

Genetic Analysis of Ventricular Arrhythmia in Young German Shepherd Dogs

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Background: Ventricular arrhythmias (VA) and sudden death are inherited in German Shepherd Dogs (GSDs).

Objectives: To estimate the genetic parameters (heritabilities and correlations) of 3 traits of VA (single premature ventricular complexes (PVCs), 2 consecutive PVCs (couplets), and 3 or more consecutive PVCs—ventricular tachycardia [VT]).

Animals: Three hundred and ninety-eight GSDs.

Methods: Prospective, observational, case control study. Dogs were phenotyped by 24-hour ambulatory ECG from 6 to 45 weeks of age. Edited ECG records included the number of incidents of (1) single PVCs, (2) couplets, and (3) VT.

Results: A data set of 1,239 Holter records from 398 GSDs was used to estimate genetic variables. Phenotypic correlations for affectedness (binarily coded 0/1) of the 3 traits ranged from 0.55 to 0.74, whereas correlations for severity (continuous values of 24-hour VA counts) ranged from 0.26 to 0.39. Estimates of genetic correlation among the severity traits were 0.06 to 0.27. Estimated heritabilities were 0.54, 0.54, and 0.46 for affectedness and 0.33, 0.69, and 0.69 for severity of PVCs, couplets, and VT, respectively. Month and year of birth and age at ECG recording had significant effects on all 3 traits. Season of ECG recording had a significant effect on the number of single PVCs, but not couplets or incidents of VT. Age of onset differed, with single PVCs appearing an average of 4 days earlier than couplets and VT.

Conclusion: These results imply a strong genetic component for this disease but suggest that differences in the 3 traits should be taken into consideration in studies to identify the underlying genes.

Key words: Canine; ECG in dogs; Holter monitoring; Inherited heart disease; Model of arrhythmias.

Spontaneous ventricular arrhythmia (VA) and sudden death occur in young German Shepherd Dogs (GSDs).^{1–5} The disorder ranges in severity from infrequent and non-life-threatening single premature ventricular complexes (PVCs) to multiple episodes of rapid polymorphic ventricular tachycardia (VT). Dogs with VT are most likely to die suddenly.^{1,6,7} No other clinical indicators of abnormality are apparent, and pathological examination of the hearts of dogs that die suddenly reveal morphologically normal hearts.¹ A window of vulnerability for the presence of VA and sudden death exists between approximately 3 and 18 months of age, with peak affectedness occurring at approximately 6–7 months of age.^{1,6} Affected dogs rarely have VA after ~24 months of age, and when they do, it is infrequent.^{1,6} The trait of VT is most commonly observed in dogs lying at rest and during rapid eye movement sleep.⁸ Because of the age and behavioral dependence of the expression of this disease, extensive observation via 24-hour ambulatory ECG monitoring (Holter monitoring) is often required to ascertain disease presence and its severity.

In people, important advances have been made in the understanding of the genetic basis of inherited arrhythmic disorders with loss of repolarization reserve including the long QT syndromes,^{9–12} Brugada syndrome,¹³ and others.^{14–19} Causative mutations have primarily been

identified in genes encoding specific sodium and potassium channel proteins involved in repolarization.^{9–19} In affected GSDs, abnormalities of ion current densities of potassium channels and calcium cycling indicate a global disruption of repolarization reserve.^{20–30} Furthermore, heterogeneous sympathetic innervation has been identified.²⁹ Given the failure to identify a singular abnormality, genome-wide analysis of linkage is more likely to be successful in identifying the causative loci for VA in GSDs than a candidate gene approach. In anticipation of conducting a full genome scan for loci linked to the disease, we performed a genetic characterization of this trait in our closed colony of GSDs with a high incidence of VA. Our goals for this study were to estimate the genetic parameters of VA traits and to characterize the progression of the arrhythmia in this population.

Materials and Methods

Dogs

Dogs were housed in an American Association for the Accreditation of Laboratory Animal Care approved facility, which conforms to the guidelines for proper animal care as described within the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were approved by the Institutional Animal Care and Use Committee of Cornell University. Data were collected on GSDs within a long-established (18 years) colony at Cornell University's College of Veterinary Medicine. Primary founders of this colony came from families with incidences of sudden death that, under subsequent analysis, were shown to have a common ancestor.¹ Additional affected and unaffected dogs have been brought into the colony over the years, in part to diversify the breeding pool. Thirty-eight backcross progeny (GSD × [GSD × greyhound]) were used for pedigree analysis, but only data from purebred GSDs were used for the quantitative analysis presented in the current study. Dogs were born during 1988–2006, and pedigree information was available for all dogs with phenotypic data. In-

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breeding coefficients were calculated for all dogs using “proc inbreed” in SAS (v. 9.1, SAS Institute, Cary, NC).

Phenotype Analysis

Ambulatory ECG (Holter monitoring) was performed on all dogs. Holter monitoring was serially performed with a median of 3.5 recordings each (eg, each dog evaluated at 11, 15, and 18 weeks of age), although dogs born between 1987 and 1997 had more extensive monitoring in order to characterize the arrhythmogenic phenotype. All ECG recordings were edited for correct beat annotation by experienced technicians. Recordings from dogs under 14 weeks of age were 15 hours rather than 24 hours in duration and the acquisition frequency was 1,000 Hz rather than 400 Hz. All records were normalized to 24 hours to enable comparison of data among all recordings. The term “record” refers to 1 dog’s ECG data recorded at 1 age. No treatment was administered before or after Holter monitoring.

Recordings were made at ages of 3–413 weeks. Because of the small number of ECG recordings on dogs under 6 and over 45 weeks of age, those records were removed from this analysis. The frequency of VA in each ECG recording was identified and 3 VA traits were categorized: single PVCs, 2 consecutive PVCs (couplets), and 3 or more consecutive PVCs (VT). Some recordings analyzed with earlier versions of software had such extensive VA that it was not possible to annotate all beats. In order to give a semiquantification of VA, thresholds for beat identification had to be made. In these records, a threshold of 5,000 PVCs was set as a maximum; such values were treated as missing data for analyses involving severity. For the analysis of age of onset, data from dogs born in 1988 and 1989 were discarded because ECG recordings were not routinely collected before 18 weeks of age in those years.

Dogs were categorized as affected or unaffected for each of the 3 traits based on their most severe record for each trait. The cutoff values for affectedness were > 100 PVCs, > 4 couplets, and > 2 VT in 24 hours. Although sudden death occurred in this population of dogs, it was not included in the analysis because not all dogs were kept beyond 6 months of age and were therefore not given the full opportunity to express this trait.

Statistical Models

Descriptive statistics and Pearson’s correlation coefficients for affectedness, severity, and age of onset of the VA traits (PVCs, couplets, and VT) were calculated by SAS (v. 9.1, SAS Institute). Significant fixed effects were identified with univariate analyses. Variance and covariance components were estimated by restricted maximum likelihood and procedures in SAS (v. 9.1, SAS Institute). Heritability (h^2) was estimated by

$$h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2),$$

where σ_a^2 is the additive genetic variance and σ_e^2 is the residual variance.

Animals often share common ancestors and consequently share identity by descent, and animal effects are commonly fitted as random effects. Unfortunately, these characteristics are not included in any of the predefined variance structures by SAS (SAS Institute) and are therefore ignored in empirical analyses. We developed an SAS macro, MTCC, on the basis of LORG (coded by Zhiwu Zhang, <http://www.people.cornell.edu/pages/zz19/research/LORG/index.html>), to incorporate the genetic relationships among the dogs. This macro computes numerical relationship among individuals from a given pedigree using half storage, extracts the numerical relationship, and assigns genetic parameters in sparse format to generate an SAS data set that can be directly used by options GDATA or LDA-TA for multiple trait data.

Affectedness (for PVCs, couplets, and VT coded as binary, unaffected/affected) was analyzed with SAS (v. 9.1) “proc glimmix,” using the model

$$\Pr(Y = 1) = \theta(\mu + \text{dogG} + e),$$

where $\Pr(Y = 1)$ denotes the probability a dog is affected; θ is the cumulative normal distribution function, which is the probit link between the linear predictor on the right-hand side and the probability for a specific outcome on the left-hand side; μ is a fixed model intercept; dogG is the random genetic effect of dog, $\sim(0, \mathbf{A}\sigma_G^2)$ where $\mathbf{A}$ is the additive genetic relationship matrix and σ_G^2 is the additive genetic variance; and e is the random residual error. Preliminary analyses included terms for sex, birth year, and inbreeding, but none of these was significant ($P > .10$), and thus they were not included in subsequent analyses.

Age of onset for the 3 severity traits was analyzed using “proc mixed” in SAS (v. 9.1) using the following animal model:

$$Y = \mu + \text{byear} + \text{trait} + \text{dogG} + \text{dogPE} + e,$$

where Y is age of onset; μ is a fixed model intercept; byear is the dog’s year of birth; trait indicates as to which trait the age corresponds to; dogG is the random additive genetic effect of dog, $\sim(0, \mathbf{A}\sigma_G^2)$; dogPE is the random permanent effect of dog, $\sim(0, \mathbf{I}\sigma_{PE}^2)$ where $\mathbf{I}$ is an identity matrix and σ_{PE}^2 is the permanent environmental variance; and e is the random residual error.

Severity traits (PVCs, couplets, or VT) were analyzed with SAS (v. 9.1) “proc mixed,” using the model

$$\log(Y) = \mu + \text{bmonyear} + \text{season} + (b_1)\text{age} + (b_2)\text{age}^2 + \text{dogG} + \text{dogPE} + e,$$

where Y is the number of PVCs, couplets, or VT in the ECG recording; μ is a fixed model intercept; bmonyear is the dog’s month and year of birth; season is the season of ECG recording (4 seasons, where January–March is season 1); fit as a covariate, age is the age in weeks of dog at ECG recording; dogG is the random additive genetic effect of dog, $\sim(0, \mathbf{A}\sigma_G^2)$; dogPE is the random permanent effect of dog, $\sim(0, \mathbf{I}\sigma_{PE}^2)$; and e is the random residual error. Preliminary analyses included terms for sex and inbreeding, but neither term was significant ($P < .10$) and therefore they were not included in subsequent analyses. Covariance components between each 2 traits were obtained from bivariate analyses.

Results

Three hundred and ninety-eight GSDs with 1,239 records were included in the analysis. Inbreeding coefficients for the dogs ranged from 0 to 0.375, with an average of 0.126. The minimum VA count recorded for all traits was 0. Across all records, the maximum recorded VA counts were 88,234 PVCs, 30,068 couplets, and 14,205 incidents of VT. Other summary statistics are presented in Table 1.

Table 1. Proportion of German Shepherd Dogs (n = 398) affected and summary statistics of ventricular arrhythmia data.

	PVCs	Couplets	VT
% Affected	69	56	51
Range	0–88,234	0–30,068	0–14,205
Mean	2,373	223	48
Standard deviation	7,731	1,527	502

PVCs, single premature ventricular complexes; couplets, 2 consecutive PVCs; VT, ventricular tachycardia.

Pattern of Inheritance

The inheritance of the arrhythmia showed no clear pattern of simple recessive or dominant inheritance (Fig 1 indicates a pedigree sample). Based on pedigree information, there was no indication of a sex bias in affectedness. Mildly affected dogs (only PVCs) produced severely affected pups with VT—some of which died suddenly—as well as mildly affected and unaffected pups. Matings between dogs with VT produced unaffected pups in addition to mildly affected and severely affected pups. Matings between affected and unaffected GSDs also produced a full spectrum of phenotypes from unaffected to severely affected. Matings between 2 unaffected dogs did not produce affected pups, although there have been few such pairings to assess in our colony.

The severity of phenotypes in GSDs × Greyhound backcross dogs mirrored the range observed in purebred GSDs (Table 2). As with purebred GSDs, there was no clear pattern of simple recessive or dominant inheritance (Fig 2). One pup in the F1 litter was affected (PVCs only) whereas the other 4 were unaffected. Two unaffected F1s (1 male and 1 female) were bred to affected GSDs to generate the backcross generation. Of 38 backcross pups, 23 (61%) were affected with at least single PVCs (2 of those dogs were classified as affected for VT—one had 3 and the other had 5 runs of VT—but they were well below the threshold for single PVCs). The most severely affected backcross pup had an ECG with over

Table 2. Proportion of Greyhound × German Shepherd Dogs backcross dogs (n = 38) affected and summary statistics of ventricular arrhythmia data.

	PVCs	Couplets	VT
% Affected	55	32	32
Range	0–103,655	0–9,979	0–13,637
Mean	3,235	234	151
Standard deviation	11,407	1,317	1,320

PVCs, single premature ventricular complexes; couplets, 2 consecutive PVCs; VT, ventricular tachycardia.

13,000 episodes of VT. Another backcross dog had the highest number of single PVCs counted in a 24-hour ECG: 103,655.

Affected versus Unaffected

Every dog was classified as affected or unaffected for each trait. Heritability estimates for affectedness were moderately high (0.46–0.54) and fairly consistent across traits (Table 3). Phenotypic correlations across traits were significantly positive, ranging from 0.55 to 0.74 (Table 3). Because of the structure and small size of the population, genetic correlations for affectedness could not be calculated.

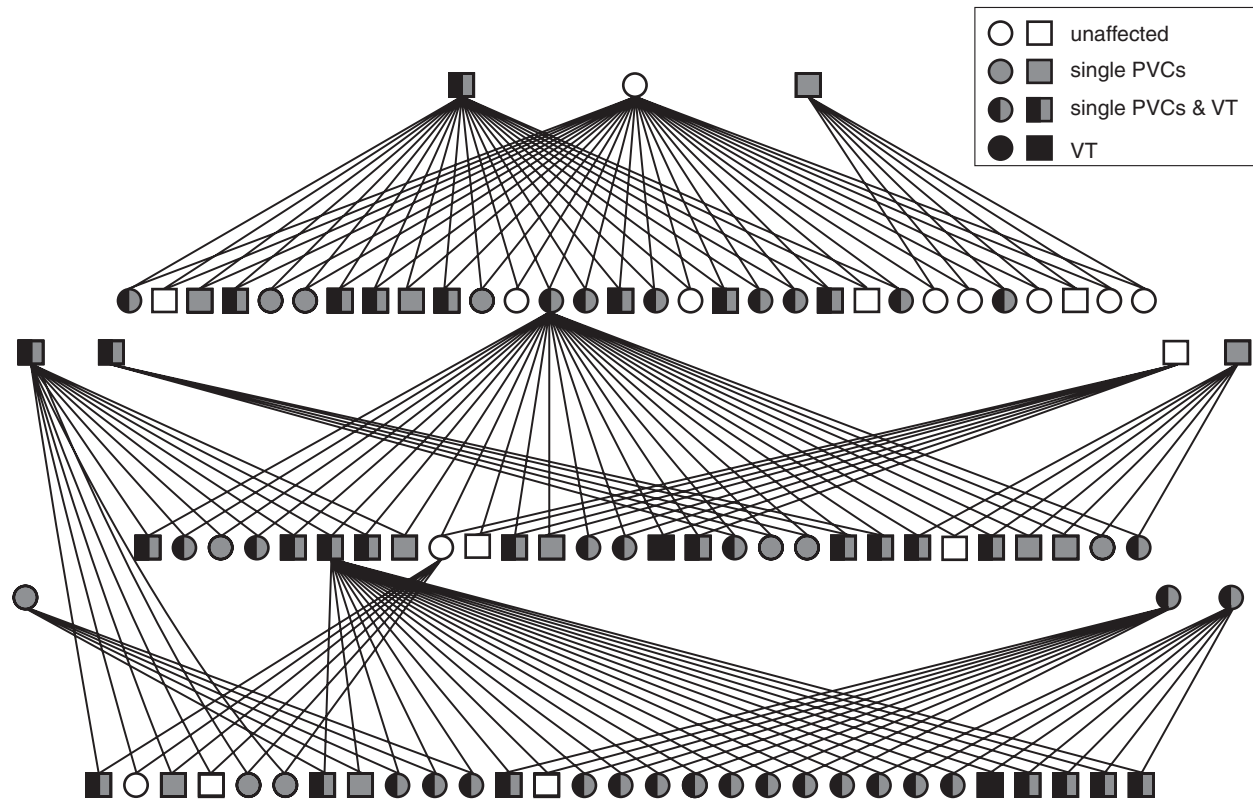


Fig 1. Sample German Shepherd Dog pedigree coded for affectedness for single premature ventricular complexes (PVCs) (gray) and ventricular tachycardia (VT) (black). Symbols that are half gray and half black indicate that the dog was affected with both single PVCs and VT. Unfilled symbols indicate unaffected status. Circles signify females; squares signify males.

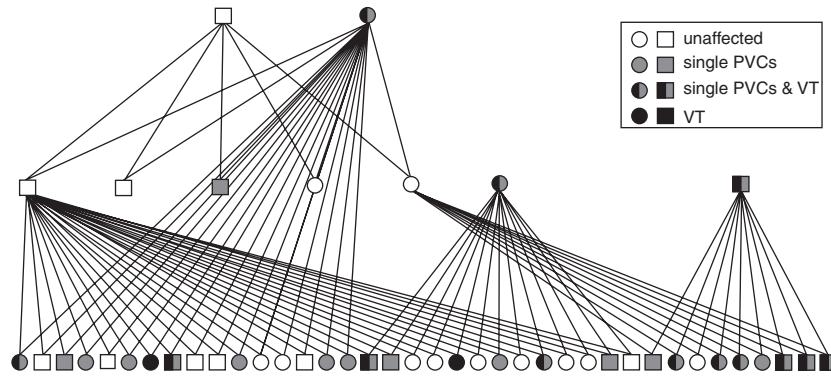


Fig 2. Backcross (GSD × [GSD × Greyhound]) family coded for affectedness for single premature ventricular complexes (PVCs) (gray) and ventricular tachycardia (VT) (black). Symbols that are half gray and half black indicate that the dog was affected with both single PVCs and VT. Unfilled symbols indicate unaffected status. Circles signify females; squares signify males. GSD, German Shepherd Dogs.

Age of Onset

Age of onset was determined for dogs affected for one or more of the VA traits. Ages of onset were not uniform across the traits ($P = .0042$). On average, onset of single PVCs (14.9 weeks) occurred more than 4 days earlier than onset of VT (15.5 weeks) and couplets (15.7 weeks). The estimate for heritability for age of onset was 0.34. Additionally, birth year was a significant fixed effect ($P = .001$), with a trend toward earlier onset in the more recent years. Phenotypic correlations for age of onset between the traits were all significantly positive, ranging from 0.47 (onsets of PVCs and VT) to 0.82 (onsets of couplets and VT).

Fixed Effects

Month and year of birth (fit as 1 variable: birth-month-year) was a significant fixed effect, as were the linear and quadratic values of the covariate age at ECG recording (Table 4) for all 3 VA severity traits. A significance level of $P < .10$ was used to determine what fixed effects would be included in the models. Neither sex nor inbreeding level was significant ($P > .10$) in any analysis. Based on univariate analysis, birth-month-year had a significant effect ($P < .002$) on the 3 traits. Season of ECG recording was significant for PVCs ($P = .074$), but not for couplets or VT. Spring (April-June) conferred the highest risk.

Age at ECG recording (linear and quadratic values) had a highly significant effect on PVC counts for all 3

traits ($P < .0001$). The regression curves for all 3 traits indicate that the incidence of VA increases with age up to a point, after which it declines at approximately 35 weeks for PVCs, 33 weeks for couplets, and 36 weeks for VT (Fig 3A–C). Not only does “improvement” come later for VT, but the rate of decrease in arrhythmic activity is slower than that for PVCs or couplets. The differences in the scale of the regression curve graphs reflect the disparity in the ranges of the trait values. It should be emphasized that whereas generalizations can be made about the age of onset, age of peak affectedness, and rates of increase and decrease in severity, each affected dog appears to have its own trajectory with regard to these attributes.

Severity of Phenotype

Phenotypic correlations for severity of VA traits ranged from 0.26 to 0.39 (Table 5). Genetic correlation estimates for VA trait severity were calculated using the averages of the respective genetic variance estimates. Likewise, the heritability estimates were averaged over all bivariate estimates obtained for the different combinations; the results are in Table 5. Estimated heritability was moderate for single PVCs (0.33), but high for couplets (0.69) and VT (0.69). Genetic correlations between the severity traits were positive, ranging from 0.06 to 0.27 (Table 5). Phenotypic correlations between the severity traits and age of onset for each trait were also estimated. The number of PVCs was negatively correlated with the ages of onset for PVCs (-0.20 ; $P < .0001$), couplets (-0.18 ; $P < .0001$), and VT (-0.15 ; $P = .0017$). That is, dogs with an earlier age of onset, for any of the 3 traits, had more single PVCs. Also, couplet severity was negatively correlated with couplet age of onset (-0.09 ; $P = .0310$).

Table 3. Heritability estimates (underlined, on diagonal) and phenotypic correlations of affectedness (below the diagonal).

	PVCs	Couplets	VT
PVCs	<u>0.54</u>		
Couplets	0.70	<u>0.54</u>	
VT	0.55	0.74	<u>0.46</u>

PVCs, single premature ventricular complexes; couplets, 2 consecutive PVCs; VT, ventricular tachycardia.

Discussion

The important new findings of this study regarding VA in young GSDs were that (1) the traits (PVCs, couplets, and VT) characterizing the disorder are not inherited

Table 4. F values and type I error probabilities (*P* values) of fixed effects and covariates.

Effect	DF	PVCs		Couplets		VT	
		F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Birth-month-year	56	1.83	.0011	2.15	.0001	2.39	<.0001
Season of ECG	3	2.32	.0743	1.18	.3144	1.33	.2631
Age at ECG—linear	1	49.59	<.0001	75.48	<.0001	33.36	<.0001
Age at ECG—quadratic	1	25.18	<.0001	42.87	<.0001	16.93	<.0001

PVCs, single premature ventricular complexes; couplets, 2 consecutive PVCs; VT, ventricular tachycardia.

by simple recessive or dominant inheritance and (2) the heritability estimates for the traits were moderate to high and phenotypic and genotypic correlations were positive, but not high.

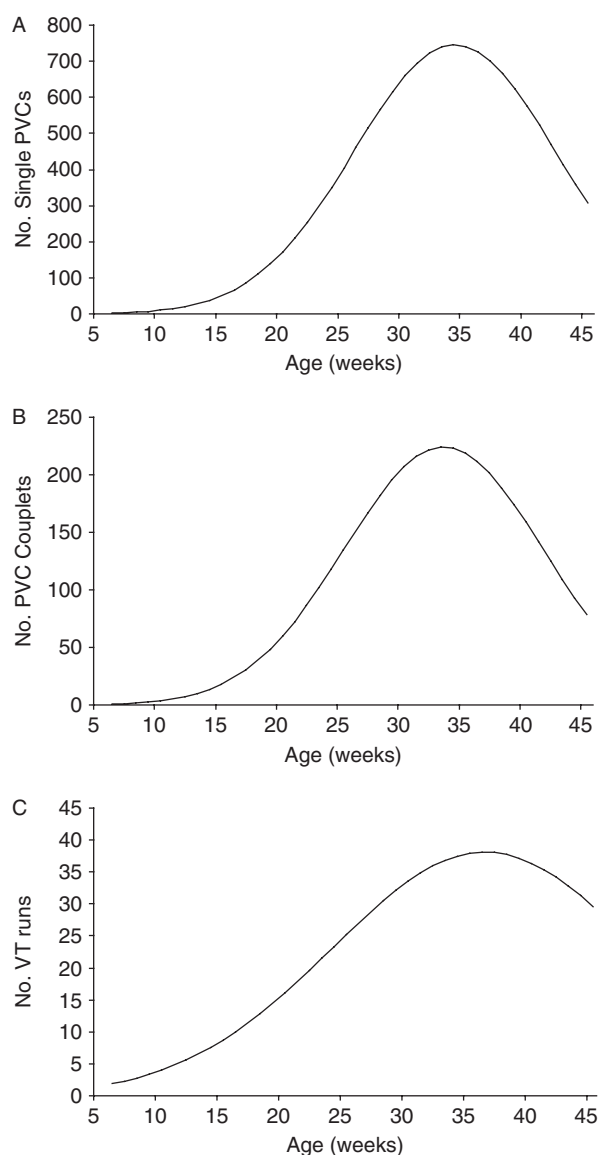


Fig 3. Regression of number of premature ventricular complex, (A) singles, (B) couplets, and (C) runs of ventricular tachycardia, on age in weeks. The differences in the scale of the regression curve graphs reflect the disparity in the ranges of the trait values.

Pattern of Inheritance: Dependence on Correct Phenotype Identification

Several factors have contributed to the difficulty in establishing that VA in GSDs (henceforth referred to as GSDIVA for German Shepherd Dog Inherited Ventricular Arrhythmias) is inherited, particularly using pedigrees of GSDs outside of the colony. First, the expression of arrhythmias is present for a limited window of time (age-dependent) and must be identified during that period or it will be missed.⁶ Previously, we reported the influence of age in the recognition of the disorder⁶; however, we had not separated out the types of VA observed. In this study, the 3 traits indicate differences with regard to age of onset and trends in severity with age. Consequently, affected dogs that are tested before 16 weeks of age or after approximately 2 years of age can be misclassified as unaffected or the severity may be underestimated. Examination of the regression plots for the increase and decline of VA as well as results from our previous studies^{1,6} suggest that screening for GSDIVA would be most effective between the ages of 25 and 45 weeks of age. This age range would most accurately differentiate affected and unaffected dogs. While performing more than one 24-hour ECG would improve the reliability of the diagnosis, it also increases the expense. Another consideration in our definition of phenotype was the selection of >100 PVCs as being affected. We could state that by placing this number too high, affected dogs are classified as unaffected. However, by placing this number lower, the converse would be true. It is inevitable in phenotyping many disorders that the line of true delineation of normal and abnormal is blurred. We have also found that a phenylephrine challenge can be used to identify affected dogs at earlier ages.²

Another difficulty in identifying VA by routine ECG is the dependence of behavior in exposing the arrhythmia,

Table 5. Heritability estimates (underlined, on diagonal), additive genetic correlations (above diagonal), and phenotypic correlations (below diagonal) of the severity of ventricular arrhythmia traits.

	PVCs	Couplets	VT
PVCs	<u>0.33</u>	0.06	0.14
Couplets	0.39	<u>0.69</u>	0.27
VT	0.26	0.34	<u>0.69</u>

PVCs, single premature ventricular complexes; couplets, 2 consecutive PVCs; VT, ventricular tachycardia.

particularly in mildly to moderately affected dogs. Holter monitoring provides an opportunity to observe VA during rest or sleep that might not be present during the excitement of ECG examination. Thus, screening for GSDIVA should be performed by 24-hour Holter monitoring and not by routine ECG recordings.

In this study, we considered different types of VA as different traits, but an important trait we were unable to assess was sudden death. Because death is the only health consequence of this disorder, it is an important factor to consider in the inheritance. We have previously made the observation that only dogs with the rapid polymorphic VT die.¹ However, the presence of VT is not an exact surrogate for sudden death. In our early work, we noted that 50% of dogs that had VT survived the first 2 years of life and subsequently lived a normal lifespan without cardiac compromise such as heart failure or syncope.¹ The reason for death in 1 dog with VT and not in another remains a mystery in terms of both the genetics and the mechanism of deterioration of VT to a fatal rhythm.^{7,28} In humans, the relationship among genetics, arrhythmias, and sudden death is also under investigation.^{14–18} Our division of the overall disease trait into multiple traits was supported by the observed differences in heritability estimates and ages of onset and the fairly low estimates of genetic correlation among the 3 traits.

Another consideration in determining the pattern of inheritance is the genetic background of dog breeds. In an earlier study,¹ outcross ($n = 5$) and backcross ($n = 11$) pups of affected GSDs and Beagles showed that none of the F1 generation was affected and only 4 of the backcross generation were very mildly affected.¹ However, in our study here, outcross and backcross to the Greyhound yielded 1 mildly affected dog in the F1 generation, whereas many backcross dogs were severely affected. Furthermore, when unaffected GSDs were crossed with affected GSDs, the phenotypic spectrum of affectedness was similar to that of mating 2 affected GSDs. Thus, both penetrance and expressivity of the phenotype are likely to be influenced by susceptibility and protective modifier alleles that differ between dog breeds.

Our results do not support a simple mode of inheritance. GSDIVA is most likely polygenic and/or multifactorial in origin. Such a conclusion is not unusual as multiple genes are thought to be at the root of other cardiac diseases of the dog.³¹ Even many inherited arrhythmias in humans that are considered to be “single gene” diseases also show genetic heterogeneity and evidence for modifier genes.^{11,13,19}

Heritability Estimates

Heritability is the proportion of phenotypic variation in a given population that derives from genetic variation rather than from environmental factors. Because heritability estimates reflect the amount of variation in genotypic effects compared with the variation in environmental effects without being absolute measurements of the respective contribution of each to a phenotype, the heritability estimates for GSDIVA reported in this study apply only to this population of dogs. However, the re-

sults provide information relative to the potential response to breeding selection. With higher heritability, as is the case with GSDIVA, the greater the impact selection against a trait in a breeding program will have in eliminating that trait in a breed.^{32,33} In addition, the relatively high heritability estimates for the traits of VA in GSDIVA indicate a greater likelihood of finding evidence of linkage to a genetic marker.

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