Section 7

Genetics

Chapter 7.1

Hereditary aspects of occipital bone hypoplasia and syringomyelia (Chiari-like malformation) in cavalier King Charles spaniels

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Introduction

Chiari-like malformation (occipital bone hypoplasia) resulting in caudal fossa overcrowding, obstruction of cerebrospinal fluid (CSF) pathways and secondary syringomyelia (figures 1 and 2) was first identified in Cavalier King Charles spaniels (CKCSs) in 1997 (Rusbridge and others 2000). After the condition was initially reported it became apparent that it was common in the CKCS breed. Affected CKCSs have now been identified across Europe, Australasia, and North America. The classic clinical sign in the CKCS is cervical and shoulder paraesthesia which results in the dog scratching at the neck/shoulders, especially when excited or on a lead. This is presumed to be a consequence of damage to the dorsal horn, decussating spinothalamic fibres and processing of sensory information. If there is ventral horn cell damage then the dog may have muscular weakness resulting in neck scoliosis or a thoracic limb paresis.

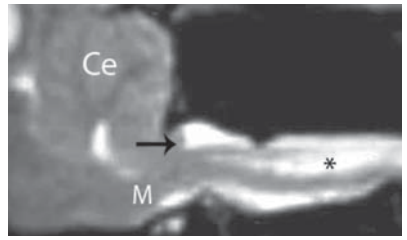


Figure 1a) Sagittal T2W magnetic resonance image of a four year old male CKCS with cervical and shoulder pain. The caudal fossa is small resulting in overcrowding of the cerebellum (Ce). There is a herniation of the caudal cerebellum into the foramen magnum (arrow), the medulla (M) is kinked and there is syringomyelia (asterisk). The cerebrospinal fluid (CSF) appears white.

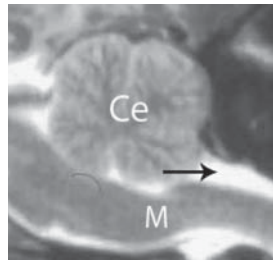


Figure 1b) Sagittal T2W magnetic resonance image of a Staffordshire bull terrier demonstrating the normal anatomy of the caudal fossa in a mesencephalic dog. Cerebellum (Ce), brain stem (M), the CSF appears white, note the large amount of fluid present in the foramen magnum (arrow) which is absent in figure 1a.

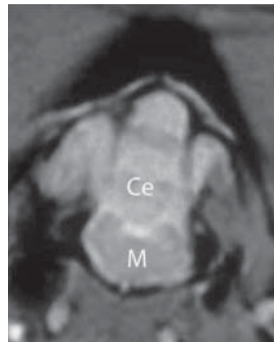


Figure 2a) Axial T2W magnetic resonance images through the cranial cervical junction of a four year old male CKCS with cervical and shoulder pain. The CSF pathways are obstructed, very little (white) CSF can be visualised around the central nervous system.

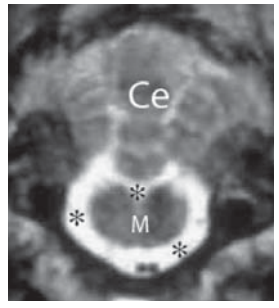


Figure 2b) Axial T2W magnetic resonance image through the cranial cervical junction in a Staffordshire bull terrier. The CSF pathways (asterisk) are unobstructed. Cerebellum (Ce) Medulla (M)

Cases with a very wide syrinx may have damage to the descending white matter tracts resulting in pelvic limb weakness and ataxia. Pain is a predominant feature of the condition and this may vary from a mild cervical hyperaesthesia to bouts of screaming after sudden changes in head position, excitement or being touched in the paresthetic region. Signs are usually recognised between six months and two

years however dogs of any age may be presented - the authors have had two ten year old dogs that were presented with neck pain and where investigation revealed cervical syringomyelia as the only explanation of the clinical signs. The condition occurs in both sexes and all coat colours. At present confirmation of the diagnosis is by MRI only. This is costly and not widely available. Many dogs have appropriate signs without confirmation of the diagnosis.

Mild cases may not require treatment or may be managed with non-steroidal anti-inflammatory drugs. A significant number of dogs have progressive signs and should be surgically managed e.g. by a suboccipital craniectomy (cranial cervical decompression) with durotomy relieving the obstruction at the foramen magnum. For dogs where surgery is not possible or successful, clinical signs may be improved by oral opioids, but by far the most effective drug is prednisolone at anti-inflammatory doses.

The modern CKCS breed was established in 1928 and in the 1930s six stud dogs were extremely popular. During the 1940s certain breed lines were extensively inbred with repeated grandfather to daughter, father to daughter, mother to son, half and full sister/ brother mating being the norm rather than the exception.

The defect in the CKCS is similar to Chiari type I malformation in humans which is characterised by underdevelopment of the occipital bone and posterior fossa with cerebellar tonsil herniation and obstruction of the foramen magnum (Karagoz and others 2002). Familial Chiari type I malformation with autosomal recessive or dominant inheritance patterns are reported with an incidence of about 2 % of total cases (Coria and others 1983, Zakeri and others 1995, Catala 1999). The Mhox gene or genes belonging to the Hox family control the development of the final shape of the occipital bone (Catala 1999). Ectopic expression of Hox-2.3 resulted in dysplasia/deficiency of occipital, basisphenoid and atlas bones in transgenic mice (McLain 1992).

This preliminary study investigated whether syringomyelia is inherited in the CKCS. The original report (Rusbridge and others 2000) had suggested a high frequency of certain names and lines within the pedigrees of affected dogs but it was not known whether this was because of the popularity of certain (champion) dogs or because these dogs passed on a genetic defect.

Materials and Methods

30 pedigrees of MRI confirmed cases (Group 1: dogs numbered 1-30) were initially scrutinised for common ancestral lines. The data was compiled on a computer programme for human genealogy - Generations C Grande Suite 8 (Sierra On-line. Inc. Bellevue, WA 98007). It generated individual pedigrees, descendant lines and enhanced hourglass pedigrees. Unlike other databases designed for dog pedigrees it had the advantage that all significant matings and relevant descendants can be viewed simultaneously. This permitted the complexity of the inter-relationships between dogs to be scrutinised. The computer programme used allowed direct descendants for any one individual to be marked so that a suspected 'founder' could be checked with reasonable accuracy.

The established population was augmented by an additional 15 cases (Group 2) with appropriate clinical signs (scratching at the shoulder/neck region, neck pain, absence of dermatological disease with or without scoliosis, thoracic limb weakness and pelvic limb ataxia) but without MRI confirmation. The sample (Group 1 and 2) included dogs from Britain, Finland, Tasmania, France, Ireland, Canada and the USA.

Information provided by the owners' pedigrees were for 3 to 5 generations. Additional pedigree information was obtained from the CKCS Book of Champions 1928-1999 (Thresh and others 2000) and The Kennel Club UK Breed Supplements -Small and Toy dog (1 Clarges Street, London, W1J 8A3). Finally a single, breed tree with affected dogs at the base and spanning 20 generations in relevant places was created. The number of dogs in the final family tree was over 1,300 CKCSs and extended back to the founder dogs for the breed. However when only 12 MRI confirmed dogs had been entered into the programme, there was sufficient framework to slot all subsequently confirmed cases to date. Pedigrees of 45 unaffected dogs were also studied (Group 3). These dogs were at least 10 years old with no clinical signs or known history of syringomyelia.

The degree of inbreeding meant that the information could not be represented meaningfully in a classic family tree for the purposes of this paper. Stud champions were sometimes used for over 12 years and sired large numbers of offspring spanning 2 or 3 generations within the same pedigree (i.e. one individual can be grandparent, great grandparent and great- great grandparent). Flow chart software (Flow chart maker; Cosmi Software. Cosmi Europe Ltd Unit 8a, Daimler Close, Royal Oak Industrial Estate, Daventry, Northants, NN11 5QJ) was used to illustrate selected information in a simplified form for this report.

Results

Analysis of the pedigree database indicated that syringomyelia had a high incidence in certain families and lines. Four key dogs, C, D, M and S, consistently occurred within the individual pedigrees but were not always present in the same pedigree. These represented four major breeding lines. Table 1 illustrates when affected dogs were descended from dogs C, D, M and S. Certain breeding lines were not represented in Dogs 5, 10, 13, 16, 18, 20, 23 and 29 from other countries, (highlighted in Table 1). This reflected the exportation of particular champion dogs from Britain. The pedigrees of dogs C, D, M and S were then studied to identify any common ancestry. All of the affected dogs could be traced back to a single common ancestor, bitch G (Figure 3). This dog died at eighteen months in 1958 and had only one recorded litter with two offspring. The direct descendants of bitch G were used to create a tree which provided a manageable tool with which to study the pedigrees. Aspects of the relationships between G, the four key dogs C, D, M and S and their descendants are featured in both Figures 3 and 4. A simplified pedigree of dogs A (non-champion) and B (champion) and their affected descendants are mapped in Figure 3. These dogs were selected because they illustrate some of the complexity of the family tree and have a high proportion of affected offspring.

Table 1

Case	Ancestor C	Ancestor D	Ancestor M	Ancestor S
1	YX	X	YX	
2		YX	YX	Y
3	Y		YX	YX
4	Y	YX	YX	YX
5*		YX	Y	YX
6	YX		X	
7	YX	YX	YX	X
8	Y	YX	X	YX
9	Y	YX	YX	YX
10*	YX	Y	Y	
11	YX			
12	Y	YX	YX	YX
13*	YX	YX	YX	X
14	YX	YX	YX	Y
15	YX	YX	YX	
16*			YX	Y
17	YX	YX	YX	X
18*	YX			
19	Y		YX	X
20*	YX		YX	
21	YX	YX	YX	YX
22	YX	Y	YX	Y
23*	YX		YX	YX
24	YX			
25	X	Y	YX	Y
26		YX	YX	X
27		Y	YX	X
28	YX	YX	YX	X
29*	YX	YX	X	
30	YX	YX	YX	X

Connection of MRI confirmed cases 1-30 to key ancestral dogs C, D, M and S.
Y- Sire of confirmed case is direct descendant, X - dam of confirmed case is direct descendant. * - non-UK dog. Note that both the dam and the sire can be traced back to C, D, S or M for each confirmed case

Figure 3 Pedigrees of two suspected carriers A and B showing relationship with G and their affected descendants

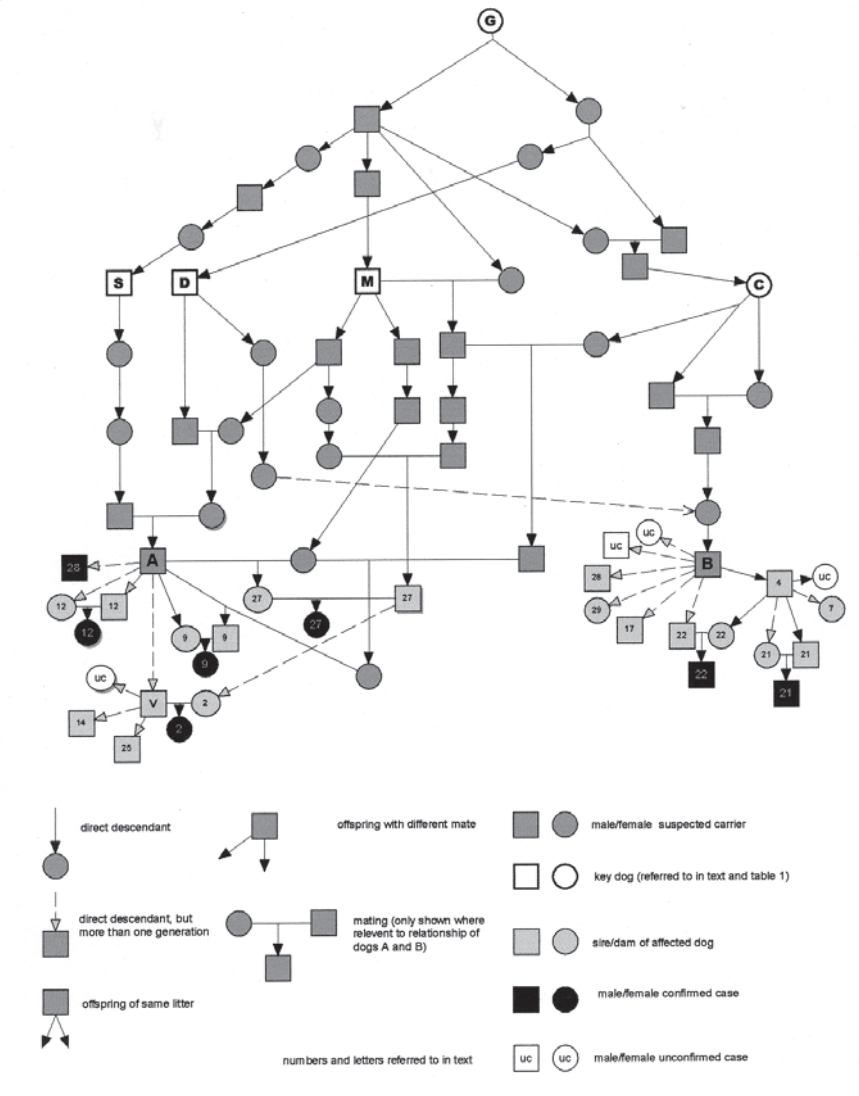
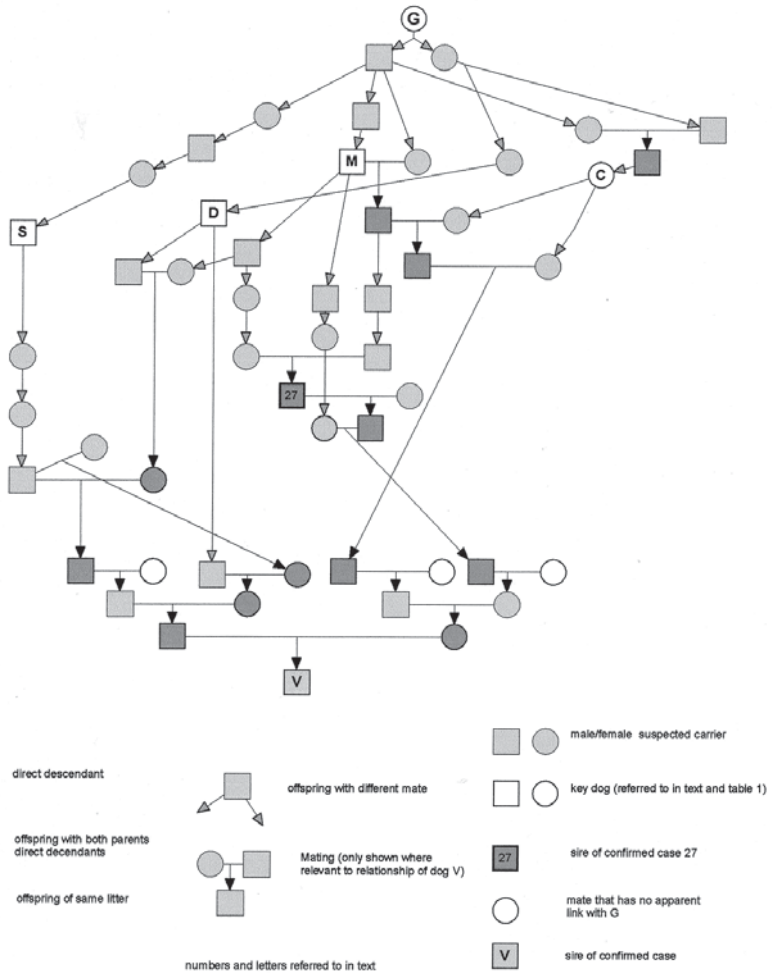


Figure 4 Pedigree of dog V showing relationship with G and "saturation" of suspected carriers in his first three generations.



The pedigree of Champion dog V is featured in Figure 4 and illustrates a phenomenon that occurs in 100% of the confirmed cases whereby all four of the grandparents are descendants of G. The degree of 'doubling up' of the descendants is colour-coded to highlight the trend. In confirmed cases the number of great-grandparents that were possible carriers range from 6 of the 8 to all 8. This was not found to be the case in control Group 3 where there remained a high degree of inbreeding but with different breeding lines (Table 2). Dog V is a suspected carrier that has sired two confirmed cases (dogs 2 and 25). He is also the grandsire of a confirmed case not included in Group 1 and an unconfirmed case. From 1989 to 2001 he had 57 recorded litters in the UK indicating how any defective gene might become widespread.

Table 2 Comparison between affected and unaffected dogs and the relationship to dogs C, D, S, and M. There are 45 dogs in each group

Descended from key ancestral dogs	Group 1 and 2	Group 3
Dam and sire	100%	60% (27/45)
All 4 grandparents	100%	17.7% (8/45)
6 Great-grandparents	100%	6.6% (3/45)
All 8 Great-grandparents	68.9% (31/45)	4.4% (2/45)

Discussion

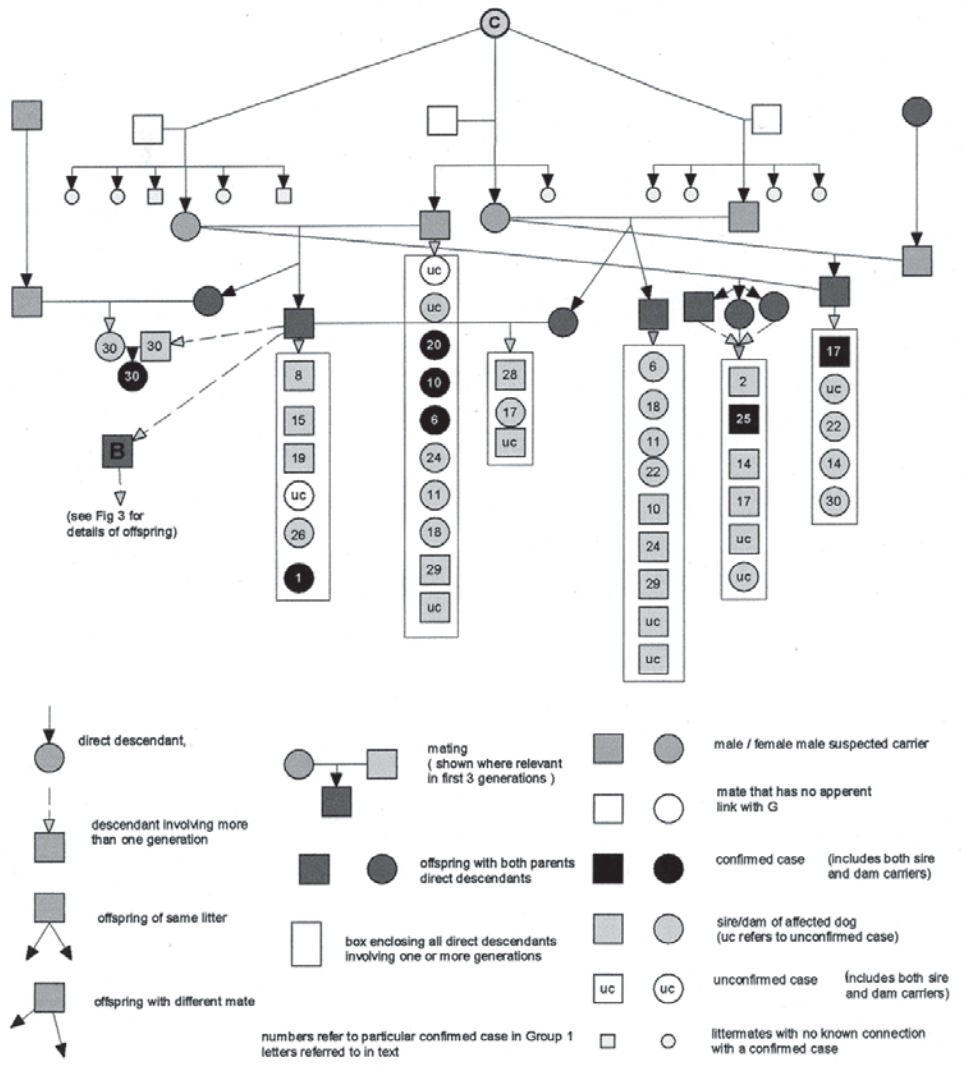
To investigate the inheritance of a disease, detailed information about an extended family is required. The ratio of affected to unaffected offspring in any given litter is usually collated to establish homozygous and heterozygous individuals and the likely mode of inheritance. In this study acquiring such information was not feasible since most puppies are sold to the pet owning public whereby they cannot be traced; additionally confirmation of the disease by MRI has only been possible in the last 7 years and has limited availability. However, analysis of the pedigrees of affected CKCS did suggest that syringomyelia had a high incidence in certain families and lines and therefore was likely to be an inherited genetic defect. All affected dogs could be traced back through both the dam and the sire to at least one of four significant ancestors – three champion dogs D, S and M and one non champion bitch C. These four dogs were descended from one bitch G. This bitch died shortly after producing her only litter of two pups and was thought a possible source of the defective gene(s) because 1) it was the earliest point in which the breed lines C, D, M and S came together, 2) it involved only one litter and 3) it offered an explanation for the high incidence of the disorder within certain parts of the extended pedigree

A number of other common ancestors were eliminated as possible 'founders' because they were successful stud dogs and therefore one would have expected an even more widespread problem earlier or because they were bitches that involved different mates and would require a more complex explanation to account for the phenomenon. For example, the sire of the single litter from bitch G had 38 recorded matings over a period of 6 years. These matings included 6 with G's dam who was matched every season for a period of 3 years resulting in twenty offspring

Aspects of the relationships between G, the four key dogs C, D, M and S and their descendants are featured in both figures 3 and 4. If bitch G carried a recessive gene(s) for occipital bone hypoplasia and this gene was passed down the generations to dogs C, D, M and S, and then to significant descendants for example dogs A, B and V, then the substantial inter breeding between descendants has the effect of increasing the chance of duplicating any defective gene(s). Figure 5 features the descendants of key bitch C (non-

champion). She died at 7 years and had 3 litters with different mates. Her family tree has been included to demonstrate how a recessive gene might build up a degree of homozygosity. This may account for the now common occurrence of cases compared to the past. The extent of inbreeding between the suspected carriers made it impossible to clearly illustrate C's family tree. In order to avoid confusion, suspected cases have been grouped into descendant boxes from stud dogs where large numbers of matings have occurred or closely bred dogs that have the same parents. The sire of affected dog 29 and dam of affected dog 22 can each be seen in two boxes because of close inbreeding. It is very possible that breeders using individuals arising from C's three different litters might not suspect any inbreeding because of the number of generations involved. In the pedigree of Case 6, Bitch C appears 6 times over a span of 5 generations.

Figure 5 Descendants of key bitch C from all 3 litters showing aspects of inbreeding



Dogs D, M, S and certain descendants from bitch C were popular dogs for breeding and most CKCSs have genetic influence from one or more. However the significance of these dogs could not be explained by mere popularity. As illustrated in Table 2, 60% of unaffected dogs have both sire and dam descended from bitch G through C, D, M and S compared to 100% of affected dogs. However, 100% of affected dogs have 6 or more great-grandparents that were direct descendants compared to 6.6% of the unaffected dogs. Therefore, it did not seem important for the development of syringomyelia if the sire and dam were descendants of bitch G through C,D,M and S, however, it was important if the majority of the great-grandparents were. All subsequently confirmed cases also fitted this pattern. These results suggest that the inheritance is more likely to be autosomal recessive i.e. both dam and sire must carry the defective gene(s), however, it also suggests a more complex pattern e.g. a variable penetrance, or that there is more than one gene involved. Without knowing how many affected and unaffected offspring there are in a litter the actual mode of inheritance cannot be determined.

It is also possible that other diseases affect the development of clinical signs. Rapid changes in intrathoracic pressure are important factors in the pathogenesis of syringomyelia (Williams 1993). Therefore, diseases which cause increased respiratory effect, e.g. an over-long soft palate, could exacerbate the development of the condition.

As so many CKCS have genetic influence from dogs C, D, M and S it would be foolish to avoid these dogs, as the resulting gene pool would be too small, resulting in more problems. As the number of descendant great-grandparents appears to be critical, the author's recommendations for breeding is to aim for no more than 5 of the great-grandparents in a potential cross to be descendants of G via dogs C,D, M and S. To implement this requires detailed knowledge of CKCS breeding and pedigrees because of the number of generations involved.

The investigation is continuing to study the mode of inheritance and the possibility of second gene. The population of affected dogs is also being compared to CKCS with idiopathic epilepsy. The authors are still seeking pedigrees of CKCSs with suspected syringomyelia from before 1993, normal CKCSs with MRI confirmation of adequate occipital bone development and CKCS with confirmed idiopathic epilepsy.

Acknowledgements

The authors are indebted to Bet Hargreaves whose in-depth knowledge of the Cavalier King Charles spaniel breed and diligence at pursuing the authors' queries was greatly appreciated. The authors are also grateful for the assistance of Malcolm Burley, Janet Ireland and Outi Kuisma-Parwar. Thanks are also due to the staff of Stone Lion Veterinary Practice for dealing with the many telephone enquires about this project.

Footnote

After this paper was published Dog V was confirmed to have syringomyelia by post mortem examination and his case history is detailed in Section 3.

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Chapter 7.2

Inheritance of occipital bone hypoplasia (Chiari-like malformation) in cavalier King Charles spaniels

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Introduction

Occipital bone hypoplasia (Chiari-like malformation) resulting in caudal fossa overcrowding and obstruction of the foramen magnum with secondary syringomyelia (SM) was first identified in cavalier King Charles spaniels (CKCS) in 1997 [1].Clinical signs are caused by damage to the cervical spinal cord and include shoulder and neck paraesthesia, forelimb weakness, cervical scoliosis and pelvic limb ataxia. A classical clinical sign is a tendency to scratch at the shoulder area especially when excited or on a lead. Pain is a predominant feature of the condition and may vary from a mild cervical hyperaesthesia to bouts of screaming after sudden changes in head position, excitement or being touched in the paraesthetic region. Most dogs also have secondary ventricular dilatation. Signs usually are recognized between 6 months and 3 years of age. However dogs of any age may be presented, and dogs with more severe disease tend to be presented earlier. The defect in the CKCS is similar to Chiari type I malformation in humans characterized by underdevelopment of the occipital bone and posterior fossa with cerebellar tonsil herniation and obstruction of the foramen magnum [2].

Like all breeds derived from a small number of individuals, the CKCS breed has little genetic variation. The problem is compounded by the repeated use of particular stud dogs; it is not unusual for a popular sire to produce over 50 separate litters and hundreds of progeny. This breeding practice encourages the emergence of recessive genetic diseases. Selection for coat colours imposes further pressures on the available CKCS gene pool. Phenotypically, 4 colour variations are recognized: ‘whole colours’, ruby (r) and black and tan (bt) where white colour is undesirable and ‘parti-colours’ – blenheim (b) (ruby and white) and tricolors(t) (black, tan and white). Red colour and parti-colors are recessive and if breeders desire these coat variations they only use particular lines. The breed has a predisposition to myxomatous mitral valve disease (MVD), [3] and breeders are advised to select for their breeding program systolic murmur-free CKCS over 2.5 years old that have systolic murmur-free parents over 5 years old[4,5]. A “clear list” is kept and published by the UK CKCS club. This practice further narrows the gene pool as dogs showing signs of MVD are removed from stud. Access to internet pedigree databases allows breeders to select for longevity.

Investigation of inheritance by segregation analysis has not yet been possible primarily because of inability to determine the number of affected versus unaffected siblings in a litter. Puppies generally are sold to the public making them difficult to trace. The disease is confirmed by magnetic resonance imaging (MRI) which has only been available routinely in veterinary medicine in the last decade, has limited availability and is expensive. It is not possible to be sure whether a dog is truly unaffected without MRI because clinical signs may not be recognized or acknowledged by the owner or breeder. Signs may be mild, develop late in a dog’s life or may not be present at all e.g. mild syringomyelia can be identified as incidental findings in CKCS having MRI for other reasons. Study of dog genetics also is made more difficult by close and repeated inbreeding. Family trees are extremely complicated, and there are typically many possible paths for the inheritance of any genetic defect. Many breeders are unwilling to admit that they have a problem in the belief that to do so damages their reputation. Response from key breeders to provide information about affected dogs in their lines, especially before 10 years ago, has been minimal. This reluctance makes it very difficult to determine the Mendelian inheritance of this trait.

Preliminary work investigating the heritable nature of this disease involved establishing a 20-generation family tree which linked 45 affected dogs. [6] All of the affected dogs had at least 6 of 8 great-grandparents that could be traced back to a common female ancestor born in 1956 whereas only 6.6% of unaffected individuals had this ancestry. These data suggested that occipital bone hypoplasia was hereditary in the CKCS and that inheritance was likely to be autosomal recessive. The aim of this study was to develop a better understanding of the inheritance of occipital hypoplasia in a defined population of CKCS.

Materials and Methods

A family tree of over 5,500 related dogs was constructed over a period of 4 years. The data was compiled

on a computer program [a] for human genealogy. Unlike databases designed for dog pedigrees, it had the advantage that all clinically relevant crosses and descendants could be viewed simultaneously when printed. This feature permitted the complexity of the inter-relationships among dogs to be more easily scrutinized. The computer program identified all directly descended individuals and generated individual pedigrees either as a tree chart or text. It also produced 'enhanced hourglass' reports for any single cross, showing other mates, with their associated offspring for any number of generations. Information provided by the owners' pedigrees was for 3 to 5 generations but these findings were enlarged to 10-25 generations with information published by the UK CKCS club [7] and UK Kennel Club[b]. Supplementary information for some dogs that involved foreign breeding lines was obtained from http://www.cavaliersonline.com. and http://www.worldpedigrees.com/xCavalier.htm. The data was analysed at various time points. The degree of inbreeding meant that the information could not be represented meaningfully in a classical family tree. Flow chart software [c] was used to illustrate selected information in a simplified form for this report. For clarity, the diagrams illustrate only those matings relevant to the investigation together with relevant descendants.

Investigation 1. Does the family tree for occipital hypoplasia with secondary SM or hydrocephalus involve a subset of, or the entire CKCS population?

Family trees for occipital hypoplasia and idiopathic epilepsy (IE) were compared. An occipital hypoplasia family tree was constructed from the pedigrees of 120 affected dogs (Table 1).

Table 1 Cavalier King Charles spaniels under study

group	characteristics
1	Confirmed SM cases 1-50
1i	Confirmed SM cases with parti-colored coats (39 cases)
1ii	Confirmed SM cases with whole-colored coats (11 cases)
2	Unconfirmed SM cases 51-120
3	40 cases diagnosed with idiopathic epilepsy (IE)
4i	10 SM cases born before 1991 (mostly deceased)
4ii	10 SM cases born after 1996

SM – syringomyelia secondary to occipital bone hypoplasia.

Group 1 (case numbers 1-50) with MRI-confirmed occipital bone hypoplasia and secondary SM (49 cases) or MRI-confirmed occipital bone hypoplasia and secondary hydrocephalus (1 case). The MRI characteristics of occipital bone hypoplasia have been described previously. [1, 6] The earliest known CKCS with MRI-confirmed SM was born in 1988. The age range of the SM population ranged from 10 months to 12 years. The dog with hydrocephalus was 3 months of age at the time of diagnosis. The dogs were born in the UK, Eire, Finland, Sweden, Canada, USA or Australia. Additional pedigree information from another 70 dogs suspected to have SM on the basis of the typical clinical signs but without MRI confirmation (Group 2 case numbers 51- 120) was used to enhance the evidence. An IE family tree was constructed from the pedigrees of 40 CKCS with IE and no clinical signs of syringomyelia (IE). The earliest pedigree dated back to 1967 (Group 3). The diagnosis of IE typically had been made by veterinary practitioners on the basis of history and clinical signs (i.e., the dogs had generalized seizures and received anti-epileptic medication).

Investigation 2. Does a relationship exist between coat colour and occipital hypoplasia?

In order to determine the influence of selection for coat variation, Group 1 was subdivided accordingly into Group 1(i) for parti-colours (b and t) and Group 1(ii) for whole-colours (r and bt). The association between coat colour and epilepsy also was examined.

Investigation 3. Has the age of onset of clinical signs changed?

Breeders claimed that when they first started to notice clinical signs of the condition 10-12 years ago, the affected dogs were older than 3 years of age. Now many dogs are less than 2 years when first presented. A subset was made up of 10 dogs born before 1991 for which full clinical information was available (Group 4i). This group was compared with the 10 youngest dogs in the study (Group 4ii) born after 1996. In addition, the natural occurrence of the disease was studied in an extended family of a dog (case number 110) identified in the previous study [6] as being a likely carrier for SM secondary to occipital hypoplasia.

Results

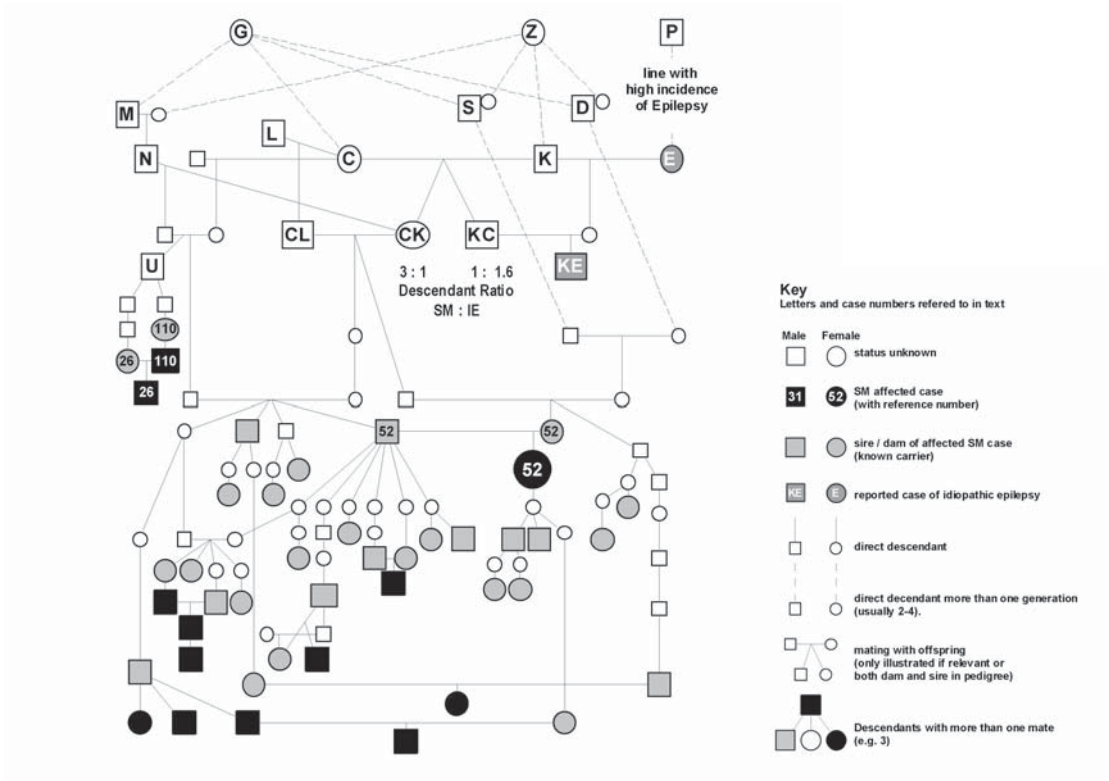
Investigation 1. Does the family tree for occipital hypoplasia with secondary SM or hydrocephalus involve a subset of or the entire CKCS population?

The CKCS breed has a narrower genetic base today than it did 15 years ago and can be traced back predominantly to a relatively small group of dogs and their closely bred descendants. All 120 dogs in this study with confirmed and suspected SM or hydrocephalus secondary to occipital bone hypoplasia could be shown to fit a similar pattern of inheritance as identified in the preliminary investigation [6] which highlighted 4 significant ancestors: C, D, M and S (Figure 1). All dogs with SM cases could be traced back through these 4 dogs, not only through both the dam and the sire, but through at least 6 of 8 great grandparents. Further investigation revealed that C, D, M and S in turn had common ancestors, namely Dam G and her mate. The study also showed another early common ancestor was Dam Z. At least 3 of 4 grandparents of all dogs with SM could be traced to Dam Z. The lines were sometimes independent from

descendant lines from Dam G. Thus, all 8 greatgrandparents of affected dogs were varying combinations of descendants of Dam G or Dam Z or (more usually) both.

All affected pedigrees fell into 2 broad groups: those descended from M with S or D; or those dominated by C. Only 1 confirmed case was descended from Dam C entirely. Dam C had only 3 different mates, which were not related to G but to ancestors common to D, M, S and Z. The relationship between G, Z, C, D, M and S is illustrated in Figure 1 which is the extended pedigree of Dam 52, the earliest known affected dog that was used in a breeding program. Two of Dam 52's sire's siblings were exported and account for affected dogs in Finland and USA. The CKCS population in these countries has a high incidence of disease as the gene pool is even smaller and the CKCS population is dominated by potential carriers of SM.

Figure 1 Family tree of dog 52 and selected descendants demonstrating relationships with i) key SM ancestors G, Z, M, S, D and C ii) dogs P, K, E, L, CL, CK, KC and KE associated with IE iii) dogs U, 110 and 26 (figure 2).



Note – Dam Z introduced into the family tree via Sire K and females that were mated with Sires D, M and S

The family tree of IE appears to be a different subset of the CKCS population although some overlap was identified. The largest concentration of IE cases was associated with Dam C when bred to Sire K (Figure 1). When descendant lines from their 2 resulting offspring CK (b) and KC (b/t), were compared, the ratio of SM:IE was 3:1 with CK and 1:1.6 with KC. The descendants of Sire CL, an offspring of Dam C and Sire L, included 8 dogs with IE. Sire L was mated to 7 other dogs and none of these descendants, to the authors' knowledge, had IE.

Although the majority of the IE group 3 had Dam G in the ancestry, only 30% of cases were descended from Dam G via dogs C, D, M or S whereas 100% of the SM group had at least 6 of 8 great grandparents descended from Dam G via dogs C, D, M or S (Table 2).

Table 2 Percentage of grandparents and great-grandparents descended via dogs C, D, M or S. Comparison between Group 1 and 2 (SM) and Group 3 (IE)

Direct descendants	Group 1 and 2	Group 3
4 grandparents	100% (100/100)	35% (14/40)
6/8 g. grandparents	100% (100/100)	30% (12/40)

Table 3 illustrates that some ancestors appear to be more influential for SM than for IE. For example, Sire P (Figure 1) is an important ancestor for both SM and IE but sires M and D are more important ancestors for SM than for IE.

Investigation 2. Does a relationship exist between coat colour and occipital hypoplasia?

Table 3 also illustrates how some dogs appeared more influential through selection for coat colours. Sires K and C are important ancestors for whole-coloured dogs with SM and for IE and might have channelled genes for SM by selection for coat variation.

The effect of colour on IE and SM is illustrated by comparison between siblings CK (b) and KC (bt), offspring from Dam C and Sire K (Figure 1 and Table 4). KC was an important ancestor in the whole-coloured lines. Thus 67% of whole-coloured SM dogs and 23% of all dogs with IE had *both* sire and dam descended from this dog. However this individual had no influence on parti-coloured with SM. KC's offspring KE had EEG-confirmed IE. By comparison, sibling CK has more influence on parti-coloured pedigrees and less on the IE pedigrees.

Table 3 Percentage of affected individuals (group 1 and 3) that are descended though the sire or dam from selected ancestors

Group	1I		1II		1(I AND II)		3	
Coat Variation	*parti-colors (b, t)*		*whole Colors(r, bt)*		*all colors*		*all colors*	
	Sire	*Dam*	*Sire*	*Dam*	*Sire*	*Dam*	*Sire*	*Dam*
Dam G (b)	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**68%**	**88%**
Dam Z (b)	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**68%**	**70%**
Sire M (b)	**100%**	**100%**	**73%**	**55%**	**94%**	**90%**	**43%**	**40%**
Sire D (t)	**80%**	**85%**	**64%**	**45%**	**76%**	**68%**	**20%**	**20%**
Sire P (bt)	**88%**	**39%**	**73%**	**91%**	**92%**	**85%**	**65%**	**73%**
Sire K (bt)	**62%**	**56%**	**100%**	**82%**	**70%**	**62%**	**50%**	**45%**
Dam C(t)	**69%**	**56%**	**100%**	**91%**	**76%**	**64%**	**48%**	**40%**

b- Blenheim, t-tricolour, r- ruby, bt – black and tan

Table 4 Percentage of affected CKCSs in group 1 and 3 *with both dam and sire descended* from siblings CK or KC demonstrating the relationship with coat variation and for idiopathic epilepsy.

Group	1i	1ii	1 (i and ii)	3
Characteristics	SM: Parti-colors (b,t)	SM: Whole Colors(r, bt)	SM: all colors	I.E. all colors
Dam CK (b)	**54%** (21/39)	**18%** (2/11)	**46%** (23/50)	**15%** (6/40)
Sire KC (bt)	**0%** (0/39)	**67%** (7/11)	**14%** (7/50)	**23%** (9/40)

Note - This gives the ratio of descendants from CK 3 SM:1 IE and KC 1SM:1.6IE
b- Blenheim, t-tricolor, r- ruby, bt – black and tan

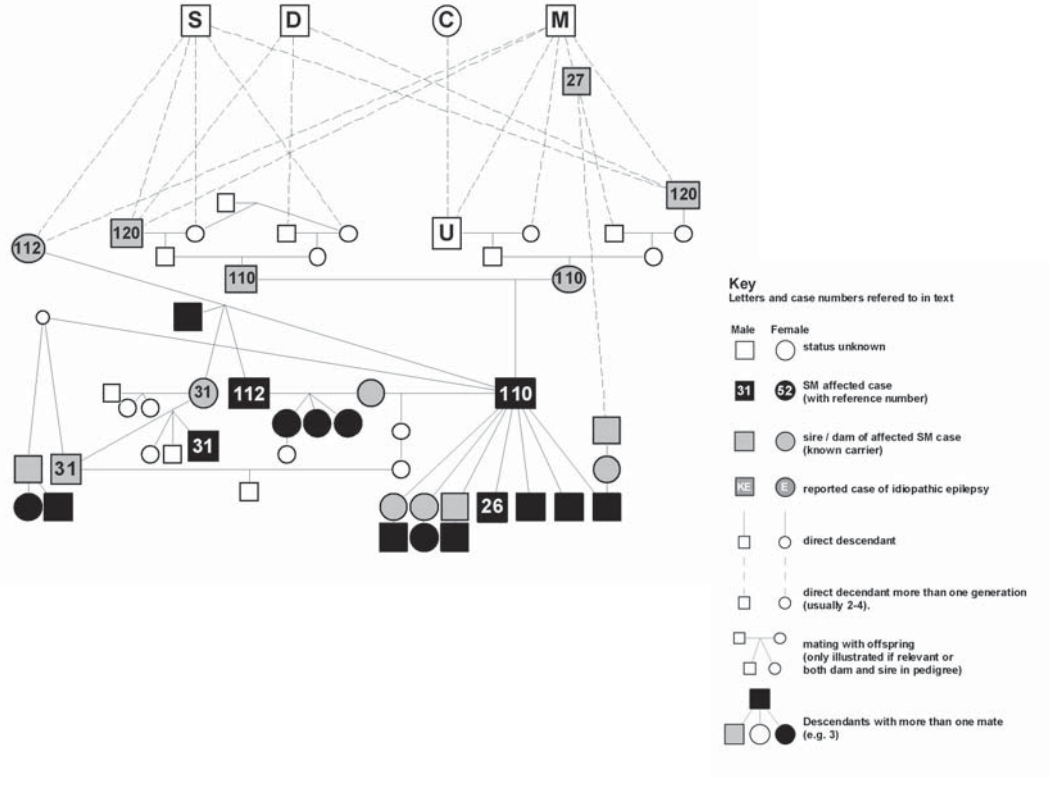
Investigation 3. Has the age of onset of clinical signs changed?

The average age of onset of the disease for Group 4i was 4.8 years (range, 3-7 years) compared to 8.9 months for Group 4ii (range, 3-15 months). Many owners with older SM dogs not included in group 4i were unsure of age of onset because it appeared to be a gradual process or the condition only was detected incidentally. Increased awareness of SM accounted for the earlier diagnosis in some dogs.

There was a tendency for increasing clinical severity and earlier onset with increased inbreeding. For example, case 47 was a severely affected female showing signs of disease from 18 months. Her sire had milder disease and was confirmed with SM at 32 months. It was common for the sire or dam to develop signs after the offspring had been diagnosed. For example dog 46 developed scoliosis at 10 months, her dam (dog 37) and her maternal aunt (dog 33) developed shoulder scratching and pain at 5 and 2 years respectively. The earliest onset of disease was in dog 41, a 3-month-old CKCS euthanized because of hydrocephalus secondary to severe occipital bone hypoplasia and foramen magnum obstruction. This bitch had 32/32 great-great-great-grandparents descended from the clinically relevant lines, which included champions known to have a tendency for shoulder scratching while walking.

Figure 2 is a case study of a family centred on dog 110 which developed mild signs of shoulder paraesthesia at approximately 18 months of age. This dog was mated to a female of unclear status. Clinical records indicate that she had a head tilt for which the cause was not determined. The consequence of the mating was a litter of 2. The male (dog 112) developed signs at 10 months and when mated to an allegedly unaffected female, the resultant puppy (dog 14) was severely affected with SM and euthanized at 6 years of age. According to the breeder, dog 112's sister had no signs consistent with SM; she was mated with a half-brother and produced dog 31 which showed signs of SM from 30 months of age. Two other matings have produced unaffected offspring so far. Other known affected dogs in the family are illustrated.

Figure 2 Family tree of dog 110 illustrating relationship with i) ancestral dogs C, D, M and S ii) Sire U and sires of affected dogs 27 and 120 iii) 12 affected descendants from 10 different matings



Discussion

The study is unusual because the complete pedigrees of dogs affected with SM secondary to occipital hypoplasia are known from the time of derivation of the breed from King Charles Spaniels in the 1930's. The investigation had many limitations that were a consequence of studying a naturally-occurring disease in a pet population. Segregation analysis has not been possible so far because of difficulty identifying whether a dog is truly affected or unaffected. In addition, the diagnosis of IE in group 3 was made, for the majority of cases, by general practitioners with limited diagnostic equipment. Therefore, conclusions on the inheritance of both diseases are limited. It is possible that SM secondary to occipital bone hypoplasia is recessive because affected individuals may be produced from apparently unaffected individuals. From a study of the extended CKCS family tree we conclude that the disease occurs as a result of repeated close breeding between 1 or more key descendants, C, D, K, M, and S - themselves descended from two dams, G and Z. All of the affected dogs born in the last 5 years are descended from C, D, K, M and S via known or suspected carriers through at least 6 of 8 great grandparents. It has not been established why, in every case, the number of potential carrier grandparents is always 3 or 4. The disease seems to require this high degree of inbreeding before becoming obvious, and this observation in turn suggests that the inheritance may be more complex than a single autosomal recessive gene. Sex-linked inheritance has not been ruled out. The ratio of male to female affected dogs is roughly equal, and the sex ratio of the database is 0.65:0.35 female:male. However in a study of 30 random selected cases it was possible to trace a possible line of inheritance to the key dogs without involving any male-to-male transmission.

Selection for coat variation and the avoidance of some lines because they carry certain diseases (e.g., heart and cataract disorders) have narrowed the CKCS gene pool. As a result, descendants of C, D, K, M, and S dominate modern CKCS pedigrees compared to 10 years ago. Occipital hypoplasia currently is so common in the breed that it could be considered "normal" for a CKCS to have some degree of occipital hypoplasia, with SM and hydrocephalus occurring in the most severely affected dogs. In ideal circumstances, a group of MRI-confirmed normal dogs would also be studied. To do so would depend on a chance finding of a normal animal that is having a diagnostic MRI for another reason and a large enough group of animals in that category has not yet been collected. In the original study [6], 45 dogs affected with SM were compared with a control group of 45 dogs over 10 years of age that showed no clinical signs of the disease. These older dogs used lines that were not as popular as those found in the pedigrees of current champions. Currently, avoiding descendants of C, D, K, M and S in a breeding program would be extremely difficult.

The SM pedigrees were compared to pedigrees of CKCS with IE because there was a concern that SM was so widespread in the population that it was possible that the whole, rather than a subset, of the CKCS population was being studied. There also was a concern that occipital hypoplasia may be directly associated with IE becasue several dogs had both diseases. Both diseases were found to be distinct subsets of the population although there was overlap especially associated with the intensely inbred lines from Dam C and Sire K where there were many half brother and sister matings. SM secondary to occipital hypoplasia is most common in blenheim and rubies which are recessive coat variations and must be bred from a more restricted gene pool. Selection for color affected the natural history of the disease because some of the champion dogs that are important ancestors for SM were popular for their tendency to "throw" a certain color. CKCS whole-color breeders commented that 15 years ago, tri-colors such as Sire D and Dam C were introduced to the ruby lines in an attempt to widen the gene pool but still deliver some whole-colors. This event appears to have been important in the history of SM by increasing the cohort of dogs with genes from both G and Z.

The disease has a tendency to be more severe in each generation (i.e. breeding mildly affected dogs can result in offspring with more severe disease with an earlier onset). There appeared to be 3 forms of the disease based on severity and age of onset: 1) neonatal form (less than 6 months of age) presenting with clinical signs relating to hydrocephalous; 2) juvenile form (6-15 months of age) initially presenting with scoliosis secondary to SM; 3) adult form (8 months-10years of age) initially presenting with shoulder scratching and pain secondary to SM.

The implications for the breed are serious. Pedigrees of the 25 top stud dogs for the last 5 years showed that not only were many very closely related, they all were descendants of suspected carriers for SM. Thirteen of these top stud dogs were directly associated with affected dogs.

The widespread tendency for heart disease in the breed must also be considered.[3,4] MVD has affected the development of SM as breeders have selected for dogs with ancestral history of longevity and late-onset systolic murmur. Unfortunately, many of these dogs had or carried a tendency for SM. Our clinical observation is that surviving dogs with SM have late onset heart disease (i.e., do not developing clinical signs until after 8 years of the age). In other words, the breeders' attempts to breed away from one disease appear to be making another disease more probable. It seems unlikely that CKCS breeders will be able to select against SM and MVD by pedigree analysis because both diseases are widespread in the population. Even if they could do so, the gene pool would be further narrowed, possibly encouraging the emergence of another disease. An appropriate way forward is to attempt to identify the causal genes in both diseases. If possible, carriers or even affected dogs then could be mated to unaffected dogs thus preserving genetic variation while reducing disease incidence.

Occipital hypoplasia with secondary SM is a valuable model of Chiari I malformation in humans both for studying the development of the disease and investigating its genetics. Chiari I malformation in humans occurs in up to 0.77% of individuals, and 75% of people with this malformation are symptomatic [8]. Familial Chiari type I malformation is reported with an incidence of about 2 % of total cases with autosomal recessive or dominant inheritance patterns [9, 10, 11] but a higher incidence is suspected [12]. The Mhox gene or genes belonging to the Hox family control the development of the final shape of the occipital bone

[11]. Ectopic expression of Hox-2.3 resulted in dysplasia or deficiency of occipital, basisphenoid and atlas bones in transgenic mice [13]. The Pax group of genes (especially Pax-1) also has been suggested as a candidate for Chiari I malformation[12]. The Pax-1 gene plays an important role in the cervico-occipital transitional zone [14].

In conclusion, the study findings support the hypothesis that occipital hypoplasia with secondary SM in CKCS is a hereditary condition. Further study is required to establish the inheritance, which is likely to be more complex than simple autosomal recessive. Selection for colour and against other diseases influences the incidence of the malformation in the population. With the advances in the canine genetic map, this family tree should be useful in further research to identify the genes contributing to the malformation in the dog, and may be useful in for research on the genetics of human type I Chiari malformation. Future studies should involve collection of DNA from extended 3 or 4 generation CKCS SM families with a view to genotyping, linkage analysis and positional gene cloning

Footnote

[a] Generations C Grande Suite 8 (Sierra On-line previously at Inc. Bellevue, WA 98007). The licence for this product has been withdrawn following a change in company ownership.

[b] The Kennel Club UK Breed Supplements -Small and Toy dog 1 Clarges Street, London, W1J 8A3

[c] Flow chart maker; Cosmi Software. Cosmi Europe Ltd Unit 8a, Daimler Close, Royal Oak Industrial Estate, Daventry, Northants, NN11 5QJ

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Chapter 7.3

Inherited Chiari-like malformation/syringomyelia in the cavalier King Charles spaniel – experiences in setting up a worldwide DNA collection

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Introduction

Like most purebred dogs, the cavalier King Charles spaniel (CKCS) has a small and decreasing gene pool. The breed was established in 1928 in response to a reward offered for recreating a spaniel similar to those depicted in portraits of the era of King Charles II (Figure 1) and the modern breed is predominantly descended from six dogs (Rusbridge and Knowler 2003).

Figure 1. CKCS (from left to right) with tricolour, blenheim and ruby colour types. The black and tan variety is not illustrated. Selection for colour affected the natural history of syringomyelia in CKCS because some of the pivotal ancestors were popular for their tendency to "throw" a certain colour.

CKCS breeders practice linebreeding which is understood (Rasmussen 2005) as a method to create a desired appearance and/or avoid known inherited diseases. A desirable ancestor is identified and descendants are often repeatedly bred together so that the common ancestor may appear several times on both maternal and paternal sides of a five generation pedigree. Once a "breeding line" is established, line- breeding is continued generation after generation with no or occasional "outcrosses" i.e. crosses to dogs with no common ancestors within a five generation pedigree. A popular stud dog can have over fifty matings to consanguineous bitches and produce hundreds of offspring which are then line-bred. Thus one individual can have a significant influence on a gene pool. While father-daughter, mother-son and full sister-brother matings are no longer acceptable more distant relationships e.g. grandfather-granddaughter, are allowed.

The complexity of the family tree makes study of canine inherited disease difficult. On the other hand, the strong familial relationships within purebred dog populations has also led to the accumulation of certain inherited diseases in nearly each breed, and the eradication of other diseases, so that many inherited diseases occur with high frequency in a breed specific manner. Recognition of the genetic background of a disease is therefore often possible in dogs, but not in other species.

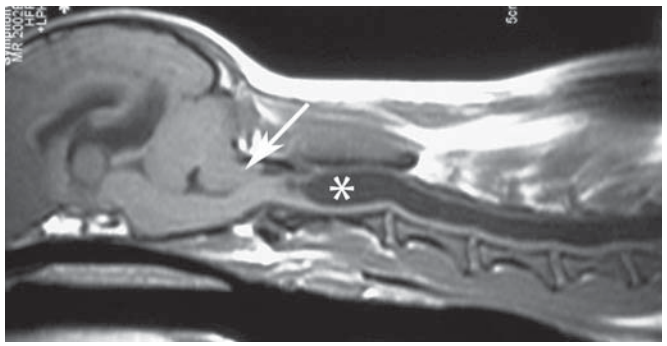
The CKCS breed has a high incidence of Chiari-like malformation (occipital hypoplasia), a condition similar to Chiari type I malformation in humans (Rusbridge et al 2000). The consequence of inappropriately small occipital bones is reduced volume of the caudal fossa i.e. the part of the skull which accommodates the cerebellum and brainstem. Cerebrospinal fluid flow is obstructed by the overcrowded cerebellum (often herniated through the foramen magnum) and brain stem. The obstruction results in fluid coalescing in cavity/cavities (syringomyelia) within the spinal cord. Some cases also have ventricular dilatation. The degree of syringomyelia is quite variable. The most severe cases have considerable spinal cord damage and are significantly disabled by 12 months of age. In contrast, some have a small subclinical syringomyelia which is only detectable by MRI or post mortem.

The classical clinical signs of syringomyelia are scratching at the neck/shoulders when walking (para-

esthesia/dyskinesia) and pain with or without cervical scoliosis, paresis and ataxia (Figure 2). At present, confirmation of the disease can only be made by magnetic resonance imaging (MRI) (Figure 3)

Figure 2 Scoliosis in a 16 month old CKCS. There is syringomyelia extending into the dorsal grey column over a number of spinal cord segments. Presumably this results in an imbalance of afferent information from the cervical neuromuscular spindles. The neck bends away from the side of the lesion.

Figure 3 Midsagittal TW1 weighted image of the brain and cervical spinal cord. Syringomyelia (asterixis) secondary to Chiari-like malformation in a 21 month female CKCS presenting with a 3 month history of yelping and a tendency to scratch at the right shoulder area. There is cerebellar herniation through the foramen magnum (arrow). This malformation is very similar to Chiari I malformation in humans



Recent data has suggested that Chiari-like malformation in the CKCS is inherited (Rusbridge and Knowler 2003, 2004). The disease has a tendency to be more severe in each subsequent generations, i.e. breeding mildly affected dogs can result in offspring with more severe disease and an earlier onset. An early observation from study of an extended CKCS family with a high incidence of syringomyelia suggests that apparently normal parents of clinically affected offspring have mild Chiari-like malformation /syringomyelia detectable by MRI only (unpublished data). A consistent observation is that all clinically affected dogs have 3-4 grandparents and 6-8 great grandparents descended from pivotal ancestors which anecdotally had produced affected offspring, some over 30 years ago (Rusbridge and Knowler 2003, 2004). Selection for colour and against other breed related diseases influences the incidence of the malformation in the population; for example, many of the pivotal ancestors were extensively used as stud dogs because they did not have early onset hereditary mitral valve disease (Rusbridge and Knowler 2004).

Identification of the causal gene(s) for Chiari-like malformation would be invaluable because it would allow development of a test to detect subclinically affected dogs and carriers for breeding purposes. It could also be useful in furthering understanding of the embryological development of occipital bone and the pathogenesis of Chiari type I malformation in humans.

Materials and Methods

After initially describing the disease, we established a database of affected dogs and their relatives. Due to the complexity of the family tree it was difficult to use existing canine genetic software. A basic program for human genealogy was used (Generations C Grande Suite 8; Sierra On-line previously at Inc. Bellevue, WA 98007) which allowed the inter-relationships among dogs to be more easily appreciated as all clinically relevant crosses and descendants could be viewed simultaneously (the licence for this product has subsequently been withdrawn following a change in company ownership). The database generated GEDCOM files (Genealogical Data Communication; Church of Jesus Christ of Latter Day Saints Family History Department) which were eventually transferred to a specifically designed program based on Microsoft Access (Microsoft Corporation USA). This database had the ability to record phenotypic variables to enable statistical and linkage analysis. The phenotypic variables included: sex; coat colour; severity of syringomyelia; clinical signs and age of onset; and presence/absence of other inherited disease.

We subsequently established a DNA collection program. Initially this was started in the UK with the support of the DNA Archive for Companion Animals, the University of Liverpool (held within the Integrated Genomic Medical Research, The University of Manchester). After initially being contacted by phone or letter, veterinary surgeons in general practice were provided with sample pots, tick-box forms detailing phenotypic information (Figure 4) and postage paid envelopes ensuring that they had an easy means by which DNA samples could be submitted (with owner consent). The DNA collection was later extended to the Netherlands, USA, Canada and more latterly Germany and France. The inclusion and exclusion criteria for DNA collection are detailed in Table 1.

Table 1

Inclusion criteria for DNA collection

1. Syringomyelia secondary to Chiari-like malformation as confirmed by MRI
2. CKCS with a normal occipital bone and no syringomyelia as confirmed by MRI
3. Selected Cavalier King Charles spaniels closely related to affected dogs and identified by project coordinators as important for linkage analysis. MRI status may not be known
4. Dogs with the stereotypical signs of syringomyelia (i.e. scratching at shoulder/neck/ear when excited or walking on the lead with or without cervical scoliosis, cervical hyperaesthesia, lower motor neuron signs thoracic limbs and pelvic limbs paresis/ataxia) but where MRI diagnosis is not possible either because of owner financial constraints or because prerequisite anaesthesia unadvisable.

All cases should have details of:

5. Clinical signs including time of onset and severity
6. Severity of MRI changes (if MRI available)
7. Presence / absence systolic heart murmur/ previous heart failure (i.e. development pulmonary oedema) and any heart medication
8. Pedigree

Exclusion criteria for DNA collection

1 Inadequate clinical records
2 Any evidence of skin disease (for cases without MRI confirmation)
3 Traumatising skin when scratching (for cases without MRI confirmation)

Results and Discussion

The family database is currently 24 generations and details over 8500 related individuals across 3 continents (North America, Australasia and Europe). MRI status is known for 193 dogs (160 affected with syringomyelia, 33 clear) and in a 12 month period we have collected over 500 DNA samples including ~ 90% of the dogs with known MRI status. It is focused around one pivotal affected stud dog and ranges over 4 generations. Ultimately genotyping, linkage analysis and positional gene cloning is planned.

Support from the breed clubs is essential in establishing a successful DNA collection program. This can be difficult to achieve as some breeders believe that highlighting health issues may reduce puppy sales and/or compromise their breeding program and thus endanger their livelihood. One of the key elements is support from senior committee club members. The election of less or more sympathetic individuals can significantly influence the ongoing success of a scheme. We found that the most successful way to ensure continuing support for research was to guarantee confidentiality and allow the breed clubs to control publicity about the disease and adopt a positive approach. This was achieved by keeping breeders and owners informed by quarterly newsletters and educational videos. E-mail was pivotal for ensuring communication both for dissemination of information and in provision of support for owners and breeders affected by the disease. Various internet support groups, some with membership of over

Figure 4 Phenotype form

DNA for Healthy Cavaliers

Syringlomyelia - Mitral Valve disease - Epilepsy

Phenotype Form

ID#_____

Send to: Clare Rusbridge- Confidential Fax: (011-44) 208 786-0525 or email: neuro.vet@btinternet.com

Pedigree Name: _____

Date of birth: _____ **Case No** _____ Owner's name _____

Color B B/T R T Gender M MN F FN Vet name/practice (practice stamp)

Sire's pedigree name _____

Dam's pedigree name _____

Date of Sampling - _____ Referring Clinician _____

Syringomyelia (please tick appropriate box)

No clinical signs ☐ Age of onset (if appropriate) – ____yrs ____mths

Shoulder scratching ☐ Neck pain ☐ Scoliosis ☐ Pelvic limb ataxia ☐ Thoracic limb weakness ☐

Was **MRI** carried out Yes ☐ No ☐ Was surgery carried out ? Yes ☐ No ☐ Date _____yrs _____mths

Occipital hypoplasia Yes ☐ No ☐
Cerebellar herniation Yes ☐ No ☐
Syringomyelia Yes ☐ No ☐
Area of spinal cord affected _____
☐ <1/3 diameter spinal cord
☐ 1/3-2/3 diameter spinal cord
☐ > 2/3 diameter spinal cord
Medullary kinking Yes ☐ No ☐
2° ventricular dilatation Yes ☐ No ☐
☐Neurologists notes attached

Details of any affected relatives _____

Mitral Valve Disease (please fill in/tick appropriate box)

Grade of murmur /6 Age of last heart clearance ____yrs _____mths Age murmur first diagnosed ____yrs ____mths

Stage of heart disease Age diagnosed _____yrs _____mths Examined by ☐Board Cert. Cardiologist ☐General Practitioner

☐Normal (0) *no murmur*
☐Mild (1) *Evidence of heart disease (i.e. murmur) but no clinical signs (heart normal size on x-ray / scan and no pulmonary oedema)*
☐Moderate (2) *As 1 plus mild cough and / or evidence of left atrial enlargement on xray/scan; no pulmonary oedema.*
☐Severe (3) *As 2 but has needed or still requires frusemide for pulmonary oedema (heart failure). Has or had clinical signs of coughing, breathlessness at exercise, some exercise intolerance*
☐Very severe (4) *On multiple drugs to control clinical signs of heart failure; unable to exercise.*

Heart medication currently receiving _____
☐Cardiologists notes / ultrasound scan results attached

Details of any affected relatives _____

Primary Epilepsy☐ Episodic Falling ☐ Compulsive disorder ☐ (e.g. fly-catching)
Age diagnosed above – Medication received -

Other (please specify) -

If applicable: Age at death_____ yrs _____mth **Cause of death** _____**age previous phenotype form** ____ yrs ___mths

I consent to DNA being extracted from my dog's sample and that this will be used entirely for research in the field of animal disease and genetics by *bone fida* scientists. I can reclaim my dog's sample at any time. **Please sign below**

200 individuals, have proved to be invaluable especially with regard to identifying other breeds with spontaneously developing Chiari-like malformation/syringomyelia. Project coordinators proved essential to focus collection of DNA by identifying particular offspring and relatives, answering queries and ensure that valuable data was not lost (Figure 5).

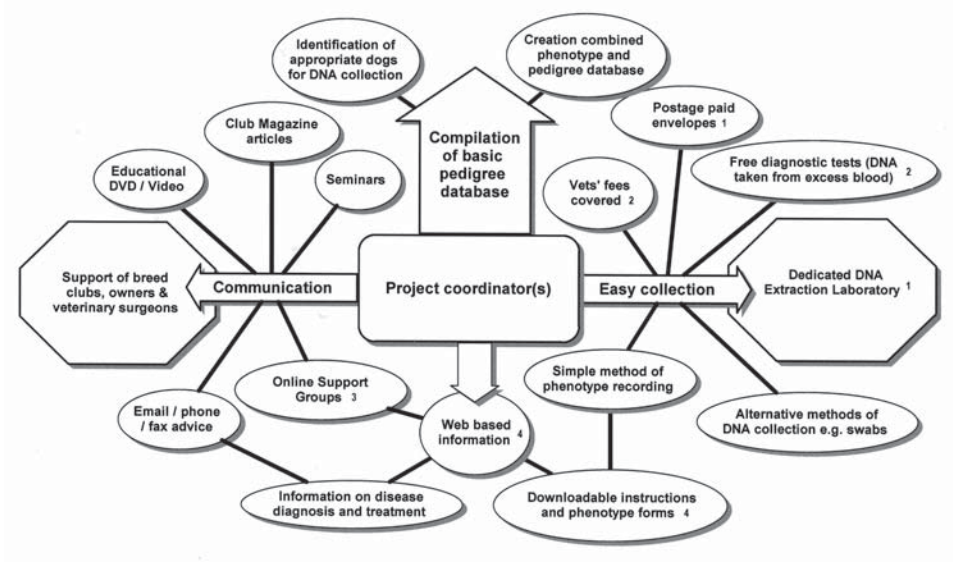


Figure 5 The key features which have ensured a successful DNA collection. Project was supported by the [1] UK DNA Archive for Companion Animals (www.liv.ac.uk/animalDNAarchive) and [2] Boehringer Ingelheim.

Internet sites / groups relating to the project

[3] http://uk.groups.yahoo.com/group/ArnoldChiari_dogs/ http://www.ourchad.20m.com

[4] http://www.cavaliers.co.uk/ http://www.thecavalierclub.co.uk/

Chiari-like malformation/ syringomyelia is a valuable model of Chiari I malformation in humans both for studying the development of the disease and investigating its genetics. Chiari I malformation in humans occurs in up to 0.77% of individuals, and 75% of people with this malformation are symptomatic (Meadows 2000). Familial Chiari type I malformation is reported with an incidence of about 2% of total cases with autosomal recessive or dominant inheritance patterns (Coria et al 1983, Zakeri et al 1995, Catala 1999) but a higher incidence is suspected (Speer et al 2000). There are a number of proposed candidate genes. Mhox gene or genes belonging to the Hox family control the development of the final shape of the occipital bone (Catala 1999) and ectopic expression of Hox-2.3 results in dysplasia or deficiency of occipital, basisphenoid and atlas bones in transgenic mice (McLain 1992). The Pax group of genes, especially Pax-1, which plays an important role in the cervico-occipital transitional zone, have also been considered as possible candidates for causative defects in human Chiari I disease (Speer et al 2000, Wilting et al 1995). However, to date it has not been possible to identify any mutations associated with Chiari I disease using human populations. The canine pedigrees being assembled here should yield crucial genetic information applicable to this very common human disease.

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Chapter 7.4

Preliminary results from syringomyelia (SM) genome wide scans in cavalier King Charles spaniel kindred and directions for future research

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Introduction

Chiari-like malformation (CM) represents an abnormality of the craniocerebral junction characterized by small volume caudal fossa with foramen magnum obstruction and secondary syringomyelia (Rusbridge and Knowler, 2003). This condition is particularly common to the cavalier King Charles spaniel (CKCS) as compared to other breeds, suggesting the involvement of genetic factors in the aetiology of this disease. Current studies suggest that syringomyelia (SM) occurs in at least 50% of dogs affected with CM and varies in severity from being asymptomatic to a severe neuropathic pain syndrome with paresis, ataxia and scoliosis. The disease has a tendency to be more severe in each subsequent generation. At present, confirmation of the disease can only be made by MRI (Rusbridge and Knowler, 2004). Pedigree analysis in a large database of over 5500 CKCS has suggested that CM in the CKCS is inherited where all clinically affected dogs share a small number of common ancestors. In fact, it has been shown that 6 out of 8 great-grandparents of all affected dogs can be traced back to two female ancestors. This small number of founders along with strict breeding criteria has led to reduced genetic variation in the CKCS breed (Rusbridge and Knowler, 2003).

In humans, while CM can be acquired following insults to the brain and cervical spine such as trauma and tumours, most cases of CM in the dog are due to a developmental defect thought to be the result of underdeveloped occipital bone(s) and caudal fossa. Morphometric studies in human patients have suggested that the fundamental defect may involve under-development of the occipital somites originating from the para-axial mesoderm (Speer et al. 2003). In hamsters, CM can be experimentally induced by administration of a single dose of vitamin A on embryonic day 8. Results from these studies suggest that the developmental defect in CM is thought to involve the somitic mesoderm at the basicranium and craniovertebral junction where a possible para-axial mesoderm insufficiency after the closure of the neural folds could lead to underdevelopment of the basichondrocranium resulting in a caudal fossa that is too small and shallow. The consequence would be overcrowding and subsequent herniation of the cerebellum into or below the foramen magnum (Marin-Padilla and Marin-Padilla, 1980).

The likelihood of a genetic basis to CM in humans is supported by many lines of evidence including familial aggregation and co-segregation with known genetic syndromes. Familial clustering has been reported in some families with CM where both vertical and male-to-male transmission was described, consistent with an autosomal dominant mode of inheritance (Speer et al. 2003). Other pedigree studies have implicated autosomal recessive mode of inheritance for CM. Most likely, the pattern of inheritance of CM is oligogenic with variable penetrance. CM has been associated with a variety of known genetic disorders including achondroplasia, Klippel-Feil syndrome, primary basilar impression and Goldenhar syndrome. The majority of these disorders affect mainly bony structures supporting the hypothesis that CM is mesodermal in origin (Speer et al. 2003).

The cellular and molecular mechanisms leading to CM are poorly understood. A number of biologically-plausible candidate genes have been proposed. The *Hox* gene family controls the development of the occipital bone and ectopic expression of Hox-2.3 results in dysplasia or deficiency of occipital, basisphenoid and atlas bones in transgenic mice (McLain et al. 1992). The *Pax* group of genes, especially *Pax-1*, plays an important role in somitic segmentation and proper sclerotomal differentiation in the cervico-occipital transitional zone (Speer et al. 2000). *Noggin* is required for growth and differentiation of the somites in the paraxial mesoderm and Noggin knockout mice show various defects affecting neural and axial-skeletal defects. *Noggin* was analysed in 33 cases of CM and no variants were identified suggesting that this gene is not a common genetic factor involved in CM (Speer et al. 2003).

The number and identity of genes predisposing to CM and associated SM have not been determined yet.

Our goal is to identify the genetic factors leading to the development of these conditions. Our specific aims are (1) genetic mapping of the CM and SM gene(s) by linkage disequilibrium analysis in the CKCS breed and in other related breeds; (2) identification of candidate genes for CM and SM using the positional candidate gene approach and (3) molecular characterization of the gene(s) mutated in CM and associated SM.

Experimental Plan and Preliminary Results

1. Genetic mapping of the CMI and SM gene(s) in the CKCS breed by linkage disequilibrium (LD) analysis:

We have constructed a genealogy of more than 10000 related CKCS dogs spanning 24 generations across 3 continents (North America, Australia and Europe). The data is stored in an Access database containing phenotypic descriptors, DNA availability, and filial relationships. Samples were ascertained through a veterinary clinic specializing in neurological diseases of dogs and also by voluntary participation of dog owners alerted of the ongoing collection effort in this breed. Once a dog was seen at the clinic for CM/SM, the owner provided pedigrees of 3 to 5 generations. When necessary the breeder was contacted to obtain the extended pedigree information. As is standard for dog breeders, dog records were tracked by using unique dog names. The collected filial data was matched to dogs in the genealogy database as well as to publicly available pedigrees of CKCS published by the UK CKCS club and UK Kennel Club. Supplementary information of some dogs of foreign breeding links was obtained from http://www.cavaliersonline.com and from http://www.worldpedigrees.com/xCavalier.htm/.We have established a wide DNA collection of over 1000 samples including mainly the CKCS breed and 30 samples from 10 other breeds affected with CM of various degrees of genetic relatedness to the CKCS (King Charles Spaniels, Brussels Griffon, Yorkshire Terrier, Staffordshire Bull Terrier, Boston Terrier, Chihuahua, Maltese terrier, a Miniature dachshunds, a Boston terrier, a Pug and a French bulldog).

Founder events and stringent breeding practices have made the CKCS breed, like other purebred dogs, a closed genetic pool, equivalent to isolated human populations used advantageously in genetic mapping studies such as the Finns, Icelanders and Bedouins. Isolated populations have limited variation in their gene pools which reduces the chances of disease heterogeneity. The higher inbreeding in isolated population also has the effect of leading to larger physical regions of genetic identity shared on chromosomal segments involved in disease expression (Varilo and Peltonen 2004). As a result, LD mapping is a promising strategy for gene mapping studies in the dog (Hyun et al. 2003). We will use this mapping strategy to identify genes predisposing to CM and to SM in the dog.

1.1. Genetic mapping of the gene(s) predisposing to CMI

CM is present in variable degrees of expression in nearly 100% of the CKCS dogs, making association studies in this single breed not feasible. CM/SM does occur with a lower frequency in other (mostly toy) breeds; however, since diagnosis is confirmed only by MRI, the exact incidence of CM in these breeds is not known. Closely related breeds are more likely to share ancestral chromosomes and hence carry the same disease allele. Studies of the evolutionary history of the dog have shown that dog breeds define distinct genetic units divided into at least 4 hierarchical groupings (Ostrander et al. 2005, Parker et al. 2004). The CKCS are clustered with mostly modern breeds including other spaniels, gundogs, hounds and terriers. Ongoing studies are focusing on defining clusters within this group. This kind of structure analysis of the dog population provides a genetic guide to the design of LD whole-genome scans. We will initially conduct a whole genome scan using dogs from the CKCS breed and other relatively modern and closely- related breeds. Studies have shown that LD in purebred dogs is ~100 times more extensive than in humans (Sutter et al. 2004). We will use allele sharing, homozygosity mapping and association testing to pinpoint a candidate region. We will next try to narrow down and delineate the minimal genetic interval containing the CM gene(s) by LD mapping and haplotype association studies using a dense SNP (single nucleotide polymorphisms) coverage between historically older but related breeds affected with CM as necessary. Analyses will be conducted by using SAS 9.1 including SAS/Genetics (SAS Institute Inc., Cary, NC, USA), as well as DMLE for LD mapping (Reeve and Rannala 2002).

1.2. Genetic mapping of the genes predisposing to SM associated with CM

Currently studies suggest that SM is present in over 50% of dogs affected with CM (Rusbridge and Knowler, 2004) making whole genome association and linkage-based studies feasible within this breed. Due to the complex inbreeding in the CKCS, a preliminary genetic analysis was necessary to evaluate the informativeness of the genetic markers and hence the feasibility of a whole genome scan in such breed. Consequently, 10 dogs were selected for genotyping with 122 markers distributed among the 38 autosomes and X chromosome. Next and with the support of the Marshfield genotyping services from the NIH, we have recently completed the genotyping of 173 CKCS dogs over 249 microsatellite markers distributed over the 38 autosomes and the X chromosome. The dogs are distributed over 34 dog pedigrees including multiple affected and unaffected sibs and half sibs with parents. The data was analysed for linkage using the program SAGE (Statistical analysis for genetic epidemiology) using the LODPAL analysis tool which allows for affected relative pair and discordant pair linkage computations. We also analyzed the genotyping data using a statistical approach testing association in the presence of linkage methods implemented in the computer program FBAT (Abecasis et al. 2000). FBAT (family based association test) provides a class of conditional tests that includes many of the established tests described for specific family structures. It handles missing parents, missing marker data, uncertain phase, continuous and discrete phenotypes as well as covariates. The main motivation for using the FBAT approach is to control for the multiple inbreeding loops present in the CKCS genealogy. This approach allows the detection of both linkage and association. Empirical evaluation of the study- specific genome wide significance criterion was conducted

using simulations with the program. Preliminary results have suggested six interesting regions on six associated chromosomes which warrant further investigation.

2. Identification of candidate genes in the minimal CM and SM interval(s)

We will search the minimum candidate genetic interval(s) for the presence of transcribed genes. As most of the dog sequence data is available *in silico*, we will define our candidate genes and their precise sequence using mostly three public databases: UCSC (http://genome.ucsc.edu), NCBI (http://www.ncbi.nlm.nih.gov) and Ensembl (http://www.ensembl.org). Comparative genomics between multiple species including human, mouse, dog and chimp is another powerful tool in identifying genes via analysis of sequence conservation. Novel genes will be analyzed for secondary structure predictions, specific sequence motifs and sequence homology to known proteins and nucleic acids. This analysis will provide some clues on the structural and functional aspects of the cloned gene and encoded protein. We will screen candidate genes chosen according to the following criteria: (1) position within the definite candidate region, (2) expression in the affected tissues and (3) predicted/known function. Candidate genes based on function are chosen if they are thought or known to be implicated in the morphogenetic processes involved in formation of the craniocerebral junction. We will first focus on sequencing the coding exons where most of the disease-associated mutations are found. We will examine promoter, untranslated and intronic regions to detect splice-site and regulatory mutations by sequence analysis. To further assess any detected splice-site or regulatory mutation(s), cellular or animal models will become necessary.

3. Molecular characterization of the gene defective in CM and in SM

Our goal is ultimately to determine the normal function of genes implicated in CM and in SM and how mutations in these genes cause the CM and SM phenotype. A mouse model (if not already available) will be generated to further study the biology of the disease. Expression studies will be performed at the RNA and protein level, both at the tissue (Northerns, RT-PCR, Westerns) and cellular levels (*in situ*, immunohistochemistry). The protein will be studied for subcellular localization and post-translational modification.

Conclusion

Identification of the genes responsible for CM with or without SM will help better understand the underlying pathogenic mechanisms for better diagnosis, prognosis and clinical management of this devastating condition. These studies will also help unravel some of the complexity involved in this malformation and in the embryonic development of the affected structures.

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