Evaluation of Tafazzin as Candidate for Dilated Cardiomyopathy in Irish Wolfhounds

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Abstract

Dilated cardiomyopathy (DCM) is a common disease in humans and dogs. Large-breed dogs and especially Irish wolfhounds belong to the frequently affected breeds. Male Irish wolfhounds show a significantly higher prevalence of DCM than females. Therefore, we evaluated X chromosome markers for linkage with DCM as well as a human candidate gene on the X chromosome. A set of X chromosomal microsatellites was genotyped in Irish wolfhound families segregating for DCM. In addition, exon and intron sequences of the tafazzin (*TAZ*) gene were assayed for polymorphisms segregating in these families. Statistical analysis of the microsatellite markers did not reveal linkage to DCM. Furthermore, all Irish wolfhounds included in this study were monomorphic for *TAZ*, and only 8 sequence differences to the Dog Genome Assembly 2.1 could be found. The results indicate that due to the lack of mutations, *TAZ* is unlikely to cause DCM in Irish wolfhounds.

Dilated cardiomyopathy (DCM) is a common cause of congestive heart failure, which is often found in humans and dogs. The disorder is characterized by an increase of myocardial mass and a reduction in ventricular wall thickness with a pronounced ventricular chamber dilatation. Some dog breeds, especially large-breed dogs such as Doberman Pinschers, Newfoundlands, Boxers, Great Danes, and Irish wolfhounds, show a high prevalence of DCM (O'Grady and Horne 1995, 1998; Vollmar 1999b, 2000). Therefore, a genetic cause seems to be likely in these breeds (Meurs 1998). Different modes of inheritance were proposed for the breeds. An autosomal dominant model was suggested for Boxers (Meurs et al. 1999) while the inheritance of DCM in Great Danes seemed to fit an X chromosome recessive mode (Meurs et al. 2001). In Newfoundlands, an autosomal dominant mode of inheritance with incomplete penetrance is likely but an autosomal recessive inheritance could not be excluded (Dukes-McEwan and Jackson 2002).

In Irish wolfhounds, the onset of the disease occurs between 3 and 7 years with a mean age of onset of 4.52 ± 1.99 years. Males are more often and earlier in their life affected than female dogs (Brownlie and Cobb 1999; Vollmar 2000) suggesting a sex predisposition. Recent complex segregation analyses have shown that the mode of inheritance can be explained best by an autosomal recessive major gene model with sex-specific allelic effects (Distl et al. 2007).

In human, 2 X chromosome–located genes could be related with DCM: dystrophin and tafazzin (*TAZ*). Other possible candidate genes like lysosome-associated membrane protein 2 (*LAMP2*) and angiotensin II receptor, type 2 (*AGTR2*) usually lead to complex syndromes and therefore were not short listed as candidate genes for DCM in Irish wolfhounds. *LAMP2* mutations seem generally lead to the Danon disease (online mendelian inheritance in man [OMIM] #300257), which is characterized by X-linked vacuolar cardiomyopathy and myopathy and is often accompanied by a mild mental retardation. Mutations of the *AGTR2* gene (OMIM #3000034) are associated with X-linked mental retardation although an increased expression of *AGTR2* could be observed in human patients with heart failure (Wharton et al. 1998), and in transgenic mice, overexpression of *agtr2* leads to DCM (Yan et al. 2003).

However, some alleles of the *dystrophin* gene cause familial DCM in human (Muntoni et al. 1993; Milasin et al. 1996; Ortiz-Lopez et al. 1997). In Doberman Pinschers, rearrangements of the *dystrophin* promoter region as universal cause of DCM have been ruled out (Schatzberg et al. 1999).

Mutations in the *TAZ* gene are known to cause Barth syndrome, endocardial fibroelastosis, and infantile DCM (OMIM 302060, 305300, and 300069). But obligate male carriers
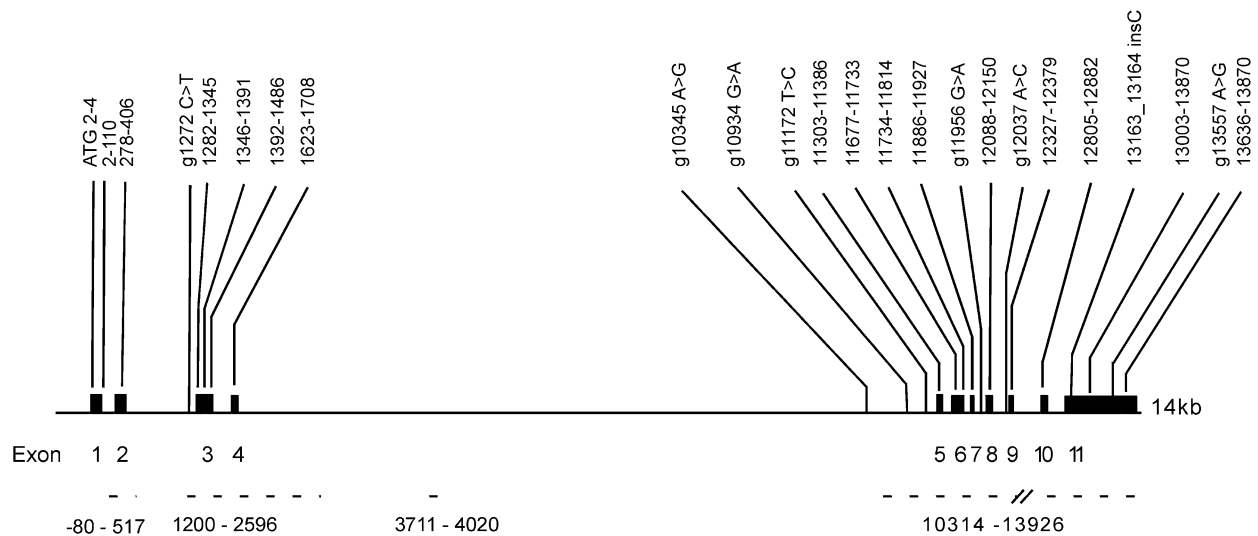
Figure 1. Genomic structure of the *TAZ* gene (LOC612975), 8 sequence differences between Irish wolfhounds, and the boxer reference sequence (Dog Genome Assembly 2.1). Exons are denoted as black boxes. The positions of the sequence differences and exons in LOC612975 are given at the top of the figure. Exons denoted with more than one size range indicate experimental transcript variants. Numbers and short lines (at the bottom of the figure) denote the parts of *TAZ*, which were sequenced for mutation analysis.

within a pedigree were observed who seemed to be less affected and were able to reproduce and transmit the disease (D'Adamo et al. 1997). Another family was reported exhibiting a wide range of cardiomyopathies from fatal infantile DCM to late-onset symptomatic and asymptomatic DCM (Ichida et al. 2001). These observations made the *TAZ* gene to a candidate gene causing a late-onset DCM in Irish wolfhounds. The role of the *TAZ* gene is still unknown. It is supposed to function as an acyltransferase in the remodelling of cardiolipin in the inner mitochondrial membrane (Neuwald 1997; Valianpour et al. 2002; Xu et al. 2003).

Here we evaluated the *TAZ* for association with the prevalence of DCM in Irish wolfhounds. This included both linkage analysis for microsatellites on the X chromosome and mutation analysis of the *TAZ* gene.

Materials and Methods

For the linkage analysis, samples of 89 Irish wolfhounds of 13 families segregating for DCM were genotyped. Out of 89 animals, 64 were affected and 25 were nonaffected. The number of genotyped individuals per family ranged from 2 to 21 dogs. The pedigrees of the families included are shown in Supplementary Figure 1. The diagnosis of DCM was based on the results of echocardiographic examinations performed by a veterinarian approved for cardiology. Echocardiographic reference values were determined for this breed, and the echocardiographic features of Irish wolfhounds with occult DCM were compared with dogs with congestive heart failure and to normal dogs (Vollmar 1999a, 1999b). The echocardiographic criteria used to diagnose DCM in Irish wolfhounds were left ventricular internal diameter systolic wider than 41 mm

and wider than 61.2 mm at end-diastole, fractional shortening below 25%, E-point to septal separation greater than 10 mm, and end-systolic volume indices greater than 41 ml/m^2. Right ventricular dilatation was diagnosed when right ventricular internal dimensions, measured during end-diastole, were wider than 36.8 mm. Left or right atrial enlargement was present when the systolic internal diameter of the atrium being examined was greater than 56 mm (Vollmar 2000).

Genomic DNA was isolated from frozen ethylenediaminetetraacetic acid–stabilized blood samples using the NucleoSpin® 96 Blood DNA Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions.

The dogs were genotyped for 8 microsatellite markers (*REN230I20*, *REN101G16*, *FH2985*, *FH2916*, *FH3027*, *FH1020*, *REN130F03*, and *FH2584*) belonging to the minimal screening set 2 (MSS2) and spanning the X chromosome from 23.4 to 115.5 Mb (Guyon et al. 2003). The 11 MSS2 markers were recommended for chromosome-wide linkage studies. We included 10 of the markers, but 2 markers *REN144O22* and D04614 failed to give reproducible results. Polymerase chain reaction (PCR) was carried out in a 12-μl reaction containing 10 ng DNA. The resulting DNA fragments were size fractionated on an automatic LI-COR sequencer (LI-COR, Lincoln, NE), and the genotypes were assigned by visual examination.

For mutation analysis, 32 Irish wolfhounds of the 11 families were included whereof 21 animals were affected by DCM and 11 animals were unaffected.

The putative exons of the *TAZ* gene were determined by comparative alignment of the canine sequence NC_006621 (125155838–125171055 X chromosome, LOC612975) versus the human *TAZ* mRNA sequence NM_000116 and canine

Table 1. DNA sequence differences in the *TAZ* gene between Irish wolfhounds and the boxer reference sequence (Dog Genome Assembly 2.1)

Mutation in the *TAZ* gene	Location of mutation
LOC612975:g.1272C>T	Intron 2
LOC612975:g.10345A>G	Intron 4
LOC612975:g.10934G>A	Intron 4
LOC612975:g.11172T>C	Intron 4
LOC612975:g.11956G>A	Intron 7
LOC612975:g.12037A>C	Intron 8
LOC612975:g.13163_13164InsC	3′UTR
LOC612975:g.13557A>G	3′UTR

expressed sequence tag (EST) (CO664816, DN866909, and CO618231) using BLAST (http://www.ncbi.nlm.nih.gov/blast/) and Spidey (http://www.ncbi.nlm.nih.gov/spidey/). The canine ESTs were identified using the EST other database under the nucleotide BLAST option. The genomic sequence was searched in the databases of the dog genome. Alignment of human mRNA was performed using the setting divergent sequences in Spidey resulting in an overall sequence identity of 77.1%. The canine ESTs covered the exons 2–11 but not exon 5.

The 11 exons of the canine *TAZ* and some nonrepetitive intron sequences were amplified using specific PCR primer pairs. The putative splice sites of the exons were within the PCR products. The oligonucleotide sequences, annealing temperatures, and PCR product sizes for the 11 amplicons are listed in Supplementary Table 1.

PCR was carried out in 30 µl containing 10 ng genomic DNA according to the standard protocol advised by the manufacturer of the *Taq* DNA polymerase (Qbiogene, Heidelberg, Germany). The subsequent sequencing of the PCR products was performed using the DYEnamic Terminator Cycle Sequencing Kit (GE Healthcare, Freiburg, Germany). The products were analyzed on a MegaBACE 1000 capillary sequencer (GE Healthcare).

Statistical Analysis

A nonparametric chromosome-wide multipoint linkage analysis using Merlin software, version 1.0.1 (Abecasis et al. 2002) was performed. The nonparametric approach does not require a priori knowledge on the mode of inheritance and on genetic parameters. Multipoint linkage was estimated through the proportions of alleles shared identical by descent for affected animals, and thus animals unaffected by DCM or unknown DCM status only contribute to the inference of haplotypes used for the multipoint test statistics. We used the test statistics Zmean and logarithm of the likelihood ratio (LOD) score under the options nonparametric linkage (NPL) all to calculate error probabilities for linkage (Whittemore and Halpern 1994; Kong and Cox 1997).

Results and Discussion

The genomic structure of the canine *TAZ* gene is similar to the human orthologous gene. For all 11 human *TAZ* exons,

homologous canine sequences could be identified. The canine *TAZ* spans approximately 14 kb and is therefore larger than the human orthologous gene (10.2 kb). Mutation analysis of the genomic sequences revealed 8 sequence differences to the boxer reference sequence (Dog Genome Assembly 2.1) but no polymorphism within the Irish wolfhounds (Table 1). This sequence homogeneity could be a result of the low genetic diversity of the Irish wolfhound population but might also indicate sequence conservation within the *TAZ* gene. The observed breed-specific sequence differences include 7 single nucleotide exchanges and one indel polymorphism. All polymorphisms were in introns or in the 3′UTR (Figure 1). These sequence differences are unlikely to affect *TAZ* expression.

Due to the high content of repetitive sequences in *TAZ* (about 50.2% of the NCBI annotated sequence LOC612975), only few intronic sequences could be included in the mutation analysis. From the nonrepetitive sequences, approximately 90% (5800 bp) were analyzed although some of the 32 Irish wolfhound samples did not allow amplification for all PCR products.

Linkage analysis showed Zmeans for 8 microsatellites between −0.43 and 0.38. The LOD scores were from −0.09 to 0.07. The maximum achievable Zmean and LOD score were 6.37 and 3.50 indicating enough power to show significant linkage. The results of our analysis supported missing linkage between DCM and *TAZ* on the X chromosome because the markers close to the *TAZ* gene (*FH2584* and *REN130FO3*) were highly informative. Some markers (*FH3027*, *FH2985*, and *REN101G16*) had only 2 or 3 alleles and low polymorphism information content and thus did not allow the exclusion of X chromosomally located genes other than *TAZ* (Supplementary Table 2).

Our results of linkage and mutation analysis indicate that the *TAZ* gene has no influence on the development of DCM in Irish wolfhounds.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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