REVIEW

An update on canine cardiomyopathies – is it all in the genes?

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Dilated cardiomyopathy is the second most common cardiac disease in dogs and causes considerable morbidity and mortality. Primary dilated cardiomyopathy is suspected to be familial, and genetic loci have been associated with the disease in a number of breeds. Because it is an adult-onset disease, usually with late onset, testing breeding dogs and bitches before breeding for a genetic mutation that could lead to dilated cardiomyopathy would be helpful to prevent disease. There is growing evidence that the genetic basis may be multigenic rather than monogenic in the majority of studied breeds. This review article describes the known genetic aspects of canine dilated cardiomyopathy and the implications of genetic tests on heart testing and the future of veterinary cardiology.

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INTRODUCTION

Dilated cardiomyopathy (DCM) is the second most common form of acquired cardiac disease in the dog after degenerative mitral valve disease and the most prevalent in large and giant breeds of dogs. Breeds reported as having the highest prevalence of DCM include Dobermanns, boxers, great Danes, Newfoundlands, Irish wolfhounds (IWH) and English cocker spaniels (Monnet *et al.* 1995, Borgarelli *et al.* 2006, Martin *et al.* 2009). Other breeds with a less common diagnosis of DCM mentioned in these studies are: German shepherd dog, Saint Bernard, Labrador and golden retriever, Neapolitan mastiff, Rottweiler, dogue de Bordeaux, Leonberger, collie, Dalmatian, greyhound and mixed breed.

DCM has been classically defined as a primary myocardial disorder causing systolic dysfunction with secondary ventricular dilation (Fig 1), normal to reduced wall thickness and increased cardiac mass resulting from myocyte enlargement (Maron *et al.* 2006). However, this definition is limited to a morphological diagnosis and does not include any of the electrocardiographic abnormalities that accompany the disease. For this reason, we prefer referring to DCM as a primary syndromic disease of the myocardium causing either mechanical dysfunction, leading to dilation and congestion, or electrical dysfunction, leading to arrhythmias and sudden death, or both mechanical and electrocardiographic abnormalities together. Indeed, DCM can be considered a syndrome causing either signs associated with congestive heart failure (pulmonary oedema, cavitary effusions) or forward heart failure signs (exercise intolerance, mucous membrane pallor, syncope) and, often, both.

Many systemic diseases can lead to a dilated, poorly contractile heart, mimicking the DCM phenotype. Examples include chronic volume overload, hypothyroidism, myocarditis and nutritional and environmental factors such as taurine deficiency and anthracycline toxicity. Tachycardia-induced cardiomyopathy (TICM) is also a cause of secondary DCM in the dog, being relatively frequent in certain breeds, such as the Labrador retriever (Perego *et al.* 2012). It is necessary to actively rule out these underlying causes of systolic dysfunction when diagnosing DCM, explaining why many authors state that DCM is a diagnosis of exclusion (Dukes-McEwan *et al.* 2003).

Primary DCM remains an idiopathic disease, but there is growing evidence that, as in humans, canine DCM has a strong genetic basis with marked familial transmission. However, as in people, the exact molecular biology mechanisms leading to its phenotypic expression are still unknown. It has been hypothesised that each breed may have its own particular genetic mutation leading to DCM. It is possible that various dog breeds share the same or similar causative genetic disorders. It is also possible that, within a single dog breed, there may be more than one genetic abnormality causing this syndrome. However, given the inbreeding practices and relative genetic homogeneity, the latter appears less likely.

In people, more than 50 loci have been associated with the suspected monogenic form of DCM, but the mutations located in these DCM-related genes account for only approximately 50% of human cases of DCM (Posafalvi *et al.* 2013). The other half is thought to be associated with multiple synergistic mutations or due to poorly understood genetic interactions (McNally *et al.* 2013). Furthermore, many authors believe that, as with many other genetic diseases, DCM has an *incomplete penetrance* (Meurs *et al.* 2012); *i.e.*, not all dogs with the same disease-causing mutation will develop the disease. Similarly, it has also been stated that DCM may have *variable expression,* referring to the wide spectrum of clinical signs and abnormalities that occur in dogs with DCM (Meurs *et al.* 2012): the same mutation may not affect all patients with the same intensity. The phenomena of incomplete penetrance and variable expression probably result from the interaction between the genetic information and the differential activation of their regulatory epigenetic factors. Different lifestyles, diet and other environmental factors may alter the way the genetic code determines not only every organ's normal physiology in each dog but also the diseases affecting them.

The two main histological findings that have been described in canine cardiomyopathies include attenuated wavy fibres and fibro-fatty infiltration of the myocardium (Tidholm & Jönsson 1997). Atrophy, attenuation and the wavy appearance of cardiomyocytes (generally surrounded by oedema) appears to be the common response of cardiac cells to various pathological stimuli. As a consequence, the presence of attenuated wavy fibres is reported to be a highly sensitive (98%) finding for DCM diagnosis in dogs (Tidholm & Jönsson 2005). The histological features seen with boxers with arrhythmogenic right ventricular cardiomyopathy (ARVC) and some Dobermanns with DCM are myocytolysis, myofibre degeneration, vacuolisation and myocyte atrophy with extensive fibrosis and fatty infiltration. Fibro-fatty infiltration provides the arrhythmogenic substrate in these breeds, and possibly others, such as the great Dane (Fig 2a, b).

The natural history and progression of DCM is not well described for all predisposed breeds, but in general, it is possible to distinguish two phases by the presence or absence of clinical signs (Wess *et al.* 2017). The preclinical phase is often a long asymptomatic period, detected only during screening programmes, careful clinical examination or preanaesthetic evaluations. The second phase is called the clinical or overt phase because clinical signs are evident. There is a wealth of differences between the predisposed breeds regarding their survival time from diagnosis, clinical manifestations, histopathology changes, inheritance patterns and age of onset.

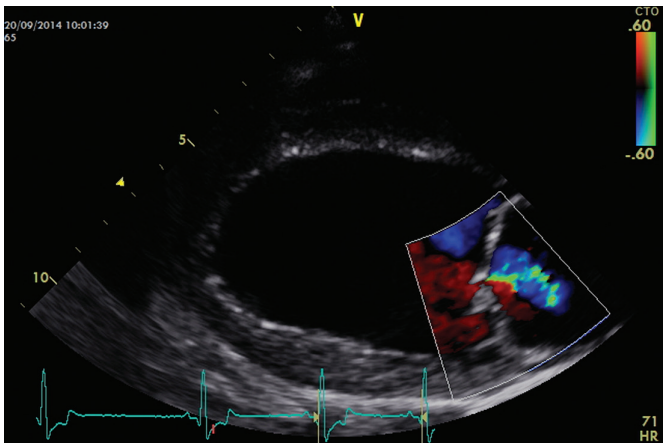

FIG 1. Echocardiographic image from a deerhound with preclinical dilated cardiomyopathy acquired from a right parasternal long axis four-chambers view. There is a dilated, rounded left ventricle. Colour flow shows a mild central jet of mitral regurgitation that occurs secondary to dilation of the mitral annulus

DOBERMANN

DCM is a highly prevalent disease in Dobermanns, which explains why this breed is often used as a model for the disease in dogs. The cumulative prevalence of DCM in Dobermann in Europe has been reported as being 58% (Wess *et al.* 2010). As DCM can occur in Dobermann between 2 and 4 years of age with a prevalence of 10%, screening is advised starting from 3 to 4 years of age (Wess *et al.* 2017). Morphological changes (*i.e.* left ventricular dilation) and electrical changes (either ventricular or atrial arrhythmias) have been described in Dobermanns with DCM and may occur together or separately. Therefore, complete screening should include a 24-hour ambulatory electrocardiogram (ECG) evaluation (Holter monitor) and echocardiography.

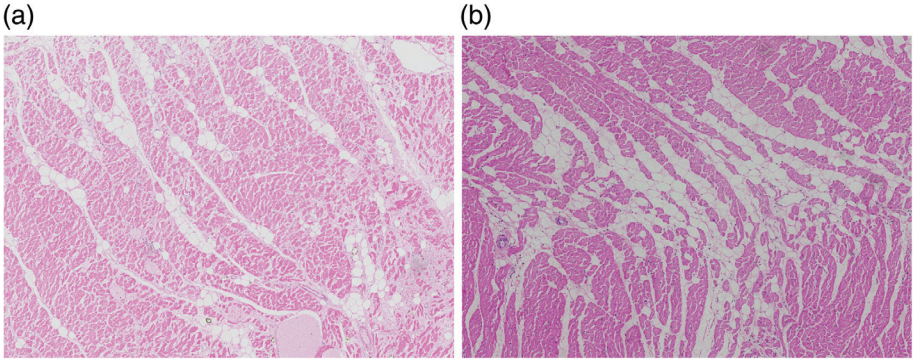

FIG 2. Haematoxylin and eosin stain from the myocardium of a great dane diagnosed with dilated cardiomyopathy. This shows areas of focal interstitial fibrosis, adipose tissue infiltration and mild myocardial degeneration on the left ventricle (a) and the right ventricle (b)

DCM was previously regarded as affecting male Dobermanns more than females (Calvert *et al*. 1997a), but a more recent study shows no overall difference in the occurrence of cardiomyopathy between male and female dogs. However, there is a difference between disease manifestations depending on the gender of the dog (Wess *et al*. 2010): male Dobermanns are more likely to develop early echocardiographic changes, whereas females are more likely to develop ventricular arrhythmias. Sudden death is a concern in the Dobermann, occurring in a third of affected dogs during the preclinical phase (Calvert *et al*. 1997b) and in a third during the overt phase (Calvert *et al*. 1997a). Sudden death is presumed to be due to ventricular tachycardia (Fig 3) leading to ventricular fibrillation and subsequent lack of cardiac output (Calvert *et al*. 1997b).

Due to the high prevalence of both hypothyroidism and DCM in some breeds, especially the Dobermann, there has been some confusion about the role of this endocrine disease in causing DCM. Although severe and chronic hypothyroidism may cause marked systolic dysfunction (potentially leading to congestive heart failure), it is now clear that, epidemiologically, hypothyroidism is not the primary cause of DCM in this breed (Calvert *et al*. 1998, Beier *et al*. 2015).

DCM is thought to be an autosomal dominant trait in the Dobermann (Meurs *et al*. 2007). Ten cardiac genes known to be associated with familial DCM in human patients have been evaluated in Dobermanns, but none of the coding regions of genes associated with DCM in humans appear to consistently account for DCM in this breed (O'Sullivan *et al*. 2011). A genome-wide association study on 141 Dobermanns from Germany and validated in an independent cohort of dogs from the UK showed that a locus on chromosome 5 contains a DCM-associated single nucleotide polymorphism (SNP), "C-allele" (Mausberg *et al*. 2011). The dogs with isolated arrhythmias (as opposed to the "dilation" group) had the highest frequency of the C-allele, but it was not possible to associate a candidate gene for DCM under this association signal.

A study carried out on an American population of Dobermanns showed that a splice site mutation in a gene encoding for pyruvate dehydrogenase kinase 4 (PDK4), a mitochondrial protein, on chromosome 14 was associated with the development of DCM in the Dobermann (Meurs *et al*. 2012). However, a later study, on a population of Dobermanns from Europe, had remarkably different results, with no association found between DCM and the PDK4 mutation (Owczarek-Lipska *et al*. 2013). This difference in results may have been due to different genetic populations within America compared with Europe.

Mitochondrial dysfunction resulting from the PDK4 mutation was studied further in a small number of American Dobermanns (Sosa *et al*. 2016). Acetyl coenzyme A is the molecule from which energy can be obtained in the mitochondria, and the sources of this molecule are mainly carbohydrates, fatty acids and amino acids. The choice of pathway between glycolysis and fatty acid oxidation is regulated by the pyruvate dehydrogenase complex (the function of which is inhibited by PDK4) as well as other factors such as energy starvation. The preferred cardiac energy source in the healthy heart is fatty acids (Grynberg &

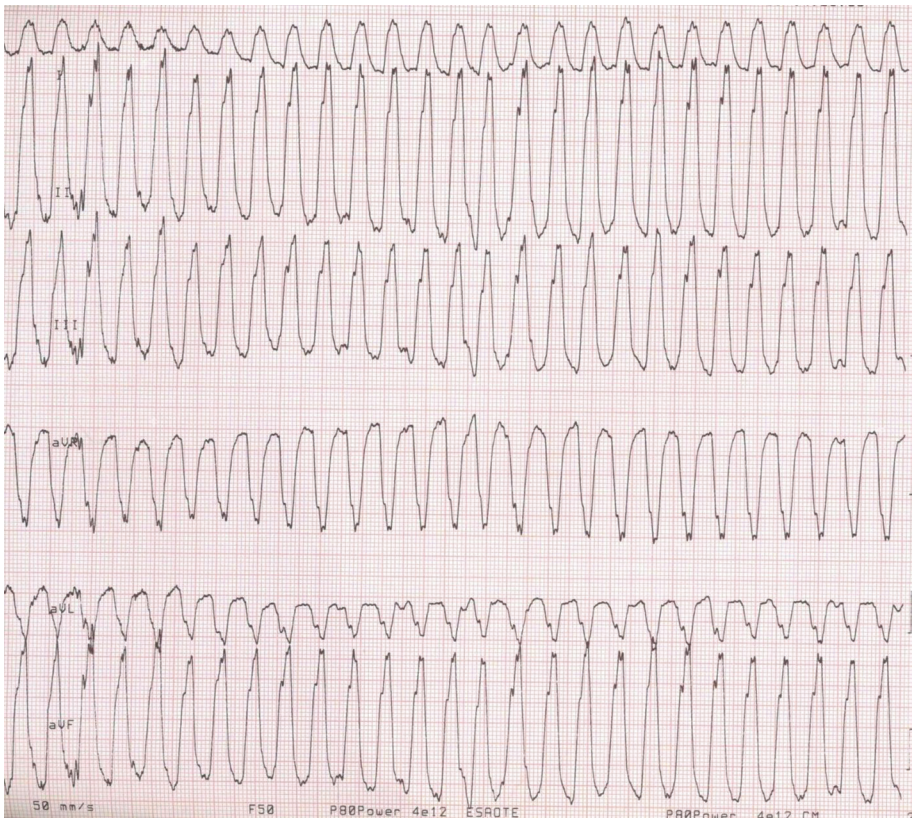

FIG 3. This electrocardiogram from a Dobermann shows a wide complex ventricular tachycardia (50 mm/s and 10 mm/mV)

Demaison 1996). It was presumed that, in Dobermanns with the PDK4 mutation, reduction of PDK4 expression would lead to deficient control of the pyruvate dehydrogenase complex, shifting metabolism away from fatty acid oxidation and increasing glycolysis (Sosa *et al*. 2016). Over time, unregulated glycolysis could potentially lead to impaired mitochondrial electron transport and predispose cells to mitochondrial damage, as observed in Dobermans with DCM and the PDK4 mutation (Meurs *et al*. 2012). The results of the American study indicated that Dobermanns with DCM and the PDK4 mutation had a lower basal mitochondrial oxygen consumption rate than did healthy Dobermanns , suggesting mitochondrial dysfunction (Sosa *et al*. 2016). Nevertheless, and especially in view of the small sample size, the significance of the PDK4 mutation as a DCM risk factor remains uncertain (Sosa *et al*. 2016).

A test for a second mutation has recently been commercialised in this breed. However, to our knowledge, the research supporting this commercial test has not been published in a peer-reviewed journal.

A meta-analysis of the available canine DCM genetic datasets showed there was an interaction between the known DCM loci (on chromosome 5 and PDK4) and an unknown X-linked locus in Dobermanns (Simpson *et al*. 2015). The study evaluated the potential multigenic contribution to the disease rather than treating it as monogenic and suggested a possible genetic basis for the known gender differences in affected Dobermanns .

Finally, microRNA (miRNA) are small, non-coding RNA molecules that are highly conserved across species. MiRNAs regulate each aspect of the translation of the genetic information and, therefore, are thought to participate as determinant epigenetic factors both in health and disease. An underpowered study evaluating circulating miRNA expression in German Dobermanns showed a non-significant trend in expression patterns in affected dogs (Steudemann *et al*. 2013). The study concluded that the technique for the evaluation of miRNAs must be improved. However, it opens up a promising new research horizon for the investigation of genetic diseases and, more specifically, for the investigation of the regulation of the genetic code transcription for DCM, regardless of whether it is a mono- or a polygenic disease**.**

BOXER

Boxers have a particular form of adult-onset cardiomyopathy that does not correspond with the classical description of DCM. "Boxer cardiomyopathy" was the name first given and was described by Harpster in 1983. He classified the disease into three categories that were thought to occur in equal proportions: (1) the preclinical phase with arrhythmias but no clinical signs, (2) the overt phase with episodic syncope during exercise or excitement and (3) the myocardial dysfunction phase with congestive heart failure and arrhythmias. Extensive myocardial histological changes consistent with myocyte atrophy, patchy necrosis and fibro-fatty infiltration affecting most extensively the right ventricle, with no (or little) left ventricular dilation, was noted. This original description is still applicable to both European and American boxers, and some authors have suggested a chronological progression from one phase to the next.

In 1999, "boxer cardiomyopathy" was compared with its homologous disease in humans, known as ARVC (arrhythmogenic right ventricular cardiomyopathy) (Wotton 1999). It was described then that boxers with ARVC have a strong familial predisposition to suffer from ventricular premature complexes (VPC) with left bundle branch block morphology, suggestive of right ventricular origin. Other ECG conduction abnormalities included notched QRS complexes and various forms of first- and second-degree atrioventricular block as well as left bundle branch block. This study described similar pathology and histology changes as described by Harpster (1983).

Evaluation of boxer families shows that ARVC is inherited as an autosomal dominant trait with age-related penetrance (Meurs *et al*. 1999). In collaboration with human ARVC researchers, convincing evidence was provided regarding the similarities with its human counterpart by producing a thorough pathological description of the disease (Basso *et al*. 2004). However, the genetics of the disease are still not fully understood. More than 20 loci have been described in human ARVC, often associated with desmosomal proteins of the intercalated disc. In people, abnormal intercalated disc conformations may lead to an increased susceptibility to environmental agents causing myocarditis, cardiomyocyte death, fibro-fatty replacement and, ultimately, ARVC (Fontaine *et al*. 1999, Calabrese *et al*. 2006).

In boxers, the cardiac ryanodine receptor (RyR2) protein and its messenger RNA expression were found to be reduced in those with ARVC. However, no genetic association with the RyR2 gene was demonstrated in this breed (Meurs *et al*. 2006). In addition, calstabin-2 messenger RNA expression was shown to be significantly down-regulated in the myocardium from ARVC boxers compared with healthy controls and Dobermanns with DCM. This was thought to be a potential mechanism related to the occurrence of ventricular arrhythmias in boxers with ARVC, but no causative mutations were found (Oyama *et al*. 2008).

The use of immunofluorescence labelling of proteins has allowed the evaluation of the molecular composition of the intercalated disc in boxers with ARVC (Oxford *et al*. 2007). This showed that ARVC causes loss of molecular integrity of the intercalated disc as a whole, loss of gap junction plaques and remodelling of other intercalated disc structures. However, no mutations were found to affect the desmosomal proteins evaluated (desmoplakin, plakoglobin, connexin-43 and plakophilin-2). The abnormalities found on the proteins from the intercalated disc were possibly more of a consequence rather than the cause of the disease. Moreover, boxers with ARVC had fewer desmosomes, adherens junctions and gap junctions compared with healthy boxers (Oxford *et al*. 2011). In addition, the ARVC boxers had abnormalities in sarcomeric structure, suggesting a novel link between ARVC and the actin-myosin contractile apparatus. Both abnormalities could result in loss of cell-to-cell attachment and decreased electrical and mechanical communication between cardiac myocytes.

Finally, a genome-wide association study revealed a significant association between an 8-base pair deletion on chromosome 17

in American boxers with ARVC compared with healthy boxers (Meurs *et al.* 2010). This mutation was associated with a reduction of messenger RNA production for the protein striatin. Immunofluorescence localised striatin to the intercalated disc region of cardiomyocytes, co-localising with the proteins desmoplakin, plakoglobin and plakophilin-2. In this study, the striatin mutation was identified in 57 of 61 ARVC boxers (either as homozygotes or heterozygotes) but was also present in nine of 38 unaffected control boxers (all heterozygotes). Boxers that were homozygous had significantly more arrhythmias than heterozygous or wild-type boxers. Moreover, when a well-characterised population of boxers from the UK was analysed, it was found that the striatin mutation was extremely common (Dukes-McEwan *et al.* 2010, Cattanach *et al.* 2015). The striatin mutation did not explain the occurrence of ARVC in UK boxers because no significant difference in proportions of genotypes between affected and healthy dogs were found. These and further discrepancies about the striatin mutation have generated some discussion (Meurs *et al.* 2014, Oxford *et al.* 2014).

To investigate this conundrum, a thorough pedigree analysis of ARVC boxers from the UK showed that affected UK individuals originated from the same American lines described by Harpster (Cattanach *et al.* 2015). The common genetic background with differing striatin results was considered sufficient to dismiss the striatin mutation as the only cause for ARVC. The study concluded that the causative mutation was very likely to co-localise with the striatin gene on chromosome 17. However, this same study recognised that boxers homozygote for the striatin mutation tended to be severely affected at early ages. This suggested that the striatin mutation may have indeed had a modulating effect on the cardiomyocyte, but it was not the aetiological agent for ARVC in boxers (Cattanach *et al.* 2015).

Lastly, it does appear that there are marked geographical differences in the occurrence of ARVC within Europe (and probably America) (personal communication). There are geographical areas where veterinary cardiologists have rarely seen boxers with right ventricular ectopic activity as a main feature of their cardiac disease. However, although the prevalence of the disease may be much lower in these regions, cases of myocardial dysfunction in boxers are also described. Therefore, it is possible that there is more than one myocardial disease affecting this breed, but it could also be that the "ARVC phenotype" is diverse.

IRISH WOLFHOUND

The prevalence of DCM in the IWH is reported as being between 24.2 and 29% (Vollmar 2000, Distl *et al.* 2007, Vollmar & Fox 2016). The onset of disease is between 3 and 7 years, with a mean of 4·52±1·99 years. DCM is more prevalent in male IWHs than females, and males are often affected at a younger age than females (Vollmar 2000, Simpson *et al.* 2016). An association has been shown between the presence of atrial fibrillation (AF) and the development of DCM in the IWH, with AF being a potential precursor to secondary DCM (Vollmar 2000, Vollmar & Fox 2016). However, not all IWHs diagnosed with AF go on to develop DCM, and some IWHs diagnosed with DCM do not have AF (Simpson *et al.* 2016). Reports suggest that between 80.5 and 87.6% of individuals diagnosed with DCM are concurrently diagnosed with AF, which provides evidence that IWHs diagnosed with AF should be closely monitored to ensure that they do not go on to develop DCM (Vollmar 2000, Simpson *et al.* 2016). Diagnosing DCM early in the disease process is important as there is evidence that treatment in the preclinical stage of DCM prolongs the period before onset of heart failure and extends survival (Summerfield *et al.* 2012, Vollmar & Fox 2016).

A gender-dependent dominant gene model is thought to play a major role in the expression of DCM in the IWH, but a monogenic model alone cannot explain the occurrence of this disease (Distl *et al.* 2007). As DCM has a higher prevalence in male than female IWHs, one study examined the role of tafazzin, a protein produced by an X chromosome-related gene (TAZ gene), which is highly expressed in the myocardium (Philipp *et al.* 2007). The TAZ gene was not shown to influence the development of DCM in the IWH (Philipp *et al.* 2007).

Using the genome-wide association technique, two studies showed that multiple loci had significant associations with the development of DCM in the IWH (Philipp *et al.* 2012, Simpson *et al.* 2016). The results of these two studies were different in that only three of the five SNPs associated with DCM in the European IWHs were associated with DCM in the UK study. Of these three SNPs, on chromosomes 1, 21 and 37, only one had the same allele associated with disease. These differences may have been due to different genetic backgrounds for both populations or due to difficulties in correctly classifying affected and control dogs. Most importantly, both studies concluded that an oligogenic inheritance of DCM is most likely to occur in IWHs and that additional unidentified genetic factors may be involved. This conclusion was derived from the fact that certain loci were associated with the disease in some individuals but not at a population level. However, when all three loci were combined, the result was the identification of a genotype that conferred a greater risk of disease (Simpson *et al.* 2016).

NEWFOUNDLAND

The prevalence of DCM in the Newfoundland has been estimated to be 10% (Dukes-McEwan 1999). Newfoundlands have the fifth highest death rate from heart disease of all breeds according to a Swedish survey of insured pedigree dogs (Egenvall *et al.* 2006). The median age of onset of clinical signs in Newfoundlands with DCM is 8 years of age, and the median age of death is 9 years (Dukes-McEwan 2000). It is thought that disease progression might be slower in the Newfoundland compared with other breeds of dog. The reported median survival time in dogs of all breeds diagnosed with mainly overt DCM was 19 weeks (Martin *et al.* 2009) compared with 6 months for Newfoundlands (Tidholm & Jönsson 1996).

The classical progression of DCM in this breed has been described as progressive reduction in contractility followed by

enlargement of the cardiac chambers, eventually leading to the development of AF (Fig 4) and congestive heart failure. Up to 77% of Newfoundlands with DCM had AF in a retrospective study (Martin *et al*. 2009). Echocardiographic studies show that Newfoundlands with left ventricular enlargement alone progress more rapidly (within 2 years) than those with depressed fractional shortening alone, which deteriorate over several years (Dukes-McEwan *et al*. 2003).

One study demonstrated predisposition to DCM in an extended family of Newfoundlands from the UK (Dukes-McEwan & Jackson 2002). Pedigree analysis showed an autosomal-dominant mode of inheritance with incomplete penetrance, although an autosomal-recessive transmission could not be excluded. A low-resolution genome scan of 48 dogs using over 200 markers showed minimal polymorphism and limited heterozygosity. A later study, using the same families of Newfoundlands plus further extended families, excluded 15 candidate genes as a cause of DCM in 74 Newfoundlands (Wiersma *et al*. 2008). Thirty-eight dogs were affected with DCM, and 36 were used as healthy controls. The conclusions of the study were that further studies, including a genome scan with a marker set of higher resolution and a larger set of samples, were warranted. Another study was equally unsuccessful at determining the genetic origin of the disease in this breed (Davidsson 2007).

Finally, plasma taurine concentration (a surrogate marker of myocardial taurine concentration) measured in 216 Newfoundlands showed that taurine deficiency occurred in 8% of dogs (Backus *et al*. 2003). Taurine is an important amino acid for myocardial function. It can be synthesised from dietary cysteine and methionine in the dog; hence, it is not considered essential in the dog. In this study, taurine-deficient Newfoundlands were older, less active and had more medical problems. Some of the taurine-deficient dogs had secondary DCM, which could be reversed after taurine supplementation. It was suggested that taurine synthesis was inadequate to meet taurine requirements in this breed. It was hypothesised that some breeds of dogs may have increased taurine loss or may have different taurine biosynthetic rates, making them more sensitive to diets with marginal taurine concentration. This shows that, in at least a proportion of Newfoundlands with poorly contractile hearts, the cardiac changes may be secondary to dietary taurine deficiency rather than due to idiopathic DCM.

GREAT DANE

The prevalence of DCM in the great Dane varies from 3.9% in referral settings (Sisson & Thomas 1995) to between 11.8 and 35.6% in prospective screening studies (Tarducci *et al*. 2003, Stephenson *et al*. 2012). The natural history, inheritance and disease progression of DCM in the great Dane has scarcely been studied. DCM in this breed is suspected to have a long preclinical phase. However, once the disease becomes apparent, great Danes may have the shortest median survival times compared with other breeds (Martin *et al*. 2010).

It was originally thought that the most common arrhythmia in great Danes was AF secondary to atrial dilation in the congestive phase of the disease (Meurs *et al*. 2001). This concept was challenged when it was found that unreported sudden death was occurring in a large proportion of sires and dams at early ages. A prospective screening study in the UK found that the proportion of dogs with AF was rather low, whereas ventricular arrhythmias

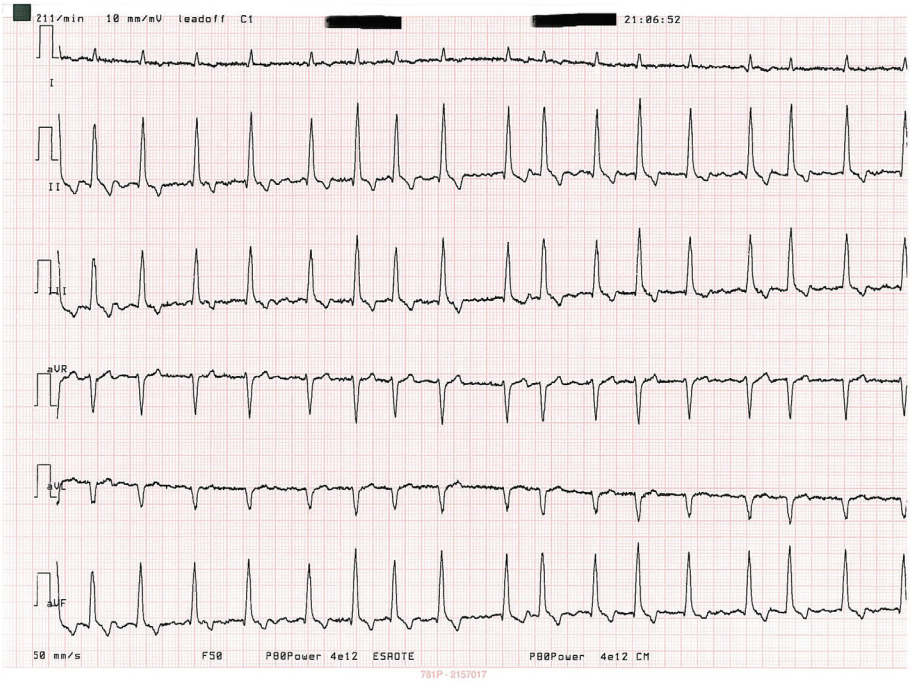

FIG 4. This electrocardiogram from a Newfoundland dog with dilated cardiomyopathy shows a fast rate (heart rate of more than 180 bpm) with irregular rhythm and no discernible P waves on any limb lead. This is consistent with atrial fibrillation (50 mm/s and 10 mm/mV)

were present in 30% of the dogs screened, and in 56% of great Danes with clinical DCM (Stephenson *et al*. 2012).

An American pedigree analysis of 17 great Danes with DCM suggested that the disease may be transmitted as a recessive trait linked to the gender-determining chromosome X (Meurs *et al*. 2001). This study concluded that affected dogs should not be used for breeding – particularly affected females – because their male offspring were at an increased risk of suffering from the disease. A larger pedigree analysis from the UK included 107 dogs (Stephenson *et al*. 2012) and found that the mode of inheritance was most consistent with an autosomal dominant trait, although polygenic inheritance could not be completely excluded.

To our knowledge, evaluation of possible genetic mutations has not been published for the great Dane. One study evaluated the presence of polymorphic markers within 14 published candidate genes for DCM in the breed (Wiersma *et al*. 2007), but no possible causative mutations were identified.

COCKER SPANIELS

Cocker spaniels have been named with relative frequency as the one small breed that suffers from DCM. The first report analysing data from 49 English cocker spaniels from a kennel with familial history of cardiac disease determined that this breed suffers from a cardiomyopathy (Gooding *et al*. 1982). Later, eight cases of young English cocker spaniels with severe congestive heart failure were reviewed and diagnosed with DCM (Thomas 1987). Moreover, some English cocker spaniels with DCM and congestive heart failure have *pulsus alternans* at the time of diagnosis (Moneva-Jordan *et al*. 2007). However, despite advanced cardiomyopathy cases being described and terminal congestive heart failure occurring in these dogs, cocker spaniels have long survival times: at the end of a small 4-year clinical trial, six of 10 cocker spaniels were still alive, and three of 10 had died of non-cardiac disease (Luis-Fuentes *et al*. 2002).

No genetic studies have been reported in this breed. In fact, it is not completely clear whether cocker DCM is a hereditary condition or whether it is secondary to a dietary deficiency of taurine and carnitine. It is also possible that there are differences between English and American cockers in that regard. In a study evaluating plasma taurine levels in dogs with DCM from different breeds, it was shown that concentration was low in 13 of 75 dogs (17%) (Kramer *et al*. 1995). This deficiency occurred in certain breeds, such as American cocker spaniels and golden retrievers, but plasma taurine concentration in breeds more commonly affected with DCM was within the reference range.

A small clinical trial including 11 American cocker spaniels with DCM showed that all dogs were found to have low plasma taurine concentrations at baseline (<50 nmol/mL) (Kittleson *et al*. 1997). The group supplemented with both taurine and carnitine showed significant echocardiographic improvement, whereas dogs receiving a placebo did not. After 4 months of supplementation, congestive heart failure treatment was discontinued in all dogs. This study has several major limitations, as acknowledged by the authors – notably it was unblinded after the first 4 months, which could have introduced investigator bias, making all the subsequent data collected less robust or inconclusive. In addition, some of the echocardiographic changes, although significant, may not have been clinically relevant or have even been real changes, *e.g*. due to intra-observer, inter-observer or day-to-day variability. As the authors mentioned in a different review article (Pion *et al*. 1998), a better designed replica of the study is needed to confirm the results. Nevertheless, based on these results, the current recommendation is to supplement American cocker spaniels diagnosed with DCM with both taurine and carnitine (Sanderson 2006). In fact, taurine deficiency as the cause of systolic dysfunction has been suspected in various breeds of dogs, including American cocker spaniels, Newfoundlands, golden retrievers, Labrador retrievers, Dalmatians, English bulldogs and Portuguese water dogs (Pion *et al*. 1998).

PORTUGUESE WATER DOGS

Portuguese water dogs have been reported to suffer from a juvenile form of DCM, causing mortality between 2 and 32 weeks of age (Werner *et al*. 2008). A genome-wide association study of 119 dogs from 16 families, including 40 affected dogs, identified an association with a locus on chromosome 8, although no causal mutation was found (Werner *et al*. 2008). It has been suggested that this juvenile form of DCM is transmitted as an autosomal-recessive trait (Alroy *et al*. 2000). The echocardiographic manifestations of DCM in this breed were reversible in some pups following oral taurine supplementation for 2 months, suggesting a correlation between infantile DCM with low plasma taurine concentrations. A later study examined this relationship between plasma taurine concentration and development of infantile DCM (Alroy *et al*. 2005). A large proportion of puppies were found to have low plasma taurine concentrations, of which several died due to infantile DCM. The results support the theory that there was a correlation between an autosomal-recessive trait and an abnormal taurine metabolism at infancy in the Portuguese water dog.

HEART TESTING SCHEMES

The purpose of heart testing schemes should be the identification of pedigree dogs with hereditable or familial cardiac diseases and their elimination from the breeding population to prevent passing the defective trait to the next generation. This relatively simple concept encounters several difficulties when it is applied in practice. The first and most obvious is that pet owners, breeders and veterinarians need to focus their efforts towards the same goals and objectives to make a scheme work. With respect to this collaboration, it appears that making a scheme compulsory may lead to greater success (Birkegård *et al*. 2016). Secondly, highly prevalent diseases should not be eradicated rapidly as this would result in breeding from a small number of dogs, thus reducing the gene pool and potentially selecting for other undesirable traits (*i.e*. bottlenecking). This is the reason why some schemes

have focussed not so much on eradicating the disease itself but on delaying the age of onset of clinical signs as a way of making the disease less severe for the population (Swift *et al.* 2017). Thirdly, congenital cardiac diseases are relatively easy to detect because these are present from birth and are commonly accompanied with obvious heart murmurs or other clinical signs, but this is not the case for some adult-onset diseases. Consequently, one cannot be sure a dog will not go on to develop DCM sometimes until after it has been used for breeding which, for obvious reasons, is inconvenient for the development of a breeding scheme. As an example, the American Orthopaedic Foundation for Animals (OFA) has proposed a scheme where "congenital clearance" remains valid for the life of the dog, whereas "adult-onset clearance" will only remain valid for 1 year from the time of the examination, and repeat examinations are necessary to continue on the clear list for adult-onset diseases (https://www.ofa.org/diseases/other-diseases/cardiac-disease). Related to this last point, the decision as to when to start testing dogs for an acquired disease is not simple. It would be onerous for breeders if tests were commenced early on in the dog's life, but it might not be effective at detecting early-onset disease if commenced too late.

Official schemes are defined as breed society- or kennel club-approved screening schemes. Currently, only a few countries have official schemes, and generally, these schemes are only applied to a few breeds and designed to reduce the prevalence of congenital heart disease. Both for congenital and adult-onset cardiac diseases, mandatory screening tests include: auscultation only or auscultation combined with spectral and colour Doppler echocardiography. No scheme includes electrocardiography or 24-hour ambulatory ECG (Holter monitor). As already stated, the obvious limitation for a disease such as DCM is that its preclinical phase cannot be reliably diagnosed by auscultation alone. Therefore, these schemes may not be enforcing the correct screening methods for the disease.

Lastly, genetic testing would be the definitive screening method if these were accurate enough to correctly identify affected dogs and exclude those that would never develop DCM. However, to date, this is not a realistic expectation. Therefore, one of the best ways to identify a disease-free population, given the problems of variable penetrance and late-onset disease, is a clear breeding history over several generations. A possible way of achieving this would be to collect and integrate retrospective information from parents and grandparents on the scheme (retrospective breeding). This emphasises the value of the collection and integration of pedigree analysis, not only for the research of familial diseases but also for any scheme aimed at breeding out late-onset familial diseases (Sargan 2015).

CONCLUSIONS

The progress over the past three decades has been significant, but the complexity of the disease mechanism is proving challenging, particularly the genetic basis of the disease. Despite numerous studies in dogs, few genetic associations have been identified. In line with many veterinary studies, one possible reason for the lack of identified genetic associations may include inadequate sample size. Other reasons include an assumption of simple Mendelian inheritance rather than more complex multifactorial heritability with multigenic interactions, difficulty characterising control and affected populations or possibly selecting young dogs as controls when they may still have the potential to develop DCM. Information from multiple loci combined could be more informative in predicting DCM than individual loci. In other words, the multiple allele effect may be important in canine DCM. Other contributing factors, including nutritional or environmental interactions, may contribute; therefore, apparently important results are not always indicative of genetic causative mutations.

Moreover, the effect of newly discovered epigenetic factors has not yet been taken into consideration. Only one fifth of the transcribed DNA is associated with protein production. The remainder is long and short non-coding portions of the DNA chain, now thought to be responsible for regulating the genetic code (Uchida & Dimmeler 2015), and suspected by many researchers to be also associated with many inherited conditions, but to the best of our knowledge, no published study in veterinary medicine has yet evaluated these.

The results of research in the Dobermann and boxer breeds appear to be the closest to identifying causal genes. The striatin interaction with ARVC, although not a reliable test marker, may be implicated in the disease in boxers. In Dobermanns, the C-allele at a locus on chromosome 5 provides opportunities for future genetic testing. The PDK4 gene requires further investigation using larger sample sizes. Work is still ongoing in breeds such as the Newfoundland and great Dane as part of the European Union funded LUPA project (http://eurolupa.org). In addition, echocardiographic reference intervals are still being developed in certain breeds with a high prevalence of DCM, such as the Scottish deerhound. This will establish breed-specific differences for normal values and ultimately breed-specific abnormalities.

To summarise, there is still a lot of work to be carried out to understand the genetic component of DCM in dogs. Further research is therefore required to fully understand the complexities of canine DCM heritability before genetic tests can be advised as part of heart testing in veterinary cardiology.

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Conflict of interest

JLA currently performs consultancy work for Idexx Laboratories Inc. and Boehringer Ingelheim.

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