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# Screening for Dilated Cardiomyopathy in Great Danes in the United Kingdom

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**Background:** Great Danes (GD) are predisposed to dilated cardiomyopathy (DCM), but little is known about progression, clinical manifestations, or inheritance in dogs in the UK. For echocardiographic screening, breed-specific reference intervals (RI) are required.

**Objectives:** To document the prevalence, clinical manifestations, and inheritance of DCM in UK GD. To establish RI for Doppler echocardiography (ECHO) in GD.

**Animals:** One hundred and seven client-owned GDs.

**Methods:** Echocardiographic screening study. Dogs were scored on ECHO and ECG variables and classified as normal (NORM), equivocal (EQUIV), or affected (AFX). Forty NORM dogs were used to determine RI for ECHO. Pedigrees from all dogs were examined for mode of inheritance.

**Results:** The prevalence of DCM in this population, based on score, was 35.6%. Significant differences in M mode left ventricular dimensions (MMLVD) were identified between male and female dogs ($P < .011$). RI for MMLVD and transformed MMLVD (allometric scaling) were lower than previously suggested. When dogs were reclassified using amended RI for MMLVD, prevalence increased to 47%. End-systolic volume index more reliably identified AFX dogs than other systolic function indices. Ventricular arrhythmias (VA) were commonly identified, with the highest prevalence in AFX dogs (54%). Pedigree analysis suggested an autosomal dominant mode of inheritance.

**Conclusions and Clinical Importance:** The prevalence of DCM in UK GD is higher than previously reported and autosomal dominant inheritance is likely. Sex or body weight-dependent RI should be used for ECHO in GD and current RI might underestimate ESVI in GD. VA might play an important role in GD with DCM.

**Key words:** Echocardiography; Inheritance; Ventricular arrhythmia.

## Introduction

Idiopathic dilated cardiomyopathy (DCM) is characterized by ventricular chamber enlargement and systolic dysfunction with normal left ventricular wall thicknesses.[1] In people, DCM is known to occur secondary to viral, autoimmune, or toxic injury, but 30–50% of cases are thought to be inherited.[2] Familial DCM occurs in a number of dog breeds, and whereas in some, causative mutations have been suggested,[a] in most cases the exact underlying mutations remain elusive.[3–6]

Great Danes (GD) are known to be predisposed to DCM, and this is one of the most common breeds identified in retrospective analyses.[7–9] Recent UK studies suggest that GD have shorter median survival times than other breeds.[10] Despite this, there are few publications examining the prevalence, natural history, or inheritance and disease progression in this breed.[6,11] DCM in GD is suggested to have an X-linked mode of inheritance[6] and the prevalence of the disease in a hospital population has been suggested to be 3.9%,[12]

**Abbreviations:**

| | |
|---|---|
| AFX | affected |
| CHF | congestive heart failure |
| DCM | dilated cardiomyopathy |
| ECHO | Doppler echocardiography |
| EF | ejection fraction |
| EQUIV | equivocal |
| ESVC | European Society of Veterinary Cardiology |
| ESVI | end-systolic volume index |
| FS | fractional shortening |
| GD | Great Dane |
| LA : Ao | left atrium to aorta ratio |
| LVIDd | left ventricular M-mode internal dimension in diastole |
| LVIDdALLO | left ventricular M-mode internal dimension in diastole after allometric scaling |
| LVIDs | left ventricular M-mode internal dimension in systole |
| LVIDsALLO | left ventricular M-mode internal dimension in systole after allometric scaling |
| MMLVD | M-mode left ventricular dimensions |
| NORM | normal |
| PEP : ET | pre-ejection period to left ventricular ejection time ratio |
| RI | reference intervals |
| ROC | receiver operating characteristic |
| SPHI | sphericity index |
| TICM | tachycardia-induced cardiomyopathy |
| VA | ventricular arrhythmias |
| VPC | ventricular premature complex |

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although 1 prospective screening study identified a higher prevalence of 11.8%.[11]

In general, dogs with DCM have a long, preclinical phase.[13] Screening during this phase encounters dogs with no echocardiographic changes and others with equivocal abnormalities, needing serial evaluation to assess progression. This is similar to the situation in relatives of human familial DCM patients, in which equivocal echocardiographic findings are documented.[14,15] To attempt early but robust identification of DCM, an European Society of Veterinary Cardiology (ESVC) taskforce proposed guidelines for the diagnosis of DCM on Doppler echocardiography (ECHO).[16] A scoring system based on M-mode left ventricular dimensions (MMLVD), left ventricular geometry, and indices of systolic function was recommended, to identify dogs in which longitudinal evaluations were indicated.

Previously, reference intervals (RI) for various ECHO parameters in healthy GD have been proposed, including MMLVD[b,17] and fractional shortening (FS).[b] Systolic function is also reported to be impaired in GD compared to other breeds.[17]

In contrast to other breeds such as the Dobermann,[18] where Holter recording is recommended as a screening tool for DCM, sudden cardiac death or hemodynamically significant ventricular arrhythmias (VA) have not been reported as part of the DCM phenotype in GD. Atrial fibrillation (AF) is the most common arrhythmia identified in GD with DCM.[6]

The aims of this screening study were therefore to (1) screen a population of GD by ECHO and ECG, and gather information on the prevalence, natural history, and inheritance of the disease and (2) identify a population of healthy dogs that could be used to determine RI for ECHO variables.

## Materials and Methods

### *Study Population*

This study was performed at the Small Animal Teaching Hospital, University of Liverpool, between 2008 and 2011. The first year of screening was undertaken as part of the DCM work package of the LUPA project.[c] Owners and breeders from across the United Kingdom were invited to bring presumed healthy dogs of at least 6 years of age for screening. To identify controls dogs for a genome wide association study, the majority of dogs screened were unrelated at parental level. In the following 2 years, these dogs were followed up and younger dogs (aged 4 years or older) were screened. In addition, relatives of dogs known to have DCM or sudden death were also screened later in the study, to determine mode of inheritance and to investigate the natural history of the disease. The study was approved by the University of Liverpool Committee on Research Ethics and by the LUPA consortium ethics work package.

### *Procedures*

All dogs underwent a full physical examination and findings were recorded. Body weight, sex, neuter status, and body condition score were recorded and body surface area was calculated.[19] Blood samples were taken for hematology and biochemistry analysis. Thyroid function testing was initially undertaken only in those dogs with clinical suspicion of hypothyroidism (in the first year of screening) and later was performed in all dogs. Dogs were excluded if any other significant congenital or acquired cardiac or systemic disease was identified.

Dogs were manually restrained in lateral recumbency for ECHO examination using an echocardiographic system[d] equipped with a 2–4 MHz multifrequency matrix transducer. The majority of the scans (85%) were performed by a single echocardiographer (HMS) who is a cardiology resident, or occasionally by one of the supervising Diplomates in cardiology (JDM [14.5%], SF). ECHO views were obtained following standard recommendations,[20,21] and stored as cine-loops of three cardiac cycles for later offline analysis.[e] MMLVD in systole (LVIDs) and diastole (LVIDd) were measured and FS calculated. MMLVD were indexed to body weight (LVIDdALLO and LVIDsALLO) by allometric scaling.[22] Measurements used to calculate the ratio of left atrium: aorta (LA : Ao) were obtained from a 2D short axis view.[23] The 2D volumetric Simpson's-derived end-systolic volume index (ESVI) and ejection fraction (EF) were calculated from end-diastolic (start of QRS complex) and end-systolic (before mitral valve opening) frames after optimizing left ventricular length and area in a right parasternal 4-chamber view. The sphericity index (SPHI) was calculated during diastole, from the maximum LV length indexed to LVIDd.[16] From continuous wave spectral Doppler of aortic outflow, obtained from the subcostal view, the pre-ejection period: ejection time ratio (PEP : ET) was determined.

A single lead ECG was simultaneously acquired during all ECHO and note made of any arrhythmia. Those dogs with identifiable arrhythmias on clinical examination or during ECHO underwent a full six-lead ECG examination. Dogs were classified as having VA if one or more ventricular premature complex (VPC) was identified during the screening period. Twenty-four hour ECG (Holter) was offered in dogs with VA and in some dogs with no arrhythmia detected. Malignant ventricular arrhythmias were classified as greater than or equal to 100 VPC in 24 hours, with the presence of couplets, triplets, or runs of ventricular tachycardia.

### *Scoring and Assignment of Groups*

Scoring was carried out as described by the ESVC Taskforce.[16] Cutoff values for ECHO parameters were determined using published data.[16,17,22] Dogs scoring 3 points or fewer were considered healthy (NORM), dogs scoring 4 or 5 were considered equivocal (EQUIV), and dogs scoring 6 or more were considered affected (AFX). For dogs with multiple ECHO examinations, the data and score from the most recent ECHO only were used in the analysis and as the final diagnosis.

### *Pedigree Analysis*

Five-generation pedigrees were obtained for all dogs included in the screening. The pedigrees from all dogs were collated into extended family trees and examined for mode of inheritance.[24]

### *Statistics*

Statistical analysis was carried out using standard commercial software.[fg] Data were examined using basic descriptive statistics and graphical methods, and were transformed as appropriate to better fit the assumptions of parametric analysis. Differences between groups were assessed with one-way ANOVA and Tukey's method for posthoc comparisons. A chi-squared test was used to assess categorical variables between groups. Significance

was set at $P < .05$. Data are presented as mean and standard deviation. Suggested RI are presented as 5th–95th percentiles.

Receiver operating characteristic (ROC) curves were generated for all ECHO variables. Separate curves were generated for each sex for LVIDd and LVIDs. Although not used within the scoring system, ROC curves were also generated for ESVI to assess the utility of this ECHO variable in the identification of AFX dogs. Optimal cutoff values to distinguish AFX dogs from NORM or EQUIV dogs were determined for each ECHO variable. The sensitivity and specificity of the cutoff values used in the scoring system were also determined, and compared to cutoff values suggested in previous studies.[11,17]

## Results

### *Population*

One hundred and seven dogs were screened over 3 years. One dog was excluded because of mitral valve dysplasia. Two dogs were excluded attributable to significant systemic disease. One dog was excluded from the normal group based on a very low FS, and the presence of ST elevation on ECG. One hundred and three dogs were therefore included in the analysis, 40 males (38 entire, 2 neutered) and 63 females (58 entire, 5 neutered). The ages of the dogs ranged from 4 to almost 12 years (48–143 months), with only 28 dogs (27%) being less than 6 years.

Thyroid function was examined in 55/103 dogs (53.4%). One NORM dog was diagnosed with hypothyroidism after screening. Another dog was being treated for hypothyroidism at the time of screening and clinical signs and serum thyroxine concentrations were well controlled. The data were re-analyzed after excluding these dogs, without any differences in the analysis, so their data were retained. No dogs were receiving cardiac medications at the time of screening.

Forty dogs were NORM, 26 dogs were EQUIV and 37 dogs were AFX. Only one dog in the AFX group was in congestive heart failure (CHF) at the time of screening. The prevalence of DCM based on score was 35.9%.

There were no significant differences in age, weight or BCS between groups. There were significant differences between groups for ECHO parameters, with the exception of LA : Ao (Table 1). There was a higher proportion of males in the AFX group compared with the EQUIV group ($P = .014$), but it was similar in the comparisons between other groups (Table 2).

**Table 1.** Statistical analysis of the differences between groups for ECHO and physical variables.

| Variable | NORM (n = 40) | EQUIV (n = 26) | AFX (n = 37) | Significance |
|---|---|---|---|---|
| Age (months) | 82.1 (22.7) | 95.3 (23.5) | 86.8 (23.5) | .107 |
| Weight (kg) | 64.3 | 61.3 | 65.6 | .134 |
| | *1.81 (0.06)* | *1.79 (0.05)* | *1.82 (0.06)* | |
| LVIDd (mm) | 50.9 (3.9)† | 51.6 (4.4)† | 59.8 (5.3) | <.001 |
| LVIDd (mm) Male | 53.6 (3.2) | | | |
| LVIDd (mm) Female | 49.5 (3.6) | | | |
| LVIDdALLO | 1.48 | 1.53 | 1.74 | <.001 |
| | *0.17 (0.028)*† | *0.19 (0.037)*† | *0.24 (0.038)* | |
| LVIDs (mm) | 36.6 | 39.8 | 47.8 | <.001 |
| | *1.56 (0.043)* | *1.60 (0.033)* | *1.68 (0.056)* | |
| LVIDs (mm) Male | 38.6 | | | |
| | *1.59 (0.032)* | | | |
| LVIDs (mm) Female | 35.6 | | | |
| | *1.55 (0.044)* | | | |
| LVIDsALLO | 0.99 | 1.09 | 1.28 | <.001 |
| | *−0.006 (0.040)* | *0.037 (0.030)* | *0.107 (0.051)* | |
| EF (%) | 53.9 (6.7) | 47.4 (8.1) | 40.9 (10.7) | <.001 |
| FS (%) | 27.8 (5.5) | 22.4 (4.9)† | 19.5 (6.3)† | <.001 |
| SPHI | 1.77 | 1.67 | 1.52 | <.001 |
| | *0.25 (0.039)*† | *0.22 (0.049)*† | *0.18 (0.041)* | |
| PEP : ET | 0.42 (0.07)† | 0.49 (0.07)† | 0.50 (0.09) | <.001 |
| EPSS (cm) | *0.53* | 0.59 | 0.92 | <.001 |
| | *−0.27 (0.128)*† | *−0.23 (0.138)*† | *−0.04 (0.141)* | |
| LA : Ao | 1.16 | 1.21 | 1.22 | .385 |
| | *0.066 (0.059)* | *0.082 (0.068)* | *0.086 (0.074)* | |
| ESVI ($mL/m^2$) | 34.4 | 41.6 | 56.0 | <.001 |
| | *1.54 (0.10)* | *1.62 (0.09)* | *1.75 (0.14)* | |

EF, ejection fraction; EPSS, E point to septal separation; ESVI, end-systolic volume index; FS, fractional shortening; LA : Ao, left atrium to aorta ratio; LVIDd, left ventricular M-mode internal dimension in diastole; LVIDdALLO, left ventricular M-mode internal dimension in diastole after allometric scaling; LVIDs, left ventricular M-mode internal dimension in systole; LVIDsALLO, left ventricular M-mode internal dimension in systole after allometric scaling; PEP : ET, pre-ejection period to left ventricular ejection time ratio; SPHI, sphericity index.

Table showing mean (standard deviation) for various ECHO and physical variables for healthy (NORM), equivocal (EQUIV), and affected (AFX) dogs. For those data that were logarithmically transformed, the untransformed mean is also shown, with the transformed data in italics. †Within each row, data that were not significantly different between the 2 indicated groups.

**Table 2.** Number of male and female dogs in each group.

| | Sex | |
|---|---|---|
| Group | Male | Female |
| NORM | 14 | 26 |
| EQUIV | 6 | 20 |
| AFX | 20 | 17 |

AFX, affected; EQUIV, equivocal; NORM, normal.

Of the NORM dogs, 11 scored 1 major criterion, and of these, 9 dogs had decreased SPHI, 1 dog had low EF (36%) and 1 had reduced FS (17%); the remaining dogs scored only minor criteria. Of the dogs in the EQUIV group, none had increased MMLVD, but all scored 4–5 points on the basis of impaired systolic function, decreased SPHI, or both.

Twenty-four dogs were screened 2 or more times during the study period (7 NORM, 8 EQUIV, 9 AFX). Three dogs moved from NORM to EQUIV, and one moved from NORM to AFX. Six dogs moved from EQUIV to AFX. Two dogs moved from EQUIV to NORM. The remaining dogs (5 NORM, 5 EQUIV and 2 AFX) did not move groups. Six of the 10 dogs that progressed (from NORM to EQUIV or AFX, or from EQUIV to AFX) had VA during ECHO.

Of the 7 NORM dogs that were scanned twice, at the second examination, 2 scored 0 points, 2 scored 2 points, and 3 scored 3 points. In 4/7 dogs, the score reduced over time, whereas in 3 of 7 dogs the score increased slightly over time. Two of 3 dogs scored 3 points on the basis of reduced SPHI. In only 1 dog was an increase in score associated with a decrease in SPHI, because of an increase in MMLVD that was still well within the previously published RI.

The prevalence of VA during ECHO was 30.0%. There was a significant difference in the proportion of dogs with VA between groups, with more AFX dogs having VA (20/37; 54%) than EQUIV dogs (5/26; 19%) and NORM dogs (6/40; 15%) ($P < .001$). Twenty dogs were Holter monitored (5 NORM, 4 EQUIV, 11 AFX) and malignant VA were confirmed in 10 dogs (1 NORM, 2 EQUIV, 7 AFX). Only 2 dogs presented with AF, one of which was in CHF.

### *Determination of Reference Intervals for GD*

Data from 40 NORM dogs (14 males, 26 females) were used to suggest RI for ECHO parameters in normal GD (Table 3) and to investigate possible differences between male and female dogs. Twelve dogs (30%) were less than 6 years old. Seven dogs had been scanned twice, 12–29 months (median 15 months) after initial screening.

Body weight ($P < .001$), LVIDd ($P = .001$), and LVIDs ($P = .011$) were significantly lower in female dogs. When allometric scaling was applied to MMLVD in diastole (LVIDdALLO) and in systole (LVIDs ALLO), however, significant differences between sexes were no longer detected ($P > .126$).

**Table 3.** Suggested reference intervals for ECHO parameters in healthy GD.

| Variable | Reference Interval (5th–95th percentiles) | Median |
|---|---|---|
| LVIDd (mm) Male | 46.7–58.7 | 53.9 |
| LVIDd (mm) Female | 42.7–56.1 | 49.8 |
| LVIDdALLO | 1.30–1.64 | 1.50 |
| LVIDs (mm) Male | 33.7–42.5 | 39.9 |
| LVIDs (mm) Female | 28.8–41.9 | 36.0 |
| LVIDsALLO | 0.84–1.11 | 1.01 |
| EF (%) | 42.1–63.9 | 54.5 |
| FS (%) | 20.0–37.0 | 27.0 |
| SPHI | 1.51–2.00 | 1.70 |
| PEP : ET | 0.33–0.55 | 0.42 |
| EPSS (cm) | 0.30–0.86 | 0.56 |
| LA : Ao | 0.91–1.41 | 1.19 |
| ESVI ($mL/m^2$) | 21.9–47.0 | 36.5 |

Suggested reference intervals for ECHO parameters in normal GD based on 5th–95th percentile range of NORM dogs.
For abbreviations, see Table 1.

Based on ROC curve analysis, MMLVD were the most reliable ECHO parameters to identify AFX dogs. PEP : ET was the least reliable parameter. ESVI was more reliable than EF or FS. ROC curve data for each variable are shown in Table 4.

The sensitivity and specificity of cutoff values for ECHO initially used by the authors were also examined (Table 5). MMLVD above these cutoff values were 100% specific. ESVI at the previously published cutoff had very low specificity. LVIDdALLO had the lowest sensitivity. The sensitivity and specificity of cutoff values used by previous authors were also examined (data not shown). These cutoff values had poor sensitivity.

### *Pedigree Analysis (n = 107)*

Twenty-two dogs belonged to 2 extended families (Fig 1). EQUIV dogs were identified within the same families as AFX dogs. AFX dogs were found in multiple generations of the same family, with both male and female dogs affected, making an autosomal mode of inheritance most likely. In both pedigrees A and B, disease appeared in each generation, making autosomal recessive conditions less likely, but there was significant inbreeding. Two affected female dogs (pedigree B) had an unaffected mother, and in both pedigrees, there was evidence of male to male transmission, excluding X-linked inheritance. Affected dogs do not all have an affected dam, excluding matrilineal (mitochondrial) transmission. Overall, the mode of inheritance was most consistent with an autosomal dominant trait, although polygenic inheritance was not completely excluded.

## Discussion

This study reports the initial findings of a large ECHO screening study of GD in the United Kingdom. We have identified a higher prevalence of DCM than

**Table 4.** ROC curve data for each ECHO variable.

| | AUC | | Optimal Cutoff | | Sensitivity/Specificity (%) | |
|---|---|---|---|---|---|---|
| Variable | Male | Female | Male | Female | Male | Female |
| LVIDd (mm) | 0.950 | 0.876 | 56.1 | 54.0 | 90.0/85.0 | 88.2/76.1 |
| LVIDs (mm) | 0.953 | 0.907 | 42.7 | 41.7 | 90.0/90.0 | 88.2/87.0 |
| LVIDdALLO | 0.904 | | 1.60 | | 83.8/77.3 | |
| LVIDsALLO | 0.948 | | 1.15 | | 89.2/89.4 | |
| EF (%) | 0.791 | | 47.5 | | 75.7/68.2 | |
| FS (%) | 0.760 | | 21.5 | | 70.3/72.7 | |
| SPHI | 0.805 | | 1.65 | | 81.1/65.2 | |
| PEP : ET | 0.690 | | 0.47 | | 64.9/57.6 | |
| EPSS (cm) | 0.885 | | 0.75 | | 86.4/86.5 | |
| LA : Ao | 0.555 | | NS | | NS | |
| ESVI ($mL/m^2$) | 0.849 | | 44.3 | | 81.1/83.3 | |

ROC curve data for each ECHO variable when NORM/EQUIV dogs are compared with AFX dogs.
For abbreviations, see Table 1.

**Table 5.** Sensitivity and specificity of previously suggested cutoff values.

| | | Sensitivity (%) | | Specificity (%) | |
|---|---|---|---|---|---|
| Variable | Cutoff | Male | Female | Male | Female |
| LVIDd (mm)[17] | 59 | 70.0 | 29.4 | 100 | 100 |
| LVIDs (mm)[17] | 45 | 80.0 | 64.7 | 100 | 100 |
| LVIDdALLO[22] | 1.85 | 24.3 | | 100 | |
| LVIDsALLO[22] | 1.26 | 51.4 | | 100 | |
| EF (%)[10] | 40 | 45.9 | | 90.9 | |
| FS (%)[10] | 20 | 54.1 | | 83.3 | |
| SPHI[10] | 1.65 | 81.1 | | 65.2 | |
| PEP : ET[16] | 0.45 | 75.7 | | 48.5 | |
| EPSS (cm)[10] | 0.70 | 89.2 | | 81.8 | |
| ESVI ($mL/m^2$)[10,31] | 30 | 100 | | 15.2 | |

Previously suggested cutoff values for ECHO variables used by the authors and the sensitivity and specificity of these cutoffs to identify AFX dogs based on ROC analysis. References for cutoff values are indicated.
For abbreviations, see Table 1.

previously reported, and a high prevalence of VA in GD. In contrast to previous reports, pedigree analysis suggests that an autosomal dominant mode of inheritance is most likely. A group of NORM dogs have been used to generate updated RI for the breed, and our data suggest that sex or body weight should be taken in to account when screening GD. This study also suggests new RI for ESVI in GD.

The prevalence of DCM in this population, on the basis of score, was 35.9%, which is significantly higher than that identified in previous studies.[11,12] This high prevalence likely relates to identification of pre-symptomatic dogs, and also to the older age of the dogs screened. This is similar to other studies that have identified higher prevalences when both symptomatic and presymptomatic individuals are assessed.[11,25–27] The large number of older dogs increases confidence in the ECHO phenotype of these dogs in comparison to other studies[11,12] and some dogs were repeatedly screened to assess progression and to ensure correct classification.

The dogs in this study were scored using previously determined RI,[16] with those for MMLVD being based on the study by Koch and colleagues.[17] In the current study, RI determined for MMLVD were lower than those previously reported.[b,17] This could be explained by differences in ECHO procedure (eg, standing versus lateral recumbency). In addition, although some dogs in previous studies were repeatedly screened, none were older than 6 years of age[a,17] and therefore it is possible that some dogs would have gone on to develop DCM later in life.

It has been previously suggested that different ECHO RI should be used for male and female dogs[b,28,29] or that body weight needs to be taken in to account.[20,22] Conversely, Tarducci and others did not identify a significant difference in MMLVD in GD between sexes.[b] Significantly smaller MMLVD were identified in female dogs in our study, although this difference was not significant when allometric scaling[22] was applied. Interestingly, if the dogs in this study are reclassified using the amended RI for MMLVD (Table 3), an additional 9 female dogs (and 2 male dogs) would be classified as AFX, making the number of AFX male (22/48; 46%) and female (26/48; 54%) dogs much more similar. This would also increase the actual prevalence of disease in this population to 46.6%.

This finding has important implications for screening for DCM in GD, as it suggests that separate RI for MMLVD should be used for males and females or that bodyweight should be taken in to account. However, the allometric scaling formulas were derived from a large population of different breeds, which might result in a large standard deviation, overestimating normal MMLVD in giant breeds. To improve its accuracy, breed-specific RI might be more appropriate.

When ROC curves were examined, MMLVD and transformed MMLVD had the highest AUC, suggesting that these variables were most useful to identify AFX dogs. Indeed, all dogs with either LVIDd or LVIDs above the RI used in this study were AFX.

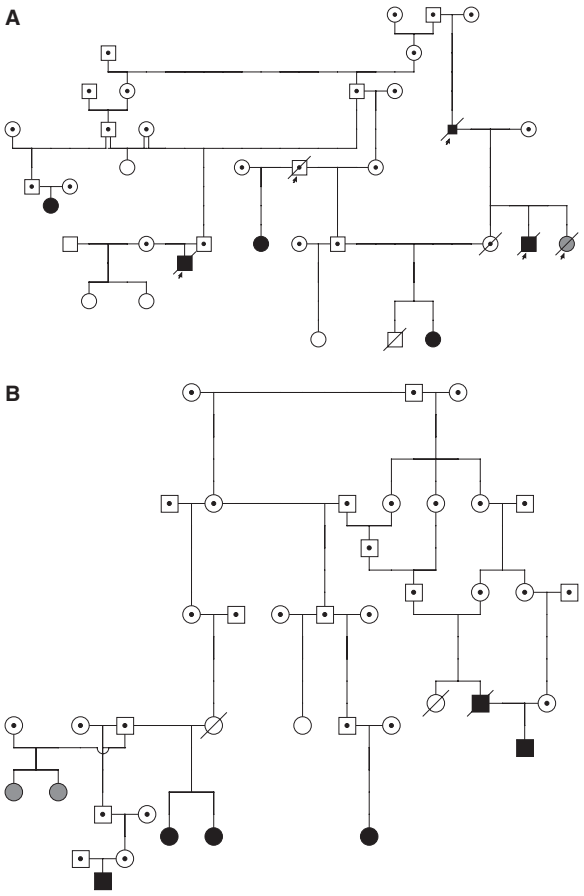


**Fig 1.** Pedigree A (**A**) and pedigree B (**B**) showing 2 extended families of Great Danes. A solid black symbol denotes an AFX dog, a solid gray symbol denotes an EQUIV dog, and a white symbol denotes a NORM dog. A dotted symbol indicates that the disease status of that dog is not known. A line through the symbol denotes that the dog has died, and an arrow indicates known cardiac-related or sudden death. Square symbols represent male dogs, circles represent female dogs. A small symbol indicates a dog that was not screened during the study, but the disease status is known.

MMLVD were a major criterion, contributing more to the score than minor criteria. It is therefore expected that the AUC were greatest for these parameters. Interestingly, however, the minor criterion EPSS had higher AUC than other major criteria.

ESVI appeared to perform better than other indices of systolic function in identifying AFX dogs. ESVI was the only ECHO variable which was not included in the ESVC score, and therefore was independent of it. Similarly, a recent study in Dobermanns has shown that ESVI may be more accurate than MMLVD in identifying dogs with DCM.[29]

The 95th percentile of Simpson's-derived ESVI in NORM dogs was 47.0 mL/m$^2$ (Table 3). This result is similar to that suggested for Dobermanns.[29] The cutoff for ESVI suggested by the ESVC taskforce[16] is 30 mL/m$^2$. This cutoff was based on the Teichholz method of estimating volumes,[30] and therefore can not be directly compared to our Simpson's-derived ESVI, or RI determined by any other method. Nevertheless, measurement of ESVI has been prospectively evaluated in normal dogs and Simpson's and Teichholz methods did show correlation, with normal dogs also having a mean Simpson's-derived ESVI of <30 mL/m$^2$ in that study.[31] In addition, the Teichholz method was more likely to overestimate volumes when ventricular geometry was perturbed.[31] A cutoff of 30 mL/m$^2$ is the only available reference interval for this ECHO variable in dogs, regardless of method of derivation.[16,31] The specificity of ESVI at this cut-off in our population was 15.2% (Table 5) suggesting that use of this cutoff might result in false positive results. It is also possible that GD may have "impaired" systolic function relative to other breeds, as has been previously proposed.[17]

SPHI at the cutoff used[16] had a low specificity for identification of AFX dogs. Some dogs (22.5%) in the NORM group had low SPHI, and 2 of these dogs remained in the NORM group on repeat screening, despite a low SPHI, suggesting that this may be normal in some dogs. SPHI is designed to take in to account the changing cardiac geometry in dogs with DCM, but can be affected by operator skill. Additional longitudinal studies are required to determine if dogs with ESVI over 30 mL/m$^2$, or decreased SPHI, progress toward a DCM phenotype.

In contrast to previous reports, VA were highly prevalent in our group of dogs (30%) and AFX dogs were more likely to have VA than other dogs. In the United Kingdom, there is a high incidence of sudden death in the GD population (unpublished data) and GD have shorter median survival times than other breeds.[10] Interestingly, dogs in the NORM and EQUIV groups also had VA, and a high proportion of dogs that showed progression of ECHO findings had VA. Additional longitudinal studies are therefore required to investigate if VA are associated with sudden death or precede ECHO evidence of DCM in UK GD.

Atrial fibrillation is common in many giant breed dogs.[32] This has led to debate about whether or not the later echocardiographic evidence of DCM is a secondary tachycardia-induced cardiomyopathy (TICM) rather than representing a primary cardiomyopathy.[16] The low prevalence of AF in this population of Great Danes means that TICM is unlikely as the cause of this phenotype, and a genuine primary dilated cardiomyopathy is present. In addition, based on Holter analysis, the mean heart rates of dogs with VA did not differ from those without (data not shown).

Analysis of pedigree data from this study indicates that an autosomal dominant mode of inheritance is most likely. This is similar to findings in other giant breeds,[4,5,33] but contrasts with results of a previous study in GD where X-linked recessive inheritance was suggested.[6] In our study, all except 1 dog was asymptomatic, whereas in a previous study,[6] freedom from disease was ascertained on the basis of lack of clinical signs, and screening was not carried out in all cases. This may have reduced identification of affected, asymptomatic dogs. In our study, the small number of

dogs and the inbred nature of the families make it difficult to be conclusive, and the lack of inclusion of all individuals in a pedigree, means that more sophisticated segregation analysis is not possible currently. The possibility also remains that different phenotypic presentation in different geographical areas is attributable to different mutations or mode of inheritance.

This study had a number of limitations. First, the majority of dogs were only screened once. Some dogs moved groups after repeat screening, indicating that ideally all dogs would have undergone repeat ECHO, to monitor progression, and to determine whether AFX dogs go on to develop clinical signs, or die of cardiac causes. Furthermore, some dogs in the NORM group had abnormalities on ECHO, and some had VA, therefore preclinical DCM was not definitively excluded in these dogs, although most were over 6 years old, reducing the risk of misclassification. VA are rarely reported in GD[6,13] and for this reason, Holter monitoring of dogs was not routinely performed in this study, which may have affected the prevalence of VA in our population.

The ECHOs were performed by different echocardiographers, which may have resulted in variability in ECHO measurements. Only two operators performed more than 99% of the ECHO, and the inter- and intraobserver coefficients of variation ranged between 0.31–5.58% (data not shown).

Although we initially attempted to screen unrelated individuals, some of the dogs in this study were related, suggesting close inbreeding. Dogs were screened only if presented by their owners, and therefore owners of dogs in certain lines may have been more likely to attend screening. Only 22 of 103 dogs belonged to the extended families studied in the pedigrees, and many dogs were completely unrelated to these lines. In addition, we later screened relatives of dogs with DCM in order to investigate the inheritance of the disease further. This might have contributed to the high prevalence of DCM identified. Owners may also have been more likely to present dogs with concerning family history or clinical signs.

The definition of AFX in this study was a score of 6 or more on a previously defined scoring system.[16] This system has not so far been prospectively evaluated, and a score of 6 might not be truly representative of a DCM phenotype. Some of the dogs scoring 6 in this study might have been affected by undetected systemic disease. As far as possible this was ruled out, but abdominal ultrasound and thyroid function testing were not carried out in all dogs. Hypothyroidism has been associated with DCM in GD.[34] More recently, thyroid function testing has been performed on all GD, so that 55 of 104 dogs were tested prior to inclusion in the study. Only 2 of 55 dogs (4%) of these dogs were diagnosed as hypothyroid and therefore the prevalence of hypothyroidism in our population appears to be low, meaning that this is unlikely to have significantly affected our results.

Receiver operating characteristic curve analysis was used to assess the utility of each ECHO variable to identify AFX dogs, but the classification of these dogs as AFX depended on score, which was itself dependent on those ECHO variables. Therefore, major criteria (scoring 3 points) may have been expected to perform better than minor criteria, which might have affected our results. Nevertheless, many individual criteria contribute to each dog's score, reducing the influence of each individual ECHO variable. The sensitivity and specificity of most individual ECHO variables were below 95%, therefore these data still support the use of a scoring system whereby many factors are taken in to account.

Unfortunately, few dogs within each pedigree were screened, and the disease status of siblings and parents of many AFX dogs remains unknown. This might have affected our pedigree data, resulting in unintentional ascertainment bias. Longitudinal screening of relatives is ongoing, and segregation analysis of the pedigrees will ideally be undertaken.

In conclusion, this study identified a higher prevalence of DCM in the UK GD population than that previously reported. Pedigree analysis of these dogs suggests that an autosomal dominant mode of inheritance is likely. VA could play an important role in GD with DCM. In addition, we suggest revised RI for ECHO variables in normal GD. Separate RI for MMLVD in males and females should be used, or narrower breed-specific RI when using allometric scaling of MMLVD. Our data also suggest that the currently available RI for ESVI might be too low, and that ESVI, calculated by Simpson's method, might be a more useful indicator of systolic function than EF or FS.

## Footnotes

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[b] Tarducci A, Borgarelli M, Bussadori C et al. Valori Ecocardiografici Negli Alani Normali. Atti Soc Ital Sci Vet (1997) 17; 593–594 (abstract).

[c] LUPA project, WP2 Cardiovascular disorders. http://www.eurolupa.org

[d] Vivid 7, General Electric Medical System, Waukesha, WI

[e] EchoPac, General Electric Medical System

[f] Minitab for Windows, Version 16.0, Minitab Inc, State College, PA

[g] SPSS for Windows, Version 18.0, SPSS Inc, Chicago, IL

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