001) line bk, angle 7 and ratio f-diameter: bc also relate to the craniocervical junction. Small differences in volume reduction may have a greater impact in compromising the CSF flow into the subarachnoid space of the miniaturised Chihuahua compared to larger breeds with more

leeway. An increase in cranial height (f-diam) was not a significant factor for SM in this breed. However, the reduction of angles 4 and 7 and the considerable reduction in the occipital crest resulted in SM dogs having a more rounded and rostrocaudal short skull. Insufficiency of the caudal skull has been previously described as a feature of SM dogs in the GB [44,249] and CKCS [63].

Affenpinschers

This study showed that syringomyelia in the Affenpinscher is associated with increased proximity of the dens to the atlas i.e. reduced line cj and this feature was unique to the breed (p = 0.009). The steeper angulation of the dens was associated with flexure of the craniocervical neural parenchyma i.e. the medulla oblongata and spinal cord (Fig 5.4 2b). This can be compared to basilar invagination (BI) in humans which a common craniocervical junction malformation associated with CMI [207]. Classical BI in humans is defined as invagination of the odontoid process into the foramen magnum with ventral brainstem compression i.e. a more severe malformation. However angular craniometric studies have distinguished subgroups of BI in humans. Type II has invagination of the dens towards the skull but not inside the foramen magnum but has a more acute angulation between the floor of the caudal fossa and the dorsal dens (clivus canal angle; in humans the clivus is the posterior skull base, i.e. posterior sphenoid and basioccipital bone, and should be in line with the dens) and greater cervical lordosis (angle between dorsal dens and dorsal surface of c7 vertebral body) [204]. It is Type II BI that seems comparable to risk factors for SM affectedness in the Affenpinscher.

Noteworthy is the relationship of SM1 with both SM0 and SM2 that appears incongruous in the Affenpinscher compared to the two other breeds in Fig 5.3B. It is possible that, in this breed, SM1 is not always an intermediate between SM0 and SM2 but associated with its own combination of conformation risk factors and SM1 does not necessarily progress to SM2 over time.

Cavalier King Charles Spaniel

Ten of the fourteen significant variables were found in the CKCS with one, line ac, unique to the breed. Line ac indicates the proximity of the spheno-occipital synchondrosis to the atlas bone. This study confirms the findings of others that the CKCS with SM have a reduced caudal fossa size [37,38,41,256] a presumed consequence of early closure of the spheno-occipital and possibly other cranial sutures[114]. Compared to other breeds including the GB [45], the CKCS has considerably greater incidence of cerebellar deformation by the supra-occipital bone and vermis herniation [43,60,256]. These findings and the coexistence of occipital dysplasia and hypoplasia [35] suggest that the CKCS may have additional predisposing risk factors for SM compared to the other breeds.

Fig 5.4 3a/b illustrates the conformation differences in association with SM. Typically a CKCS with SM has an increased f-diameter compared to those without SM (p value <0.001) but in this chosen example both dogs 3a and 3b have a similar f-diam \sim 47mm, thereby negating this variable. SM affected

dog 3b has a reduced occipital crest and flattened supraoccipital bone. The atlas is more rostral and the dorsal atlanto-occipital membrane is vertically orientated. The cerebellum is deformed and flattened by the supraoccipital bone. There is only a small herniation of the cerebellar vermis into the spinal canal. Note the acute angle the sphenoid bone makes with the basioccipital bone of the SM affected dog 3b at the spheno-occipital synchondrosis. The superimposed red triangle of this dog on the blue triangle of unaffected dog 3a appears rotated caudally at point X. This elevated angulation of the sphenoid bone is called the 'sphenoid flexure' in this study. Additionally, in this exemplar, the dens of dog 3b is both closer to the basioccipital bone and has greater angulation. This is comparable to an increased clivo-axial angle in humans and is called the 'cervical flexure' in this study. In humans ventral brainstem compression is associated with considerable pain and normalization of the clivo-axial angle with surgical intervention is linked with clinical improvement [257].

Reduced occipital crest and supraoccipital bone

An interesting observation in this study was the reduction of the occipital crest in SM dogs in all three breeds (Fig 6). Point'd' in the framework grid is the junction of the occipital crest and the supraoccipital bone and its placement is an intrinsic part of angle 3, 6, and 7. Underdeveloped occipital bones have long been considered to play an influential role in CM and developing SM [258]. Unlike the other occipital bones, the supraoccipital bone ossifies by both intramembranous and endochondral means. This bone is derived from cephalic paraxial mesoderm, and possibly neural crest [199,259], whereas the remaining occipital bones are somatic mesoderm origin [113]. A histopathological study of the neonatal supraoccipital bone in the CKCS showed that foetal tissue bone was poorer quality with significantly reduced number of osteoblasts and chrondrocytes with increased osteoclasts and apoptosis compared to controls. In contrast, the adult CKCS supraoccipital bone had poor cellularity compared to controls. Furthermore the adult supraoccipital bone showed histological signs of active remodelling and it was hypothesised that this could alter the capacity to accommodate the mechanical pressure from the growing brain [196]. The four occipital bones that surround the foramen magnum and form part of the skull base, together with the sphenoid and the petrous temporal bones, are cartilaginous endochondral bone and mesodermal in origin (the chondrocranium) [171] unlike the bones of the skull vault are membranous and neural crest in origin (the dermatocranium) [167].

Changes in angulations which impact on the caudal cranial fossa

Another interesting finding in this investigation is the changes in angulations associated with SM and reduction of caudal cranial fossa volume; 1) 'cervical flexure' associated with changes in dens angulation and medullary elevation (kinking) at the craniocervical junction described in the Affenpinschers, 2) 'sphenoid flexure' at the spheno-occipital synchondrosis described in the CKCS. Change in craniocervical morphology as a consequence of a shortened and flattened clivus (i.e. sphenoid and basioccipital complex) is well described in humans and explained by different timings of

sclerotome development. The fusion of the basioccipital and occipital sclerotomes occurs before the cervical sclerotomes and a lordotic skull base angle "forces" retroflexion of the cranial cervical segments and results in a dens that points up and back into the neural parenchyma [203]. However, whether a similar situation occurs in dogs is yet to be established.

Summary

This study used a range of morphometric measurements to characterize the hindbrain and craniocervical junction and successfully compared the phenotypic variables of Chihuahua, Affenpinscher and CKCS dogs with and without SM. It used refined morphometric techniques developed to investigate the genetic basis of CM in the GB. A total of 14 of the 23 variables considered were significant for SM and included the five traits found in the GB analysis, suggesting elements of a common aetiology. Different combinations and values of traits distinguished unique differences between the three study breeds. Two changes in angulation, the 'sphenoid flexure' rostral to the spheno-basioccipital synchondrosis and 'cervical flexure' which extended caudally to C2, were common to all three breeds. These flexures were associated with occipital bone hypoplasia and reduced caudal cranial fossa volume and, together or individually, they introduced risk factors to the severity for SM by compromising CSF flow dynamics and/or neural parenchyma compliance. The complexity of quantifying the phenotype involved evokes the need to develop software in the form of a digital mapping tool that might be used to identify dogs at risk of SM and CM associated pain. Such a tool might assist with the diagnosis of different traits predisposing SM in order to consider alternative surgical management, for example, a ventral rather than dorsal decompression. Furthermore, the objective morphometries might provide estimated breeding values for screening breeding dogs and reduce the risk of SM through selective breeding.

Chapter 6

General Discussion

This study undertook a range of morphometric measurements to characterise the hindbrain and craniocervical junction and successfully compared the phenotypic variables of Chihuahua, Affenpinscher and Cavalier King Charles Spaniel (CKCS) dogs with and without Syringomyelia (SM). It used refined morphometric techniques developed to investigate the genetic basis of Chiai-like malformation (CM) in the Griffon Bruxellois (GB). A total of 14 of the 23 variables considered were significant for SM and included the five traits found in the GB analysis, suggesting elements of a common aetiology. Different combinations and values of traits distinguished unique differences between the three study breeds. Two changes in angulation, the 'sphenoid flexure' rostral to the sphenooccipital synchondrosis and 'cervical flexure' which extended caudally to C2, were common to all three breeds. These flexures were associated with occipital bone hypoplasia and reduced caudal cranial fossa volume and, together or individually, they introduced risk factors to the severity of SM by compromising CSF flow dynamics and/or neural parenchyma compliance. A reduced angle 3 was most consistently significant in all the studies and in all breeds in association with SM, The point of this imaginary angle 3 links the alignment of the supraoccipital bone with that of the basioccipital skull base across the foramen magnum. Such an anomaly has a direct bearing on the critical components at the craniocervical junction to ensure an effective hydrodynamic transmission of CSF.

Chapters 3.4 and 5 each concluded with a discussion of the results and their relevance and relationship to the research of other groups. In this chapter, an attempt is made to draw together those findings in seeking practical outcomes that help diagnosis and assist appropriate pain management. The morphometric mapping of DICOM TW1 and TW2 mid sagittal images of the brain and cervical region of GB, CKCS, Affenpinscher, Chihuahua and GB crosses revealed the complex nature of CM/SM and its relationship with aspects of brachycephalic conformation and miniaturisation. An initial definition that CM is a condition characterised by a mismatch between the volumes of the caudal fossa and its contents so that the cerebellum and brainstem are herniated into or through the foramen magnum should now be amended. Although a reduced caudal fossa is fundamental in its association with impaired CSF flow, a definition of CM should not be restricted to that configuration. Brachycephaly should now be recognised as contributing to canine CM together with the additive anatomical anomalies of the atlas and axis bones at the craniocervical junction. Furthermore, interference with drainage of CSF within the ventricular system of the whole brain and the subarachnoid spaces overlying the brain may result in intracranial pressures that may damage neural parenchyma. Thus, this dissertation supports the concept that neuropathic pain, as the primary clinical sign of CM and secondary SM, is not only associated with damage to the spinal cord dorsal horn but also the consequences of increased CSF pressure within the cranial cavity and the anomalies at the craniocervical junction involving the first two cervical vertebrae.

Natural variation and clinical significance of CM

The initial definition of CM, which was defined by the shape of the cerebellum, was made based on limited data over a decade ago. Although SM is considered secondary to CM, some Griffons have SM without CM according to the BVA grading [94]. This can be explained within the definition which takes account of the mismatch of the brain with the whole skull and not just the cerebellum and caudal fossa. There is a need to account for the prevalence of CM in clinically unaffected dogs which may be a result of natural variation [43]. In this thesis CM1, intermediate between CM0 (without CM) and CM2 (with cerebellar herniation), remains something of a paradox. The BVA define CM1 as the cerebellum lacking a rounded shape with indentation by the supraoccipital bone but with a signal consistent with CSF between the vermis and foramen magnum. A desirable outcome for characterising CM would be the means to distinguish between pathogenic and natural variations. In particular, CM morphometries that have been used in this thesis can be related directly to SM and it should be possible to predict which CM variants might develop SM.

In accordance with customary scientific practice, investigators of secondary SM have usually examined any particular variable using the reduced (cranial) caudal fossa as the CM model (Schmidt *et al.*, 2014; Shaw *et al.*, 2013; Driver *et al.*, 2010; Cerda-Gonzalez, Olby & Griffith, 2015; Dewey *et al.*, 2004; Loughin, 2016; Harcourt-Brown *et al.*, 2011; Rusbridge *et al.*, 2000). A recent quantification of brain herniation on MRI has identified both a caudal transtentorial herniation and a foramen magnum herniation [261]. In contrast, the morphometric mapping technique used in this dissertation evaluates several interrelated variables simultaneously and explores the manner and the degree of changes in both the caudal fossa and the craniocervical junction i.e. the additive contribution of any deviation made towards CM and acknowledges the accumulative effects that might produce 'late onset' of SM. Fig 6.1 provides a schematic summary of the key morphological traits identified in this dissertation comparing dogs with and without SM. It illustrates the relationships between the variables that have been investigated. Dogs may have one or more of the features which predispose to SM and it's severity and any individual elements, such as medullary kinking, that are not always associated with SM in isolation, can be additive in reducing the overall caudal cranial fossa. Fig 6.1 Schematic framework of selected CM traits (red lines) and 'normal' traits (blue lines) to illustrate underlying unifying pathophysiological processes.



Key:

(a) dorsum of spheno-occipital synchondrosis

(b) basion of basioccipital bone

(c) rostral edge of the dorsal lamina of the atlas

(d) junction between the supraoccipital bone and the occipital crest

(f) centre of "best fit" occipital lobe circle placed on the cranial baseline (abi) and extended to encompass the occipital lobes. The centre of the circle is f and indicates the height of the caudal cranial fossa.

(g) point at which the optic nerve deviates into the optic canal

(k) extended line from point b along the best fit line of the ventral medulla oblongata to where it changes angle to the spinal cord (degree of medullary elevation/kinking).

The genetic and endocrinal basis of craniosynostosis is pivotal in skull modelling especially with respect to brachycephaly [112]. A more recent study explored breed-specific patterns of cranial suture and synchondrosis closure in relation to the preaxial angle (proxy for airorhynchy) in domestic dogs. Investigating the closure of 18 sutures which they compared with the wolf, the group identified a correlation between patterns of closure and skull shape in the domestic dog [253]. There are ten bones that make up the cranium (6 unpaired and 4 paired) and the timing of suture closure between them is genetically predetermined. The ultimate shape of the calvaria would be dependent on the nature of all these sutures. In rats, when the spheno-occipital synchondrosis was surgically removed it drastically changed the pattern of growth of the skull but tampering with the sutures of the vault did not. Compared to controls, the exorcized experimental rats had observable differences in the angulation of the skull base, an increased curvature of the cranial roof and a marked forward displacement of the occipital condyles. Other changes included a ventral and forward rotation of the plane of the foramen magnum [262]. It is hypothesised that the changes in angulation at the spheno-occipital and the spheno-presphenoid synchondroses ("sphenoid flexure") occur during early embryonic development.

Furthermore increased vaulting of the dorsum are part of the same process of compensatory changes in skull dimensions and brain shape described for the observed changes in the GB dog with craniosynostosis [44,94] and head shape [63,249] and can also result in changes to cervical conformation.

A schematic figure was devised to portray hypothetical changes in skull and brain conformation in the study of Griffons [94] and, for convenience, this illustration is provided here as Fig 6.2. Example 'e' provided an explanation as to why line ae (between the spheno-occipital synchondrosis and dorsal edge of the cerebellum) lengthened when the expectation might have been that it would shorten with underdevelopment of the supraoccipital bone. The figure illustrates how the cerebellum becomes invaginated under the occipital lobes as a natural consequence of a reduction of both the supraoccipital (dorsoventral) and basioccipital bones (cranial base).

Fig 6.2 Diagrammatic illustrations to show the hypothetical effects of compensatory changes in skull dimensions and brain shape when there is craniosynostosis or shortening of certain skull bones.



The components of this figure were created using Adobe PhotoshopTM which enables manipulation of shapes with fixed points facilitating a simple representation of the distortion. a) Normal; the skull is represented by a black box and brain by a grey circle with reference points w, x, y and z. Asterisk represents a fixed reference point outside brain. b) Uniform rostrocaudal shortening (black arrows). There is a compensatory increase in height of the brain (white arrow). Lines xy, yz (yellow) and wy (blue) increase and angles wyz and xyz decrease c) Cranial base shortening (black arrow); asymmetrical shortening results in an axial tilting of the brain. There is also a compensatory increase in height (white arrow). Line xy and yz increases (yellow), wy shortens (blue) and angle wyx and xyz decrease. d) Caudal dorsoventral shortening (black arrow). There is a compensatory lengthening of the brain and rostral increase in height (white arrow). Line wy increase (blue), xy and yz shorten (yellow), angle xyz decreases e) cranial base and caudal dorsoventral shortening. When the cranial base and the caudal part of the skull are shortened the circular model brain becomes increasing ellipsoid and there is greater axial tilt with an increase in height rostrally (white arrow). The ventorostral part (olfactory bulbs) is displaced ventrally, the dorsocaudal part (occipital lobes) is displaced caudally and the ventrocaudal part (hindbrain) is displaced rostrally. Line yz shortens and angle xyz decreases. However distance to the external reference point (asterisk) increases (red dotted line). This illustration is offered as an explanation for the increase in line AE and BC in this study and also for the change in brain shape with increasing CM affectedness

It is accepted that small differences in skull shape are not extreme to be pathogenic. Fig.6.3 explores the radiographs of two Australian terriers, included in the first GB investigation where they were used to illustrate a mesaticephalic breed [94]. Bitch A was CM0 and dam of the mixed breeding investigation in Chapter 3, the other, Bitch Q, was CM1. The radiographs have been resized with fixed ratios for comparative purposes and the skull outlines then superimposed (Stages 1-3). Differences between the two skulls are highlighted by aligning either i) the skull bases and occiputs (stage 4) or ii) the orientation of the muzzle and dorsum of the calvaria (stage 5). Essentially Bitch Q with CM has a proportionately reduced skull dorsoventrally with a shorter muzzle and steeper stop (nasion) so that the eye orbit is displaced outwards (eye appears bigger). The MRI status confirms that the cerebellum is displaced through the foramen magnum. There is a difference in the angle that the nasal bone makes with base of the skull and the occiput but it is not exaggerated. In this example of breed brachycephaly (reduced muzzle) the homogeneous changes are unlikely to have a significant impact on CSF flow cause any CM discomfort or SM because there appears little reduction in the length of the skull base, but a change in its angulation.



Fig 6.3 Comparison of two radiographs of Australian terriers with and without CM

Obviously, there is a need for further investigation, but it seems that it is possible that CM, as defined by the existing BVA/KC scheme, includes a range of variation and it should be possible to distinguish between these and symptomatic Chiari-like syndromes.

Is the characterisation of CM using morphometric mapping useful?

A key aspect of the characterisation of CM is to be able to distinguish between what appears to be natural minor variations in the shape of cerebellum and features that compromise the neural parenchyma in such a way as to cause pain or destruction of tissue. An important distinction between the characterisation of CM in this dissertation and those of other research groups investigating CM is a different focus of attention for analysis. In this manuscript, it is the 'occipital circle' that is most central to CM, not the cerebellum, and it offers a proportionate measurement for comparing the relative distances and juxtaposition of neural parenchyma. Although the diameter of the circle ('f-diameter') is a reflection of the height of the calvaria from the skull base, the 'best fit' circle defines the shape of the caudal skull and this has been demonstrated as significant for SM in the Griffon [44] and the CKCS [63]. It outlines the occipital lobes and their position relative to the cerebellum and interparietal bone. In dogs with painful CM and SM, the cerebellum is invaginated under the occipital lobes (Fig 4.3 and Fig 5.5). The caudal skull 'slope' in a spatial sense can be relative to the increased doming of the calvaria which is considered a direct result of brachycephaly. The 'sphenoid flexure' can also be viewed as part of the process of brachycephaly, with more acute angulation relative to the basioccipital associated with CM pain and SM in Chapter 4. Also, the f-diameter is a highly significant trait in all the morphometric studies and in the genetic studies and associated with CFA2.

Application of morphometric mapping

The conclusion in Chapter 4 described linking the lines and angles generated by the morphometric mapping as a unique 'signature' for each dog. In order to demonstrate how these may be used in a practical sense, the superimposed 'signatures' in Fig 4.3 have been extracted and enlarged as Fig 6.4, with fixed point 'a' (spheno-occipital synchondrosis) now represented by * and angles/lines relative to the * suture are numbered 1-7. The approximate positions of the dens and atlas are marked.

Another example of how the CKCS 'signatures' can be viewed in a practical sense is provided in Fig 6.5 which is not schematic but makes comparison with other Toy breeds. It illustrates the morphometries of six exemplar sagittal MRIs of whole brains which include the three 'best fit circles' used in Chapter 4. DICOMs are curtesy of Fitzpatrick Referrals. There are three CKCSs with and without symptomatic CM and SM (left side) and three 'other breeds' (weight matched) on the right side. The 'signatures' are accentuated with colour codes identified in the key. Dog A ('normal' CKCS) and dog B (Highland terrier) have a similar size occipital circle ~ 48.7 mm, and dogs D (French Bulldog) and E (CKCS with CM/SM) are similar with ~50.6mm. Dog C (CKCS with CM pain) has the smallest occipital circle with 43.7mm and Dog F (Poodle with hydrocephalus) has the largest circle with 52.75mm.

Fig 6.4 Superimposed 'signatures' of four exemplar CKCS dogs aligned at fixed point * (Ch.4)



Key. **Blue**=control, **yellow**= CM pain, **pink** =CKCS case 1, **red**=CKCS case 2 These dog signatures provide a crude overview of the characteristics of CM pain and SM dogs compared to the control 'normal' CKCS (blue). They highlights the sphenoid elevation (2), the dorsocaudal rotation of the occipital lobes for the SM dogs (3 and 6), the reduced basi-occipital bone (5) of the CM pain dog (yellow) and SM case 2 (red), the proximity of the atlas and dens to the basi-occipitals and the reduced supra occipital (6) for both SM cases. Angulation of the dens and atlas are not represented in the figure.

Fig 6.5 Morphometric mapping on six exemplar sagittal MRIs of whole brains with measurements of three 'best fit circles' comparing CKCS (with and without CM and SM) and other breeds (without CM or SM).



Left side CKCS; blue = no CMSM, yellow = CM pain, red= CMSM. Right side 'other breeds' (aqua).

In order to facilitate comparison, Fig 6.5 now matches the six dogs in Fig 6.4 above by superimposing their 'signatures' after the occipital circle f has been resized to the same diameter but retaining ratios and aligned at point a* (sphenoid-occipital synchondrosis).



Fig 6.6 Superimposed 'signatures' of six exemplar dogs in Fig 6.5.

'Signatures' of six exemplar dogs have been resized with same 'f-diameter' with ratios retained and superimposed so they are aligned at point * (sphenoid-occipital synchondrosis). Other toy breeds = aqua, CKCS; blue = no SM, yellow = CM pain, red = CMSM. Note that the red and yellow symptomatic signatures have the greatest deviation at b,c, d, e and i relative to others.

It can be seen that, notwithstanding the disparity of skull shapes of the 'other breeds' (aqua), the signatures for the symptomatic CKCS with CM pain (yellow) and CM/SM affected (red) possess the most extreme positions in comparison and to them and the CKCS without CM/SM (blue).

Multifactorial basis of CM/SM

The case has been made throughout this thesis that CM severity is 'additive' in nature and was noted in all three investigations (Chapters 3-5). This supports a genetic basis of incomplete penetrance that has been cited in human CM/SM [16,31,218,263] and in dog [229]. Gene mutations can effect all aspects of cell function from the protein-coding genes to regulatory signalling pathways and structural growth. Epigenetic modification, such as DNA methylation, becomes an additional layer of complexity. Indeed, the heritability estimate of SM in CKCS implies that about 37% of the condition is due to genetic factors [230]. A study that focused on the genetic link between human CM0 and CM-I suggests that additional epigenetic and/or environmental factors are likely to play an important role in the development of CM0 versus CM-I type [34].

Purebred dogs are becoming increasingly accepted as naturally occurring models in the genetic studies of human disease [264]. In human CM, the more serious disorders CM-II, III and myelomeningocele involve the disruption of primary neurulation[16]. Congenital defects of neural tube closure (neural tube defects; NTDs) are among the commonest and most severe disorders of the foetus and newborn. A number of cellular functions are essential for neural tube closure. There are > 200 genes is known to

cause NTDs in mice [265]. Defects of the cytoskeleton, cell cycle and molecular regulation of cell viability have been investigated using mouse NTD mutants. These have revealed some key signalling pathways for NTDs [150] Research studies of gene identification in NTDs have mainly adopted a candidate gene approach such as those on the genes of the folic acid pathway (vitamin B12) since this plays an important role in the development of the neural tube [266].

Chapter one related how embryonic growth and development of the brain and skull are in harmony with each other. The entire process is exquisitely synchronised at the intracellular level of transcriptional and regulatory genes to the macro responses of local environmental influences. In the mouse, the first neural tube closure is initiated at the hindbrain/cervical boundary (6 somite stage) and spreads both rostrally and caudally from this site [135,150]. The pons and cerebellum (Metencephalon) and the medulla oblongata (Myelencephalon) that encompass the hindbrain are bounded externally by the ring of four occipital bones that comprise the foramen magnum. The distorting forces generated by both an increase in size of the developing brain and the internal hydrostatic forces of its ventricular system shape these bones, as part of the cartilaginous chondrocranium. The final size and shape arises from remodelling by endochondral reabsorption and sutural growth [16,267]. A single genetic irregularity at this stage of development would be far more potent or wide reaching, influencing later development as the embryo grows.

Paraxial mesodermal insufficiency and miniaturisation

In humans, CM-I can be just part of a spectrum of abnormalities most of which are mesodermal in origin. A 'reduction' in the caudal cranial fossa might indicate that there is either insufficiency in growth e.g. the plausible paraxial mesodermal insufficiency' theory postulated by early research [268]. If there were a mesodermal insufficiency then this would have a widespread manifestation, not localised, and possibly implicated in 'minaturisation' of some components of the head. The cephalic paraxial mesoderm forms the supra-occipital [153]. Couly *et al*, describes the occipital as a whole as a giant vertebra enlarged to form a cupula in which the brain rests. In this model, the neural arch is represented by the exo-occipital and the supra-occipital and the corpus of the vertebra is represented by the basioccipital [153]. The cranial base is angled at the level of the hypophyseal fossa where the rostral prechordal and caudal chordal parts meet. In the early embryo this angle is obtuse but by the time of ossification it should have flattened. Consequently inadequacy of cartilage growth will result in a short cranial base with increased angulation [262]. In humans, the clivus (basioccipital)–supraocciput angle is a useful parameter to differentiate various causes of foetal ventriculomegaly and in particular CM-II [193].

The examplar MRIs of Chihuahuas, with and without SM, provided in Fig 5.5, were selected deliberately because both had atlanto occipital overlapping, a common condition in this miniaturised breed (A-M Kiviranta *et al* in press) Conversely, Fig 6.7 provides MRI examples of two Chihuahuas

with and without SM that do not include atlanto occipital overlapping. These illustrate two insufficiencies in the CM/SM affected dog (1b) compared to dog 1a without SM which may have both been caused by paraxial mesodermal insufficiency i) deficiency of clivus (aqua arrow) ii) reduced or absent interparietal bone (yellow arrow) so the occipital lobes become more ventrally positioned overlying the cerebellum (white *). This example illustrates how an overall insufficiency might be allied with 'minaturisation' in a specific manner that might result in CM/SM. Such a hypothesis clearly needs further investigation.

Fig 6.7 Sagittal MRI of Chihuahua with and without SM and their morphometric overlays.



lb compared to lb. Supraoccipital reduced, atlas is closer to basi-occipital so angle 3 is increased, lines ai, bi and bc are shorter but not ab, angles 1, 2, 3, 4, 6, 7 and 8 smaller but not angle 5. Sphenoid more acute angle to occipitat at synchondrosis (X). Cerebellum invaginated under occipital lobe *.

The morphometric grid of Chihuahua 1b with SM (red) has been superimposed on the blue morphometric grid of Chihuahua 1a without SM.

Role of Supraoccipital bone in CM/SM

Occipital hypoplasia has been a key identifying features of SM secondary to CM [227] and the findings in this thesis suggest that the supraoccipital plays a highly significant role. A macroscopic analysis of neonatal CKCS and control dogs of similar size and head shape, Giejda also found that the height but not the width of the supraoccipital bone was significantly shorter in the CKCS cohort. Furthermore, the histology of both the basi-occipital and supra-occipital showed that the latter had marked apoptosis in the cell population of chondrocytes which would result in decreased bone deposition [196]. Pathological changes with a lower cellularity were also identified in the single case of an adult supraoccipital bone [35]. As part of an investigation into the development of CM in the CKCS, Giejda studied the histology of the supraoccipital bone in a variety of neonatal dogs and identified two parts: superior-membranous and inferior cartilaginous [196]. Giejda observed a sinus in neonatal dogs between the two parts of the bone and this sinus was located on the internal surface of the occipital protuberance implying that the two parts ossified and fused at birth. Histomorphometric analysis confirmed an irregular and concave bone with reduced numbers of trabeculae (bony spicules which form a meshwork of intercommunicating spaces), compared to the controls. In other words, the quality of the supraoccipital bone in the CKCS foetus was impaired. In the human foetus, it has been shown that cerebellar growth commences later than the cerebral hemispheres, whereas the growth of the bony posterior fossa appears to be in advance and independent of cerebellar growth [269]. It is credible, therefore, that any reduction in the proportions of the bony posterior fossa does not inhibit growth of the cerebellum which would develop 'normally' but becoming impacted or herniated within the reduced caudal fossa. Indeed, it has been demonstrated that CKCS have an enlarged cerebellum relative to the volume of the entire brain [37].

CM-I and CM-0 are considered to be congenital bony malformation i.e. skeletal, rather than neural in origin [29,270], although failure of the pontine flexure to form normally may lead to elongation of the brainstem and CMI and CMII [271]. Although there are considerable similarities between human CM-I and canine CM, an important difference between the two is the neuraxis and the fact that man is bipedal. The skull of man has evolved to be supported by the neck in a different plane to that of the dog. Furthermore, selective (functional and aesthetic) dog breeding has resulted in dogs that carry their heads at different angles in order to have their eyes set in a particular manner [110].

Brachycephaly

A frequent misconception relates to the use of the human term 'brachycephalic' or 'short head' based on the cephalic index. Canine brachycephaly is recognised not only by the reduction in craniofacial bones (craniofacial index) but also in changes in the palate position [108]. An extensive study of canine skulls collected over a period of years made by the German anatomist Nussbaumer showed a continuum of airorhynchic dogs arranged in order of severity. These were suggestive of multifactorial origins but not necessarily similar to those of humans and caution taken comparing with the canine CM model.

Most of the genetic research into CM/SM has focused understandably on the hindbrain but now the research findings in this dissertation have revealed that brachycephaly is also implicated. Genetic mapping of head conformation associated with brachycephaly has identified several candidate genes [109,272]. One example of a gene associated with brachycephaly is Fibroblast Growth Factor (*FGF*). This signalling transducer plays an important role in osteogenetic cell proliferation (*Fgfr2*) and differentiation (*Fgfr1*) in the developing skull vault [166]. Genetic diseases and syndromes that are associated with CM-I and arise from mutations include Crouzon syndrome (Chapter 3) and Apert's syndrome (*Fgfr2*) and Achondroplasia (*Fgfr3*). Using mutant mice, Eswarakumar and colleagues[172], showed that one variant *Fgfr2IIIc* altered the sutures in the skull vault with a domed forehead and shortening and angling of both the frontal and nasal bones similar to the 'sphenoid flexure'. Interestingly, homozygous *Fgfr2IIIc* 'loss of function' mutant mice also exhibited dwarfism in addition to abnormal (brachycephalic) skull shape [273]. *Fgfr2IIIc* plays a role in endochondral ossification whereby the onset of mineralization is retarded in the mutants, and the growth of the skull base and axial and appendicular skeletons are reduced. This outcome was associated with decreased areas of both proliferating chondrocytes and ossification zones in these endochondral bones, leading to premature

loss of skull base sutures and smaller than normal long bones and vertebrae including C1 and C2. Any phenotypic comparison between mouse and dog head shape and miniaturisation is clearly subjective, but the experimental study illustrates how single mutations can bring about a range of differences in skull shape and structure similar to those that pose a risk to CM/SM.

In the embryo, the neural crest cells are involved with the development of the craniofacial skeleton. The *Tcof1* gene encodes the nucleolar protein Treacle, which in neuroepithelial cells, is necessary for the development of neural crest cells that migrate to form the craniofacial mesenchyme. Research using *Tcof1* haploinsufficent mouse mutants resulted in a underdevelopment of the craniofacial bones [274]. Compared to wild-type controls, the mutants exhibited a domed cranial vault and shortened frontal and nasal bones similar to brachycephaly in the Pug and French Bulldog. Interestingly, working on Treacher Collins syndrome, a craniofacial disorder that results from mutations in *Tcof1*, J Dixon and M.J Dixon found that the penetrance and severity of the clinical features exhibited a wide variation in the phenotype when mice with different genetic backgrounds were used [275]. This indicated that factors in the different genetic backgrounds contribute extensively to the *Tcof1* phenotype and may play an additive role in brachycephaly in several different breeds but does not involve shortening of the skull base and risk of CM/SM. More pertinently, however, an investigation into the coupling of osteoblasts and osteocytes by Connexin43 (Cx43) and Runx2, two genes that interact to regulate gene expression of cortical bone. An in vivo study using knockout mice have revealed altered interparietal geometry in compound hemizygotes for the two genes. This provides an example of the genetic influence of osteoblast differentiation and proliferation that can contribute to aspects of the skull phenotype by altering bone shape [276].

Another candidate gene for brachycephaly is *SMOC2* which is involved in the utilization of cryptic splice sites (disadvantageous sites in the genome that are dormant or used only at low levels unless activated by mutation of nearby authentic or advantageous splice sites [277]). *SMOC2* has been shown to intract with *FGF4* retrogene insertion, previously associated with appendicular chondrodysplasia and also known to reduce neurocranium size. Using 86 landmarks made on 374 skull CT images of a wide range of dogs to capture morphological variation, the research team identified a genetic variation which disrupted the activity the SMOC2 gene which accounted for 36% of face length variation in dogs [278].

Brachycephaly in the context of reduced skull base and displacement of neural parenchyma may result in compression of the midbrain adjacent to the occipital lobes and block the cerebral aqueduct with subsequent increase in intracranial pressure and dilation of the lateral ventricles .

Compromised CSF flow dynamics

Results presented in Chapter 5 demonstrated that the craniocervical junction was a significant risk factor for the Chihuahua and Affenpinscher breeds which compromised CSF flow dynamics. This is the bony gateway linking the subarachnoid spaces of the brain and the spinal cord and altered conformations affects the CSF dynamics. Impedance studies have been made in humans [279,280] and morphometric measurements of the human clival angle have demonstrated that a wider angle is correlated with a decrease in the width of the foramen magnum, but not the height [168]. These variants are associated with fetal ventriculomegaly [193]. Although the morphometric mapping generated in the characterisation of CM does not differentiate any ratio of parenchyma to ventricular cavities, the values do represent the combined effect of any anomalies and identifies differences that might help diagnosis. For example a large f-diameter in in itself may be indicative of a bigger dog or increased volume with hydrocephalus but when used as a ratio with the distance across the foramen magnum (line bc), the basioccipital to the atlas (line ac) or, it can suggest risk of CM pain or SM.

A unifying hypothesis for hydrocephalus, Chiari malformation, syringomyelia, anencephaly and spina bifida has been proposed by Williams is that they are caused by inadequate venous drainage compliance [281]. A significant relationship between reduced venous pressure and parenchymal 'overcrowding' of the caudal cranial fossa in the pathophysiology of SM has been identified in the CKCS [100]. and narrowing of the jugular foramen [256]. Osseous abnormalities of the reduced caudal cranial fossa and at the craniocervical junction is thought to set up hydrostatic differentials between the spinal cord and the subarachnoid space [84,230,].

In Chapter 4, it was demonstrated that that it is not only an increase in the height of neural parenchyma (f-diameter) that is important as a risk factor for CM/SM but its position, angulation and overall shape. The brachycephalic skull, a risk factor for dogs with SM, would have a brain with less surface area ratio to volume than its elliptical (normal) counterpart would. For a given volume, the object with the smallest surface area is a sphere. Low olfactory bulb angles and ventral olfactory bulb orientations were associated with brachycephalia. Positioning of the olfactory bulbs, cribriform plate, and ethmoid turbinates are related [283]. The CSF drains from the subarachnoid space along the olfactory nerves to the nasal lymphatics, which in turn, empties into the cervical lymph nodes [284]. Thus, the meninges covering the cerebrum and cerebellum, the falx cerebri, tentorium cerebelli, ventrally rotated olfactory bulb and cribriform plate all proffer a reduced surface area to brain volume ratio in the CM dog. The arachnoid villi function as a valve regulating the drainage of CSF back into the venous sinus and it is suggested that, within the confines of the skull the phasing of the cardiac cycle may be affected, compromising the CSF flow mechanisms within the brain. Canine congenital ventriculomegaly is often an asymptomatic, incidental finding in small, toy, and brachycephalic dogs [285]. Human hydrocephalus is associated with much smaller sinuses than normal and Bateman et al suggests an elevation in venous pressure may explain the lack of CSF absorption into the arachnoid granulations in

chronic hydrocephalus [286]. Ventriculomegaly has been shown to be associated with SM in the dog [248] and a lack of CSF absorption might also create an imbalance of pressure gradients within neural parenchyma during CSF pulsations associated with the cardiac cycle, thus predisposing to SM.

A hypothesis that a reduction in the skull base and compensatory displacement of the cerebellar parenchyma during embryological development leading to progressive impairment of CSF flow dynamics might be extended further to include rostro-caudal doming. Fig 6.9 has been adapted from an illustration by Saunders (1983) in Miller's Anatomy of the Dog, Figure 16.16: Ventricular system of the dog brain to emulate brachycephalic shortening of the skull base. Using PhotoshopTM software tool for 'skew' and not fixed ratio. The 'normal' image has been reduced at its bottom edge, simulating the effect of rostro caudal shortening on the brain (bottom image) with reduced supra-occipital caudally which produced the automatic compensation rostrally.

Fig 6.9 'Natural' distortion of the ventricular system of the dog with bilateral reduction of the skull base and supraoccipital bone with compensatory increase in cerebral height that might occur during brachycephaly and CM.



Ventricular system of the dog brain adapted from Miller's Anatomy of a Dog. Top: 'normal dog' Bottom: using the 'distortion' tool in PhotoshopTM.image has been reduced equally on both sides at the ventral/bottom edge only to simulate brachycephalic skull shortening. Notice how the olfactory bulb now appears rotated with frontal lobe flattening and airorhynchy appears. As a result of the change in the angles shape and dimensions, the more spherical shape of th brachycephalic brain has less surface area to volume ratio and a change in a more ventral position and angulation of the olfactory tract and brainstem.

This conservation of volume but not shape is supported by research into human intentional cranial deformations which modified the thickness and shape of the skull but retained their volumes [287] This results in the reduced more acute angulation of the olfactory tracts, airorhynchy 'sphenoid flexure' (Chapter 4), occipital lobes overlying the compacted cerebellum and the ventricular system presents less surface area for the choroid plexus in the roof of the 3rd and 4th ventricles. Ventriculomegaly associated with SM has been related to a reduced caudal fossa [248]. However, the impact of brachycephaly may be a more important instigator and unifying factor towards changing the dimensions of the cranial caudal fossa, compression of the midbrain, angulation of the olfactory tracts and brainstem than currently envisaged by research in CSF flow dynamics.

Artificial Selection for CM and risk of SM

For a variety of reasons associated with modern life styles, the demand for 'doll' type dogs as a pet has never been so high and breeders have responded to this desire. The traditional head conformation of the CKCS with a shallower stop was not regarded as 'brachycephalic' and like the breed standard of the GB (appendix) has given way to the 'baby face' of the modern breed type and accompanying risk of CM/SM [16]. Particularly appealing are 'large eyes' due to the increased palpebral aperture and, in some cases, shallow orbits. Fig 6.10 illustrates the difference between two Australian Terriers described in the previous section.

Fig 6. 10 Eyes of Australian terriers with and without CM



The ostensibly 'larger' eyes of the dog with CM appear more baby-like and appealing

The mixed breed and backcross project of Chapter 3 showed it is possible, through selection, to retain a longer skull base and reduce the length of the muzzle which is a desired breed characteristic for the GB. This is illustrated in Figs.6.11 and 6.12 (opposite page). The radiographs of three generations have been inverted to highlight the skull shape. The occipital crest (interparietal bone) is less apparent in GB grandfather but the greater hind skull of mother, which had been backcrossed to a GB, is retained in the F2 (abfg2).

Fig 6.11 Radiographs of three generation of mixed breed GB family illustrating changes in the hind skull



The radiographs have been resized so that the distance from the base of the skull (white line) immediately, to the most dorsal point of the cranium rostral to the acoustic bulla is equal (aqua line). The pink (length of muzzle) and blue length of calvaria) lines of GB P1 have been superimposed onto the radiographs of the F1 hybrid and F2 backcross (abfg2) The muzzle length (pink) of F2 dog is shorter than F1 but slightly longer than P1. Both P1 and F1 dogs have an undershot jaw inherited independently ([112]. The aqua shaded hindskull of P1 is smaller compared to that of dogs F1 and F2 dogs that are similar to each other.

All four P1 (brachycephalic GB dog F, siblings A and G and chondrodystrophic and mesaticephalic Australian terrier B) are all FCI Breed Champions and therefore acknowledged distinguished examples of their breed type. As expected for complex traits involved in body shape and hair-coat, all F1 hybrids (ab) showed intermediate forms of the parents. They had relatively longer muzzle with a less pronounced undershot jaw than their brachycephalic GB sire (A). In the F2 backcross generation, male abfg1 has both head and body conformation faults for the Breed standard with a wider head than GB, more pronounced 'stop' and longer muzzle than his sibling. The eyeball has normal orbital coverage. By comparison, F2 backcross abfg 2 most resembles the GB breed standard with the nasal planum level with the lower eyelid (flattened yellow triangle) and reduced orbital coverage resulting in the characteristic "large" eyes of the GB. The ear and body conformation is "set" correctly for a GB (similarities with purebred GB sire fg1). The four dogs affected with SM in the study all exhibited reduced caudal skull development compared to their relatives.



Second generation (F2) backcross offspring (left) with purebred Griffon sire -photo courtesy of Henny van den Berg



Fig 6.12 Facial features of three generation pedigree of F2 Backcross and F1 and F2 body conformation.

P1 = first parents F1 = first filial generation F2 = second filial generation. Australian terrier (Dam B) has a longer muzzle which is well below eyes (yellow triangle) and upright ear pinna then the brachycephalic GB. Sire A has greater palpebral aperture so the eyes appear proportionally larger to the face. F1 Hybrid: ab4 eyes are "smaller" (more orbital coverage) than the GB with muzzle and ear placement and body conformation intermediate between parents and incorrect for the GB.

Fig. 6.13 is a follow up illustration of 3-dimensional modelling using Mimics Materialise[™] software of brain and upper spinal cord of the two F2 mixed breed siblings abfg1 and abfg2 in Chapter 3. The images have been resized, with fixed ratio retained, for comparison and the olfactory bulb rotation typical of brachycephalic breeds is apparent on the shorter muzzled female sibling abfg2. However the aqua arrows also indicate that the cerebellum is additionally displaced rostro dorsally.



Fig 6.13 Three dimensional image of brain and cervical spinal cord of F2 backcross siblings

yellow* = frontal sinus, blue* = occipital crest in male abfg1 absent in abjg2. Aqua arrow brachycephalic features of rotated olfactory bulb in abfg2. White line = equal distance on both images but abfg1 male appears shoter than female abfg

The mixed breeding heritage of the backcross has introduced greater genetic variation with a degree of forebrain flattening in the male compared to the ventral rotation of the olfactory bulb seen in the more brachycephalic female sibling (abfg2). The siblings were given the same CM1 grade by the BVA/KC but have very different skull conformations. Clearly the above analysis is based on a few dogs and more research is needed to clarify exactly where, on the spectrum of CM, any pathogenic risk lies and any heritable traits involved in purebred dogs. This will provide less ambiguity between what is natural variation in the shape of the cerebellum for brachycephalic breeds and greater clarity about pathogenic configurations. Additionally, it should be possible to predict the risk of SM in young dogs so that breeders are able to make their breeding selections earlier.

The CM/SM research undertaken in this thesis into has been successful because of the excellent working relationship between concerned breeders and pet owners and veterinarians. A good example of this was the Syringomyelia International Symposium Veterinary Satellite Meeting for dog owners, veterinary surgeons and nurses at Rugby School on October 2007 sponsored by the Ann Conroy Trust Charity (Appendix - publication)

Conclusion

The aim of this thesis was to characterise the phenotype and severity of CM/SM in selected toy breeds. A definition of Canine Chiari-like malformation was previously based on information over a decade ago and subsequent worldwide interest has led research to provide a greater insight and raised awareness about CM and SM and their relationship with each other. The complexity of CM/SM makes a succinct revised definition of CM particularly challenging. However the findings in this thesis suggest that a description of the condition should embrace bone reduction and displacement around the whole brain and spinal cord to include spheno-occipital synchondrosis angulation, reduced occipital crest and rostral displacement of the axis and atlas with increased odontoid angulation. Furthermore, the affiliation of such abnormalities with neuropathic pain is crucial. The introduction of this thesis highlighted the welfare concerns that arose from CM/SM and the purpose of the characterisation of CM was to make a contribution towards addressing those concerns. Through provision of an accurate phenotype for symptomatic CM and SM, these studies provides the opportunity for precise genotyping and diagnosis that can be used in future practical applications and offer a valuable contribution to dog welfare.



Australian terrier (Bitch B) with her Griffon hybrid 'Graussie' puppy

Photo courtesy of Henny van den Berg

Limitations of the study

Mixed breed study (Chapter 3)

The mixed breed study was not a scientific experiment but part of a breeding program with much loved pets. It took advantage of an accidental mating between two different breeds and data is limited by the goodwill of the owner Henny van den Berg. The expense and effort of maintaining so many dogs was considerable and the puppies that were rehomed as pets were not available for screening as originally planned. For economic reasons MRI screening was limited in terms of field of view and sequences and did not include MR images of the entire brain so that measurements of the forebrain were not available. Since offspring were screened at one year, this was a prohibitive factor investigating SM which can be late onset.

Although the study sample size was small, the five foundation dogs are part of a worldwide GB pedigree database of over 300 dogs with known CM and SM status and confirm relatives in Europe, Australia and the USA. The study cohort also includes all three races of Griffon Bruxellois (red rough coat), Petit Brabancon (red smooth) and Griffon Belge (Black and Tan rough coat) with varying degrees of CM and SM affectedness.

Mating decisions were entirely those of breeder and co-author Hv/dB based on 40 years' experience and as a Dog Show Judge for 24 years. Such a program has to take into consideration not only other health issues (eyes heart, patella luxation, etc.) but the availability of suitable dogs. An outcross with another breed is not supported by any Griffon Breed Clubs or the Kennel Club (UK). The financial cost for screening litters over a relatively short period of two and a half years, limited the total number of puppies that could be included in the study, despite additional funding provided by public donations (Syringomyelia DNA Research).

Finding suitable CM free dogs in breeds with a high prevalence of CM can be difficult for breeders. This was the reason why the F1 backcross was not mated to a CM normal dog. The worldwide GB breed population has a very small gene-pool and it can be extremely difficult to find suitable outcross dog. Furthermore, overuse of the limited MRI screened dogs shown to be clear of CM and SM (popular sire syndrome) would further reduce the gene pool[235,288]. A mixed breed outcross has the advantage of reducing inbreeding depression and disease incidence[289].

Morphometric mapping of CKCS (Chapter 4)

Although allowance was made for the variable quality images by selecting the most obvious landmarks, the initial placement of a single line on the curvature the cranial base was sometimes difficult. In order to overcome this, a large number of inter-related measurements were made to mitigate any deviation.

Since robust phenotyping is paramount for intended genetic studies, screenshots were also made of each dogs 'signature' for reference so they could be checked visually for consistency.

The study was somewhat limited by the number of entire brain MRI images available for the control and CM pain cohort. 35% of the latter group were less than 5 years of age and may go on to develop SM, but these dogs were age matched. Screening asymptomatic dogs over 5 years is not common and the BVA/KC/ CM/SM Health breeding scheme [80] does not require MRI of the whole brain. Furthermore, it relies on the asymptomatic assessment by the owner and some clinical and/or behavioural signs of pain may not be recognised. Despite ruling out other clinical causes in the CM pain cohort, the interpretation of the behavioural signs of pain remains subjective and intermittent signs may be overlooked and others over-interpreted.

Craniometric analysis in Chihuahua, Affenpinscher and CKCS (Chapter 5)

Although this retrospective study included MR images from different sources the landmarks selected were those most easily identified irrespective of machine resolution and had been verified in previous studies. The effect of different positioning in the MR coil with respect to sternal or dorsal recumbency was not considered an issue because the head was fully extended in both positions. The poorer signal to noise ratio of low-field MRI inevitably makes interpretation of SM1 challenging but variability was limited by having the same interpreter for all the evaluations (CR) and more than one when possible.

The number of Affenpinschers affected with SM and number of young dogs of unconfirmed SM status compared to the other two subgroups was a limitation in the study. However, it was considered important to include all dogs possible therefore dogs without SM were age matched with dogs with SM. Affenpinschers are not as popular as CKCS and Chihuahua but the low numbers of reported symptomatic dogs may also be due to differences in head conformation in the breed that reduce the risk of SM.

Future Studies

Diagnostic tool

Following successful private funding in the name of a pet CKCS called Hannah and a Pet Plan Scientific Primer Grant, a post graduate has now been appointed to carry the CM phenotype characterisation forward using innovative machine learning and image analysis to quantify risk factors for Canine Chiari-like Malformation and syringomyelia. This technique has the advantage of removing observer bias. Once devised, the 'tool' can be validated and it is anticipated that this will provide a grading system to identify the severity of particular traits in a summative manner. The existing pool of data can be exploited to keep costs to a minimum and extended to other breeds. More research is needed to investigate the relationship of SM1 with both SM0 and SM2 – is it an intermediate stage or not necessarily progress to SM2 over time.

Genetics

A key strategy for the characterisation of CM is that the significant traits to be used in conjunction with the simultaneous DNA and RNA collection of the dogs in the study and the ongoing genetic studies in collaboration with Drs Kibar and Rouleau working at CHU Sainte Justine Research Centre, Montreal University on the human CM-I [79]. There is an acceptance that CM-I is similar to canine CM and that dogs provide a spontaneously occurring, natural model for the disorder. So far only two breeds have been involved, namely the GB for CM and the CKCS for SM. However DNA has already been collected from the i) Chihuahua and Affenpinscher study ii) mixed breed family and it is hoped that these will be used to investigate other aspects of this condition.

Hereditary studies

Pain suffered by dogs is a welfare concern but if it is a result of a hereditary condition, even if complex form, it may be possible to alleviate the misery and reduce the incidence by breeding selection.

- This is the opportunity to continue the research on skull morphology that was initiated with the Mixed Breed project (Chapter 3). Keeping a longer skull base but shortening the facial bones to reduce risk of CM/SM in other brachycephalic breeds.
- The large family databases of both CKCS and GB can be used to investigate the inheritance of different traits and identify any combinations of conformation that are less risky than others. This may allow breeders to use dogs which might have removed dogs from the gene pool.
- iii. Data exists to investigate the relationship between painful CM and SM in a family which has both disorders and which may elucidate the traits that differentiate between them.
- iv. Use of the morphometries to investigate the mode of inheritance of CM and SM

Conformation studies

- i. A study already underway in collaboration with the Swedish Kennel Club, supported by funding from Charity Cavalier Matters, to investigating the head shape conformation in the CKCS. It is intended to match the eternal head shape of these dogs with the internal morphometries in order to provide an additional tool for breeding selection
- ii. The extent to which CM encompasses a heterogeneous grouping of disorders is not fully understood and the 'additive' nature of complexity can result in different traits predominating in different breeds and even within the same breed. There is an opportunity to extend the morphometric mapping in other breeds predisposed to CM/SM such as the King Charles spaniel and Maltese for which a collection of suitable MRI images exist.

Pathomorphological studies

- i. A study already initiated is one that is looking at the different skull and brain conformation between scratchers and non-scratchers in the CKCS that can be correlated to the size and position of the syrinx
- ii. Further collaborative research with University of Helsinki investigating CM/SM pain in Chihuahua
- Craniometrics developed in this thesis can offer support for mathematical modelling of CSF production and flow in the brain and spinal cord associated with brachycephaly and CM
- iv. Histological and embryological studies of the brain of breeds with and without SM to investigate the potential role of neural non-compliance.

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Appendix

Tables

Hir	ndbrain st	udy	Whole brain study			
Function	1 2		Function	1	2	
L5 (aeb)	0.089	0.283	line bk	0.188	0.351	
L7 (bdi)	0.143	0.03	L2 (fac)	-0.133	-0.047	
L9 (jcb)	0.057	-0.068	n-diameter	0.226	-0.378	
(Constant)	-8.437	-4.924	Ellipticity	-0.363	0.219	
			Olfactory Bulb Angle	0.078	0.038	
			(Constant)	23.538	-10.499	

Table 4.4 Canonical Discriminant Function Coefficients

Table 5.2. Canonical Discriminant Function Coefficients for 1) breeds 2) SM used in the scatterplots (Fig 5.1).

Canonical Discriminant Function Coefficients for 1) breeds 2) SM							
	1.Breeds	Function 1	Function 2		2. SM	Function 1	Function 2
u	f-diam	0.192	-0.563		f-diam	0.285	0.211
aratic	bc	0.284	1.545		L3 (dib)	0.087	-0.052
seps	id	0.009	0.25		L4 (jcb)	-0.055	0
standardized coefficients used in the	ac	0.225	-0.003		(Constant)	-17.383	-4.784
	fg	0.208	-0.074				
	cj	0.156	0.456				
	<i>L</i> 1(afg)	0.072	-0.064				
	L3 (dib)	-0.03	0.093				
	<i>L</i> 6(dba)	-0.044	0.176				
	f-diam:bc	0.465	7.86				
	f-diam:cd	0.295	-1.134				
Un	(Constant)	-27.317	-34.26				

			Chihuahua			Affenpinscher			CKCS		
I	nt	S	Trait	Function 1	Function 2	Trait	Function 1	Function 2	Trait	Function 1	Function 2
nica	ction cient	L3 (dib)	-0.076	0.113	f-diam	0.619	0.298	line id	0.305	0.591	
Cano	scrin	run oeffi	L4 (jcb)	0.124	0.116	line cj	-0.718	0.953	f-diam:ai	5.548	0.523
0	Di	ĊŬ	(Constant)	2.281	-14.466	(Constant)	-19.318	-20.817	(Constant)	-18.807	-18.979
	ds	as	SM			SM			SM		
Functions at	ns at	ntroi		Function 1	Function 2		Function 1	Function 2		Function 1	Function 2
	ction	Ce	SM0	0.734	0.096	SM0	-0.496	-0.176	SM0	-0.673	0.142
	Fun	dno	SM1	0.313	-0.195	SM1	0.116	1.068	SM1	-0.349	-0.406
	ć	5	SM 2	-0.803	0.031	SM 2	1.463	-0.25	SM 2	0.632	0.035

Table 5.3. Canonical Discriminant Function Coefficients used in the scatter plots (Fig 5.2).

Table 6 3. UK and USA GB Breed Standard for head compared to facial dysmorphiccharacteristics of Crouzon Syndrome. [226]

Features	UK Griffon Bruxellois Breed Standard (since 2009*)	USA Brussels Griffon Breed Standard	Crouzon syndrome (branchial arch syndrome)
Head	Fairly large in comparison to body, rounded but in no way domed, moderately wide between the ears.	Large and round, with a domed forehead. The stop deep.	Brachycephaly (short and broad)
Nose	Always black and definite stop between muzzle and skull.	Very black, extremely short, its tip being set back deeply between the eyes so as to form a lay-back.	Beak like nose (Psittichorhina)
Eyes:	Black-rimmed, very dark, round, clear and well-spaced. Moderately large; size should be in proportion to size of skull.	Set well apart, very large, black, prominent, and well open.	Bulging eyes set wider than normal
Ears:	Semi-erect, high set, the smaller the better.	Small and set rather high on the head. May be shown cropped or natural. If natural they are carried semi-erect.	Low set ears and high prevalence of ear canal malformations
Mouth	Slightly undershot with even teeth, not showing teeth or tongue. Relatively short, wide muzzle, neat lips with good turn up.	Jaws must be undershot. The incisors of the lower jaw should protrude over the upper incisors. The lower jaw is prominent, rather broad with an upward sweep.	Concave face and protruding chin (hypoplastic maxilla)

* Following concerns about the welfare impact of the conformation of some pedigree dogs many breed standards were modified by the Kennel Club (UK) so as not to encourage features that might prevent a dog from breathing, walking and seeing freely. Typically these changes were the insertion of downplaying words such as "slightly", relatively" and "moderately.

U-tube link to Movie Morph <u>here</u> (<u>https://youtu.be/TcPdH9Gcnqo</u>)

Legends for Morph Movies

Morph Movie A1. Morphing morphometric 'signatures' of four exemplar sagittal whole brain T2w MRI provided in Fig 4.4; Control, CM pain and two conformation cases of the SM cohort. The movie highlights the dynamic changes of the skull conformation and brain parenchyma associated with progressive brachycephaly and airorhynchy, shortening of the basicranium and supraoccipital bones and the proximity and angulation of the atlas and dens. Using a 'fixed ratio' image tool, the occipital circle of the four exemplar MRI images has been standardised and the baseline abi aligned, the movie graphically illustrates the concertina flexure of the dog morphometric signatures with changed CM and SM status. As the video morphs from the control dog to one with CM pain that the nasal bone and hard palate become closer so that the hard palate becomes more horizontal, the nasal cavity and frontal cavity reduce in volume and the rostral forebrain is flattened. As the model progresses into SM case 1 the nasal and rostral forebrain changes become more extreme. In addition to the forebrain changes, the hindbrain is pushed caudally and the craniocervical junction kinks as a consequence of the cervical vertebral being closer to the skull with flattening of the supraoccipital bone. Consequently there is a "concertina" flexure of the brain with a compensatory increase in height of the cranial fossa (asterisk). In SM case 2 the concertina flexure is more extreme caudally (X) with the cerebellum invaginated under the occipital lobe and the olfactory bulbs are much reduced in size and ventrally displaced.

Morph movie A2: Chihuahua 1a with atlanto-occipital overlapping (AOO) but without SM that morphs to Chihuahua 1b with AOO and SM. The basioccipital and supraoccipital bones shorten. Angles 1 and 2 in the midbrain become smaller and the occipital crest reduces in size so that the cerebellum is deformed and invaginated under occipital lobes. The dens becomes more angled and moves closer to the foramen magnum resulting in a ventral flexure of the spinal cord. This conformational change is called 'cervical flexure'. The obtuse angle 3 is associated with AOO.

Morph movie A3: Affenpinscher 2a without SM that morphs to Affenpinscher 2b with SM. The basicranium shortens and the midbrain angles 1 and 2 become smaller so that the cerebellum is deformed and flattened against the flattened and shortened supraoccipital bone and is invaginated under the occipital lobes. The occipital crest is also reduced. The dens appears to move closer towards the foramen magnum with greater angulation resulting in 'cervical flexure' of the spinal cord

Morph movie A4: CKCS 3a without SM that morphs to CKCS 3b with SM. Both basioccipital and supraoccipital bones shorten. As the basicranium shortens, the midbrain angles 1 and 2 become smaller so that the cerebellum is deformed and flattened against the straightened supraoccipital bone and is invaginated under the occipital lobes. The basisphenoid bone is flexed dorsocaudally ('spenoid flexure). The dens and atlas move closer to the foramen magnum with a change in angulation of the spinal cord (cervical flexure) elevating the medulla oblongata (medullary kinking).

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Programme graphic compilation by S. P. Knowler: Syringomyelia International Symposium Veterinary Satellite Meeting for dog owners, veterinary surgeons and nurses. Rugby School, October 2007 sponsored by the Ann Conroy Trust Charity. **Delegates Notes**

Veterinary Satellite Meeting

For breeders, dog owners, veterinary surgeons & nurses

Rugby School, Friday 26th October 2007 17.00 - 21.00 hrs









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Welcome...

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The Ann Conroy Trust welcomes you to Syringomyelia 2007

It i. It is with great pleasure that we welcome you to Rugby, to join this meeting of specialists in the management of syringomyelia. This event has been organized by the Ann Conroy Trust and is endorsed by the Society of British Neurological surgeons, the Spine Society of

Europe and the University of Birmingham.

Even today, syringomyelia can extract a heavy toll from its sufferers and yet they and their families are frequently frustrated by the apparent lack of knowledge about the condition amongst physicians in general. They become frightened by the threat of developing progressive neurological disability and the

thought that they may have to face up to major neurosurgery. It is for this reason that Ann Conroy, herself a syringomyelia victim, set up the charity which now bears her name, in 1980. Her intention was to help support fellow suferers and their carers and to promote education and



research in the field of syringomyelia and its associated disorders. Since those early days the charity has developed and now provides a variety of services to both patients and specialists who treat syringomyelia. The charity sees dissemination of knowledge about these conditions as one of its principal aims, which is precisely why we are proud to have you join us at this symposium.

Tony Kember, Chairman ACT

The Mayor welcomes you to Rugby

As Mayor I am proud that the Borough of Rugby has been chosen as the setting for this important International Symposium, and extend to all involved a warm welcome to this historic location.

As I am sure you are aware, the town of Rugby is famous the world over as the birthplace of rugby football - which of course led to the spectacular event that is the Rugby World Cup, the 2007 staging of this tournament having just concluded in France.



Rugby is, however, less well-known as the place where Sir Frank Whittle did his pioneering work on the development of the jet engine, which had its test run in the Borough. Here too the hologram was invented, and helium gas first identified. Add to this much of the development work in radar - there is obviously something in the air that fuels innovation.

I hope therefore that the air in Rugby will encourage you all to be innovative in thought and word during the Symposium, and that you will leave here with an increased commitment to your vital work.

I wish you a most enjoyable and productive time in Rugby.

The Headmaster welcomes you to Rugby School

I am delighted to welcome you all to Rugby School for what I hope will prove to be a very successful symposium.

Rugby School has every claim to be the leading co-educational boarding school in the United Kingdom. We are an institution that has always valued the teaching of science and one of Rugbyis many claims to fame is that for over two hundred years this school has been at the



forefront of science education in this country. For example, in the 1960's Geoff Foxcroft was instrumental in the development of the Nuffield A Level Physics course. More recently John Taylor has been the key figure in the shaping and piloting of a new AS Level, Perspectives on Science, which challenges pupils to think critically about the profound and exciting historical, philosophical and ethical questions raised by science.

Enjoy your visit to the home of the great game of Rugby Football and I hope that your time here proves to be extremely worthwhile.

Patrick Derham, Headmaster





Sue Peach, Mayor

RUGBY SCHOOL



Welcome to Syringomyelia 2007

Although a relatively rare clinical entity, syringomyelia continues to fascinate neurosurgeons and other specialists worldwide. To describe it as an enigmatic condition is as valid today as was the case half a century ago. This is despite advances in imaging technology, which nowadays permit an accurate diagnosis to be made at an early stage, in most cases. Indeed, as a result of such advances, we now face new challenges such as the appropriate management of the increasing number of apparently idiopathic syrinx cavities that are being detected. Further, some of the old questions remain unanswered, such as just what is the pathophysiological basis of CSF accumulation within the substance of the spinal cord?



The first important monograph on the subject of syringomyelia was published in 1973. There have been four other dedicated texts since, most recently in 2004. It is now seven years since the last international meeting on the subject was held, in Kobe, Japan. In that time we have seen advances made in a number of areas and we have achieved general agreement over some aspects of surgical technique. There nevertheless remain differences of opinion over many aspects of treatment and there are still several questions regarding case selection. There are particular issues relating to paediatric practice, including the relationship between spinal deformity and syringomyelia. We are not fully conversant with the natural history of all forms of syringomyelia. Nor are we particularly effective in our management of those symptoms of syringomyelia that persist even after anatomically effective treatment of the syrinx cavity. Altogether, it is timely that we now meet at Syringomyelia 2007.

This symposium brings together many specialists, from a variety of disciplines, from around the world. We are especially pleased to welcome our invited lecturers, who will deliver keynote addresses as introductions to each of the main sections of the symposium. We are equally grateful to those of you who have submitted original work for presentation. We have papers from major institutions and authors of international renown as well as work from smaller units, altogether reflecting experience gained in day-to-day neurosurgical practice around the world. We also have a number of experts from fields outside neurosurgery, including mathematicians and physicists, physiologists, radiologists and pathologists. We are also joined by colleagues from the world of veterinary medicine. It is my earnest hope and expectation that we can combine our experiences and will indeed define the present state of understanding of syringomyelia and its related disorders, discuss openly controversies in practice and provide direction for future research.

Graham Flint, Chairman, Syringomyelia 2007.

Welcome to Veterinary Syringomyelia 2007

iTo be able to cure, with some degree of certainty, a disease that, during the time it lasts, is extremely excruciating, is an addition, however small, to the utility of our profession.i (Dr John Fothergill 1773)



Syringomyelia 2007 is a unique event not least because of the eclectic mix of delegates. Neurosurgeons, neurologists, geneticists, scientists, pain management specialists, veterinary surgeons, mathematicians, physiotherapists, nurses, dog owners and breeders have all been brought together by a common interest - to better understand the enigma of syringomyelia. Some may wonder what relevance canine syringomyelia has to the human condition (and vice versa!). However we have much to learn from each other. The canine condition is a naturally occurring model of Chiari type I malformation and globally there are hundreds of affected pet dogs. Such a large population offers huge potential - from elucidating the genetics of the disease to finding a better way of managing central neuropathic pain. From the veterinary perspective I

am sure that I speak for many of my colleagues in saying how excited and privileged we are to be able to participate in this conference with so many world renowned experts. Understanding of the management of canine syringomyelia is just in its infancy and often we look towards the human equivalent for new direction. So welcome to all delegates - this conference should prove a stimulating and enlightening experience for us all.

Clare Rusbridge, Chair, Veterinary Syringomyelia 2007



Contents

Ask the Experts.....14

Breeder/Pet Owners Questions......page insert

List of Delegates......page insert

Delegate notes sponsored by Intervet Compiled by Penny Knowler, Stone Lion Veterinary Centre, Wimbledon, UK



RESEARCH • PERFORMANCE • INTEGRITY



Programme of Events

Venue:	McCready Theatre	RTELIA CHIAR
17.00	Canine syringomyelia - an Introduction Dr. Clare Rusbridge, Stone Lion Veterinary Centre, UK -Sponsored b	y Ann Conroy Trust
17.20	Human chiari malformation and syringomyelia Mr. Graham Flint, Queen Elizabeth Hospital, Birmingham, UK	
17.40	The search for the gene(s) predisposing to Chiari I malformation wis syringomyelia Dr. Guy Rouleau, University of Montreal, Canada	th
18.00	Breeding for Health in pedigree dogs: optimization of breeding strategies to reduce inherited disease Dr Sarah Blott, Animal Health Trust- Sponsored by Hills	Hill's
18.20	Experiences in surgery for canine syringomyelia Dr. Dominic Marino, Long Island Veterinary Specialists, USA - Sponsored by Novartis	
18.40	Coffee break	
19.00	Syringomyelia Question Time - Ask the Experts Chaired by Dr. Bruce Fogle -Sponsored by Pfizer Animal Health	Pfizer
	Questions collated by Sandy Smith and Carol Fowler (pet owners) Sue Robinson, Veronica Hull (breeders) - Sponsored by Boehringer-Ingelheim	Boehringer Ingelheim
20.00	Buffet supper	
21.00	Close	

21.15 Coaches to hotels



Speakers...



Clare Rusbridge, BVMS PhD DipECVN MRCVS -European and RCVS Specialist in Veterinary Neurology Stone Lion Veterinary Centre, 41 High St, Wimbledon, London SW19 5AU, UK

Clare graduated from the University of Glasgow in 1991 and then enjoyed a year in the USA as a small animal intern at the University of Pennsylvania. She was fortunate to have the opportunity to spend some weeks at North Carolina Veterinary School Neurology department where she first became interested in

Veterinary Neurology. She then spent a year in general small animal practice in Cambridgeshire. In 1993 she joined the Royal Veterinary College, completing a 3 year BSAVA/Petsavers residency in Neurology and then spent a year as a Staff Clinician in Neurology. In 1996 she was board-certified by the European College of Veterinary Neurology. Since August 1997 she has operated a neurology referral service at the Stone Lion Veterinary Referral Centre in Wimbledon gaining Royal College of Veterinary Surgeons Specialist status in 1999. She became interested in Chiari-like malformation / syringomyelia in 1995 and has continued to research this disease focusing on the genetics, pathogenesis and treatment. She gained a PhD in this subject from Utrecht University in February 2007. Her other professional interests include other causes of neuropathic pain (in particular feline orofacial pain syndrome), epilepsy, feline neurology, and inherited diseases.



Graham Flint, BSc, MB, FRCS, Consultant Neurosurgeon, Queen Elizabeth Hospital, Birmingham, UK.

Graham is a graduate of Birmingham medical school, UK where he qualified in 1975. As a student he won the Russell memorial prize in nervous diseases, in 1974 and again in 1975. He also obtained an honours BSc degree in anatomical studies in 1972. He became a fellow of the Royal College of Surgeons of England in 1980. Following basic surgical training he was accredited in

neurosurgery in 1987. He was appointed to his present post, as consultant neurosurgeon at the Queen Elizabeth hospital, in Birmingham, in 1992. He is an honorary senior lecturer at the University of Birmingham and an examiner for the UK Intercollegiate Specialty Board in neurosurgery. In the early part of his consultant career Graham specialised in the treatment of spinal disorders but since 1995 he has run the syringomyelia service established in Birmingham by the late Bernard Williams. Via this field Graham has since developed a practice dealing principally with disorders of CSF circulation, including a special interest in neuroendoscopy. In 2005 he brought together a group of UK based neurosurgeons with an interest in syringomyelia. Graham sees Syringomyelia 2007 as the natural progression of this project, with the assembly of an international group of specialists, from a variety of disciplines, which can discuss and share their knowledge, understanding and uncertainties about syringomyelia and its related conditions.





Guy A. Rouleau, M.D., Ph.D. FRCP(C) Canada Director, Ste-Justine Hospital Research Center and The Center for the Study of Brain Diseases, University of Montreal

Dr Guy Rouleau graduated in medicine at the University of Ottawa in 1980, completed his Neurology Residency at McGill University in 1985 and a PhD in Genetics at Harvard in 1989. He is the Director of both the Research Centre of the CHU Sainte-Justine and the Centre of Excellence in Neuromics of the University of Montreal and is the leader of 'Genome Canada', a national research initiative which is involved in the identification and characterisation of genes.

Dr Rouleau is Professor of Medicine at the University of Montreal and holds the Canada Research Chair in the Genetics of the Nervous System. He specializes in the genetics of different brain diseases and has been honoured in many ways including the Award of Excellence Department of Medicine, University de MontrÈal and the Michael Smith Award (Canadian Institutes of Health Research)

Dr Rouleau has published over 350 original papers and 30 Book chapters, Reviews and Editorials



Sarah Blott, B.Sc, M.Sc, Ph.D Department of Genetics, Centre for Preventive Medicine Animal Health Trust, Lanwades Park, Kentford Newmarket, Suffolk, CB8 7UU

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Dr Sarah Blott is a Research Group Leader at the Animal Health Trust and is interested in the development of breeding schemes for companion animals that combine state-of-the-art knowledge in quantitative genetics with molecular genetic markers. She has an MSc in Animal Breeding and a PhD in Quantitative Genetics, completed at the Roslin Institute (Edinburgh). Following the award of a Wellcome Trust Travelling Fellowship, she went to the University of LiËge, Belgium to work with Professor Michel Georges on methods for fine mapping of complex trait loci in dairy cattle. In 2002 she joined Sygen International plc, one of the world's leading animal breeding companies, as a Senior Research Scientist and was responsible for data analysis on several research projects in the areas of gene mapping and high throughput gene expression analysis. She has also successfully managed multi-partner European Commission research projects and was a Visiting Scientist at the University of Cambridge Vet School in 2005.



Speakers...(continued)



Dominic J Marino, , DVM, DACVS, CCRP Chief of Staff, Long Island Veterinary Specialists 163 South Service Road, Plainview, NY 11803,

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ANIMAL HEALTH

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Dr Dominic J Marino graduated with honours from Auburn University in Alabama, where he received both his Bachelor of Science and Doctor of Veterinary Medicine degrees. He completed a one-year internship in small animal medicine and surgery at the University of Georgia and a threeyear comprehensive residency in small animal orthopaedic, soft tissue and neurosurgery at the Animal Medical Centre in New York City. Dr. Marino was honoured with both the Martin and Beatrice Weiser Outstanding Research Award and the North Shore Animal League Petering Scholarship Award during his residency. He is board certified by the American College of Veterinary Surgeons and is the former head of Orthopaedic/Neurosurgery service at the Animal Medical Centre in New York City. Dr Marino has published many scientific articles, and authored chapters in veterinary medical textbooks. He lectures extensively on many surgical topics including soft tissue, orthopaedic and neurosurgery. Dr. Marino served as chairman of the Eastern Veterinary Orthopaedic Society for several years and is on the National Advisory Board of North Shore Animal League America. He is currently the chairman of the Department of Surgery, and the Chief of Staff at Long Island Veterinary Specialists in Plainview, NY. Dr. Marino serves as the surgical consultant to: The Humane Society of New York, NY; Nassau University Medical Centre, Uniondale, NY; North Shore Animal League America, Port Washington, NY; Bide-A-Wee Animal Adoption Centre, Wantagh, NY; The Guide Dog Foundation, Smithtown, NY; Sweet Briar Nature Preserve, Smithtown, NY; and is an active executive board member of the Police Surgeons Benevolent Association.



Presentation Abstracts

Dr. Clare Rusbridge,

Consultant veterinary neurologist and neurosurgeon, Stone Lion Veterinary Centre, Wimbledon, London

Canine syringomyelia - an Introduction

Syringomyelia (SM) is emerging as a common canine spinal cord disease particularly in toy breeds such as the cavalier King Charles spaniel (CKCS), King Charles spaniel and Griffon Bruxellois. The pathogenesis is not fully understood. An important contributory factor is mismatch between brain and skull volume (Chiari-like malformation - CM). Compared to other toy breeds, the CKCS is overwhelmingly overrepresented suggesting a genetic predisposition. Studies have suggested that 95% of CKCS have CM and as many as 50% have SM with the proportion of affected dogs increasing with age. Compared to unaffected CKCS, CKCS with SM-related clinical signs have a smaller caudal fossa (i.e. the hind chamber of the skull) to total brain volume ratio. However other unidentified anatomical or environmental factors are likely to contribute to the development of SM. Studies comparing intracranial dimensions have not demonstrated a significant difference in caudal fossa size in CKCS with and without SM. In other breeds such as the Griffon Bruxellois, SM may be present without CM.

Recent studies suggest 35% of SM-affected dogs have clinical signs of pain. Some signs such as vocalising after sudden changes in posture are likely due to obstruction of cerebrospinal fluid (CSF) flow. SM also results in a neuropathic pain syndrome probably due to damage to the areas of the spinal cord processing information about pain and other sensations (spinal cord dorsal horn). Affected dogs behave as if they experience allodynia, appearing to dislike touch/contact to certain areas of skin. Signs of pain are positively correlated with syrinx width i.e. dogs with a wider syrinx are more likely to experience discomfort, and dogs with a narrow syrinx may be asymptomatic, especially if the syrinx is symmetrical and not involving the spinal cord dorsal horn.

Dogs with a wide syrinx may also scratch at a localised area of skin. It is hypothesised that this behaviour is in response to unpleasant sensations (i.e. dysaesthesia). Scoliosis (spinal curvature) is also common where there is a wide syrinx. This is thought to be due to damage to the part of the spinal cord which processes information about body position (spinal cord dorsal horn).

The main treatment objective is pain relief. Cranial/cervical decompression surgery is successful in reducing pain and improving neurological deficits in approximately 80% of cases and approximately 45% may still have a satisfactory quality of life two years postoperatively. However the syrinx appears persistent in many cases and the clinical improvement is probably attributable to improvement in CSF flow through the foramen magnum. In some cases fibrous tissue adhesions over the foramen magnum result in re-obstruction and 25% to as many as 50% of cases eventually deteriorate. Medical treatment plays an important role in management of canine SM. The most commonly used drugs are those that reduce CSF pressure (e.g. furosemide, cimetidine or omeprazole); non steroidal anti-inflammatory drugs, neurogenic analgesics (e.g. gabapentin, pregabalin, and amantadine) and corticosteroids. Prognosis for SM managed medically is guarded especially for dogs with a wide syrinx and/or with first clinical signs before 4 years of age. A small case series found 36% were eventually euthanatized due to pain. However 43% survived to be greater than 9 years of age (average life expectancy for a CKCS is 10.7 years). Most dogs retain the ability to walk although some may be significantly tetraparetic and ataxic.



Graham Flint, BSc, MB, FRCS, Consultant Neurosurgeon, Queen Elizabeth Hospital, Birmingham, UK.

Human Chiari and Syringomyelia

The German pathologist Hans Chiari described four types of anatomical abnormalities at the region that we refer to as the craniovertebral junction. Only two of these are seen with any frequency and they are referred to as Chiari I and Chiari II malformations. The alternative term hindbrain hernia encompasses both of these abnormalities.

Chiari I hindbrain hernias are seen predominantly but not exclusively in adults, with an average age of presentation in the early part of the forth decade. They are thought to develop as a result of a mis-match between the size of that part of the brain known as the cerebellum and the portion of the cranial cavity that contains it, which is referred to as the posterior fossa in humans, this being equivalent to the caudal fossa in quadrupeds. The condition can be likened to a person having shoes that are too small for his or her feet. Chiari II hindbrain hernias are part of a more profound anatomical disturbance which includes a group of spinal deformities known collectively as spina bifida. Hydrocephalus is also present in the majority of these cases.

Hindbrain hernias of either type produce symptoms it two main ways. In the first place they put pressure upon the brain stem and upper spinal cord. This may generate a variety of symptoms including disturbances of vision, sensations of vertigo, difficulties with speech and swallowing, sensations of imbalance and sensory disturbances and feelings of weakness in the limbs. Chiari malformations also impair free movement of cerebrospinal fluid (CSF) across the craniovertebral junction and this, in turn, can have two main consequences. In the first place the individual may experience onset of severe headache when coughing, sneezing, straining or bending over. Secondly, the condition of syringomyelia may develop.

Apart from in certain exceptional circumstances, surgical treatment of hindbrain hernias is seldom mandatory. The presence of an associated syringomyelia cavity will tend to tip the balance in favour of some form of intervention but an operation is not always needed. It depends, ultimately, on how troublesome and intrusive the symptoms are for the affected individual.

Drug treatment, using substances that decrease the rate at which CSF is formed, may produce useful relief from symptoms in a minority of cases. Very occasionally we encounter spontaneous resolution of a hindbrain hernia or syringomyelia cavity. For most affected individuals, however, effective treatment consists of a major brain operation, taking the form of a craniovertebral decompression. This procedure is generally very good at reducing, if not eliminating the headaches caused by Chiari malformations. Associated syringomyelia cavities will also collapse down, to a greater or lesser degree, in most cases.

Even when neurosurgical intervention produces a satisfactory anatomical result, with an opening up of the CSF pathways across the craniovertebral junction and collapse of an associated syrinx, a patient may continue to be troubled by various symptoms. Body pains and unpleasant, abnormal sensations often persist and sometimes worsen. Such symptoms are usually only partially responsive to medical treatments or other measures.



Guy A. Rouleau, M.D., Ph.D. FRCP(C) Director, Centre for the Study of Brain Diseases, Montreal, Canada

The search for the gene(s) predisposing to Chiari I malformation with syringomyelia

Zoha Kibar1, Penny Knowler2, Marie-Pierre DubÈ3, Clare Rusbridge2 and Guy A. Rouleau4

- 1 Department of Obstetrics and Gynecology, CHU Sainte Justine ResearchCenter, University of MontrEal, MontrEal, QuEbec, Canada;
- 2 Stone Lion Veterinary Centre, Wimbledon Village, UK;
- 3 Institut de Cardiologie deMontrÈal, Centre de recherche, UniversitÈ de MontrÈal, MontrÈal, QuÈbec,Canada;
- 4 Center for the Study of Brain Diseases, CHUM Research Center -Notre Dame Hospital, University of MontrÈal, MontrÈal, QuÈbec, Canada

Chiari I malformation (CMI) represents a common congenital abnormality in humans affecting the craniocerebral junction where there is herniation of the part of the brain into the spinal canal, often in association with syringomyelia (50-70%), which is an abnormal fluid filled cavity in the spinal cord. CMI occurs in 1 in 1200 of people, with a higher frequency in females versus males (3:2). The most common symptoms of CMI include headache (81%), brainstem or spinal cord dysfunction including scoliosis (30%) and syringomyelia (65-80%), ocular disturbances (78%), otoneurological disturbances (74%) and lower cranial nerve signs (52%). In advanced cases, CMI can lead to severe neurological deficit and permanent nervous system damage. CMI is thought to result from genetic and environmental factors. The developmental defect in CMI is thought to be the result of an underdeveloped occipital bone. CMI in humans is similar to a common condition in the cavalier King Charles spaniels (CKCS) breed, Chiari-like malformation (CM) that is characterized by underdevelopment of the occipital bone with foramen magnum obstruction and secondary syringomyelia (SM). This high incidence in a specific breed as compared to other breeds suggests the involvement of genetic factors in the etiology of this disease. Pedigree analysis in a large database of over 5500 CKCS has suggested that CM/SM is inherited where all clinically affected dogs share a small number of common ancestors. To date, no genetic factor predisposing to CMI has been identified in either humans or dogs.

The CKCS is the only known naturally-occurring animal with a high frequency of CMI. We constructed a genealogy of more than 10600 related CKCS dogs spanning 24 generations across 3 continents (North America, Australia and Europe) from over 600 MRI confirmed dogs. A molecular genetic study of 173 CKCS dogs identified six chromosomal regions with potentially significant results: chromosomes 4, 5, 9, 11 and 15. We are currently performing additional studies in additional dogs. Confirmed chromosomal regions will be further studied, with the aim of identifying the causative mutation(s) and gene(s). Identification of the CM/SM gene(s) will allow the development of a genetic test for the identification of carriers for breeding purposes with the ultimate aim of reducing or eliminating this devastating condition in the CKCS breed.

In parallel, we have initiated the recruitment of a cohort of human CMI patients where we will investigate the human equivalent(s) of the dog CM/SM gene(s) for involvement in human CMI. The identification of a gene that contributes to the etiology of CMI will provide an important step to the understanding of the underlying causes of the disease and the development of better therapeutic strategies. The finding of a predisposing gene may also lead to the development of simple and accurate diagnostic tests for better counselling and clinical management of CMI patients and their relatives. Furthermore, this study will help unravel some of the cellular mechanisms implicated in the embryonic development of the craniocerebral junction.



Sarah Blott, B.Sc, M.Sc, Ph.D Department of Genetics, Centre for Preventive Medicine, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU

Breeding for Health in pedigree dogs: Optimization of breeding strategies to reduce inherited disease

Today's dog breeders face difficult, sometimes conflicting, decisions when it comes to selecting which dogs to mate together. They need to breed dogs that conform to the breed standard and are of desirable temperament while at the same time selecting against undesirable traits such as inherited disease. Optimization of breeding strategies will help breeders to avoid inherited disease in their own dogs and reduce the prevalence of disease in the breed as a whole. In addition, diversity within the population will be maintained so that the long-term risk of new diseases arising is minimized. The study will focus on complex diseases such as syringomyelia, where the disease is influenced by many genes as well as the environment. Information from pedigrees, phenotypic records (e.g. MRI scans) and DNA markers will be combined to generate breeding values for individual dogs. This will allow breeders to make more informed decisions about which dogs to mate. The use of optimization techniques will allow selection against disease to be balanced with the need to avoid compensatory over-emphasis on lines considered disease free to date. The initial study will generate breeding values for risk of syringomyelia in the Cavalier King Charles Spaniel, and the information required for the maintenance of breed diversity. The longer term aim is to develop a computerized mate selection program that breeders can use via the internet, allowing them to assess the suitability of proposed matings.



Dominic J Marino, DVM, DACVS, CCRP Chief of Staff, Long Island Veterinary Specialists 163 South Service Road Plainview, NY 11803 USA

Experiences with Syringomyelia, A Neurosurgeon's Perspective.

Foramen magnum decompression (FMD) is the preferred mode of therapy for Chiari type I malformation of people. Surgery is often successful, but 8-30% of patients require re-operation, due to excessive scar tissue formation at the FMD site. Both the occipital portion of the skull and scar tissue result in compression of nervous tissue and abnormal cerebrospinal fluid dynamics, perpetuating the secondary formation of a syrinx and resultant neurologic deterioration. Syrinx formation in the cervical region is well documented in both human and veterinary patients since the cervical region is easily incorporated in the cervical magnum decomplete MRI studies from the brain to the sacral spinal cord. Of the 19 dogs, 19 (100%) had cervical, 9 (47.4%) had cervical / thoracic, 5 (26.3%) had cervical / thoracic / lumbar syrinx formation. A FMD procedure for Canine Chiari-Like Malformation (CLM) has been described; the success rate of this procedure is approximately 81%. Similar to humans, about 25% of canine patients require re-operation due to excessive post-operative scar tissue formation. In our experience, worsening clinical signs associated with scar tissue impingement typically occur within 3 months of surgery.

A cranioplasty procedure developed for Chiari type I patients at the Chiari Institute has substantially reduced the frequency of post-operative scar tissue compression at the FMD site. The purpose of this report is to describe a modification of that procedure for dogs with CLM and report on the results of long-term follow-up. Twenty-one dogs with MRI confirmed CLM underwent FMD with cranioplasty and had greater than 6 months follow up (median 11.2 months). Seventeen of 21 dogs were Cavalier King Charles spaniels, the 4 remaining dogs were a Pug dog, Chihuahua, Pomeranian and a Maltese. There were 10 male dogs, and 11 female dogs. Mean age was 3.2 years (range, 10 mos-8yrs, 2 mos). Following the FMD procedure previously described, a cranioplasty was performed. Guide holes for either 1.5 mm diameter (1.1 mm drill bit) or 2.0 mm (1.5 mm drill bit) titanium screws were made in the occipital bone, around the edge of the FMD defect. Self-tapping titanium screws were inserted into the holes for an approximate depth of 2-3 mm. A skull plate was fashioned using titanium mesh and polymethylmethacrylate (PMMA), and fixed to the back of the skull, using the titanium screw heads as anchor posts for the PMMA. There were no intraoperative complications. Postoperative complications included a mild head tilt and ataxia in 1 dog (re-operated cases), and the need for narcotic pain medications for about 2-3 weeks in 3 dogs (persistent neck pain). All but one of the dogs experienced clinical improvement following the procedure, although one has required a second decompression distal to the cranioplasty secondary to scar tissue formation. Mean follow-up time was 1 year (range, 6 to 16 mos). Post-operatively, 2 dogs experienced a lack of improvement and 1 was euthanized due to recurrent pain around the head and neck. This latter dog also had severe PSOM and it was undetermined if the pain were due to CLM or the ear disease. Nine dogs had follow up MR scans after surgery to evaluate the effect of surgery on both syrinx length and volume. At a median 5 months (range, 2-13 months) the syrinx length and volume were decreased 20% and 21% respectively. Cranioplasty appears to be well tolerated in dogs with CLM and may contribute to the reduction in syrinx size. Although cranioplasty appears to represent an improvement in the surgical management of CLM, it has not provided a solution. Intermittent exacerbation of clinical signs associated with excitement can be expected in some patients.



Syringomyelia Question Time Ask the Experts

Chaired by

Dr B R B Fogle MBE DVM MRCVS

Sponsored by Pfizer Animal Health



Bruce Fogle is the principal of the Portman Veterinary practice in London and has written over 50 books, including most recently "A Dog Abroad" which further details his Scandinavian travels with his charismatic Golden Retriever Macy. He is the resident Vet on the Jeremy Vine Show and has been the invited speaker at symposia and Veterinary Facilities in 24 countries and is a professional consultant to several international pet food and insurance companies, the Encyclopaedia Britannica and Microsoft Encarta. He has a monthly column in Dogs Today and is a regular contributor to many newspapers and journals.

Dr Fogle is the co-founder and vice Chairman of the Guide Dogs for the Deaf and was honoured with the MBE for his services to the Deaf. Bruce is a founding member for the Society for Companion Animal Studies in the UK and the Delta Society in the USA

We are extremely grateful to the following for coordinating the questions:

Pet owners: Sandy Smith and Carol Fowler

Breeders: Sue Robinson, Veronica Hull - Sponsored by Boehringer-Ingelheim



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