Primary Lens Luxation in Australian Tenterfield and Miniature Bull Terriers is Due to An Old *ADAMTS17* Mutation and is an Additive Trait

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Abstract: Primary Lens Luxation (PLL) is an inherited disease common in Terrier breeds. A truncating mutation in the *ADAMTS17* orthologue on CFA03 is reported to cause PLL in 17 breeds, mostly Terriers. This study investigated the mode of inheritance and penetrance of PLL, age of the *ADAMTS17* mutation, and other possible causes of PLL in Australian Tenterfield Terriers and Miniature Bull Terriers. The *ADAMTS17* mutation and 30 nearby microsatellites on CFA03 were genotyped in 66 Australian Tenterfield Terriers, and 74 Miniature Bull Terriers. Lifetime risk of developing clinical PLL in homozygote and heterozygous animals, penetrance of PLL in general population, and recurrence risk of the disease for offspring and siblings of an index case was estimated. The effect of the *ADAMTS17* mutation on the risk of PLL was dose and age dependent, and best fitted an additive model. Primary lens luxation was fully penetrant in homozygotes over 6 years and incompletely penetrant with a significant risk of PLL in heterozygote animals. Microsatellite haplotype testing estimated the mutation to be an old mutation, consistent with its appearance on several different background haplotypes and suggesting it entered the tested Tenterfield Terrier population at least three times, and the tested Miniature Bull Terrier population once. Finite polygenic model and survival analyses suggested that the *ADAMTS17* mutation is the main cause of PLL in these breeds, and if the PLL phenotype is controlled by factors other than this mutation, such as a second locus or an environmental effect, the effect is much less than that of this disease associated SNP.

Keywords: *ADAMTS17* mutation, Miniature Bull Terrier, Mode of inheritance, Penetrance, Primary Lens Luxation, Tenterfield Terrier.

INTRODUCTION

Primary Lens Luxation (PLL) is an inherited disease in which the lens comes free from its attachments and moves anteriorly or posteriorly in the eye. In Tibetan Terriers, Miniature Bull Terriers, and Shar Peis, lens luxation is due to an abnormal arrangement of the zonular fibres of the ciliary processes, which hold the lens in its normal position in the eye [1, 2]. The disease is common in Terrier breeds such as the Tibetan Terrier, Miniature Bull Terrier and Jack Russell Terrier; but has also been reported in non-Terrier breeds such as the Chinese Shar Pei and Australian Cattle Dog. Primary Lens Luxation is usually first diagnosed in dogs when they are 2-6 years old, with the average age being 4.7-5.2 years [3, 4].

A truncating mutation in the canine *ADAMTS17* orthologue [Entrez Gene ID: 488708] was recently shown by ourselves and others to cause PLL in Jack Russell Terriers, Miniature Bull Terriers and Lancashire Heelers [5]. This mutation has been also identified in 14 additional breeds,

mostly Terriers or breeds with Terrier co-ancestry, but also in some breeds with more diverse origins such as the Australian Cattle Dog. However, this mutation was not detected in PLL affected animals in some other breeds including the Shar Pei and Brittany Spaniel [6]. This GT to AT splice donor site mutation located at the 5' end of intron 10, results in the skipping of exon 10 during transcription, a shift in the normal reading frame and a premature stop codon. Primary lens luxation is reported to have an autosomal recessive mode of inheritance in these breeds. However in Farias' 2010 study, 23 of 196 dogs with PLL were heterozygous for the *ADAMTS17* mutation, and 12 were homozygous for the normal allele [5]. Gould *et al.*, also suggested an increased but low risk of PLL in *A/G* heterozygote animals [6].

The mode of inheritance and penetrance of the *ADAMTS17* mutation, and whether other genetic or environmental factors contribute to the development of clinical PLL is not well known yet. The aim of this study was to further investigate the mode of inheritance and penetrance in PLL using *ADAMTS17* mutation data, estimate the age of the mutation, and to investigate evidence for other genetic and environmental factors affecting the expression of the *ADAMTS17* mutation in Australian populations of

Tenterfield Terrier and Miniature Bull Terriers. Here we report that the *ADAMTS17* mutation has a dose and age dependent penetrance, and an additive mode of inheritance in Australian Miniature Bull Terriers and Tenterfield Terriers. It is the main cause of PLL in those breeds, and is probably an old mutation.

MATERIAL AND METHODS

Animal Ethics

The University of Queensland Animal Ethics Committee approved this study SVS/089/907[nf], SVS/095/08, SVS/ 284/10/QCCC/ACAHF/KIBBLETRUST (NF), as did The University of Queensland Human Ethics Committee 2007000223.

SELECTION OF DOGS AND gDNA EXTRACTION

Sixty-six Tenterfield Terriers and 74 Miniature Bull Terriers from a population in which PLL was segregating were enrolled in the study. Veterinary ophthalmologists examined the dogs' eyes after pupillary dilation with 1% Topicamide (Alcon-Couvreur N.V., Puurs, Belgium) and using indirect ophthalmoscopy, applanation tonometry and slit lamp biomicroscopy. Diagnostic criteria included any evidence of PLL including the presence of an aphakic crescent, iridonesis, elevated intraocular pressure, anteriorly displaced vitreous or vitreous syneresis. Pedigrees were collected if available. Genomic DNA was extracted from EDTA blood using a salt extraction protocol [7], and QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer instructions.

GENOTYPING THE *ADAMTS17* MUTATION

Genotypes for the *ADAMTS17* SNP were performed as in [5].

GENOTYPING MICROSATELLITES ON CFA03

Thirty microsatellites on CFA03 were amplified. Thirteen of these microsatellites were located in the region between 40 Mb to 45 Mb, the region containing the canine *ADAMTS17* mutation which is located at 43.52 Mb. Eight of 30 were from a previous study [8], and 22 were from the available databases [9-12] (Supplementary Material **1**). Microsatellites were amplified using primers fluorescently labelled using a M13 tailing method [13]. All microsatellites were initially amplified in singleplex PCR and results compared with those obtained from multiplex PCR to ensure consistency. Singleplex PCRs contained 10-30 ng DNA, 1 x PCR Buffer, 0.2 µM of each dNTP, 0.05 µM forward and 0.1 µM reverse primers, 0.1 µM labelled M13 primer, and 1 U HotstarTaq® DNA Polymerase (QIAGEN, Hilden, Germany) in a 10 µl reaction volume. The M13 primer was labelled with 6FAM™, VIC®, NED™ and PET® dyes (DS-33 GeneScan™ Installation Standard, Applied Biosysems, Foster City, USA). Microsatellites were amplified at 95ºC for 15 min; followed by 35 cycles of 94ºC for 1 min, 55ºC for 1 min, and 72ºC for 1 min; and one cycle of 72ºC for 10 min.

Seven multiplex PCRs were performed (Supplementary Material **1**). Ten µl PCRs contained 10-30 ng DNA, 1x Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), variable concentrations of forward and reverse primers, and 0.2-0.3 µM of each labelled M13 primer. The M13 primer was labelled with 6FAM™, VIC®, NED™ and PET® dyes. Microsatellites were amplified at 95ºC for 15 min, followed by 35 cycles of 94ºC for 30 s, 55ºC for 90 s, and 72ºC for 1 min; and one cycle of 72ºC for 10 min. Labelled PCR products were genotyped using 3130XL Genetic Analyzer (Applied Biosystems, Foster City, USA), and analysed with software Genemapper version 3.7 (Applied Biosystems, Foster City, USA).

LINKAGE AND ASSOCIATION ANALYSES

Sib-pair64 software [14] was used for non-parametric linkage analysis and also association analysis between the microsatellites and the PLL phenotype, and to determine segregation of PLL linked markers with the *ADAMTS17* mutation. Superlink1_7.linux32 [15] was also used for parametric linkage analysis using microsatellites and disease phenotype data.

INVESTIGATION OF MODE OF INHERITANCE AND DISEASE PENETRANCE

Kruskal Wallis testing for mean age at diagnosis was performed using Sib-pair64 software to investigate the effect of different genotypes of the PLL causative *ADAMTS17* biallelic SNP on the age of onset of disease. Survival analysis was performed using the Kaplan Meier method, Weibull model, and gamma distribution parametric model in R-2.10.0 software [16]. Single major locus recurrent risk was calculated using Sib-pair64 software, both to further investigate the mode of inheritance and penetrance of PLL, as well as to evaluate the risk of recurrence and risk to offspring from varying types of matings between affected and unaffected animals.

ESTIMATION OF THE AGE OF THE *ADAMTS17* MUTATION

Superlink1_7.linux32 was used to analyse microsatellite haplotypes to allow estimation of the age of the *ADAMTS17* mutation. This calculation was based on the number of recombination events in the haplotypes. The DMLE programme (Version 2.3) [17] was also used to estimate the age of the *ADAMTS17* mutation, based on the recombination pattern observed in the flanking microsatellite markers.

INVESTIGATION OF CAUSES OTHER THAN THE *ADAMTS17* MUTATION IN PLL

Finite polygenic model analysis using Sib-pair64 and survival analysis using R-2.10.0 were performed to investigate the effect of factors of than the *ADAMTS17* mutation in PLL.

RESULTS

PLL Status

Sixty-five Tenterfield Terriers and 65 Miniature Bull Terriers had clinical PLL phenotype recorded as diagnosed by a veterinary ophthalmologist, with 10 additional animals (1 Tenterfield and 9 Miniature Bull Terriers) having no phenotype recorded.

PLL PHENOTYPE WAS LINKED TO MICRO-SATELLITES ON CFA03

Parametric linkage analysis supported linkage between 39.05 - 48.6 Mb on CFA03 and the PLL phenotype in both breeds. Maximum LOD scores obtained using dogs of both breeds in the analysis were 1.54 for CamC0.045 (43.5 Mb on CFA03), and 1.40 for ZUBECA4 (42.8 Mb on CFA03). Similarly, non-parametric linkage analysis also supported linkage between 41.5 - 45.3 Mb on CFA03 and PLL in both breeds, with a maximum LOD score of 1.46 for CamC0.045 and ZUBECA4. Association analysis also showed that allele *422* of CamC0.045 was associated with PLL (empirical $P = 2 \times 10^{-6}$).

SEGREGATION OF LINKED MICROSATELLITES AND THE *ADAMTS17* MUTATION

Association analysis showed the *ADAMTS17* SNP allele *A*, consistently segregated with CamC0.045 allele *422* (LD $r^2 = 0.96$). The frequency of the *A-422* haplotype in the study population was 0.41 (0.38 in the Tenterfield Terrier samples, 0.51 in the Mini Bull Terrier samples).

MODE OF INHERITANCE AND PENETRANCE OF PLL

The prevalence of clinical PLL in this population of Tenterfield Terriers was 12% (8/65), and 23% (15/65) in Miniature Bull Terriers. Altogether, 23 dogs had clinical PLL with 15 being *A/A* (65%) for the *ADAMTS17* SNP and 8 being *A/G* (35%). While 7.5% of clinically unaffected animals were *A/A*, all *A/A* dogs over 6 years old had clinical PLL (Table **1**).

Kruskal Wallis testing showed significant differences between groups of animals with different PLL status according to their *ADAMTS17* SNP genotype ($P = 0.0016$) (Table **2**). Twenty-three dogs in this study were *A/A* homozygotes, with 8 dogs clinically unaffected by PLL. However, all of these *A/A* unaffected dogs were young. The mean age of *A/A* unaffected animals was 21 months, whereas the mean age of *A/A* affected animals was 54 months.

The mean age of diagnosis with PLL was 54 months in *A/A* homozygote animals and 72 months in *A/G* heterozygote animals. The mean age of *A/G* heterozygotes with no clinical signs of PLL was 49 months, compared with 72 months in heterozygous animals with clinical PLL.

Survival analysis using non-parametric Kaplan-Meier estimate analysis showed that the estimated lifetime risk of clinical PLL for *A/A* homozygotes was higher than in *A/G* heterozygote dogs (Fig. **1**). No dogs were diagnosed with PLL at 2 years of age, but by 3 years of age PLL had been diagnosed in 10% of *A/A* homozygotes. Homozygous *A/A* dogs over 6 years of age showed 100% disease penetrance. In heterozygote animals, penetrance of the disease phenotype was less than 5% in animals aged 3 years, and 60% in animals aged 10 years (Fig. **1**). There was zero penetrance in *G/G* homozygote dogs in the study population. Survival analysis using the parametric Weibull model produced similar results to those obtained from non-parametric Kaplan-Meier estimate analysis (data not shown). Ascertainment corrected survival analysis under a Gamma distribution parametric model also gave similar results to those from the nonascertainment corrected Kaplan-Meier and Weibull model analysis (data not shown), so we believe our penetrance estimates to be robust to model misspecification.

Table 1.   Proportions of *ADAMTS17* SNP Genotypes in PLL Clinically Affected and Clinically Unaffected Dogs (Miniature Bull Terriers and Tenterfield Terriers Combined)

ADAMTS17 SNP Genotype/PLL Status	A/A	A/G	G/G
PLL affected	65.2% (15/23)	34.8% (8/23)	0% (0/23)
PLL unaffected (all ages)	7.5% (8/107)	47.7% (51/107)	44.8% (48/107)
PLL unaffected > 6 years	0% (0/21)	61.9% (13/21)	38.1% (8/21)

Table 2.   Kruskal Wallis Testing for Mean Age at First Diagnosis of Clinical PLL

PLL Status/ *ADAMTS17* SNP Genotypes *	Number of Dogs	Mean age of Diagnosis in PLL Affected and Unaffected Dogs	Standard Deviation of Mean Age of Diagnosis in PLL Affected and Unaffected Dogs
Unaffected-*A/A*	8	21	16
Unaffected-*A/G*	51	49	33
Unaffected-*G/G*	48	47	33
Affected-*A/A*	15	54	11
Affected-*A/G*	8	72	28

This test compared the mean age of diagnosis of clinically affected and unaffected animals according to their genotypes for the *ADAMTS17* SNP ($P = 0.0016$) (4 degrees of freedom and H = 17.45). The *P* value indicates that the differences between mean ages are significant between different groups of animals according to their PLL status and *ADAMTS17* SNP genotypes.
* *A* = mutant allele causing PLL in Miniature Bull Terriers and Tenterfield Terriers, *G* = wild type allele
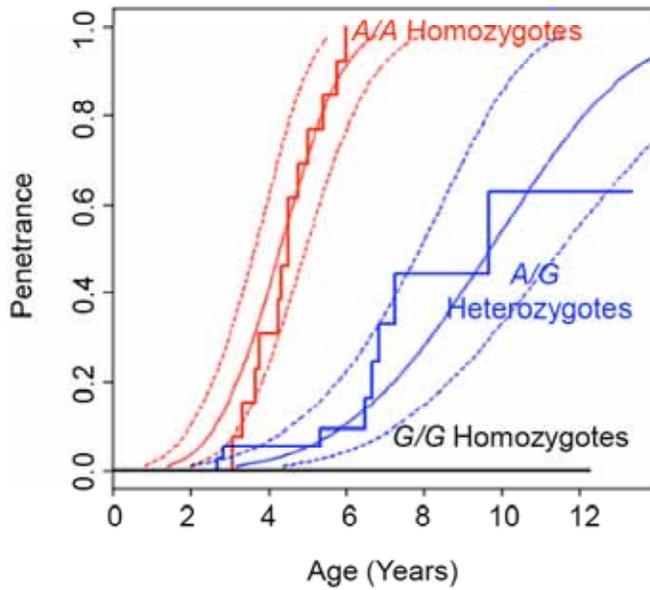
Fig. (1). Penetrance of the *ADAMTS17* SNP in the clinical phenotype calculated using age at first diagnosis. This plot shows survival analysis curves created by R-2.10.0 software, reflecting the lifetime risk of developing PLL due to the *ADAMTS17* mutation in years of age at the time of first diagnosis. This plot was produced using the non-parametric Kaplan-Meier estimate and parametric Weibull model. Step curves are the empirical age at onset curves, created using non-parametric Kaplan-Meier estimate analysis. The continuous lines show the lifetime risk estimates obtained using Weibull model survival analysis in *A/A* homozygote animals (red line), *A/G* heterozygote animals (blue line) and *G/G* homozygote animals (black horizontal line). Maximum and minimum 95% confidence intervals for age-at-first diagnosis created using Weibull model analysis are shown with non-continuous curves.

Only 10 *A/G* heterozygote animals were over 7 years old in the study population, resulting in a wide estimated confidence interval for these animals (Fig. **1**). Therefore, for subsequent risk calculations, a penetrance value of 45% was used, which was the value calculated from dogs up to seven years of age. Under this model (mutation frequency 0.41, lifetime penetrance 100%, 45%, 0%), the recurrence risk to offspring and siblings of an index case was predicted to be 0.53 (see Table **3**).

Table 3. Estimated Risk to Offspring from Various Mating Types, for the Development of Clinical PLL

Mating	Proportion of Offspring from Matings	Risk to Offspring of Clinical PLL
UnA[1] x UnA	0.67	0.12
Aff [2]x UnA	0.29	0.26
Aff x Aff	0.04	0.57

This table shows the estimated proportion of offspring with clinical PLL from various types of matings, obtained by single major locus recurrent risk calculation using Sib-pair64 .
[1]UnA; clinically unaffected by PLL
[2]Aff; clinically affected by PLL

ESTIMATION OF THE AGE OF THE *ADAMTS17* SNP

Eight of 15 Miniature Bull Terriers with clinical PLL were homozygous and seven were heterozygous for the disease associated SNP allele, and nine of these animals shared a common 9.6 Mb haplotype, with a core 600 Kb haplotype consisting of the *ADAMTS17* SNP and two of the closest flanking microsatellite markers shared by all except one animal (where there was an apparent mutational event). The common extended haplotype was also seen in some affected Tenterfield Terriers, so is likely to represent the ancestral background haplotype. The other risk haplotypes were consistent with a pattern of six ancestral recombination events in this 9.6 Mb interval. Using the DMLE+ program to analyse these data, we estimated the age of the mutation at 26 (21-28) generations old.

Similar haplotype analysis in Tenterfield Terriers in the same linked region showed that 7 of 8 animals with clinical PLL were homozygous for allele *422* at the nearest marker CAMC03.045. However, unlike the Miniature Bull Terriers, haplotype homozygosity was not present in the entire region. Eighteen PLL associated haplotypes were identified in this region, all containing allele *422*. Three of these haplotypes were major haplotypes, with the other 15 variants likely to have occurred with recombination events within these main haplotypes. Despite this, the estimated age of the mutation was not greatly different, at 27 (23-29 generations).

Given that there are multiple obligate recombination events in the 600 Kb interval between the two nearest flanking markers to the *ADAMTS17* mutation, this precludes the mutation being young. This is also implied by the same mutation being present in multiple dog breeds [5]. The estimated mutation age is quite sensitive to assumptions about how rapidly the breeds have expanded in size: 76 generations (95%CI, 53-118), assuming the breeds expanded at 10% per generation, and 23 generations (95%CI, 25-73) assuming 25% expansion per generation. Based on breed records, the latter expansion rate seems more plausible [18]. Assuming the mutation is relatively neutral with respect to reproductive fitness, and has not been actively selected for as a correlated trait by breeders, we can also estimate mutation age based on the mutation frequency. We used effective population sizes of 50 and 100 animals, based on those estimated for Terrier breeds in Australia [19]. Under the same expansion estimates, we obtain estimates of 10 generations (25% expansion) and 24 generations (10% expansion). Overall, these analyses would suggest that this mutation entered the Australian population base early in the twentieth century.

INVOLVEMENT OF OTHER GENETIC OR NONGENETIC FACTORS IN PLL

Finite polygenic model analysis when combining data from both breeds indicated that additive quantitative trait locus variance and additive polygenic variance decreased after including the *ADAMTS17* SNP in the analysis (Table **4**). This indicated that the *ADAMTS17* mutation accounted for much of the PLL phenotype, decreasing the likelihood of involvement of a second locus in PLL.

Goodness of fit measures for mixed effects survival analysis models that excluded or included the *ADAMTS17*

Table 4.　The PLL Phenotype is Largely Due to the *ADAMTS17* SNP as Determined Using the Finite Polygenic Model Analysis

Variance/Likelihood	Without inclusion of *ADAMTS17* SNP	With inclusion of *ADAMTS17* SNP
Additive QTL[1] variance	18.328804 (51.6%)	0.146914 (3.5%)
Additive polygenic variance	0.792643 (9.8%)	0.456353 (11.7%)
Environmental variance	0.047852 (38.6%)	0.046973 (84.7%)
Modal model log likelihood	-504.372	-391.767
Mean model log likelihood	-483.808	-398.259
C.V.[2] Log likelihood	4.63%	2.35%

This table shows the variance of the quantitative trait PLL in the combined Miniature Bull Terrier and Tenterfield Terrier populations with and without the effect of the *ADAMTS17* SNP. Additive quantitative trait locus variance and additive polygenic variance decreased significantly after including the *ADAMTS17* SNP, indicating that *ADAMTS17* SNP is the main cause of PLL.
[1] Quantitative trait locus
[2] Coefficient of variation

SNP as a fixed effect, were not significantly different ($P = 1.0$ for null, 0.878605 for integrated and 1.000 for penalised analysis), indicating that the *ADAMTS17* mutation could account for most (or all) of the familial aggregation of the PLL phenotype.

DISCUSSION

Primary Lens Luxation is due to a mutation in the gene *ADAMTS17* on CFA03 in 17 canine breeds, mostly Terriers and breeds with Terrier co-ancestry, but also in some breeds with more diverse origins [5, 6]. Despite this, the actual mode of inheritance of PLL remains unclear. The current study findings suggest PLL shows dose and age dependant effects, consistent with an additive allelic model. Thus on average, animals homozygous for the mutation are expected to show the disease earlier in life (mean age of 4-5 years) compared to heterozygous animals (mean age 6 years or more). Survival analysis showed that penetrance of the PLL mutation increased with age, with full penetrance in *A/A* animals over 6 years, and incomplete but up to 60% penetrance in *A/G* animals by 10 years of age. Survival analysis with and without ascertainment correction produced the same results, suggesting the penetrance value calculated for the PLL mutation is unlikely to be biased due to animal selection in this study.

In calculating the risk of disease in the general population, we used the penetrance estimate of 45% in heterozygous animals up to age 7 years. This was because there were only 10 *A/G* heterozygote animals over the age of 7 years in our study population, resulting in a wide 95% confidence interval for *A/G* heterozygote animals over this age. Due to the lack of published data on the actual prevalence of PLL in Miniature Bull Terriers or Tenterfield Terriers, the estimated risks for the disease in the general population are projected values.

In our recent study in Jack Russell Terriers, Miniature Bull Terriers and Lancashire Heelers, 9.4% (23/196) of PLL clinically affected dogs were *A/G* heterozygotes [5]. Thus in PLL in these breeds, the estimated penetrance for heterozygote animals is lower than expected in a purely additive codominant disease. However, most of the animals

in this study were under 7 years, and so may develop disease with age. Gould *et al.,* also suggested an increased but low risk of PLL in heterozygote animals [6]. However, in the current study, a penetrance of as high as 60% was calculated in heterozygous animals up to 10 years of age, and a penetrance of 100% in homozygous animals over the age of 6 years. This suggests that the mode of inheritance may best fit an additive model. Such modes of inheritance have been reported in inherited diseases such as Parkinsonism in humans [20].

Gene additive effects, gene interactions, and interactions between genes and the environment can also affect the mode of inheritance in genetic diseases. Thus, an alternate hypothesis to additive inheritance could be that a second or modifying locus is associated with PLL, or environmental factors could interact with genetic factors to alter penetrance and the clinical picture of disease.

One of the important results of this study was the relatively high risk of heterozygote animals developing clinical PLL, a disease which is commonly painful. Reducing the prevalence of clinical disease in recessive diseases may involve breeding carrier or affected dogs to unaffected homozygous dogs to produce unaffected homozygote and/or carrier pups. However, when carrier animals have a relatively high risk of developing a potentially painful disease this strategy may be problematic. Further, the frequency of the PLL disease allele in some populations may also be high, making removal of all PLL affected and carrier animals impractical in these breeds due to the resultant loss of genetic variability in the breed. Hence, control of PLL may include maintenance of as many breeding lines as possible, by breeding as many unaffected homozygous animals as possible to related breeds with a low PLL mutation prevalence, and screening the progeny for inherited diseases. This strategy allows breeding away from PLL whilst minimising the risk of unveiling other genetic problems.

In this study, calculations estimating the lifetime risk of developing clinical PLL in homozygous and heterozygous animals, the estimated penetrance of PLL in general population, and the recurrence risk for offspring and siblings

of an index case were performed. These data provide clinicians with indications of the risk of, and likely clinical onset of, clinical PLL in both homozygote and heterozygote Miniature Bull Terriers and Tenterfield Terriers in Australia. It also identifies problems in breeding with animals carrying this mutation and proposes methods to address this issue.

In the Miniature Bull Terriers one main disease related haplotype was present in dogs in this study, supporting the age of the mutation in this population being approximately 100 years. Miniature Bull Terriers are believed to have their origins in the Old English White Terrier, the Dalmatian and the Bulldog, and were first documented as a breed in the late nineteenth century. As the Bulldog and Dalmatian have not been reported to suffer from PLL, the extinct Old English White Terrier could thus be the originating breed for the mutation in the Miniature Bull Terrier population in this study [21-23].

In the Tenterfield Terrier sample, we inferred that the mutation was introduced by multiple founders, each time with the disease associated mutation being on a different background haplotype. Maximum likelihood estimation of founder haplotypes is difficult because of the persistent linkage disequilibrium seen in our pedigree founders, so our conclusions should be a little tentative. In two older haplotypes the disease associated region had been reduced to a very small region around allele *422*, consistent with more recombination events occurring in many generations over a longer time frame. These haplotypes may have their origins in the founders of the Tenterfield Terrier breed in Australia, although the breed has only been registered, and so had a closed breeding pool, for approximately two decades [21]. The third haplotype in the Tenterfield Terrier population was the commonest haplotype in the affected Miniature Bull Terriers.

This study found that the *ADAMTS17* mutation was the main cause of PLL in the tested populations. However, difficulties in determining the mode of inheritance for PLL suggest a possible role for other genetic or nongenetic factors in the disease phenotype. Such factors may include other *ADAMTS17* alleles, a second mutation with a lesser effect on the disease phenotype or environmental effects. In support of this, clinical PLL has been reported in *G/G* homozygote dogs in several breeds including Miniature Bull Terriers, Lancashire Heelers, Jack Russell Terriers, Tenterfield Terriers, Rat Terriers, Jagdterriers, and Wire-haired Fox Terriers, suggesting genetic heterogeneity is possible in these breeds [5, 6]. Further, Gould *et al.,* reported that in 16 other breeds with PLL, the *ADAMTS17* causative mutation was not present, suggesting a genetically distinct form of the disease is possible in those breeds [6].

As the previous study reporting the PLL associated mutation in the canine *ADAMTS17* gene did not sequence the introns and regulatory sequences [5], mutations in introns or regulatory sequences may not have been detected. Such sequence variants, as well as synonymous and non synonymous coding sequence changes, may affect the disease phenotype by altering RNA splicing or gene expression [24-26]. Thus, a hypomorphic allele of the *ADAMTS17* SNP could be associated with a milder phenotype in compound heterozygotes, as evidenced by later

disease onset and decreased penetrance. Hence, sequencing of *ADAMTS17* introns, and 3´ and 5´ untranslated regions in dogs with varying clinical PLL status and PLL associated *ADAMTS17* mutation genotypes may further our understanding of disease pathogenesis. Determination of *ADAMTS17* expression levels using RT-PCR and protein quantitation on ocular tissues from animals with PLL could also provide evidence of involvement of various alleles of the *ADAMTS17* SNP in PLL development.

Candidates for a modifying locus in PLL include an *ADAMTSL* gene, as encoded proteins from these genes have been suggested to interact with the ADAMTS proteins and have a regulatory effect on their activities [27]. Future GWAS using SNPs located throughout the whole canine genome may help to investigate the involvement of secondary or modifying loci in the development of PLL. However, an effect from environmental factors could also change the penetrance of the disease in *A/G* heterozygote animals. Finally, it also may be speculated that a second mutation in the *ADAMTS17* gene which is segregating with the PLL associated allele in heterozygote animals, could result in reduction of expression of the PLL associated allele, causing a milder phenotype in affected heterozygotes.

Mutations in *ADAMTS17* in humans are associated with Weill-Marchesani syndrome (WNS) like syndrome, which is characterised by lens dislocation and short stature [28]. Interestingly, one of the reported mutations in humans with WMS-like syndrome is a homozygous *G>A* splice-site donor mutation in intron 12 of the gene which produces a truncated transcript, similar to the mutation in the canine orthologue which causes PLL. In contrast, mutations in the coding regions of the *ADAMTS17* gene were excluded from association with primary lens instability in the cat, and the *FBN1* gene has been suggested as a potential candidate gene in this species [29], suggesting that genetic causes of inherited lens dislocation may be different in dogs and cats.

CONCLUSIONS

Inheritance of PLL in Australian Miniature Bull Terriers and Tenterfield Terriers is likely to most closely follow an additive model, with heterozygotes at significant risk for developing clinical PLL. The *ADAMTS17* PLL associated SNP is likely to be an old mutation in the Terrier breeds, and has been introduced several times into the Australian Tenterfield Terrier population. If the PLL phenotype is controlled by factors other than the *ADAMTS17* SNP, such as a second locus or an environmental effect, the effect is much less than that of this disease associated SNP. These findings support continued monitoring of carriers of the PLL mutation for evidence of lens luxation, as well as the mutation being an old one present in many dog breeds.

CONFLICT OF INTEREST

Declared none.

SUPPORTIVE/SUPPLEMENTARY MATERIAL

Supporting information is available on the publishers Web site along with the published article.

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