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Sudden Death Associated with QT Interval Prolongation and KCNQ1 Gene Mutation in a Family of English Springer Spaniels

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Background: A 5-year-old, healthy English Springer Spaniel died suddenly 4 months after delivering a litter of 7 puppies. Within 4 months of the dam’s death, 3 offspring also died suddenly.

Hypothesis: Abnormal cardiac repolarization, caused by an inherited long QT syndrome, is thought to be responsible for arrhythmias leading to sudden death in this family.

Animals: Four remaining dogs from the affected litter and 11 related dogs.

Methods: Physical examination and resting ECG were done on the littermates and 9 related dogs. Additional tests on some or all littermates included echocardiogram with Doppler, Holter monitoring, and routine serum biochemistry. Blood for DNA sequencing was obtained from all 15 dogs.

Results: Three of 4 littermates examined, but no other dogs, had prolonged QT intervals with unique T-wave morphology. DNA sequencing of the KCNQ1 gene identified a heterozygous single base pair mutation, unique to these 3 dogs, which changes a conserved amino acid from threonine to lysine and is predicted to change protein structure.

Conclusions and Clinical Importance: This family represents the first documentation in dogs of spontaneous familial QT prolongation, which was associated with a KCNQ1 gene mutation and sudden death. Although the final rhythm could not be documented in these dogs, their phenotypic manifestations of QT interval prolongation and abnormal ECG restitution suggested increased risk for sudden arrhythmic death. The KCNQ1 gene mutation identified is speculated to impair the cardiac repolarizing current I Ks, similar to KCNQ1 mutations causing long QT syndrome 1 in humans.

Key words: Long QT Syndrome.
to evaluate surviving littermates and related dogs for phenotypic evidence of LQTS and a causative mutation.

**Materials and Methods**

**Study Design**

Observational case-control study.

**Clinical Evaluations**

The affected litter consisted of 5 males and 2 females. Data could be obtained on 2 females (pups “C” and “R”) and 2 males (pups “E” and “T”). Nine related dogs also were evaluated, including the litter’s sire, the dam of the affected litter’s dam (proband), 2 full- and 1 half-sister to the proband, and other members of the dogs’ extended family (Fig 1). Initially, a complete history, physical examination, resting ECG (30–40 second), thoracic radiographs, routine serum biochemistry, indirect blood pressure measurement, echocardiogram with Doppler, and subsequently, 24-hour Holter recording were done on pup C. All of these tests, except radiographs, also were done on pup E.

Tests on other littermates and related dogs included, at minimum, a history, physical examination, and resting ECG. Pup T was directly examined by another veterinary cardiologist who performed a complete echocardiographic examination with Doppler. Pup R was evaluated by a local veterinarian; routine serum biochemistry also was performed. Holter recordings could be obtained on 5 dogs: pups C, E, R, T, and 1 related ESS (dog AS, Fig 1). Blood was collected on these 13 and 2 additional dogs (P, proband’s sire and B, full brother; Fig 1) for DNA sequencing in a search of a mutation in a candidate gene (KCNQ1) associated with a cardiac repolarizing current. DNA samples also were obtained from 99 unaffected, unrelated dogs (controls) of various breeds. After baseline data collection, pups C, R, and E were treated with a beta-blocker (atenolol 12.5 mg PO q12h) because this treatment has been useful in some people with LQTS. All dogs were housed and cared for by their owners. All testing was done in accordance with accepted clinical practice and principles of the NIH Guide for Care and Use of Laboratory Animals.

Heart rate and standard complex measurements were determined from resting ECGs. Corrected QT intervals (QTc) were calculated using Van De Water’s formula and average HR.21,22 QT interval and preceding TQ interval durations were measured from hard-copy resting ECGs at 50 mm/s. Ratios of QT to preceding TQ interval were calculated for each beat. To evaluate ECG restitution, mean QT/TQ ratio and the percentage of beats with QT/TQ interval were calculated for each beat. To evaluate ECG resting ECGs at 50 mm/s. Ratios of QT to preceding interval and preceding TQ interval durations were measured from calculated using Van De Water’s formula and average HR. QT was determined from resting ECGs. Corrected QT intervals (QTc) were calculated using the Wilcoxon Signed Rank test when data were not normally distributed, and using a t-test for comparisons where the test for normality was satisfied. Mean values ± standard deviation are reported. P < .05 was considered significant. Analyses were done by SAS 9.3.a

**DNA Analysis**

Sequences of exonic, untranslated, and splice site regions of the canine KCNQ1 gene (ENSCAFG00000010231) were obtained from the canine genome using the UCSC genome browser of the Broad CanFam3.1 assembly. Amplification primers were designed by Primer 3 software (http://frodo.wi.mit.edu). Amplification primers for exon 8 of the KCNQ1 gene, the exon containing the LQTS mutation, were as follows: forward 5′ catcaggtagtagtg; reverse 5′ cttccacacctaatctact.

Standard PCR amplifications were carried out using AccuPrime GC-rich buffer A,b 2 unit/μL AccuPrime GC-rich DNA polymerasec, 20 mM of each amplification primer, and approximately 100 ng of template DNA. Samples were denatured for 3 minutes at 95°C followed by 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and finally 72°C for 10 minutes. The annealing temperature was optimized (55°–60°C) to accommodate the respective primer.

Residual amplification primers and dNTPs were removed from the PCR product using a single-step enzymatic cleanup kit.d Amplicons then were sequenced on an ABI 3730XL sequencerd The nucleotide sequences were evaluated for sequence change among ESS dogs, control dogs, and the published normal canine sequence (http://genome.ucsc.edu).

A base pair change was considered possibly causative for long QT syndrome if it: was present in dogs with QT prolongation but not other dogs or the published canine sequence, changed a conserved amino acid, and changed that amino acid to a different

![](image.png)

**Fig 1.** ESS family pedigree. Squares represent males; circles, females. Tested individuals identified by letters. Shaded symbols indicate LQTS-affected dogs. Open symbols indicate normal QT duration—except those with an asterisk represent dogs of unknown phenotype. A diagonal line indicates sudden death.
polarity, acid/base status, or structure. Any amino acid change identified was evaluated with the PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) program to predict possible impact of the amino acid substitution on the protein. PolyPhen2 also was used to determine level of conservation using the multiple sequence alignment function (UniProtKB/UniRef100 Release 2011_12). UniProtKB was utilized to evaluate the sequence annotation and determine which functional region of the protein the amino acid substitution affected (http://www.uniprot.org/).

Finally, the normal and altered sequences were evaluated for changes that occur in the secondary structure normal with 4 protein modeling software programs, GOR4 (http://npsa-bil.ipcb.fr/cgi-bin/npsa_automat.html), SIFT (http://sift.jcvi.org), SWISS-MODEL Workspace (http://swissmodel.expasy.org), and Protean 3D.

**Results**

A month after the adult female ESS died, a male pup (5 months old) died suddenly after being released from its crate and running across the room. A second pup (male, 7 months old) died suddenly while playing outdoors. Pup T, evaluated at 8 months of age; other available dogs were evaluated within the next 3 weeks. A third male (8 months old) had SD during play shortly before evaluation. There was no history of episodic weakness, syncope, or other abnormality in any dog, including those that died. Routine vaccinations and parenteral nutrition had been given according to standard recommendations. Dogs with SD were not receiving other drugs at the time of death.

Physical examination findings on pups C, E, and R, and 7 related dogs were normal. A soft systolic left basilar murmur was heard in pup T. The litter’s sire and one of the sire’s half-sisters also had soft systolic murmurs. Other physical findings were unremarkable. Blood pressure in pups C and E was normal (not available in others).

Resting ECGs showed normal sinus arrhythmia (9 dogs) or regular sinus rhythm (3 dogs) with HRs from 80 to 150 beats/min. Pup T had sinus tachycardia (180 beats/min) when evaluated at 8 months of age; other available dogs were evaluated within the next 3 weeks. A third male (8 months old) had SD during play shortly before evaluation. There was no history of episodic weakness, syncope, or other abnormality in any dog, including those that died. Routine vaccinations and parenteral nutrition had been given according to standard recommendations. Dogs with SD were not receiving other drugs at the time of death.

**Results of KCNQ1 gene sequencing showed that the 3 dogs with QT prolongation, but no other dogs, had a heterozygous single base pair change from cytosine to adenine in codon 1 of exon 8 (Fig 4). This changes a highly conserved amino acid in the protein transcript from threonine (T), a nonaromatic, neutral hydrophilic amino acid, to lysine (K), a basic positively charged amino acid at position 377 (Genbank KF439050; KCNQ1_T377K). Altered protein structure is predicted (Fig 5). The protein structure analysis program GOR4 predicted an increase in the extended strand and a decrease in the random coil within the domain containing the mutation. The SWISS-MODEL Workspace program predicted a 3-dimensional model of the normal and mutant KCNQ1 C-terminus cytoplasmic protein region, which further confirmed reduction in random coil and increased extended strand structure. Commercial software (Protean 3D) was used to further analyze the SWISS-MODEL Workspace output file with the following results: Chou-Fasman analysis of secondary structure indicated removal of a beta region and extension of the neighboring alpha region in the mutant protein; Kyte-Doolittle hydrophathy analysis indicated decreased hydrophobicity in the mutant protein; and Lehninger charge density evaluation
indicated creation of a positive charge region in the mutant protein.

After initial data collection and in addition to beta-blocker treatment, avoidance of strenuous activity and excitement was recommended for the 3 affected dogs. Nevertheless, Pup C died suddenly after going outside within 2 months of initial evaluation (9 months of age). Pup R’s owners decided to discontinue atenolol after a few weeks; SD occurred while running at 15 months of age. Pup E developed progressive unprovoked aggression toward children and was euthanized per owner request at 20 months of age. No cardiovascular abnormalities were found at necropsy.

Discussion

This study represents the first report of familial LQTS in the dog, with the identified KCNQ1 gene mutation apparently arising de novo in the dam of the affected litter. The clinical presentation was similar to LQTS in humans in which the first sign often is SD. It was characterized phenotypically by prolonged QT intervals in the absence of any pharmacologic manipulation or evidence of cardiac or other systemic disease. None of these ESS showed any indication of familial atrial standstill or ventricular dysfunction.

The QT interval duration, extending from the onset of the QRS complex to the end of the T wave, is determined by ventricular cell AP duration in aggregate. Factors that delay repolarization prolong the QT interval. Electrophysiologically, this includes reduction in outward K⁺ flux during phases 2 and 3, especially of either the slow (Iₖ,slow) or rapid (Iₖ,fast) components of the Iₖ (delayed rectifier) current, or enhanced inward Na⁺ or Ca⁺⁺ current during depolarization.¹⁰ Repolarization delay predisposes to potentially lethal arrhythmias from triggered activity, reentrant mechanisms, or both.²⁴ Decreased outward K⁺ current, by prolonging phase 2 and allowing Ca⁺⁺ channels to partially recover from inactivation, can facilitate development of early after-depolarizations that may trigger premature ventricular depolarization. In addition, regional disparity in ventricular repolarization and refractoriness facilitates development of reentrant ventricular tachycardia.

The polymorphic ventricular tachycardia known as Torsades de Pointes (TdP) is commonly associated with LQTS. TdP often degenerates to ventricular fibrillation.¹⁰,²⁴ QT duration varies with changes in HR, largely reflecting underlying cardiac autonomic influences. Therefore, QT duration is considered in the context of HR. Several equations have been developed to describe the QT-HR relationship or to “correct” QT interval duration for HR, although these all have limitations. In people, QTc usually is calculated using the RR interval from the preceding beat (ie, instantaneous HR). In dogs, respiratory sinus arrhythmia generally produces marked variability in RR intervals. However, QT duration remains essentially unchanged during respiratory sinus arrhythmia despite large changes in

Fig 2. ECGs from an affected (A, pup E) and normal (B) ESS at HR of 120/min. Note prolonged QT duration (270 msec; QTc 310 msec) and large biphasic T waves in A. Leads marked, 50 mm/s, 1 cm = 1 mV.
beat-to-beat intervals because more beats than occur during a respiratory cycle are required for ionic conductance to reach a new steady state for the new HR (so-called “QT memory”). Although not perfect, we used Van de Water’s correction formula, with mean RR interval calculated from mean HR (over 6–10 seconds), to normalize the QT interval to an RR interval of 1000 msec (HR of 60 beats/min):

\[ QTc = \frac{QT}{C0}0.087(\text{RR}-1000), \] with QT and RR in msec. Commonly used correction formulas in people (eg, Bazett’s or Fridericia’s) tend to overestimate QTc at higher HRs and underestimate QT prolongation at lower HRs. Although, as in people, there may be no clear cut-off between normal and abnormal QTc in the larger canine population, the QTc calculations in these dogs showed a clear distinction between unaffected and affected dogs.

The utility of QTc formulas for distinguishing arrhythmogenic repolarization abnormalities from physiologic variation is limited however, because they do not account for physiologic changes in autonomic state or QT hysteresis on the QT-RR relationship. During increased vagal influence (eg, exhalation), there is little change in QT duration at long RR intervals (slow HR). However, as vagal tone decreases and sympathetic influence increases, the QT-RR relationship becomes steeper. This creates a dynamic oscillation between flatter and steeper QT-RR relationships, which QTc formulas cannot reliably predict.

A newer technique for assessing arrhythmia vulnerability involves the generation of beat-to-beat QT-RR plots (QT “clouds”) from 5- to 10-minute ECG recordings to differentiate physiologic variation in the QT interval caused by changing HR and autonomic influence from impaired repolarization. This beat-to-beat method also is used to evaluate ECG restitution by plotting QT to preceding TQ intervals. ECG restitution is the ability of the heart to recover from 1 beat to the next. It describes the “work” phase (QT interval) in relation to the preceding “rest” phase (TQ interval). This also is a dynamic relationship that varies with autonomic state as well as abnormal repolarization delay. The mean QT/TQ ratio and percent heartbeats with QT/TQ ratio >1 are also ECG biomarkers of restitution. In the normal unstressed heart, “rest” time exceeds “work” time and the QT/TQ ratio is <1. However with exercise (tachycardia) or other stress, this ratio may be >1 because the TQ shortens to a greater extent than does the QT. Sustained periods of QT/TQ >1 are thought to represent inadequate recovery time.

![Fig 3. ECGs from affected pup C, QT duration 270 msec (A); and pup R, QT duration 260 msec (B). 50 mm/s.](image-url)
between beats and increased vulnerability to arrhythmia. QT prolongation, especially with increased repolarization heterogeneity, magnifies this risk. The percentage of beats with QT/TQ >1 reflects the relative time spent on the restitution curve during which electrical stability is questionable. Healthy people have <25% of beats with QT/TQ >1; normal dogs appear to have fewer, and 5% was reported in 1 study. An increase in mean QT/TQ ratio and higher percentage of beats with QT/TQ >1 have been correlated with increased arrhythmogenicity in people and have been observed experimentally in dogs after administration of arrhythmogenic substances.

Although we were unable to apply beat-to-beat methods in our dogs because we lacked extended ECG recordings on all dogs, as well as the required software, we attempted to generate “snapshots” of ECG restitution using brief resting ECGs of consistent duration and recording conditions. We acknowledge that sinus arrhythmia might be a confounding factor in our brief recordings, however, most dogs had fairly regular sinus rhythm at the time. Furthermore, the ECGs would have

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**Fig 4.** A heterozygous C to A nucleotide change was identified in the 3 dogs labeled “affected”. The 3 normal dogs match the reference sequence and are homozygous for the wild type C in position 2, codon 1, exon 8 of the KCNQ1 gene.

**Fig 5.** Protein models (SWISS-MODEL) of the KCNQ1 gene, C-terminus region, demonstrating normal protein sequence (A) and mutant protein sequence (B). Note loss of random coil and increased extended strand in (B) compared to normal (arrows).
been long enough to encompass 3–5 respiratory cycles, allowing measurements at different levels of autonomic tone. Although this approach has not been fully validated, and the duration of our ECGs was limited, it did identify clear differences between groups. The increased mean QT/TQ ratios and markedly increased percentage of heartbeats with QT/TQ >1 despite lower HRs in LQTS dogs is consistent with prolonged repolarization and increased risk for lethal arrhythmias. Indeed, the dogs had 100% of heartbeats with QT/TQ >1 did experience SD.

Inherited long QT syndrome in people has a prevalence estimated at 1:2,500–5,000 individuals, with 13 LQTS forms now reported.10 About 70–75% of human LQTS cases result from mutations in 3 major genes: KCNQ1, KCNH2 (hERG), and SCN5A.9,20 Respectively, these genes code for the alpha subunits of the slow-activating delayed rectifier potassium current (I\(_{\text{Ks}}\)), the rapid-activating delayed rectifier potassium current (I\(_{\text{Kr}}\)), and the fast inward sodium current (I\(_{\text{Na}}\)). Approximately, 35% of positive LQTS genetic tests in people are caused by loss-of-function mutations in the KCNQ1 gene, causing LQT1.20 To the authors’ knowledge, no examples of familial LQTS have been documented previously in dogs or other animals. The missense mutation in KCNQ1 discovered in these 3 affected ESS dogs, which changes a highly conserved amino acid, appears to have effects similar to those in people with LQT1, although this specific mutation has not been reported in people.

Common clinical manifestations in humans with LQTS include syncope and a high risk of TdP, which is the first cardiac event at an earlier age than females. However, the risk for SD in affected females becomes greater than for males during adulthood, with risk reversal occurring during the mid-teens.19,24 Although the number of dogs reported here is too small for statistical significance, the dam of the affected litter was ostensibly normal until SD at 5 years of age, whereas 3 male pups were the first to die.

Beta-blockers are helpful in preventing syncope and sudden death in people with LQT1.19 Improved ECG restitution, as implied by the decreased percentage of heartbeats with QT/TQ >1 in pup E after atenolol, is thought to decrease arrhythmia risk. Other recommendations include exercise restriction, and avoidance of hypokalemia and drugs known to prolong the QT interval.

This study has a number of limitations, especially the fact that the dam and 3 pups were not available for study. Also, because ECG recordings were not available at the time of SD, we could not document an arrhythmia as the cause of death. Unfortunately, necropsy examinations were not available on dogs with SD. However, none of the 5 littermates with SD or their dam showed any evidence of decreased exercise tolerance or other signs of underlying myocardial disease. Likewise, clinical and echocardiographic findings on the pups examined did not indicate the presence of myocardial disease. Despite the large percentage of affected dogs in this litter, the total number of affected dogs was small. Nevertheless, the findings reported here are important because this family of ESS represents the first report of inherited LQTS in dogs, along with an associated, novel KCNQ1 mutation.

Footnotes

a SAS Institute Inc, Cary, NC
b Qiagen, Valencia, CA
c ExoSAP-IT, Affymetrix, Santa Clara, CA
d Applied Biosystems, Foster City, CA
e DNASTar, Madison, WI

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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