# An *ADAMTS17* Splice Donor Site Mutation in Dogs with Primary Lens Luxation

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**Purpose.** To identify the genetic cause of isolated canine ectopia lentis, a well-characterized veterinary disease commonly referred to as primary lens luxation (PLL) and to compare the canine disease with a newly described human Weill-Marchesani syndrome (WMS)–like disease of similar genetic etiology.

**Methods.** Genomewide association analysis and fine mapping by homozygosity were used to identify the chromosomal segment harboring the PLL locus. The resequencing of a regional candidate gene was used to discover a mutation in a splice donor site predicted to cause exon skipping. Exon skipping was confirmed by reverse transcription-polymerase chain reaction amplification of RNA isolated from PLL-affected eyes and from skin fibroblast cultures from PLL-affected dogs. An allelic discrimination assay was used to genotype individual dogs at the splice donor site mutation.

**Results.** The PLL locus was mapped to a 664-kb region of canine chromosome 3 containing regional candidate gene *ADAMTS17*. Resequencing *ADAMTS17* revealed a GT→AT splice-donor-site mutation at the 5′ end of intron 10. The predicted exon 10 skipping and resultant frame shift were confirmed with RNA derived from PLL-affected dogs. The *ADAMTS17* mutation was significantly associated with clinical PLL in three different dog breeds.

**Conclusions.** A truncating mutation in canine *ADAMTS17* causes PLL, a well-characterized veterinary disease, which can now be compared to a recently described rare WMS-like disease caused by truncating mutations of the human *ADAMTS17* ortholog. (*Invest Ophthalmol Vis Sci.* 2010;51:4716–4721) DOI:10.1167/iovs.09-5142

Ocular lenses are held in place behind the pupil by zonular fibers that link the capsule of the lens near its equator to the surrounding ciliary muscle.[1] Stretching or rupture of the zonular fibers can result in the displacement of the lens, producing a condition known as ectopia lentis. The disease can be caused by trauma or by a variety of conditions that distort normal ocular anatomy, such as glaucoma and intraocular tumors.[2] Individuals with certain rare heritable metabolic diseases, including homocystinuria (OMIM 236200), sulfocystinuria (OMIM 272300), and hyperlysinemia (OMIM 238700), may also have ectopia lentis.[2] In addition, ectopia lentis can occur in conjunction with Marfan syndrome (OMIM 174700) and Weill-Marchesani syndrome (WMS; OMIM 277600 and 608328), two distinct disease entities that can result from mutations in the fibrillin 1 gene (*FBN1*) or at other loci.[3–5] Mutations in *FBN1* can also cause autosomal dominant isolated ectopia lentis[6] (OMIM 129600), and a mutation in *ADAMTSL4* has recently been identified as a cause of autosomal recessive isolated ectopia lentis (OMIM 225100).[7]

In veterinary medicine, isolated ectopia lentis, known as primary lens luxation (PLL; OMIA 1152), has been recognized as a canine familial disorder for more than 75 years.[8] PLL is encountered at high frequency in several terrier breeds and in some other breeds with probable terrier coancestry.[8–13] In most instances, PLL goes undetected until one of the lenses is grossly displaced from the patellar fossa.[9–11,14] Concurrent iridodonesis usually signals subluxation in the opposite eye. Gross displacement of the lens in the second eye often follows weeks or months later.[9–11] Ultrastructural abnormalities of the zonular fibers are already evident at 20 months of age,[14] long before the initial luxation that typically occurs when the dogs are 3 to 8 years old.[10,11] This suggest that PLL results from defective development or maintenance of the zonular fibers, or both.

We previously mapped the PLL locus to a segment of canine chromosome 3 (CFA3).[13] Here we demonstrate that a mutation in a splice donor recognition site in regional candidate gene *ADAMTS17* is a cause of PLL. During the preparation of this manuscript, Morales et al.[15] reported that truncating mutations in human *ADAMTS17* cause a WMS-like disease (OMIM 613195),

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characterized by ectopia lentis and short stature. In the current report, we have focused the discussion on a comparison of the human and canine disease phenotypes resulting from truncating mutations in *ADAMTS17*.

## Materials and Methods

All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. DNA samples used in this study were collected from North American, European, and Australian dogs. In North America, samples from individual members of the Jack Russell Terrier ($n$ = 398), Miniature Bull Terrier ($n$ = 90), and Lancashire Heeler ($n$ = 8) dog breeds were obtained from DNA collections kept as part of the University of Missouri Animal DNA Repository (http://www.caninegeneticdiseases.net/) or the Canine Health Information Center DNA Bank (http://www.caninehealthinfo.org/dnabank.html). Many of the DNA samples were collected from dogs with PLL; however, we also collected DNA from breed members with normal eyes. In Europe, DNA samples were collected from individual, privately owned Jack Russell Terriers ($n$ = 27), Miniature Bull Terriers ($n$ = 126), and Lancashire Heelers ($n$ = 115) recruited specifically to investigate the genetic cause of PLL. All European dogs had been examined by a veterinary ophthalmologist at least once and either had PLL or were older than 5 years of age and had been declared free of clinical signs consistent with PLL. DNA samples were also collected from Miniature Bull Terriers from Australia ($n$ = 65). All but seven of the Australian samples came from dogs that had been examined by a veterinary ophthalmologist.

Genomewide association mapping was performed with a whole-genome genotyping kit (CanineSNP20 BeadChips; Illumina, San Diego, CA) and DNA samples from 48 Jack Russell Terriers (28 cases and 20 controls). Fine mapping was performed with samples from 49 Miniature Bull Terriers (38 cases and 11 controls) and from 40 Lancashire Heelers (22 cases and 18 controls) using 768 single nucleotide polymorphism (SNP) markers located between 20 Mb and 70 Mb on CFA3 and assayed with a genotyping array (Golden-Gate platform; Illumina).

RNA was obtained from the lenses and ciliary bodies of eyes enucleated from PLL-affected dogs with secondary glaucoma or from dogs unaffected by PLL but with other ocular disorders that required globe removal. Ocular tissues were preserved in an RNA stabilization solution (Ambion RNALater; Applied Biosystems, Foster City, CA) and frozen at −70°C until use. Total RNA from lens and ciliary body material was extracted (RNeasy kit; Qiagen, Valencia, CA) according to the manufacturer's instructions. Cultured canine fibroblasts from PLL-affected and normal dogs provided a second source of RNA. These fibroblast cultures were derived from skin biopsies taken from live dogs under local anesthesia, as specified in a protocol approved by the University of Missouri Animal Care and Use Committee. Biopsies consisted of a 2 cm × 2 cm piece of skin containing epidermis, dermis, and at least some subcutaneous tissue. The tissue was diced, and cells were allowed to adhere to plastic culture flasks before incubation in fibroblast culture medium consisting of medium (Gibco MEM Alpha; Invitrogen Corp., Carlsbad, CA) plus 20% fetal bovine serum and 1% penicillin/streptomycin at 37°C in 5% $CO_2$ atmosphere. Fibroblasts were expanded to approximately 12 million cells per sample and preserved, frozen, in culture medium containing 10% dimethyl sulfoxide. Thawed fibroblasts were grown to confluence, trypsinized, and washed with 1× PBS. Total RNA was isolated from fibroblasts with an RNA purification kit (SurePrep TrueTotal; Fisher Scientific, Pittsburgh, PA) according to the manufacturer's instructions.

Resequencing the coding regions of all 22 canine *ADAMTS17* exons was accomplished after PCR amplification of genomic DNA (primers are listed in Table 1). RT-PCR amplifications of fibroblast RNA were performed with primers designed against sequences in exon 9 (5′-TGTCAGGAGAGTGGGTAAAGG-3′) and exon 11 (5′-GGACGTATCTCCTTCCACCAG-3′). Purified PCR and RT-PCR amplification products were sequenced with a DNA analyzer (3730xl; Applied Biosystems). Genotypes at *ADAMTS17:c.1473+1* were determined with a TaqMan allelic discrimination assay.[16] The PCR primer sequences were 5′-CACCAACGCCACCTTCTG-3′ with 5′- GATGCTCCTTCCTGTGTCAGT-3′, and the probe sequences were 5′-VIC-AAACATGGAGATAAGCAG-MGB-3′ for the mutant allele and 5′-FAM-ACATGGAGGTAAGCAGMGB-3′ for the wild-type allele.

## Results

### Genomewide Association Mapping

Genomewide association analysis was performed under an autosomal recessive model of inheritance with DNA from PLL-affected ($n$ = 28) and control ($n$ = 20) members of a large Jack Russell Terrier family. Under this analysis, the null hypothesis

**Table 1.** PCR Primers for *ADAMTS17* Resequencing

| Target | Forward Primer Sequence/Reverse Primer Sequence | Amplicon Size (bp) |
|---|---|---|
| exon 1 | GTTGGGTTTGTCCGCAGC/GGGAGGGCAGGAGGAAGGTG | 261 |
| exon 2 (5′) | GCTGACGCGTCTCCTCTCTCCC/CCGCCTCCTCCACCTCGAA | 285 |
| exon 2 (3′) | CCCCGGACCCCGAAAGC/GCGACTAAGCGACGGGCAGA | 334 |
| exon 3 | GACATGAGGACCAGGCCAGAG/GGCGTGTACTGTCAATCATGCTG | 327 |
| exon 4 | GCCAAGGACTGCATGAACATCTCT/GCTGGGCAAAAGACAAGAAC | 290 |
| exon 5 | AGTTTTTACCCAACATCTTCCTCTGT/ACTCAATGGCAACTAGGAGACTTGAA | 266 |
| exon 6 | TTCCCCAATCCCCAATTTGT/GCCTGAATTTCTAGCAAACTGCCTT | 279 |
| exon 7 | CTATGTGCAGGATGACCAATCAAGC/ACCAGGTCTCCAGATTATCACAGGG | 238 |
| exon 8 | TCCCACGTTCTGAAGAGGATGTGTT/AAAGTATGTGGCAGCACTGTTTCAA | 261 |
| exon 9 | TGAAAGCCTGTAGCATAAGTGTCCCT/GGCCAGCTCAGAGTTCTAAGAGATTC | 314 |
| exon 10 | CTTCTTGCCTTCTCTTGACTGACAGA/CGACAGAAGCCAGATGCTCCTT | 285 |
| exon 11 | GTGACCTGTATATGGTGTTCTAGGCA/CCAGTGGAGCTTTAGGCACTATTGAG | 265 |
| exon 12 | GGATTCATTGGAGCTGTGTGG/CCCTGATCAAGGCACCCTAGA | 249 |
| exon 13 | GCTGGGATTGCAGAGATACCATGTG/TAGGAGGTGTTTACATTCTTGGAGGA | 391 |
| exon 14 | ATGCACCTGCACCTTACATGGC/TTCTGGGGTGAGGGAGC | 265 |
| exon 15 | TGGGCCCATTGGACAAGTC/TGCAGCTGCAGTATCCCCTTGA | 301 |
| exon 16 | CTCTGATGCACCCCTCAGGACA/CCCCTTGCAACCTCAGGCTT | 294 |
| exon 17 | GCCAATTCAGCAAGCCTGTAAG/GCCCTGGGAAGAGCGTCT | 296 |
| exon 18 | GCATCAGTGAATGGCCTTTC/CTTAGCAACAAGAGAACATGGCAT | 329 |
| exon 19 | CTCCCCACCCAGTTATTCTCTTC/GTTGGGATCCCTGTGGACAAGACC | 324 |
| exon 20 | ACATGAGCTGAATCAGACCGGAT/CGCAGGGTGATGGCTGAGCTTC | 283 |
| exon 21 | GGCCCAGGAGCAGCTACATTG/GAACTCCTTAGCCAGTGGGGACT | 339 |
| exon 22 | ATGTCAGACCATAGGCCATCTCT/TAGCCGTGTCATCAGGGTTC | 324 |

of no association specifies expected genotype class frequencies to be in Hardy-Weinberg equilibrium. Under the alternative hypothesis of recessive inheritance, affected dogs are all homozygous for one SNP allele, and all normal dogs either are heterozygotes or are homozygotes for the alternate allele. To discriminate between these hypotheses, we constructed an index based on the expected genotype frequencies under the alternate hypothesis and found the expected value and variance of the index under the null hypothesis. These were used to construct a *t*-test for each SNP, which produced nominal *P* that we converted to $-\log_{10}(P)$ values to facilitate the genome-wide association analysis plots in Figure 1. This analysis identified a single 4-Mb segment of CFA3 with strong associations between SNPs and PLL (Fig. 1A). This chromosomal region overlapped the PLL locus region previously mapped with DNA samples from Miniature Bull Terriers and Lancashire Heelers (Fig. 1B).[13] Inspection of the genotypes from individual Jack Russell Terriers revealed that 26 of the 28 affected dogs were homozygous at seven consecutive CFA3 SNPs from 43,292,397 to 44,045,545 bp on build 2.1 of the canine genome reference sequence (http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9615), whereas only 1 of 20 control dogs was homozygous for this 7-SNP haplotype. These SNPs were flanked by SNP markers that were heterozygous in some affected dogs, thus defining a 1.2-Mb target region from 42,932,098 to 44,147,699 bp on CFA3 containing 8 genes (Fig. 1C).

## Fine Mapping

Of the 768 genotyped SNPs located between 20,112,571 and 69,975,205 bp on CFA3, 593 SNPs were informative in 60 PLL-affected Miniature Bull Terriers and Lancashire Heelers and 29 members of these breeds with healthy eyes. The strongest associations were obtained for SNPs located between 41,227,703 and 49,013,128 bp (Fig. 1D). Within this region, 36 of the 38 PLL-affected Miniature Bull Terriers and 17 of the 22 PLL-affected Lancashire Heelers were homozygous for a 21-SNP haplotype from 43,175,187 to 43,790,361, whereas only one of the control dogs, a Lancashire Heeler, was homozygous for the entire haplotype. The haplotype was flanked by markers that were heterozygous in some affected dogs at 43,153,038 and

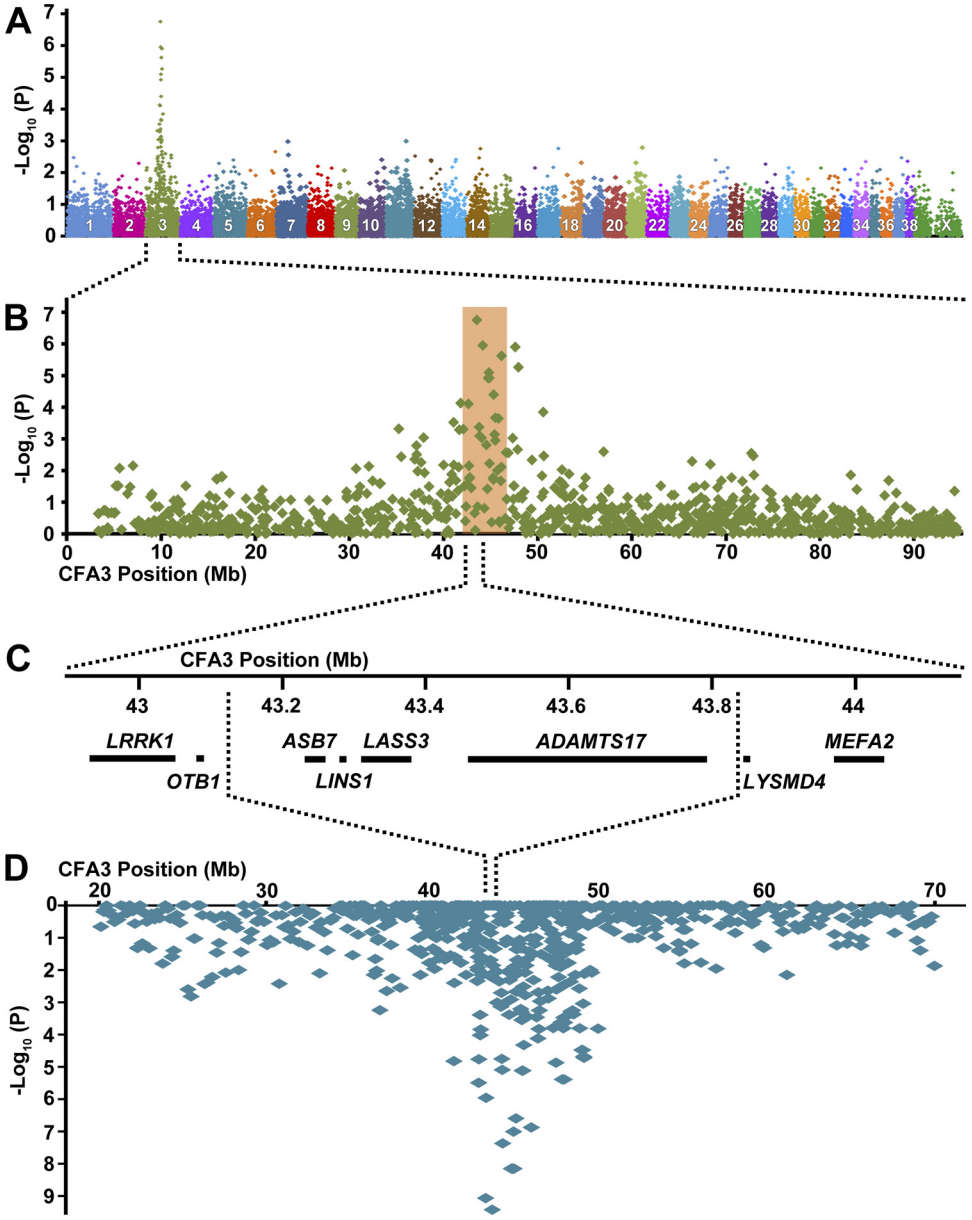


**Figure 1.** Summary of the results from mapping the PLL locus. (**A**) Genome-wide association analysis of case and control samples from Jack Russell Terriers in which $-\log_{10} P$ values were determined with a recessive model. (**B**) The $-\log_{10} P$ values for the CFA3 SNPs from (**A**) are plotted versus their CFA3 positions on an expanded horizontal axis. *Orange rectangle*: position of the PLL locus previously mapped with samples from Miniature Bull Terriers and Lancashire Heelers.[13] *Dotted lines*: beginning at 42.9 Mb and 44.2 Mb, these lines define a region of SNP homozygosity among samples from affected Jack Russell Terriers. (**C**) Plot of the chromosomal positions of the eight genes within the region of homozygosity of affected Jack Russell Terriers. *Dotted lines*: beginning at 43.1 Mb and 43.8 Mb, these lines define a region of SNP homozygosity among samples from affected Miniature Bull Terriers and Lancashire Heelers. (**D**) The $-\log_{10} P$ values for CFA3 SNPs, determined from Miniature Bull Terriers and Lancashire Heelers, are plotted versus CFA3 chromosomal position.

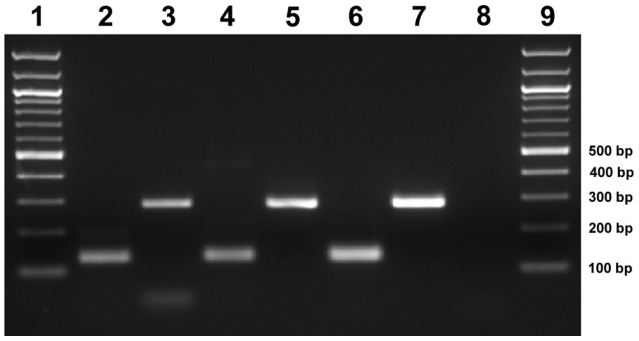


**FIGURE 2.** Agarose gel electrophoretograms containing RT-PCR amplicons produced with RNA from A/A homozygous PLL-affected dogs and G/G homozygous control dogs. A band at 284 bp was expected from the consecutive splicing of exon 9 to exon 10 to exon 11; a band at 133 bp was expected from the splicing of exon 9 to exon 11. Sources of RNA include lens tissue from an affected Jack Russell Terrier (*lane 2*), lens tissue from a control English Springer Spaniel (*lane 3*), ciliary body tissue from the same affected Jack Russell Terrier (*lane 4*), ciliary body tissue from the same control English Springer Spaniel (*lane 5*), cultured fibroblasts from a different affected Jack Russell Terrier (*lane 6*), cultured fibroblasts from a control Jack Russell Terrier (*lane 7*), and a no-template control (*lane 8*).

43,816,982 bp, thus refining the CFA3 target region to 664 kb, which contained four genes (Fig. 1C).

## Resequencing and Transcript Analysis

Among the four genes in the CFA3 target region, *ADAMTS17* was considered the best candidate because it encodes a protein structurally similar to that encoded by *ADAMTS4L*, a gene associated with autosomal recessive ectopia lentis.[7] Resequencing the 22 *ADAMTS17* coding exons from PLL-affected Jack Russell Terriers and control dogs revealed two sequence variants in the gene from affected dogs: a silent C→T transition at c.396 (numbering as in EMBL XM_545825.2) and a G→A transition at c.1473+1, which destroys the splice donor recognition site in intron 10. Because there are no potential cryptic splice donor sites in the vicinity, the G→A transition was expected to cause exon 10 skipping,[17] resulting in a frame shift and a premature termination codon. This was verified by RT-PCR, with primers designed against exons 9 and 11 and used to amplify *ADAMTS17* transcripts in total RNA from PLL-affected and control dogs. Agarose gel electrophoretograms of RT-PCR amplicons derived from G/G homozygous control dogs contained a single band at approximately 284 bp, as was expected from the splicing of consecutive exons (Fig. 2). Sequence analysis of this amplicon confirmed the respective splicing of exons 9 to 10 to 11 and also revealed that exon 11 was preceded by an alternative splice acceptor sites such that three base pairs from the 3′ end of intron 10 were included in about half the transcripts (Fig. 3). The RT-PCR amplification of RNA from the A/A homozygous dogs produced a single band at approximately 133 bp (Fig. 2). Sequence analysis confirmed that exon 9 was spliced to the alternate splice site acceptors that precede exon 11, causing the expected frame shift (Fig. 3). The bands produced with RNA from the PLL-affected dogs were nearly as bright as those produced from equivalent amounts of the control RNA, indicating that a readily detectable amount of the mutant transcript escaped nonsense-mediated mRNA decay.[18]

## Association between Genotype and Phenotype

DNA samples from 829 individual dogs were genotyped at *ADAMTS17:c.1473+1.* Table 2 shows the number of normal and PLL-affected dogs and the percentage of dogs affected for each breed and genotype. There was a strong association between PLL and the A/A genotype (Fisher's exact 2 × 3 test; $P = 2.9 \times 10^{-43}$ for the Miniature Bull Terriers, $P = 4.7 \times 10^{-57}$ for the Jack Russell Terriers, $P = 3.0 \times 10^{-16}$ for the Lancashire Heelers, and $P = 3.14 \times 10^{-120}$ for all tested dogs; Table 2). Nonetheless, 15 of 176 dogs with the A/A genotype had normal eyes when last examined. The average age for these 15 dogs was 2.4 years (range, 1–10 years). Of the 196 dogs with PLL, 161 were A/A homozygotes, 23 were A/G heterozygotes, and 12 were G/G homozygotes. Among the Jack Russell Terriers with the G/G genotype, the percentage that exhibited PLL was very similar to the percentage of A/G Jack Russell Terriers with PLL. Similarly, with the Lancashire Heelers, the percentage affected was similar for that with the G/G genotype and that with the A/G genotype. In contrast, PLL was five times more common among Miniature Bull Terriers with the A/G genotype than among Miniature Bull Terriers with the G/G genotype (Table 2).

## DISCUSSION

All SNPs with strong associations with PLL in Jack Russell Terriers were clustered in a region of CFA3 that overlapped the PLL locus previously mapped in Miniature Bull Terriers and Lancashire Heelers,[13] indicating that the disease was an allelic trait in all three breeds. Fine mapping led to the identification of *ADAMTS17* as the most credible candidate gene and to the discovery of a G→A transition at *ADAMTS17:c.1473+1* that destroys the exon 10 splice donor recognition site. RNA from PLL-affected dogs with the A/A genotype contained *ADAMTS17* transcripts with the skipping of exon 10, a frame shift, and a premature termination codon between the metalloprotease domain and the disintegrin-like domain. Homozygosity for the A allele was strongly associated with PLL in all three dog breeds. Taken together, these findings implicate the A allele at the beginning of intron 10 of *ADAMTS17* as the most common cause of canine PLL in the three breeds examined. Nonetheless, the 15 A/A homozygotes with normal eyes and the 35 PLL-affected dogs that were not A/A homozygotes (Table 2) deserve comment. All but one of the A/A homozygotes with normal eyes were younger than 6 years of age, whereas

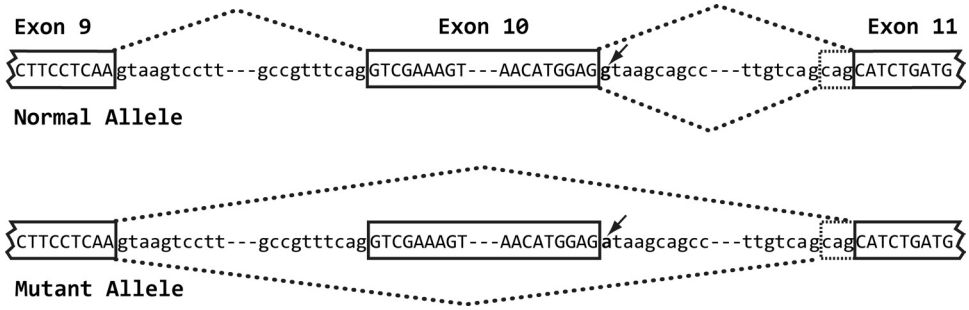


**FIGURE 3.** Exon splicing patterns of transcripts from normal and mutant *ADAMTS17* alleles. In mRNA from the normal allele, exon 9 is spliced to exon 10, which is spliced to each of the two alternative splice acceptor sites at the 5′ end of exon 11. In RNA from the mutant allele, exon 10 is skipped and exon 9 is spliced to the alternative exon 11 splice acceptor sites. *Arrows*: position of the transition at *ADAMTS17:c.1143+1*.

**TABLE 2.** Breeds, Genotypes, and Phenotypes for the 829 Dogs Studied

| | Breed | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Miniature Bull Terrier | | | Jack Russell Terrier | | | Lancashire Heeler | | | All Dogs | | |
| Genotype | A/A | A/G | G/G | A/A | A/G | G/G | A/A | A/G | G/G | A/A | A/G | G/G |
| PLL | 85 | 16 | 2 | 55 | 2 | 6 | 21 | 5 | 4 | 161 | 23 | 12 |
| Normal | 11 | 96 | 71 | 3 | 79 | 280 | 1 | 46 | 46 | 15 | 221 | 397 |
| Total | 96 | 112 | 73 | 58 | 81 | 286 | 22 | 51 | 50 | 176 | 244 | 409 |
| % PLL | 89 | 14 | 2.7 | 95 | 2.5 | 2.1 | 95 | 9.8 | 8.0 | 91 | 9.4 | 2.9 |

with PLL the initial lens luxation can occur in dogs as old as 10.[10] Many of these dogs are expected to develop PLL as they grow older.

Twelve of 409 (or 3%) of the G/G homozygous dogs had PLL. These dogs might have had one or more of the alternative forms of PLL with a divergent breed distribution and a distinct zonular histopathology, previously described by other investigators.[11,12] The true prevalence of non-*ADAMTS17*–associated PLL in these breeds is probably much lower than 3% because efforts were made to include as many PLL-affected dogs as possible in this study. If all the PLL in dogs with the A/G and G/G genotypes resulted from factors unrelated to *ADAMTS17*, the within-breed percentage of dogs affected with PLL should have been similar for each genotype. As indicated in Table 2, similar percentages of affected dogs were found for A/G and G/G Jack Russell Terriers (2.5% and 2.1%, respectively) and for A/G and G/G Lancashire Heelers (9.8% and 8.0%, respectively). In the Miniature Bull Terriers, however, the percentage of PLL-affected dogs was more than five times higher among dogs with the A/G genotype than among dogs with the G/G genotype (14% vs. 2.7%, respectively; Table 2). This suggests that compared with the G/G homozygotes, the A/G heterozygotes may be at increased risk for PLL and that this putative increased risk may depend on the breed or genetic background of the dog.

*ADAMTS17* is one of 19 known mammalian members of the ADAMTS family of genes that encode secreted metalloproteases that proteolytically modify extracellular structural proteins.[19,20] The products of ADAMTS genes are modular proteins composed of an N-terminal signal peptide, a prodomain, a metalloprotease domain, a disintegrin-like domain, a central thrombospondin type 1 domain, a cysteine-rich domain, and a spacer domain, followed by a variety of C-terminal structures that, in the case of ADAMTS17, consist of four thrombospondin repeats and a PLAC domain. The thrombospondin units are thought to facilitate the binding of ADAMTS proteins to components of the extracellular matrix.[20] Certain ADAMTS gene products are required for normal growth and for the normal development of organs such as the adrenal gland, kidney, uterus, and heart.[21–23] ADAMTS gene products have been implicated in the pathogenesis of important human diseases, including metastatic cancer and arthritis.[24,25] Mutations or allelic differences in a variety of ADAMTS genes have been associated with a diverse set of diseases or phenotypes, including Ehlers-Danlos syndrome type VIIC (OMIM 225410),[26] WMS,[5] osteoarthritis,[27] osteoporosis (OMIM 166710),[28] thrombotic thrombocytopenic purpura (OMIM 274150),[29] and abnormal pigment distribution.[30]

Morales et al.[15] recently reported WMS-like symptoms in patients from the Arabian Peninsula who were homozygous for 1 of 3 distinct truncating mutations in *ADAMTS17*. These patients shared the decreased stature and ocular abnormalities (lenticular myopia, ectopia lentis, spherophakia, and secondary glaucoma) characteristic of WMS patients; however, unlike WMS patients, they did not have brachydactyly, joint stiffness, or congenital heart abnormalities. The ocular pathology was attributed to a zonular fiber deficiency that allowed the lens to round up and impinge on the anterior chamber; nonetheless, broken or missing zonular fibers were not detected.[15]

Although the report of Morales et al.[15] may encourage future discoveries of additional WMS-like patients with *ADAMTS17* mutations, the current understanding of this newly recognized WMS-like disease phenotype is based on clinical findings from eight children or young adults (average age, 20.4 years; range, 10–36 years[15]) who might have been in the early stages of a lifelong and potentially progressive degenerative disease. In contrast, PLL has been recognized for more than 75 years,[8] has diminished the eyesight of hundreds or thousands of members of several dog breeds, and has been the subject of several scientific reports and reviews.[8–14,31–34] Results obtained from past and future studies of PLL may contribute to our understanding of the WMS-like disease, and vice versa.

None of the previous reports about PLL indicate that the skeletal sizes of affected dogs are any different from those of unaffected members of the same breed.[8–14,31–34] Nonetheless, if dogs with preclinical PLL are slightly smaller than their littermates, selection for decreased size might have inadvertently contributed to the high frequencies of the A allele in these small-dog breeds. Future comparisons of anatomic dimensions from adult littermates with different *ADAMTS17* genotypes could be used to determine whether PLL has both skeletal and ocular components, as is reported to occur in the WMS-like disease.[15] The ocular manifestations of truncating *ADAMTS17* mutations appear to be less severe in human patients than in dogs with PLL. All eight human patients had subluxated lenses and distorted but unbroken zonular fibers,[15] whereas, PLL is usually diagnosed after zonular fiber breakage and full luxation of the initial lens.[9–11] Nonetheless, with PLL, a usually undetected period of subluxation can precede the initial luxation.[9–11] The eight young human patients with WMS-like disease may be at a comparable early subluxation stage of their disease. It remains to be seen whether their zonules will eventually detach, leading to full lens luxations as typically occurs in canine PLL.

The WMS-like disease described by Morales et al.[15] appears to be a recessive trait. Most, but not all, earlier reports indicate that PLL is also recessive[10,14,31–34]; however, the overabundance of PLL-affected heterozygous Miniature Bull Terriers (Table 2) opens the possibility that individuals with a heterozygous *ADAMTS17* mutation may be at an increased risk for disease. This could be attributed to haploinsufficiency, or, alternatively, the gene product from the mutant allele may have a dominant negative effect on zonular development or maintenance. In spite of the premature termination codon in the transcript from the mutant allele, the comparable brightness of the wild-type and mutant bands in the RT-PCR gel (Fig. 3) indicates that a substantial amount of the mutant transcript escapes nonsense-mediated mRNA decay. Morales et al.[15] also noted that all three truncating *ADAMTS17* alleles of patients with WMS-like disease produced stable transcripts.

Cells in the nonpigmented or inner layer epithelium of the ciliary body produce and secrete fibrillin 1, which is the major structural component of the zonules.[35,36] Fibrillin 1 transcription decreases with age but never completely stops,[36] suggesting that there is continuous but low-level replacement and remodeling of the zonular fibrillin 1. The association of zonular fiber deficiency with a mutation likely to eliminate or alter ADAMTS17 function suggests that normally functioning ADAMTS17 is required for the development or maintenance of normal zonular fibers. Dagoneau et al.[5] proposed that fibrillin 1 might be a substrate for ADAMTS10. Our results suggest that fibrillin 1 might also be a substrate for ADAMTS17. Similar disease processes may underlie both PLL and the adult-onset recessive human ectopia lentis associated with mutations in *ADAMTSL4*.[7] Like the ADAMTS genes, the ADAMTSL genes encode proteins with thrombospondin type 1 domains, cysteine-rich domains, spacer domains, and C-terminal structures with thrombospondin repeats and PLAC domains; however, the ADAMTSL proteins lack the metalloprotease and disintegrin-like domains characteristic of the ADAMTS proteins.[20] Hirohata et al.[37] have suggested that the function of the ADAMTSL proteins is to regulate ADAMTS protein activities. It appears that ADAMTSL4 may regulate ADAMTS17 activity because mutations in the genes that encode either protein can result in zonular fiber deficiency.

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