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Inherited Occipital Hypoplasia/ Syringomyelia in the Cavalier King Charles Spaniel: Experiences in Setting Up a Worldwide DNA Collection

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Abstract

Inherited diseases commonly emerge within pedigree dog populations, often due to use of repeatedly bred carrier sire(s) within a small gene pool. Accurate family records are usually available making linkage analysis possible. However, there are many factors that are intrinsically difficult about collecting DNA and collating pedigree information from a large canine population. The keys to a successful DNA collection program include (1) the need to establish and maintain support from the pedigree breed clubs and pet owners; (2) committed individual(s) who can devote the considerable amount of time and energy to coordinating sample collection and communicating with breeders and clubs; and (3) providing means by which genotypic and phenotypic information can be easily collected and stored. In this article we described the clinical characteristics of inherited occipital hypoplasia/syringomyelia (Chiari type I malformation) in the cavalier King Charles spaniel and our experiences in establishing a pedigree and DNA database to study the disease.

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 re-creating a spaniel similar to those depicted in portraits of the era of King Charles II (Figure 1), and the modern breed is descended from six dogs (Rusbridge and Knowler 2003). CKCS breeders practice line breeding, 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," that is, crosses to dogs with no common ancestors within a five-generation pedigree. A popular stud dog can have over 50 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. Although father–daughter, mother–son, and full sister–brother matings are no longer acceptable, more distant relationships—for example, 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 occipital hypoplasia, a condition similar to Chiari type I malformation in humans (Rusbridge et al. 2000). The consequence of an

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

overly small occipital bone is reduced volume of the caudal fossa, that is, the part of the skull that accommodates the cerebellum and brainstem. Cerebrospinal fluid flow is obstructed by the overcrowded cerebellum (often herniated through the foramen magnum) and brainstem. 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 magnetic resonance imaging (MRI) or postmortem.

The classical clinical signs of syringomyelia are scratching at the neck/shoulders when walking (paresthesia/dyskinesia) and pain with or without cervical scoliosis, paresis and ataxia (Figure 2). At present, confirmation of the disease can only be made by MRI (Figure 3).

Recent data has suggested that occipital bone hypoplasia in the CKCS is inherited (Rusbridge and Knowler 2003, 2004). The disease has a tendency to be more severe in each subsequent generations, that is, 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 occipital hypoplasia/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 that anecdotally had produced affected offspring, some over 30 years ago (Rusbridge and Knowler 2003, 2004). Selection for color 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).

Figure 2. Scoliosis in a 16-month-old CKCS. There is syringomyelia extending into the dorsal gray 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.

Identification of the causal gene(s) for occipital hypoplasia would be invaluable because it would allow development

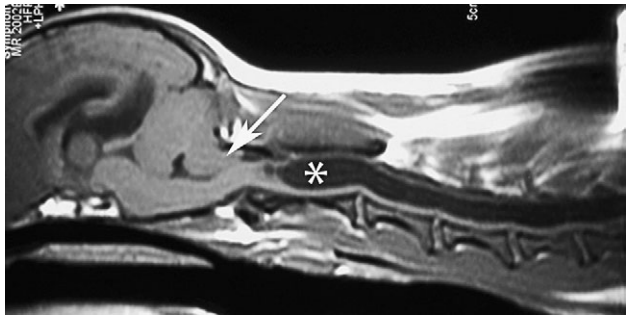

Figure 3. Midsagittal TW1 weighted image of the brain and cervical spinal cord. Syringomyelia (asterixis) secondary to occipital hypoplasia in a 21-month female CKCS presenting with a 3-month history of yelping and a tendency to scratch at the right shoulder area. There is cerebellar herniation through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

DNA for Healthy Cavaliers

Syringomyelia - 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**

Figure 4. Phenotype form.

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 Online previously at Inc. Bellevue, WA) which allowed the interrelationships among dogs to be more easily appreciated because all clinically relevant crosses and descendants could be viewed simultaneously (the license 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. This database had the ability to record phenotypic variables to enable statistical and linkage analysis. The phenotypic variables included: sex; coat color; 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 United Kingdom with the support of the DNA Archive for Companion Animals, the University of Liverpool (held within the Integrated Genomic Medical Research, 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, United States, 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 occipital hypoplasia as confirmed by MRI.
2. CKCS with a normal occipital bone and no syringomyelia as confirmed by MRI.
3. Selected CKCS 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 hyperesthesia, 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 anesthesia unadvisable.

All cases should have details of:

1. Clinical signs including time of onset and severity.
2. Severity of MRI changes (if MRI available).
3. Presence /absence systolic heart murmur/previous heart failure (i.e., development pulmonary edema) and any heart medication.
4. Pedigree.

Exclusion criteria for DNA collection:

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

Results and Discussion

The family database is currently 24 generations and details over 8,500 related individuals across three 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 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 four 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 because 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 crucial 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 200 individuals, have proved to be invaluable especially with regard to identifying other breeds with spontaneously developing occipital hypoplasia/syringomyelia. Project coordinators proved essential to focus collection of DNA by identifying particular offspring and relatives, answering queries, and ensuring that valuable data was not lost (Figure 5).

Occipital hypoplasia/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 et al. 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

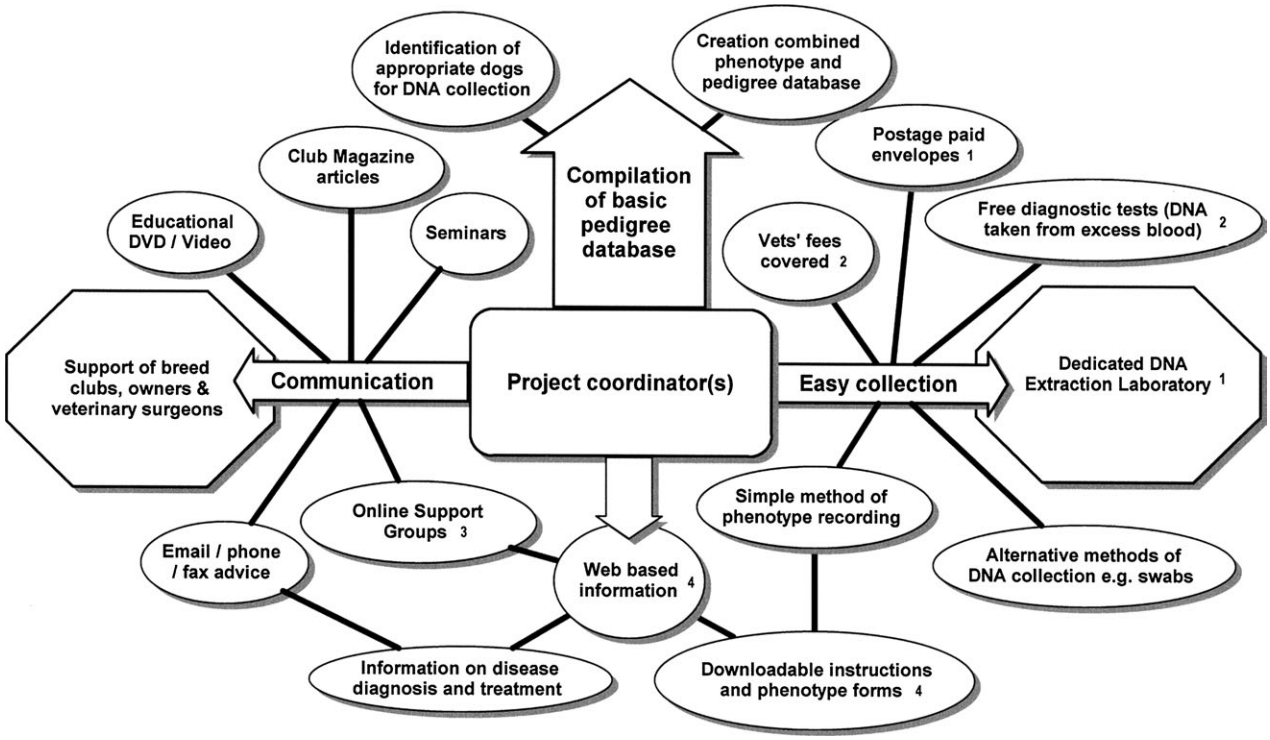

Figure 5. The key features that have ensured a successful DNA collection. Project was supported by [1]The UK DNA Archive for Companion Animals (www.liv.ac.uk/animaldnaarchive) and [2]Boehringer Ingelheim. Internet sites/groups relating to the project: [3]http://uk.groups.yahoo.com/group/arnoldchiari_dogs, www.ourchad.20m.com, and [4]www.cavaliers.co.uk and www.thecavalierclub.co.uk.

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, especiallyPax-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 common human disease.

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