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# Facial changes related to brachycephaly in Cavalier King Charles Spaniels with Chiari-like malformation associated pain and secondary syringomyelia

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## Abstract

**Background:** Recent studies including an innovative machine learning technique indicated Chiari-like malformation (CM) is influenced by brachycephalic features.

**Objectives:** Morphometric analysis of facial anatomy and dysmorphia in CM-associated pain (CM-P) and syringomyelia (SM) in the Cavalier King Charles Spaniel (CKCS).

**Animals:** Sixty-six client-owned CKCS.

**Methods:** Retrospective study of anonymized T2W sagittal magnetic resonance imaging of 3 clinical groups: (1) 11 without central canal dilation (ccd) or SM (CM-N), (2) 15 with CM-P with no SM or <2 mm ccd (CM-P), and (3) 40 with syrinx width ≥4 mm (SM-S). Morphometric analysis assessed rostral skull flattening and position of the hard and soft palate relative to the cranial base in each clinical group and compared CKCS with and without SM-S.

**Results:** Sixteen of 28 measured variables were associated to SM-S compared to CM-N and CM-P. Of these 6 were common to both groups. Predictive variables determined by discriminant analysis were (1) the ratio of cranial height with cranial length ($P < .001$ between SM-S and CM-N) and (2) the distance between the cerebrum and the frontal bone ($P < .001$ between SM-S and CM-P). CM-P had the lowest mean height of the maxillary area.

**Conclusions and Clinical Importance:** CKCS with CM-P and SM-S have cranial brachycephaly with osseous insufficiency in the skull with rostral flattening and increased proximity of the hard and soft palate to the cranial base. Changes are greatest with CM-P. These findings have relevance for understanding disease pathogenesis and for selection of head conformation for breeding purposes.

**Abbreviations:** BOAS, brachycephalic obstructive airway syndrome; ccd, central canal dilation; CI, Confidence Interval; CKCS, Cavalier King Charles Spaniel; CM, Chiari-like malformation; CM-N, dogs without central canal dilation or syringomyelia with no clinical or behavioural signs of pain; CM-P, dogs with clinical and behavioral signs of pain with no syringomyelia or with a central canal dilation less than 2 mm wide; CSF, cerebrospinal fluid; DA, discriminant function analysis; DICOM, Digital Imaging and Communications in Medicine; FS, feature selection; ICC, intraclass correlation coefficient; ML, machine learning; MRI, magnetic resonance imaging; SM, syringomyelia; SM-S, dogs with syrinx width ≥4 mm and with SM specific signs of phantom scratching, scoliosis, paresis or proprioceptive deficits.

**KEYWORDS**
Brachycephalic obstructive airway syndrome, Cerebrospinal fluid, Craniosynostosis, Dysmorphia, Hard palate, Soft palate

## 1 | INTRODUCTION

Brachycephalic conformation is a risk factor for syringomyelia (SM) secondary to Chiari-like malformation (CM) in the Cavalier King Charles Spaniel (CKCS).[1,2] Recent characterizations of CM include cranial osseous reduction and neural parenchymal displacement resulting in a compensatory increased cranium height, rostral forebrain flattening, olfactory bulb reduction and ventral rotation, reduced caudal cranial fossa, and abnormalities of craniocervical junction.[3,4] Cavalier King Charles Spaniels with SM-S have a range of possible conformation anomalies depending on the severity of craniocervical junction incongruities, the proximity of the dens, increased airorhynchy with a smaller, more ventrally rotated olfactory bulb. There have been many studies examining the rostral cranial cavity and craniocervical junction, which have been reviewed,[4,5] but this study investigates the orofacial region.

Diagnosis of CM/SM in dogs is challenging because SM is not always associated with clinical signs[6,7] and dogs with CM alone could have behavioral and clinical signs of pain.[8] Compared with dogs with SM-S and CM-N, CKCSs with CM-associated pain (CM-P) have the shortest basioccipital bone and greatest forebrain flattening with compensatory increased cranial fossa height and displaced parenchyma, however, without (presumed) compromise of cerebrospinal fluid (CSF) channels and SM.[9] CM might be more appropriately considered a brachycephalic obstructive CSF channel syndrome rather than a single malformation.[5] The complexity of the existing morphometries of CM/SM and the corresponding interference of CSF circulation has inspired the development of a machine learning (ML) technique for diagnosis. The process aligns the midsagittal magnetic resonance imaging (MRI) image for each subject in the cohort to a reference image from an average dog from the normal (control) cohort (reference subject). This is done at a pixel level, resulting in a deformation field that maps each aligned image to the reference image. The reference subject is chosen by calculating the mean value of annotations from a previous study[9] and identifying the subject whose annotations are numerically closest to the mean. After this, the process of "feature selection" is carried out to select the most relevant pixels for separating subjects into controls and phenotype groups. This has been accomplished in 2 phenotypes: (1) CKCS with and without SM whereby the markers related to SM were consistently clustered over 4 levels of granularity, corresponding to sella turcica/presphenoid bone region and ventral soft palate, and (2) CKCS with CM-P and no SM where the biomarkers have some commonality with SM but included a specific area just rostral to the sella turcica at the opening of the optic canal, the olfactory bulb, corpus callosum, and the soft palate.[10]

The CM-P anatomical deviations of these features were hypothesized to be associated with brachycephaly and this study was motivated to investigate changes in facial anatomy associated with clinically relevant SM (defined as SM associated with signs of myelopathy) and CM-P in CKCS, including investigation of features identified by the machine learning technique.

## 2 | MATERIALS AND METHODS

### 2.1 | Study cohort

Retrospective review of available medical records at Fitzpatrick referrals between September 2013 and 2017 was searched for CKCS that were presented for diagnostic investigation of neurological signs or pain or for prebreeding screening for CM/SM under the Kennel Club / British Veterinary Association health scheme.[11] Inclusion in the study required sagittal T2-weighted MRI images of the cervical spinal cord and head including the nasal cavity acquired by 1.5 T MRI unit (Symphony Maestro Class, Siemens, Enlargen, Germany). The search identified 206 dogs and the medical records and MRI of these dogs were evaluated by author CR for the following: age at MRI; final diagnoses; clinical and behavioral signs of pain defined in a previous study[12]; and maximum transverse diameter of the central canal dilation or syrinx (if present). Dogs were excluded if they did not include the rostral head or if the diagnosis was equivocal; for example, if an alternative explanation of pain was identified. Syringomyelia is a late onset disease and, therefore, young dogs might not express a true MRI phenotype.[13] Thus, CM-affected dogs (clinically normal or with clinical signs of pain) without SM aged less than 4 years old were excluded. Dogs with a milder SM phenotype (transverse width of 2-3.99 mm) were also removed, as a previous study has suggested that specific clinical signs associated with SM are seen in dogs with a SM transverse width of 4 mm or more.[8]

A total of 66 CKCS (32 females, 34 males) were identified, of which 40 dogs had SM and 26 dogs did not. Excluded from the study were 140 dogs. After identification of the study cohort, all of the MRI images were anonymized and randomized by the author FS so that those analyzing the MRI were blinded to the phenotype. The study cohort was subsequently divided into 3 clinical groups for statistical analysis with abbreviations CM for dogs without SM; N = clinically normal, P = pain; S = SM-specific clinical signs of phantom scratching, scoliosis, paresis, or proprioceptive deficits:

1. Control group (CM-N; n = 11): CKCS that had MRI when age over 4 years old (mean/SD = 6.1/1.) with mean weight 11.3 kg (SD = 3.0) with CM but no MRI evidence of central canal dilation

(ccd)/SM, no clinical or behavioral signs of pain or other signs of CMSM*.

2. CM pain group (CM-P; n = 15): CKCS that had MRI when age over 4 years old (mean/SD = 6.1/1.7) with mean weight 10.4 kg (SD = 2.1). Chiari-like malformation but no SM or a ccd of less than 2 mm. Clinical and behavioral signs of pain when orthopedic, neurological, and MRI examination had not identified another cause of pain with a final diagnosis of CM-P according to previously defined criteria*.[12]
3. Clinically relevant SM group CKCS (SM-S; n = 40): CKCS with signs of myelopathy with a neurolocalization corresponding to site of SM (variable phantom scratching, scoliosis, paresis, proprioceptive deficits) and a syrinx with a transverse diameter of 4 mm or more with age range 0.7-10.6 years (average/SD = 5.5/2.5), mean weight 9.9 kg (SD = 2.2).

Asterisk in the above list represents clinical or behavioral signs of pain that are defined as vocalization (spontaneous, on picking up or after movement especially when recumbent and during the night), spinal pain, and changes in activity and behavior, which suggested avoidance or pain when jumping up or doing stairs, behavior change (aggression, withdrawn, anxious, described as more timid) and sleep disturbance.[12,14,15]

## 2.2 | Morphometric mapping

Because the study aim was broad, morphometric mapping was divided into 2 separate studies, 1 focusing on anatomical features relating to the soft palate and the other relating to the hard palate and performed independently by 2 investigators using imaging software available to them. Conformity between the studies was sort by including a similar framework to standardize the variables: the height of the cranium perpendicular to the basicranium and overlapping "points of interest" of forebrain flattening and olfactory bulb ventral rotation investigated in both studies.

## 2.3 | Soft palate study

This study used Digital Imaging and Communications in Medicine (DICOM) reading software Mimics Materialise Innovation Suite Research v18 (Mimics Materialise, Technologielaan 153 001 Leuven, Belgium). Fifteen measurements, recorded by the author SPK, are summarized as follows and illustrated in Figure 1.

1. Soft palate—size (area, length, width) and alignment of the soft palate relative to the hard palate and skull base.
2. Forebrain flattening—distance between the forebrain parenchyma and outer surface of frontal bone.
3. Midbrain—distance between the olfactory lobe and sella turcica.
4. Points of interest—distances from the interface between the hard and soft palate (P) were taken to 5 points of interest (lime green in Figure 1): A (spheno-occipital synchondrosis); B (basion of the basioccipital bone); U (rostral edge of the ethmoid plate); V (dorsal sella turcica); distances to 2 points of interest from the caudal end of the palate (Q) were measured: B and C, the rostral end of the atlas. Measurements A, B, and C are similar to previous

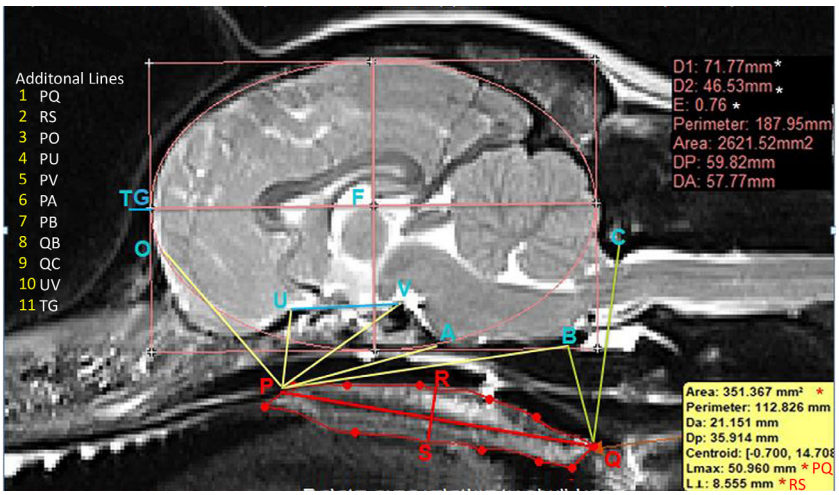


**FIGURE 1** T2 weighted midsagittal magnetic resonance imaging of a Cavalier King Charles Spaniel illustrating 17 cephalometric measurements made of the soft palate and frontal bones using Digital Imaging and Communications in Medicine reading software Mimics Materialise. Best-fit ellipse* (pink, with box parameters): D1*—maximum length of ellipse; D2*—maximum height of ellipse; E* ellipticity—the degree of deviation from a circle or sphere of an elliptical or ellipsoidal shape; F—ellipse centroid; G—rostral point of maximum length of ellipse. Soft palate area* (red, with annotated yellow box): P—interface of hard and soft palate; Q—most caudal point of soft palate; PQ*—maximum length through the polygon centroid (Lmax, yellow box); RS*—width at right angles through centroid; ($L_{\perp}$, yellow box)*—calculated by the Mimics Software program. Other points of interest (aqua letters): A—spheno-occipital synchondrosis; B—basion of the basioccipital bone; C—rostral edge of dorsal lamina of atlas; O—most rostro-dorsal point of olfactory bulb; T—external surface of the frontal bone extended from point G; U—rostral edge of the ethmoid plate; V—dorsal sella turcica. (Points A, B, and C were similar to a previous study.[9])

studies.[3,4,9] Finally, 3 other distances were measured: (1) between U and V, and (2) between the outer surface of the frontal bone T and the maximum length of best-fit ellipse (G).

Standardization of variables using the maximum height of the cranium perpendicular to the skull base was achieved with a "best-fit" ellipse (Figure 1) aligned on skull base, which encompassed the maximum length (D1) and height (D2) of the brain parenchyma. To further assess brachycephaly in this study, the automatically configured value for ellipticity (E) of the calvaria was noted, that is, the degree of deviation from a circle or sphere of an elliptical or ellipsoidal shape (pink lines).

A single midsagittal MRI can never ensure that the thickness and length of the palate are fully represented. Mimics Materialise software was used to overcome this caveat by outlining the area of the soft palate on a midsagittal image as a polygon, which generated values for its area, perimeter, and length and width through the centroid of the polygon.

## 2.4 | Hard palate study

This study used DICOM reading software E-Film (https://www.ibm.com/uk-en/marketplace/efilm-workstation). Standardization of the study cohort to investigate the relative position of the hard palate, position of olfactory bulb, and nasal cavity was achieved as follows (Figure 2): an extended (pink) line drawn (wx) along the skull base (as in soft palate study) with 2 perpendicular lines from (1) spheno-occipital synchondrosis to the dorsal surface of the cerebral hemisphere (ki), (2) most rostral point of the forebrain parenchyma (ed), where "e" marks the intersection, and "d" marks the dorsal extent of the olfactory bulb. Parallel to skull baseline wx, (pink) line "yz," through intersection d, extending beyond the frontal bone.

### 2.4.1 | Hard palate

Distances below the skull baseline wx to the hard palate were measured at c, f, and g (hard and soft palate interface), with perpendicular lines extending from points a, d, and l, respectively. The maxillary "area" was estimated by lines ad × de.

### 2.4.2 | Points of interest

"a" marks the external surface of the frontal bone enclosing the frontal sinus, which provides a measure of the distance from the cavarium and degree of "frontal flattening." "d" marks the rostral edge of forebrain neural parenchyma at most dorsal point of olfactory bulb. A line extended from "d" to point "h" on skull baseline "wx" to encompass the entire olfactory bulb.

## 2.5 | Statistical analysis

IBM SPSS v25.0 was used for statistical analysis and *P*-values <.05 were considered significant.

Intraclass correlation coefficient (ICC) was used to validate (1) the reliability of using 2 different DICOM reading software packages by comparison of the height of the cranium perpendicular to the basicranium and (2) interrater reliability of the measurements for each

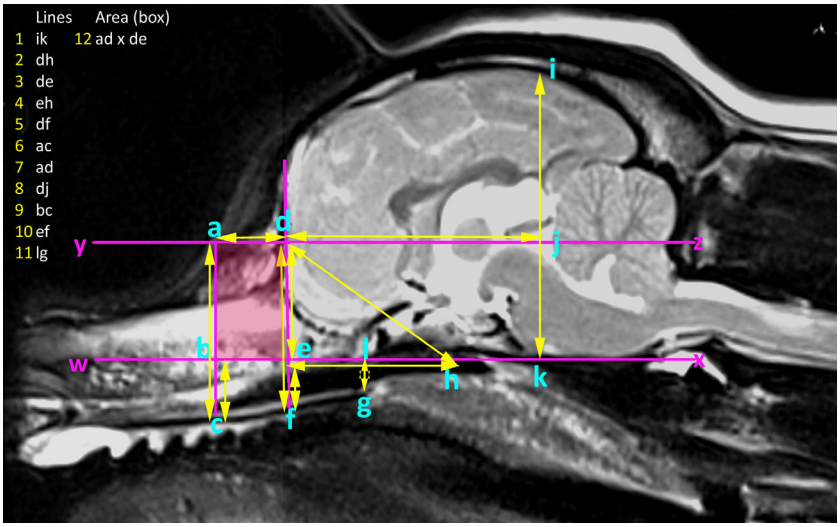


**FIGURE 2** T2 weighted midsagittal magnetic resonance imaging of a Cavalier King Charles Spaniel illustrating the cephalometric measurements (12) of the nasal, maxillary, and hard palate using Digital Imaging and Communications in Medicine reading software E-Film. Reference framework (pink lines) wx is the basicranium and yz parallel at the level dorsal to olfactory bulb. Four bisecting vertical lines perpendicular to baseline: abc—where a is the outer surface of the nasal bone on line yz, b intersection with line WX, and c hard palate; def—where d is the most rostral edge of cerebrum at line yz, e intersection with line WX, and f hard palate; lg—where l is perpendicular from line wx to g, junction of hard and soft palate; ijk—where ik is the perpendicular height of the calvaria from line wx, with j intersecting line yz representative maxillary "area" encompassed by lines ad × de

researcher (4 measurements from 10 dogs were repeated and the results compared).

The cranial height of the dog was selected to standardize the traits because it was measured independently in both studies and is more robust than, for example, body weight, which depends on diet and exercise. Thus, each line variable value was divided by D2 in the soft palate study and ik in the hard palate study) and recorded as a ratio (ratio R). The study cohort was analyzed using 2 different approaches:

1. Comparison of 3 clinical groups (CM-N, CM-P, and SM-S): one factor analysis of variance (ANOVA) and post hoc Bonferroni. *P*-values were considered significant: <.05 for ANOVA and with Bonferroni correction, <.02 for the *t* test. Because segregated traits associated with CM and SM have been shown to be additive to the severity, Discriminant Function Analysis (DA) was applied to the data in order to examine the relationships between the significant variables in more depth. DA is helpful to ascertain the most important phenotypic trait variables that distinguish between each group.

**TABLE 1** Significant variables (16) identified comparing SM-S with CM-N and with CM-P

| | SM affected versus control (SM-S versus CM-N) | | | SM affected versus CM pain (SM-S versus CM-P) | | |
|---|---|---|---|---|---|---|
| | **Dependent variable** | **Mean difference** | ***P* value** | **Dependent variable** | **Mean difference** | ***P* value** |
| Significant for both group comparisons (n = 6) | Cerebral ellipticity (E) | −4.005 | <.001 | Cerebral ellipticity (E) | −2.483 | .01 |
| | Cranial ratio | −8.1395 | <.001 | Cranial ratio | −4.5573 | .01 |
| | Line TG-R | 3.8211 | <.001 | Line TG-R | 3.0646 | .001 |
| | Line PQ-R | −10.7031 | .05 | Line PQ-R | −12.5131 | .005 |
| | Line PV-R | −5.3402 | .02 | Line PV-R | −4.8862 | .02 |
| | Line PB-R | −9.6191 | .001 | Line PB-R | −6.3819 | .02 |
| Significant for 1 group comparison (n = 10 [6 + 4]) | Cranial height D2 | −1.75467 | .04 | Line PO-R | −4.6213 | .04 |
| | Cranial height lk | 0.2107 | .03 | Line ac-r | −8.41 | .004 |
| | Line dj-r | −7.4161 | .01 | Line ad-r | 5.826 | .006 |
| | Line UV-R | −3.6367 | .004 | Line df-r | −7.4839 | .02 |
| | Line PA-R | −6.1274 | .01 | | | |
| | Maxillary area (ad × de) | −1.47052 | .01 | | | |

*Note*: 16/28 significant associated with SM affected (SM-S) CKCS. Six variables were significant compared to both CM-N and CM-P, 6 additional variables comparing CM-N and 4 additional variables comparing CM-P. Lower case letters indicate hard palate study; upper case letters indicate soft palate study.
Abbreviations: CM-N, dogs without central canal dilation or syringomyelia; CM-P, dogs with clinical and behavioral signs of pain with Chiari-like malformation associated pain with no syringomyelia or central canal dilation with is less than 2 mm wide; n, number; SM, syringomyelia.

**TABLE 2** Categorized significant variables (18/28) identified in independent "*t*" test comparing Cavalier King Charles Spaniel with SM (SM-S) and no SM (CM-N + CM-P)

| **Brachycephalic morphometries** | | | **Soft palate morphometries** | | | **Hard palate morphometries** | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | ***t*** | ***P* value** | **Variable** | ***t*** | ***P* value** | **Variable** | ***t*** | ***P* value** |
| Cerebral ellipticity (E) | 4.67 | <.001 | Line PQ-R | 3.7 | <.001 | Line df-r | 2.3 | .02 |
| Cranial height D2 | −3.09 | .003 | Line PO-R | 2.97 | .004 | Line ac-r | 3.32 | .001 |
| Cranial height (ik) | −2.92 | .005 | Line PU-R | 2.3 | .02 | Line lg-r | 2.46 | .02 |
| Cranial length/height ratio | 4.71 | <.001 | Line PV-R | 3.56 | .001 | | | |
| Line UV-R | 3.38 | .001 | Line PA-R | 3.25 | .002 | | | |
| Line TG-R | 4.49 | <.001 | Line PB-R | 4.02 | <.001 | | | |
| Line ad-r | 2.77 | .01 | | | | | | |
| Line dj-r | 2.8 | .01 | | | | | | |
| Maxillary area-R | 3.25 | .002 | | | | | | |

*Note*: The variables have been grouped into 3 columns relating generally to their anatomical association. Lower case letters indicate hard palate study; upper case letters indicate soft palate study.
Abbreviations: CM-N, dogs without central canal dilation or syringomyelia; CM-P, dogs with clinical and behavioral signs of pain with Chiari-like malformation associated pain with no syringomyelia or central canal dilation with is less than 2 mm wide; SM, syringomyelia.

2. CKCS with and without SM (SM-S verus CM-N and CM-P): independent sample *t* test with Levene's test for equality of variance and any significant variables entered into stepwise logistic regression modeling to confirm the results, odds ratios (ORs) and their 95% confidence intervals (CIs) being reported.

## 3 | RESULTS

The intraobserver reliability test revealed an ICC value of 0.93 for the soft palate study and 0.99 for the hard palate study with a 95% CI, which was considered narrow.

The ICC analysis of variable maximum height in both studies (Figure 1, white lines D2 and ik) yielded a significance of <0.001 and ICC = 0.853 and confirmed that measurements made by 2 different software programs were similar enough that the variables in the 2 studies could be combined (28 variables in total) for statistical analysis in 2 ways—first, comparing the 3 clinical groups with each other and second, comparing CKCS with wide SM and no SM.

### 3.1 | Comparison among 3 clinical groups CM-N, CM-P, and SM-S

Single ANOVA analysis with post hoc Bonferroni-associated revealed no significant variables that distinguished between SM-N and SM-P but a total of 16 of 28 significant variables comparing SM affected CKCS (SM-S) with other groups: 6 were significant for both CM-N and CM-P, and 10 compared with either CM-N group (N6) or CM-P group (N4; Table 2). (The means and standard deviations for the 20 variables are provided in Supporting Information S1 and S2.)

Four of the 6 common variables (ellipticity; $P < .001$); cranial length/height ratio ($P < .001$) line TG-R, rostral flattening ($P < .001$) and line PB-R, the distance between the hard and soft palate interface to basion of basioccipital ($P = .001$) each had greater numerical significance comparing SM-S group to CM-N than to the CM-P ($P = .01$, $P = .01$, $P = .001$, and $P = .02$, respectively). However, variable PV-R ($P = .02$, distance from the rostral point of the hard palate to sella turcica) and PQ-R ($P = .005$, palate length through the centroid) had greater numerical significance for CM-P than CM-N when both compared to SM-S ($P = .05$ and $P = .02$, respectively). The 6 additional variables comparing SM-S with CM-N were increased cranial height (D2, $P = .04$ and lk, $P = .03$), shortened rostral cranial fossa (dj-r, $P = .01$), and reduced distance between olfactory bulb and sella turcica (UV-R, $P = .004$), distance from the hard/soft palate interface and sella turcica (PA-R, $P = .01$) and the maxillary area ad-r × ae-r, $P = .01$). The 4 additional variables comparing SM-S with CM-P indicated a reduced mean distance with CM-P dogs between the hard and soft palate interface and the rostral olfactory bulb (PO-R, $P = .04$) and between the frontal bone and the hard palate (ac-r, $P = .004$ and df-r, $P = .004$) and between the cranium and the frontal bone (ad-r, $P = .006$).

The CM-P group was intermediate between CM-N and SM-S in terms of rostro-caudal shortening of the cranium, that is, both facial (ad-r) and cranial length (dj-r) but the CM-P group had the greatest

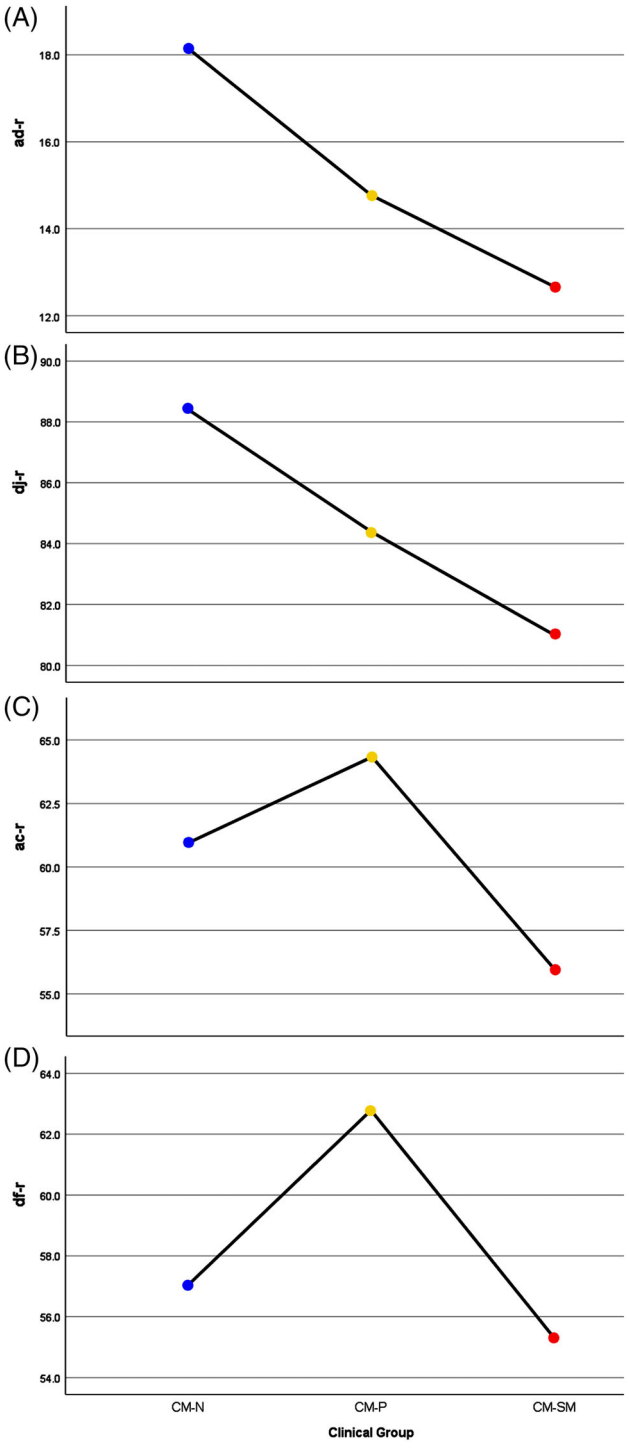


**FIGURE 3** Means plots in 3 clinical groups for rostro-caudal facial and cranial length and dorsal ventral facial length. Plots 1 and 2: rostro-caudal facial (ad-r) and cranial length (dj-r) CM-P group mean (yellow) can be intermediate between CM-N (blue) and SM-S (red). However, this is not the case with Plots 3 and 4: rostral-caudal facial-cranial shortening (ad-r and dj-r) which relate to distance between the hard palate and the frontal bone (ie, dorsoventral height of muzzle). Abbreviations: CM-N, dogs without central canal dilation or syringomyelia; CM-P, dogs with clinical and behavioral signs of pain with Chiari-like malformation associated pain with no syringomyelia or central canal dilation with is less than 2 mm wide; SM, syringomyelia

dorsoventral reduction of the muzzle, that is, df-r (distance between the hard palate and the frontal bone, variables df-r and ac-r; Table 1, Figure 3).

Using the independent "t" test to compare group CM-N versus CM-P, there was only 1 significant variable line df-r ($P = .02$) indicating that a reduced distance between the hard palate and the frontal bone was particularly associated with CM-P.

When all the significant variables are entered in discriminate analysis, 2 functions resulted:

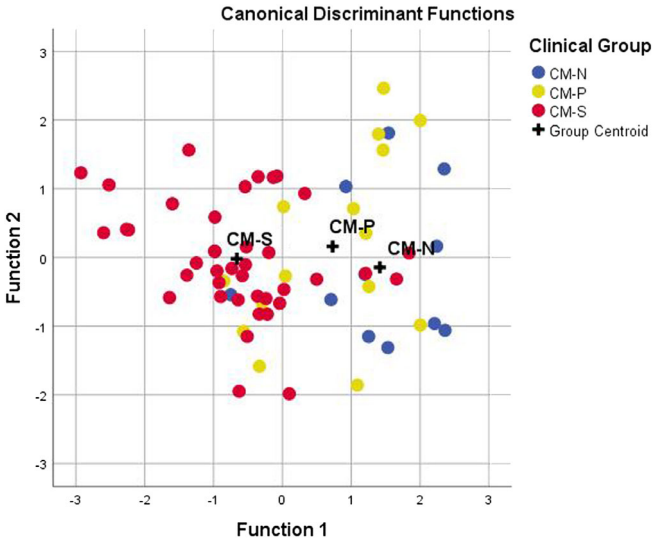


**FIGURE 4** Canonical discriminate functions of Cavalier King Charles Spaniel (CKCS) with clinical groups CM-N, CM-P, and SM-S. Average 75.8% correctly classified with SM-S highest separation of 92.5% and CM-N with 54.5% and 46.7% for CM-P. The horizontal separation has the greatest statistical importance hence dogs with larger TG-R and cranial length/height ratios (CM-N) are represented on the right hand of the graph SM-S centroid with a more spherical shaped cranium and increased "stop" (reduced TG-R) CKCS is furthest on the left. Abbreviations: CM-N, dogs without central canal dilation or syringomyelia; CM-P, dogs with clinical and behavioral signs of pain with Chiari-like malformation associated pain with no syringomyelia or central canal dilation with is less than 2 mm wide; SM, syringomyelia

1. Function 1 = 0.247 Line TG-R + 0.139 cranial length/height ratio (constant −21.082).
2. Function 2 = 0.270 Line TG-R − 0.142 cranial length/height ratio (constant +18.763).

This indicated that Line TG-R and the cranial length/height ratio were the best variables for distinguishing between the 3 clinical groups CM-N, CM-P, and SM-S. Overall, 75.8% of original grouped cases correctly classified with the predicted clinical group membership for CM-N as 54.5%, 46.7% for CM-P, and 92.5% for SM-S. Figure 4 plots values for each variable for Function 1 against Function 2 thus providing a pictorial representation of the dogs in each group relative to one another.

### 3.2 | Comparison of CKCS with SM (SM-S) and without SM (CM-N + CM-P)

After combining the measurements of both investigations, the independent "t" test identified 18 of 28 significant variables. These have been organized in Table 2 to indicate their anatomical association: brachycephaly, 9 significant variables allied with foreshortening of the muzzle and cranium; soft palate, 6 associated significant variables; and hard palate with 3 associated variables.

Stepwise logistic regression revealed that rostral skull flattening (Line TG-R) dominated any model (OR = 1.529 [95% CI: 1.22-1.92]), but when removed, line PB-R (distance between the hard and soft palate interface (P) and basion of basioccipital bone (B) dominated the model and yields OR = 1.15 (95% CI: 1.06-1.25).

## 4 | DISCUSSION

This is the first investigation known to the authors to measure orofacial structures involving the hard and soft palates with respect to CM and SM. It takes account of a data-led ML technique and was strengthened by using 2 available imaging software packages, E-Film and Mimics Materialise.

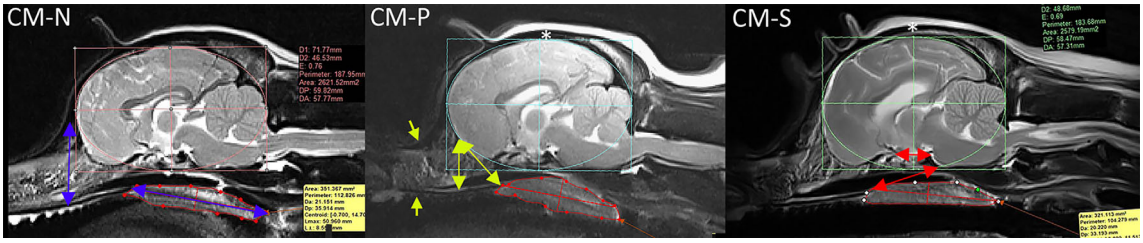


**FIGURE 5** Key differences in hard and soft palate in 3 clinical groups CM-N, CM-P, and SM-S. CM-N (blue arrows) has the least brachycephalic head, a gentle stop (see text for definition) with greatest maxillary area between the hard palate and frontal bone and the longest soft palate length. CM-P (yellow arrows) has the least distance between the hard palate and the cranium with ventrally displaced olfactory lobes and pronounced stop. SM-S (red arrows) has the greatest reduced middle cranial fossa and distance between the hard and soft palate interface and basicranium. White* (CM-P and SM-S) indicates compensatory cranial doming. Abbreviations: CM-N, dogs without central canal dilation or syringomyelia; CM-P, dogs with clinical and behavioral signs of pain with Chiari-like malformation associated pain with no syringomyelia or central canal dilation with is less than 2 mm wide; SM, syringomyelia

Canine brachycephalic conformation typically includes foreshortening of the facial skeleton (muzzle) and not necessarily incudes the cranium.[16-18] The results of discriminant analysis gave 75.8% correctly classified grouping with SM-S highest separation of 92.5%, together with the predictive statistics of ML, consolidate the concept that a deep stop is a risk factor for SM-S and CM-P. A summary of these most important differences among the 3 groups with respect to the position and size of the hard and soft palate is provided in Figure 5.

It should be noted that the "stop" is the pronounced angle between the nasal and maxilla bones and the frontal bones, which is a defining feature of domesticated mesaticephalic and brachycephalic dogs and by contrast is not present in wolves.[19] In some brachycephalic dogs, this stop is an indented cone-shaped depression between the eyes, which cannot be easily measured. We have hypothesized that this reduction in midfacial bony tissue is a paramount feature of CM but not SM and could be a driving force for expression of other traits such as a miniscule frontal sinus[20] and reduced, ventrally orientated olfactory bulb.[21]

The reduced dorsoventral muzzle (Figure 3, lines ac-r and df-r) and the development of the deep stop (Figure 4, Line TG-R) in the research findings suggest that dogs with clinical signs (CM-P and SM-S) do not compromise turbinates' in the same manner as the airways of brachycephalic dogs such as bulldogs with reduced foreshortening of the muzzle. However, this might compromise the CSF circulation and drainage particularly in the area of the cribriform plate, olfactory lobes, and forebrain, which could result in clinical signs of pain.

Oropharyngeal anomalies related to respiratory functional impairment particularly brachycephalic obstructive airway syndrome (BOAS)[22-26] did not investigate their relationship with CSF circulation. The olfactory bulb has a significant role in CSF circulation through the glymphatic system (astroglial-mediated interstitial fluid bulk flow) and the CSF absorption through the nasal turbinates,[27-29] and because both CM and SM are disorders of the CSF circulation, any facial anatomical anomalies which influence CSF production or absorption will help elucidate understanding for these conditions.

Conformational features identified in Tables 1 and 2 compliment previous research on skull and brain conformation associated with CMSM[1,4,5,9] but the results also validate the findings of ML study with respect to SM and CM-P markers. Changes in the relative position and size of the soft palate and rostral skull flattening could be useful in providing further diagnostic indicators for CMSM. Although a thickened soft palate has been linked to brachycephaly[30,31] and to otitis media with effusion[23] in the CKCS, in this investigation, a thickened palate was not significant. Soft palate hypoplasia is a rare condition,[32] and brachycephalic dogs typically have elongated soft palates with thickened superficial epithelium, extensive edema of the connective tissue, and mucous gland hyperplasia with several muscular alterations.[33] However, this is a secondary change to microtrauma or associated with genetic predisposition[31] and it would appear from the results of the study that it is the proximity of the palate to the cranium that is significant with CM-P and SM-S, not the structure itself. This relative position for both hard and soft palate with respect to increased airorhynchy[34] supports the view that CM-P and SM-S involve early embryological changes in the pervasive osseous reduction associated with para-axial mesodermal insufficiency associated with CMSM[35,36] and craniosynostosis that have already shown to exist with human CM/SM.[37] Crouzon syndrome, in particular, affects both the bones of the midface and cervical spine.[38,39] Such oropharyngeal changes in CM-P and SM-S dogs might well compromise CSF circulation in the skull both rostrally and caudally simultaneously causing disruption and could contribute to BOAS/sleep disordered breathing.[40]

## 4.1 | Limitations of study

The study was limited by the small cohort size of the CM-N and CM-P groups. This was in part because of the strict inclusion criteria of age and size of ccd in order to reduce the variables in investigation. The average weight of the CKCS in a recent study was 10.5 kg[41] Although CM-N dogs heavier than average with 11.3 kg and dogs with CM-SM lighter (9.9 kg) the sample sizes are too small to make assumptions.

The study was strengthened by fact that 2 different researchers analyzed the data using different techniques but resulted in similar findings. Furthermore, the data were grouped and analyzed with SM (n = 40) and without SM (n = 26), which increased statistical strength.

In this retrospective study of DICOM images only, it was not possible to ensure consistency with all operating theater variables. Although all dogs were positioned in MRI in dorsal recumbency, a limitation of the study was that the soft palate might have been compressed by endotracheal tube thereby altering its shape.

## 4.2 | Clinical relevance/impact

Dogs with clinically relevant CM/SM are more likely to have brachycephalic features of the rostral skull flattening with reduction of nasal tissue and a well-defined stop. This evidence not only enhances our understanding of the disease and "at risk" head conformation but could also impact on the assessment of MRI and disease diagnosis. It suggests the whole skull should be analyzed and not just the hindbrain currently required in prebreeding screening. This information has implications not only for breeders and pet owners but also for the veterinary profession to raise awareness about the welfare aspects of breeding. Furthermore, an increased risk for SM and painful CM might not be confined to brachycephalic breeds but other miniaturized purebreeds and hybrids that have gained in popularity as pets.

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## CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Authors declare no IACUC or other approval was needed.

## HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study

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## SUPPORTING INFORMATION

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