Evaluation of ADAMTS17 in Chinese Shar-Pei with primary open-angle glaucoma, primary lens luxation, or both

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OBJECTIVE
To evaluate the coding regions of ADAMTS17 for potential mutations in Chinese Shar-Pei with a diagnosis of primary open-angle glaucoma (POAG), primary lens luxation (PLL), or both.

ANIMALS
63 Shar-Pei and 96 dogs of other breeds.

PROCEDURES
ADAMTS17 exon resequencing was performed on buccal mucosal DNA from 10 Shar-Pei with a diagnosis of POAG, PLL, or both (affected dogs). A candidate causal variant sequence was identified, and additional dogs (53 Shar-Pei [11 affected and 42 unaffected] and 95 dogs of other breeds) were genotyped for the variant sequence by amplified fragment length polymorphism analysis. Total RNA was extracted from ocular tissues of 1 affected Shar-Pei and 1 ophthalmologically normal Golden Retriever: ADAMTS17 cDNA was reverse transcribed and sequenced, and ADAMTS17 expression was evaluated by quantitative reverse-transcription PCR assay.

RESULTS
All affected Shar-Pei were homozygous for a 6-bp deletion in exon 22 of ADAMTS17 predicted to affect the resultant protein. All unaffected Shar-Pei were heterozygous or homozygous for the wild-type allele. The variant sequence was significantly associated with affected status (diagnosis of POAG, PLL, or both). All dogs of other breeds were homozygous for the wild-type allele. The cDNA sequencing confirmed presence of the expected variant mRNA sequence in ocular tissue from the affected dog only. Gene expression analysis revealed a 4.24-fold decrease in the expression of ADAMTS17 in ocular tissue from the affected dog.

CONCLUSIONS AND CLINICAL RELEVANCE
Results supported that the phenotype (diagnosis of POAG, PLL, or both) is an autosomal recessive trait in Shar-Pei significantly associated with the identified mutation in ADAMTS17. (Am J Vet Res 2018;79:98–106)

Primary open-angle glaucoma and PLL have been reported to occur as autosomal recessive traits in several dog breeds.1–7 The development of POAG, an optic neuropathy associated with increased intraocular pressure in the presence of an apparently normal iridocorneal angle, has been reported in Beagle, Norwegian Elkhound, Basset Hound, Basset Fauve de Bretagne, and Petit Basset Griffon Vendeeen breeds.1,5,6,8,9 Primary lens luxation describes a spontaneous dislocation of the crystalline lens from the hyaloid fossa of the eye as a result of breakdown of the lens zonules and is mainly found in terrier breeds.3 Primary glaucoma and PLL have both been reported in Chinese Shar-Pei, but how these 2 breed-related diseases interrelate remains to be defined.4,7,10,11 Primary glaucoma has not been well characterized in Shar-Pei; anecdotally, the condition appears to be of the open-angle rather than closed-angle form.

To the authors’ knowledge, all forms of canine inherited POAG and PLL for which mutations have been identified to date are associated with independent homozygous mutations in ADAMTS10 or in ADAMTS17, which are genes known to be important in physiologic and pathologic ocular processes. Two mutations in ADAMTS10 and 3 mutations in ADAMTS17 have been reported to be associated with POAG, and 1 mutation in ADAMTS17 has been reported to be associated with PLL, in various canine breeds.2–6,12,13 These 2 genes are also more generally implicated in connective tissue disorders, often being associated with > 1 phenotype in affected individuals. In Beagles, the same ADAMTS10 mutation associated with POAG is associated with reduced scleral rigidity, and

ABBREVIATIONS

<table>
<thead>
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<tr>
<td>ADAMTS17</td>
<td>Amplified fragment length polymorphism</td>
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<tr>
<td>ADAMTS10</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>Cycle threshold</td>
</tr>
<tr>
<td>dNTP</td>
<td>Deoxynucleotide triphosphate</td>
</tr>
<tr>
<td>PLL</td>
<td>Primary lens luxation</td>
</tr>
<tr>
<td>POAG</td>
<td>Primary open-angle glaucoma</td>
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<tr>
<td>qRT-PCR</td>
<td>Quantitative reverse-transcription PCR</td>
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in people, multiple ADAMTS17 mutations are each associated with multiple ocular phenotypes including ectopia lentis, spherophakia, myopia, and glaucoma in affected individuals along with systemic abnormalities including joint stiffness, brachydactyly, short stature, and cardiac abnormalities.12,14–20

The purpose of the study reported here was to investigate ADAMTS17 as a candidate gene for POAG and PLL in Shar-Pei. A secondary aim was to develop a DNA test that could be used to identify genetic variants significantly associated with either or both diseases in this breed.

Materials and Methods

Dogs, records review, and sample collection

Electronic records of the Animal Health Trust Canine Genetics Research Department sample database were searched for details of submitted DNA samples (buccal mucosal swab specimens) obtained from Shar-Pei of any age and either sex in which POAG, PLL, or both was diagnosed by a certified veterinary ophthalmologist. These dogs were categorized as affected Shar-Pei. Dogs with other concomitant ocular conditions were eligible for inclusion. Clinical notes in the database were reviewed, and age, sex, diagnoses, gonioscopic appearance of iridocorneal angle, and any other reported ocular anomalies were recorded.

Shar-Pei ≥ 72 months of age that underwent ophthalmic examination by 1 board-certified veterinary ophthalmologist (JACO) between November 8, 2016, and November 23, 2016, and were found to be free of clinical signs of POAG and PLL were enrolled in the study as unaffected Shar-Pei. The examination included slit-lamp biomicroscopy,2 direct3 and indirect4 ophthalmoscopy, and rebound tonometry.4 Buccal mucosal swab specimens were collected from each dog. The minimum age of 72 months was selected because genetically affected dogs would be expected to show clinical signs of POAG, PLL, or both by this age.6 Age and sex of unaffected Shar-Pei were recorded.

Archived DNA samples (extracted from buccal mucosal swabs) from dogs of breeds other than Shar-Pei were used to assess whether any identified potential causal variant sequences were present in the wider canine population. No specific inclusion criteria were employed for these dogs; none had received ophthalmic examinations, and the number of samples used was determined by convenience alone.

Ocular tissue was obtained from 2 dogs. One of the dogs was an 8-year-old Shar-Pei in the affected group that had a diagnosis of POAG and underwent unilateral enucleation on welfare grounds. The other dog was a 12-year-old Golden Retriever that had been humanely euthanized for reasons unrelated to the study; prior to euthanasia, this dog underwent a complete ophthalmic examination to rule out the presence of POAG, PLL, or other suspected inherited ocular disorders. Enucleated globes from the affected Shar-Pei and the ophthalmologically normal Golden Retriever were used for confirmatory genetic analysis.

All dogs in the study were client-owned pets, and ophthalmic examination and buccal mucosal swabbing were performed after informed owner consent was obtained in writing. All experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic and Vision Research and approved by the Animal Health Trust’s Research and Ethical Approval Committee.

Buccal mucosal swab samples from individual dogs were placed in 2-mL polypropylene microcentrifuge tubes and stored at −20°C for up to 9 years until testing. The DNA was extracted from swabs with a commercially available kit5 used in accordance with the manufacturer’s instructions. The enucleated eyes were transected along the sagittal plane, immersed in 30 mL of RNA stabilizing solution,6 and stored at −80°C for up to 2 years until use. The iridocorneal angle tissues included ciliary body, iris, trabecular meshwork, and sclera and were isolated by microsurgical dissection with the aid of an operating microscope7; RNA was extracted immediately prior to use with a commercially available kit8 used according to the manufacturer’s instructions.

ADAMTS10 and ADAMTS17 candidate variant genotyping

An initial genetic investigation was performed on the first 10 samples from affected Shar-Pei received by our laboratory. These were genotyped for all 6 previously described canine POAG-associated and PLL-associated genetic mutations.2,3,5,6,12 Genotyping was performed by Sanger sequencing methodology, allelic discrimination, or AFLP, the choice of which was dependent on the nature of the mutation that was tested for (Appendix 1).

ADAMTS17 amplification and resequencing

Primer pairs were designed21,22 to amplify the coding sequence and flanking splice sites for all 23 canine ADAMTS17 exons by use of gene sequences derived from the Ensembl genome browser23 (Appendix 2). The PCR assays were carried out with 12 µL reaction mixtures consisting of 0.6 U of rapid-start DNA Taq polymerase,4 1X PCR buffer,3 200 µM dNTP mix,10 0.83 µM forward and reverse primers,10 and 10 ng of template genomic DNA. The thermal cycling program used was as follows: initial denaturation at 95°C for 10 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at an optimized temperature (56° to 58°C, determined by online software8) for 30 seconds, and extension at 72°C for 1 minute; and a final extension phase at 72°C for 10 minutes. Prior to sequencing, agarose gel electrophoresis was performed on PCR products for each primer pair to ensure adequate amplification. Exons 1 and 2 failed to amplify by this method.
Next-generation sequencing of the remaining ADAMTS17 exons was performed after PCR assay amplification of genomic DNA for the initial 10 samples from affected Shar-Pei. Multiplex PCR assays were carried out with 17-µL reaction mixtures consisting of 0.09 U of rapid-start DNA Taq polymerase, 21X PCR buffer, 200 µM dNTP mix, 0.2 µM each of forward and reverse primers, and 10 ng of template genomic DNA. The thermal cycling program was as follows: 95°C for 5 minutes; 30 cycles of 95°C for 30 seconds, annealing at an optimized temperature for 60 seconds, and 72°C for 1 minute; and 72°C for 5 minutes. Libraries for ADAMTS17 sequencing were prepared through the use of a commercially available kit. Sequencing of DNA from the initial 10 affected dogs was performed on a benchtop sequencing platform, generating a dataset of 75-bp paired-end reads.

Sanger sequencing was performed for exons that failed to amplify by initial PCR assay as assessed with agarose gel electrophoresis (exons 1 and 2) or that could not be sequenced with the benchtop analyzer following multiplex PCR assay (exon 16). Sanger sequencing was performed following PCR amplification as described. An additive to aid amplification of GC-rich regions (1X; 2.40 µL) was included for exons 1 and 2. The same primers used for initial PCR amplification were also used for the subsequent Sanger sequencing reactions, which were performed with forward and reverse primers used separately. Sanger sequencing reactions were performed with a commercially available kit. The 6-µL reaction mixtures consisted of 0.2X reaction mix, 1.7X sequencing buffer, and 0.27 µM forward or reverse primer and were prepared according to the manufacturer’s instructions. The thermal cycling program was 96°C for 30 seconds, followed by 45 cycles of 92°C for 4 seconds, 55°C for 4 seconds, and 60°C for 110 seconds. Sequencing products were separated on a benchtop genetic analyzer. Next-generation sequencing results were also confirmed for these 10 affected dogs by Sanger sequencing.

Next-generation output read alignments were examined with an open-source software program, and the data were manually browsed for variants. Sanger sequencing results were analyzed with a sequence handling and analysis software program.

For any variant predicted to alter protein sequence, the level of conservation of wild-type protein sequence among selected species was visually assessed. To enable this, the relevant regions of the ADAMTS17 protein sequence for 44 different species (including Canis lupus familiaris) were downloaded from a genome browser and aligned by use of a multiple sequence alignment tool before visual inspection for conservation of the relevant amino acids.

### Genotyping for the ADAMTS17 candidate causal variant

Genotyping for a candidate causal variant identified in exon 22 by resequencing of ADAMTS17 was performed with an AFLP approach for all dogs included in the study. Primer pairs were designed to amplify the candidate causal variant with gene sequences derived from the University of California-Santa Cruz bioinformatics site. The primers selected were as follows: a tailored forward primer (ie, a primer including an added, nonspecific utility sequence), 5′-GCAGCAAAATTAGGCCTCTTGTCCGACTTAT3′; a reverse primer, 5′-TTGTGTAGTCAGGACCTCTCT-3′; and a third fluorescence (FAM)-labeled tail primed, 5′-FAM-TGACGGGCGACAAATG-3′. The expected amplicon size was 287 bp. Amplification was carried out by PCR assay. Reactions (12 µL) consisted of 0.5 U of rapid-start DNA Taq polymerase, 1X PCR buffer, 200 µM dNTP mix, 0.17 µM forward primer, 0.42 µM of reverse primer, 0.5 µM of the fluorescence-labeled primer, and 10 ng of template genomic DNA. The thermal cycling program was 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds; and 72°C for 30 minutes. Dogs were genotyped for the candidate variant by capillary electrophoresis of 5′-FAM-labeled PCR products on genetic analyzers, and output data were scored by use of a genotyping software package. On the basis of results for the entire genotyping data set, a P value for association of the candidate variant with affected status (ie, presence of POAG, PLL, or both) was calculated by the Fisher exact test through the use of an open-source whole genome association analysis tool with a value of P < 0.05 accepted as significant.

### Synthesis and sequencing of ADAMTS17 cDNA

To confirm the candidate causal variant was present in coding genomic ADAMTS17 DNA and that it affected mRNA transcription or sequence, cDNA was reverse-transcribed from the iridocorneal angle tissue RNA obtained from 1 affected Shar-Pei and 1 ophthalmologically normal Golden Retriever and then sequenced. The cDNA was synthesized with a reverse transcription kit used in accordance with the manufacturer’s instructions. A pair of primers spanning the potential mutation identified by resequencing was designed in accordance with cDNA sequences derived from the genome browser database. Primer selection was as follows: forward, 5′-CGAGGACTATTCAGGCTGCTA-3′; reverse, 5′-TCTTGTATCTAGGACCTCTCT-3′. The expected amplicon size for the control sample cDNA was 194 bp. Sanger sequencing of the target cDNA region and analysis of results was performed as described for genomic DNA, with an annealing temperature of 60°C.

### ADAMTS17 expression analysis

To investigate the potential effect of the candidate causal variant on the level of ADAMTS17 gene expression, relative quantification was performed by qRT-PCR assay. Assays were designed for the target ADAMTS17 gene and for a ubiquitously ex...
pressed housekeeping gene (TBP) for comparison. For ADAMTS17, the forward primer was 5′-GGTCTCACATTTGGGCTTTACCC-3′, and the reverse primer was 5′-CTTACGACTCTGACTGAG-3′; a 5′FAM-labeled probe with the sequence ATGCCATTGCTGGGC was used. For TBP, the forward primer was 5′-AGGCAGGGAAAAATGACCAGAG-3′, the reverse primer was 5′-GGGACATCTACATACAGTCT-3′, and the probe was 5′-TCAAGATTCAAGAATGTTGG-3′. The qRT-PCR assay was carried out in triplicate for iridocorneal angle tissue cDNA from the affected Shar-Pei and from the ophthalmologically normal Golden Retriever (used as a control sample). Mean Ct values were calculated from the triplicate samples for ADAMTS17 and TBP from both dogs. The difference between ADAMTS17 and TBP Ct values (∆Ct) was calculated for each sample. The difference between the mean ∆Ct values (derived from 3 samples) for the 2 dogs (∆∆Ct) was then calculated. From the ∆∆Ct values, the fold change in ADAMTS17 expression in tissue from the affected dog versus that in the control sample was calculated (2−∆∆Ct), and a 2-tailed t test was performed with a value of P < 0.05 considered significant.

Results

Review of the sample database identified buccal mucosal swab specimens collected from 21 Shar-Pei affected with PLL, POAG, or both. The amount of clinical information recorded for dogs from which samples were obtained varied substantially (Supplementary Table S1, available at avmajournals.avma.org/doi/suppl/10.2460/ajvr.79.1.98). However, 10 dogs had a diagnosis of PLL with secondary glaucoma, 9 had a diagnosis of POAG with secondary buphthalmos and lens subluxation, and 1 had a diagnosis of POAG in one eye and PLL in the other eye. The record for 1 dog indicated that the veterinary ophthalmologist could not decide between a diagnosis of POAG and PLL. The unaffected Shar-Pei group comprised 26 females (11 sexually intact and 15 spayed) and 15 males (10 sexually intact and 5 neutered). The mean ± SD age of these dogs at the time of examination was 93.5 ± 24.7 months.

The unaffected Shar-Pei group included 42 dogs (26 females [11 sexually intact and 15 spayed] and 15 males [10 sexually intact and 5 neutered]). The mean ± SD age of these dogs at the time of examination was 93.5 ± 24.7 months.

A total of 95 dogs of breeds other than Shar-Pei were selected for genotyping, including 31 breeds represented by ≤ 5 dogs each. This group comprised 49 males and 46 females; mean ± SD age was 64.1 ± 50.3 months.

Initial ADAMTS10 and ADAMTS17 candidate variant genotyping

The initial genetic investigation of samples from 10 affected Shar-Pei revealed that all were homozygous for the wild-type alleles at the chromosomal locations of both previously described canine POAG-associated variants for ADAMTS10.2,12 All 10 dogs were also homozygous for the wild-type alleles at chromosomal locations for the 4 previously described PLL-associated4 and POAG-associated5,6 ADAMTS17 variants.

ADAMTS17 resequencing and genotyping by AFLP

Visual scanning of the ADAMTS17 sequence read alignments revealed only 1 variant that segregated for potential mutations associated with POAG, PLL, or both in Chinese Shar-Pei. Both images depict the site of a 6-bp deletion in exon 22 of ADAMTS17; Shar-Pei that had a diagnosis of PLL, POAG, or both (affected dogs) were homozygous for the wild-type canine sequence; horizontal gray bars represent consensuses with the wild-type nucleotide sequence, white bars indicate deleted nucleotides, and vertical black marks depict the position of nucleotide centering at the time of screen capture. The location of the deletion was chromosome 3: bases 40,935,387 through 40,935,393 (determined by use of Ensembl CanFam3.1). In panel A, DNA sequence from an affected Shar-Pei is compared with the wild-type canine sequence: horizontal gray bars represent consensus with the wild-type nucleotide sequence, white bars indicate deleted nucleotide sequence, and vertical black marks depict the position of nucleotide centering at the time of screen capture. The location of the deletion was chromosome 3: bases 40,935,387 through 40,935,393 (determined by use of Ensembl CanFam3.1). In panel B, the red box delineates the same region in DNA from an affected Shar-Pei and an ophthalmologically normal (unaffected) Shar-Pei in the study.

![Figure 1](image1.png)

**Figure 1**—Representative screen capture images of output from a DNA sequence viewing program used after next-generation sequencing (A) and electropherograms produced by Sanger sequencing (B) in a study to evaluate the coding regions of ADAMTS17 for potential mutations associated with POAG, PLL, or both in Chinese Shar-Pei. Both images depict the site of a 6-bp deletion in exon 22 of ADAMTS17; Shar-Pei that had a diagnosis of PLL, POAG, or both (affected dogs) were homozygous for the wild-type canine sequence; horizontal gray bars represent consensus with the wild-type nucleotide sequence, white bars indicate deleted nucleotide sequence, and vertical black marks depict the position of nucleotide centering at the time of screen capture. The location of the deletion was chromosome 3: bases 40,935,387 through 40,935,393 (determined by use of Ensembl CanFam3.1). In panel A, DNA sequence from an affected Shar-Pei is compared with the wild-type canine sequence: horizontal gray bars represent consensus with the wild-type nucleotide sequence, white bars indicate deleted nucleotide sequence, and vertical black marks depict the position of nucleotide centering at the time of screen capture. The location of the deletion was chromosome 3: bases 40,935,387 through 40,935,393 (determined by use of Ensembl CanFam3.1). In panel B, the red box delineates the same region in DNA from an affected Shar-Pei and an ophthalmologically normal (unaffected) Shar-Pei in the study.

![Figure 2](image2.png)

**Figure 2**—Depiction of DNA sequence for the ADAMTS17 region of interest identified in Figure 1 and corresponding predicted protein sequence. Sequence is shown for representative samples from an unaffected (top) and an affected (bottom) Shar-Pei. The box indicates the bases present in the unaffected dog and absent in the affected dog, and the arrow indicates the site of the deletion, which was predicted to result in the loss of 2 valine residues in the resultant protein sequence.
gated with disease in the first 10 affected Shar-Pei tested. The variant was identified by next-generation sequencing as a homozygous 6-bp deletion in exon 22 (Ensembl CanFam 3.1 chromosome 3: bases 40,935,387 through 40,935,393), and this result was confirmed by Sanger sequencing (Figure 1). The variant represented an in-frame deletion predicted to result in the loss of 2 valine residues in the ancillary domain of the resultant protein (Figure 2). These residues were found to be highly conserved in this domain across species (Figure 3).

The AFLP genotyping for the remaining 11 affected Shar-Pei revealed that all were homozygous for the same variant. Genotyping of the 42 unaffected Shar-Pei revealed that all were either homozygous (n = 18) or heterozygous (24) for the wild-type allele. The association between the deletion and affected status (ie, diagnosis of POAG, PLL, or both) calculated on the basis of results for the entire genotyping dataset (n = 63 Shar-Pei) was significant (\( P = 3.79 \times 10^{-14} \)). Finally, the 95 dogs of breeds other than Shar-Pei that were genotyped by the same method were found to be homozygous for the wild-type allele.

**ADAMTS17 cDNA sequencing and gene expression analysis**

Examination of sequencing alignments confirmed that the identified 6-bp deletion was present in ADAMTS17 cDNA derived from iridocorneal angle tissue of the affected Shar-Pei and absent in that of the ophthalmologically normal Golden Retriever. This confirmed that the deletion occurred in coding genomic DNA and that the variant sequence was transcribed into mRNA.

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**Figure 3**—Comparisons of protein sequence for a selected region of the ancillary domain of ADAMTS17 for 44 species. The 2 valine residues (delineated in red) predicted to be lost as a result of the 6-bp deletion identified in the ADAMTS17 gene of affected Shar-Pei were highly conserved among species.

- **Platyfish**
- **Amazon Molly**
- **Tilapia**
- **Spotted Gar**
- **Zebrafish**
- **Stickleback**
- **Cod**
- **Anole Lizard**
- **Coelacanth**
- **Xenopus**
- **Opossum**
- **Tasmanian Devil**
- **Turkey**
- **Chinese Softshell Turtle**
- **Zebra Finch**
- **Flycatcher**
- **Duck**
- **Chicken**
- **Lemoy**
- **Sloth**
- **Hedgehog**
- **Hyrax**
- **Tarsier**
- **Microbat**
- **Megabat**
- **Kangaroo Rat**
- **Lesser Hedgehog Tenrec**
- **Armadillo**
- **Rat**
- **Mouse**
- **Sheep**
- **Elephant**
- **Rabbit**
- **Horse**
- **Ferret**
- **Panda**
- **Dog**
- **Dolphin**
- **Guinea Pig**
- **Marmoset**
- **Cow**
- **Olive Baboon**
- **Bushbaby**

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Mean $\Delta C_T$ for ADAMTS17 relative to the housekeeping gene TBP was 3.76 for the sample from the affected Shar-Pei and 1.68 for that from the Golden Retriever, resulting in a $\Delta \Delta C_T$ of 2.08. This corresponded to a 4.24-fold decrease in ADAMTS17 expression in the ocular tissue from the affected dog ($P < 0.001$).

**Discussion**

Primary glaucoma and PLL have previously been reported in Chinese Shar-Pei, but to the authors’ knowledge, there has been no evidence previously reported in the literature to indicate whether these 2 conditions are interrelated.7,11 We selected ADAMTS17 as a candidate gene for investigation in Shar-Pei with POAG, PLL, or both (ie, affected dogs) because this gene has previously been associated with POAG and with PLL in other dog breeds. Our results showed that all 21 affected Shar-Pei in the study were homozygous for a 6-bp deletion in exon 22 of ADAMTS17, and all 42 unaffected Shar-Pei were either homozygous or heterozygous for the wild-type allele. This provided evidence that the phenotype (POAG, PLL, or both) is inherited as an autosomal recessive trait in Shar-Pei, which is in line with all other previously published ADAMTS17 mutations associated with POAG or PLL in other dog breeds.3–8

It remains unclear whether this single mutation could cause both PLL and POAG. In the authors’ opinion, the most likely explanation for both conditions to have been diagnosed in dogs with this genotype is that it can be difficult to differentiate between them. Either POAG or PLL can cause increased intraocular pressure and lens subluxation, with an apparently normal globe size in some dogs on initial clinical examination.26 There was evidence for this in the present study; 1 ophthalmologist was unable to decide between a diagnosis of POAG and PLL in an affected dog, and another ophthalmologist diagnosed POAG in one eye and PLL in the other eye of an affected dog. However, in the latter dog, buphthalmos of both eyes was detected, and the authors believe that the observed posterior lens luxation could have been attributable to secondary lens zonule rupture, which would be more consistent with a primary diagnosis of POAG than with PLL. Furthermore, after the initial genetic investigation, an author (JACO) had the opportunity to examine 2 of the dogs that had a previous diagnosis of PLL; in this author’s opinion, both dogs were affected by POAG with secondary lens instability. Both eyes in each dog were subjectively buphthalmic and had iridodonesis without anterior lens displacement. Detailed gonioscopic examination was only possible for 1 eye of 1 dog at that time and revealed an open iridocorneal angle. Thus, although it is possible that both phenotypes exist and are associated with the identified ADAMTS17 variant in this breed, it is also possible that POAG is the only primary disease which, as described in other breeds, is often seen as a chronic disease with secondary buphthalmos and resultant lens subluxation or luxation as a result of lens zonule stretching.5,6 In terriers with PLL, the lens is usually acutely displaced into the anterior chamber, causing glaucoma through obstruction of the pupil, the iridocorneal angle, or both.60 In Shar-Pei, however, the most common clinical sign of PLL has been reported to be iridodonesis, with some dogs also having buphthalmos.7 This is not consistent with the classic clinical signs of PLL and is more consistent with a chronic disease process.4,6 However, we can only speculate as to whether confusion between these conditions commonly occurs, and further characterization of these conditions is warranted.

An alternative, and perhaps less likely, explanation is that POAG and PLL are 2 distinct phenotypes in Shar-Pei that are genetically indistinguishable. In this scenario, the ADAMTS17 variant would have a deleterious effect on the lens zonules and on aqueous outflow pathways of the eye. It has been shown that individual mutations in ADAMTS17 are associated with multiple ocular phenotypes that exist simultaneously in people, including ectopia lentis (equivalent to PLL in dogs) and glaucoma, and these conditions likely relate to the function of the protein the gene encodes.17–20 Until POAG and PLL in Shar-Pei can be further characterized, we suggest they might be considered together as POAG-PLL in this breed.

Proteins of the ADAMTS family are secreted enzymes that have important roles in extracellular matrix degradation and turnover and are involved in the assembly, stability, and anchorage of microfibrils that are known to be essential components of mammalian aqueous humor outflow pathways and lens zonules.27–31 The effect that the mutation identified in the present study has on protein function remains unknown. The 6-bp deletion in exon 22 is an in-frame deletion predicted to result in the loss of 2 valine residues in the ancillary domain of the resultant protein. This domain is thought to determine substrate specificity and binding to the extracellular matrix.30 In people, mutations affecting this domain have been shown to be strongly associated with ectopia lentis and other ocular phenotypes.17,29,32 Our qRT-PCR assay data were also consistent with reduced ADAMTS17 expression in ocular tissue from an affected dog. Thus, it is possible that the mutation has deleterious qualitative (ie, functional) and quantitative effects on the protein produced. The significant association between the variant sequence and affected status (diagnosis of POAG, PLL, or both) in this study and the finding that these residues are highly conserved in the ancillary domain of the ADAMTS17 protein across species support that the deletion is causative of POAG-PLL in Shar-Pei.

Although detailed phenotypic characterization was beyond the scope of the present study, the AFLP genotyping used represents a test for a genetic mutation significantly associated with POAG-PLL in Shar-Pei, and the existence of this test can enable longitudinal clinical assessment of Shar-Pei that are homozygous for the variant allele, and thus enhance our
understanding of the pathogenesis of these ocular abnormalities in the breed. Monitoring of genetically affected dogs for early signs of disease could also allow earlier interventions, which may improve the prognosis for vision; however, at this time, the most appropriate treatment remains unknown. The DNA test may also enable breeders to eliminate the variant allele, and potentially the disease, from the Shar-Pei breed over time, while allowing judicious breeding of carriers that have other, desirable genetic characteristics that are advantageous to retain.

Acknowledgments

Funded by the Dog Welfare Trust and Dogs Trust. Funding sources did not have any involvement in the study design, data analysis and interpretation, or writing and publication of the manuscript.

The authors declare that there were no conflicts of interest.

Footnotes

4. TonoVet. Care Finland, Vantaa, Finland.
5. QiAmp DNA blood Midi Kit, Qiagen, Manchester, England.
8. Qiagen RNeasy Midi Kit, Qiagen, Manchester, England.
12. Qiagen PCR buffer, Qiagen, Manchester, England.
17. Q solution, Qiagen, Manchester, England.
19. ABI 3130xl, Applied Biosystems, Forest City, Calif.
25. Quantitect Reverse Transcription Kit, Qiagen, Manchester, England.

References


**Appendix 1**

Previously described mutations in canine *ADAMTS10* and *ADAMTS17*, the breeds and ocular phenotypes of dogs in which they were identified, and the methods used to test for their presence in DNA samples from 10 of 21 Chinese Shar-Pei with a diagnosis of POAG, PLL, or both (affected dogs) in a study to evaluate the coding regions of *ADAMTS17* for potential mutations associated with these conditions.

<table>
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<tr>
<th>Breed</th>
<th>Ocular phenotype</th>
<th>Gene</th>
<th>Exon or intron</th>
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<th>Nature of mutation</th>
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<td>Basset Fauve de Bretagne</td>
<td>POAG</td>
<td>ADAMTS17</td>
<td>Exon 11</td>
<td>c.1552 G &gt; A</td>
<td>Missense</td>
<td>Allelic discrimination</td>
<td>Oliver et al 6</td>
</tr>
<tr>
<td>Petit Basset Griffon Vendeen</td>
<td>POAG</td>
<td>ADAMTS17</td>
<td>Intron 12</td>
<td>Large-scale rearrangement</td>
<td>Inversion</td>
<td>Allelic discrimination</td>
<td>Forman et al 5</td>
</tr>
</tbody>
</table>

G > A = G-to-A transition.
## Appendix 2

Primer pairs used for ADAMTS17 resequencing.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer sequences (5′–3′)</th>
<th>Amplicon size (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>Forward: TGATTTACACGTTGGTTTGA</td>
<td>436</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Reverse: AGCTGGAATCTGAGTGCGAGAGA</td>
<td></td>
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</tr>
<tr>
<td>Exon 2 (5′ site)*</td>
<td>Forward: GCTGGACCGCTCTCGGTCGGA</td>
<td>285</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Reverse: CCGCTCTCCACCTCCCTCC</td>
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<td></td>
</tr>
<tr>
<td>Exon 2 (3′ site)*</td>
<td>Forward: CCCCCCCGACCCGGAAAGCG</td>
<td>334</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Reverse: GCGACTAAGGCGGCGGAGGCGGAN</td>
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<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>Forward: ACATGAAGGACCGCGAGGACGA</td>
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</tr>
<tr>
<td></td>
<td>Reverse: AGGGCCGCTGCTACACATGAAATG</td>
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<td></td>
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<tr>
<td>Exon 4</td>
<td>Forward: TTTCACTGCTGCGCCCTGC</td>
<td>591</td>
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</tr>
<tr>
<td></td>
<td>Reverse: GACCCCGACACTGAAACTACA</td>
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</tr>
<tr>
<td>Exon 5</td>
<td>Forward: CCACACTTCTCCCTGTTCC</td>
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<tr>
<td></td>
<td>Reverse: AGAGGAGGAGGCGAGGTGC</td>
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<tr>
<td>Exon 6</td>
<td>Forward: CATGACGCTCTGTCGCCAG</td>
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<tr>
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<td>Reverse: TTACTGATGAGGAGTCGCA</td>
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<tr>
<td>Exon 7</td>
<td>Forward: ATCCGCAGAGGCGAGGTGC</td>
<td>293</td>
<td>56</td>
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<tr>
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<td>Reverse: GTCGACATCCTCAGAAG</td>
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<tr>
<td>Exon 8</td>
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<tr>
<td></td>
<td>Reverse: GTAATGCTTGGTGTTCCA</td>
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<tr>
<td>Exon 9</td>
<td>Forward: TCTGCGGATGTGCCGCGG</td>
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<tr>
<td></td>
<td>Reverse: TTCTGGCAATCGGAGGGTC</td>
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<td>Exon 10</td>
<td>Forward: TCCAGGCACTGACGTCCTTC</td>
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<tr>
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<td>Reverse: AACACGCGAAGAAGGCTGAG</td>
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<tr>
<td>Exon 11</td>
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<td>Reverse: CGGATGAAAGCTGCAAGA</td>
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<td>Exon 12</td>
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<tr>
<td></td>
<td>Reverse: GTGTTGAGGAGTGGAGG</td>
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<tr>
<td>Exon 13</td>
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<td></td>
<td>Reverse: ACTCTCCAGAGTGGCTAGC</td>
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<tr>
<td>Exon 14</td>
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<td>Reverse: GCCAGTTCCTCCATTTCAGC</td>
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<td>Reverse: GTTCTGAGGGAGGACGAGG</td>
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<td>Exon 19</td>
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<td></td>
<td>Reverse: GCAAGCGAGGCTGCTCTCCGAG</td>
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<td>Exon 20</td>
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<td>Exon 21</td>
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<td>Exon 23</td>
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<tr>
<td></td>
<td>Reverse: TCAGGCTACGCTGAAACGA</td>
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</tr>
</tbody>
</table>

*The 2 primer pairs amplified overlapping regions of this exon.*