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A variant in *OLFML3* is associated with pectinate ligament abnormality and primary closed-angle glaucoma in Border Collies from the United Kingdom

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Abstract

Purpose: Canine primary closed-angle glaucoma (PCAG) is a complex disease caused by multiple genetic factors. A c.590G>A variant in *OLFML3* was recently reported to be a candidate for pectinate ligament abnormality (PLA) and PCAG in the Border Collie. We investigated the association of this variant with PLA and PCAG in Border Collies from the United Kingdom.

Methods: The *OLFML3* variant was genotyped in 106 Border Collies comprising 90 with normal eyes (controls) and 16 with PLA (n = 11) and/or PCAG (n = 5) (cases). Genotyping was performed in an additional 103 Border Collies to estimate variant frequency within the population. To investigate the association of the variant with disease in other breeds, genotyping was performed in 337 non-Border Collies with PLA and/or PCAG.

Results: Of the 90 controls, 71 were homozygous for the wild-type allele, two were homozygous for the variant, and 17 were heterozygous. Of the 16 cases, three were homozygous for the wild-type allele, 11 were homozygous for the variant, and two were heterozygous. The association of the variant allele with disease was significant ($P = 1.1 \times 10^{-9}$). We estimated the frequency of this variant to be 4.4% within the United Kingdom Border Collie population, and it was not identified in clinically affected dogs of any other breed.

Conclusions: This study confirms the association of the *OLFML3* variant with PLA and PCAG in Border Collies from the United Kingdom. DNA testing for the variant and selective breeding can reasonably be expected to result in a reduction of PLA and PCAG prevalence in the breed.

KEYWORDS

Border Collie, goniodysgenesis, *OLFML3*, pectinate ligament abnormality, pectinate ligament dysplasia, primary glaucoma

1 | INTRODUCTION

Canine glaucoma is described as primary if it occurs in the absence of any antecedent identifiable ocular disease process.[1] Instead, primary glaucoma is caused by unidentifiable and inherent abnormalities in the aqueous humor outflow apparatus of the eye.[1] In adult dogs, primary glaucoma is subdivided into primary open-angle glaucoma (POAG)

and primary closed-angle glaucoma (PCAG) on the basis of the appearance of the iridocorneal angle (ICA). In POAG, the ICA appears open and with normal pectinate ligament anatomy early on in the disease process even in the presence of pathologically elevated intraocular pressure.[2] PCAG, on the other hand, is associated with pectinate ligament abnormality (PLA), a form of goniodysgenesis, in several breeds of dog.[3-12]

The increased prevalence of primary glaucoma in certain dog breeds implies a genetic etiology. In all breeds investigated thus far, POAG has been shown to be inherited as an autosomal recessive disease associated with mutations in either *ADAMTS10* or *ADAMTS17*, both of which are genes involved in the formation and maintenance of the extracellular matrix.[13-17] PCAG, in contrast, is thought to be a complex disease being caused by multiple genetic and environmental factors and, to date, no causal variants have been proven.[18-21] The Border Collie is a breed that may be affected by PLA and PCAG and is currently under investigation for these conditions by the United Kingdom's hereditary eye disease scheme (BVA/KC/ISDS Eye Scheme).[22] In this breed, PLA prevalence is relatively low in the United Kingdom and anecdotal reports of PCAG are rare.[22] Recently, a variant in *OLFML3* has been reported to be a candidate for PLA and PCAG in the Border Collie in a study using DNA samples from dogs collected worldwide.[23] The variant is a guanine to adenine base change in *OLFML3* (c.590G>A) on canine chromosome 17. This is a missense variant which is predicted to change arginine to glutamine in OLFML3 protein (p.Arg197Gln). The purposes of this study were (a) to assess the association of the reported *OLFML3* variant with PLA and PCAG in a cohort of Border Collies from the United Kingdom, (b) to estimate the frequency of the variant within the United Kingdom Border Collie population, and (c) to assess whether the variant is present in dogs with PLA and PCAG of other breeds.

2 | MATERIALS AND METHODS

2.1 | DNA samples and animals used

DNA samples from 546 dogs were used. Of these, 443 dogs (106 Border Collies, 26 Leonbergers, 108 Basset Hounds, 50 Dandie Dinmont Terriers, 72 Flat Coated Retrievers, and 81 Welsh Springer Spaniels) had undergone ophthalmological examination. The Border Collies were subdivided into those without PLA or PCAG (controls) and those with PLA and/or PCAG (cases) to investigate the association of the variant with disease. The non-Border Collies were composed only of dogs with PLA and/or PCAG to assess whether the variant was present in individuals of these breeds with disease. An additional cohort of 103 Border Collies that had been collected for a different and unrelated study and that had not undergone ophthalmic examination was used to assess *OLFML3* variant frequency within the United Kingdom Border Collie population. All DNA samples were from privately owned pet dogs originating from the United Kingdom. Samples were collected following fully informed and written owner consent and with approval of the Animal Health Trust's Research and Ethical Approval Committee (24-2018E). Samples were collected in the form of buccal mucosal swabs, and DNA was extracted using the QIAmp® DNA Blood Midi Kit (Qiagen) using a protocol developed by the Animal Health Trust and based on the manufacturer's instructions.

2.2 | Examination procedure

To ensure that dogs were as representative of the UK population as possible, several steps were undertaken. Gonioscopy screening sessions were held in different locations around the UK and at different types of event, including dog shows, "fun days" and breed health information days. The gonioscopy screening was promoted by a variety of different mechanisms, including correspondence from the Kennel Club to the owners of Kennel Club registered dogs, via breed club websites and via social media. All dogs that were volunteered for screening were accepted, regardless of their age, ancestry, or Kennel Club registration status. Pedigree information was not available for all of the dogs included in the study and so it was not possible to formally assess relatedness of the dogs.

Pectinate ligament abnormality status of dogs was based on the results of gonioscopy as previously described.[22,24,25] Briefly, gonioscopy was performed bilaterally in conscious dogs following application of 0.5% proxymetacaine (Bausch & Lomb, Chauvin Pharmaceuticals Ltd.) to the ocular surface using a 19 mm Koeppe goniolens (Ocular Instruments) filled with 2 mg/g carbomer gel (Viscotears; Alcon) before placing onto the cornea. The entire 360° of the ICA was then examined using a handheld slit-lamp biomicroscope (Keeler PSL Classic) for the presence of PLA which was quantified according to the percentage of ICA circumference affected, estimating this to the nearest 5%. Regions of ICA were judged to be affected by PLA where they exhibited abnormally broad pectinate ligament fibers or solid sheets of tissue as previously described.[8,26] Under the BVA/KC/ISDS Eye Scheme and at the time this study was conducted, dogs were considered to be "affected" if greater than 20% of the entire ICA circumference was affected by PLA.[26] The same examiner, a diplomate of the European College of Veterinary Ophthalmologists (JO), performed all examinations to reduce the influence of subjectivity. Case/control designation was as follows:

2.3 | Controls

Controls were defined as dogs with PLA affecting ≤20% of the ICA of each eye.

TABLE 1 Primer details for amplification of target region of *OLFML3* and Sanger sequencing

Forward primer	Reverse primer	Annealing temperature	Product size
ATGAAGATCCTGAAGCGGTTT	ATGAGTTGCAACGTGTTCTCC	60°C	295 base pairs

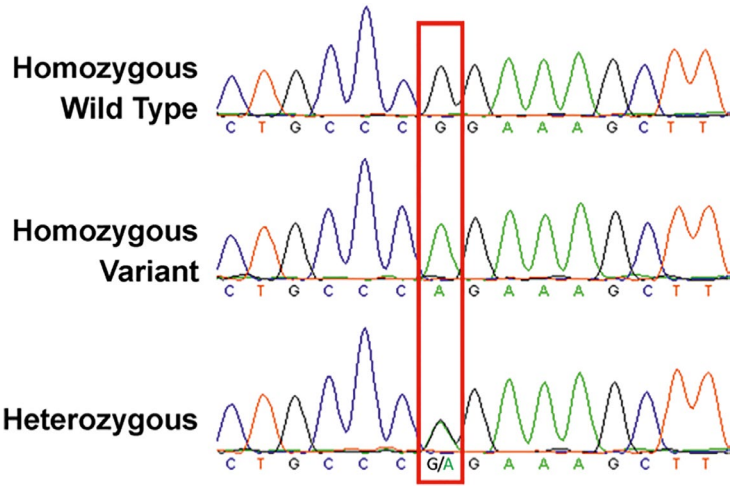

FIGURE 1 Electropherogram Sanger sequencing traces of the investigated region of *OLFML3* as viewed in Staden Gap4 software (http://staden.sourceforge.net/). The site of the c.590G>A *OLFML3* variant is delineated by a red box (chr17:51,786,924). Dogs were designated as homozygous for the wild-type allele (G/G) if only guanine was present at this site (top trace), homozygous for the variant allele (A/A) if only adenine was present (middle trace), and heterozygous if both guanine and adenine were present (G/A) (bottom trace)

2.4 | Cases

Cases were subdivided into PLA cases and PCAG cases. PLA cases were defined as dogs with PLA affecting >20% of the ICA of each eye. PCAG cases were defined as dogs in which PCAG was diagnosed in one eye with an intraocular pressure >50 mm Hg (arbitrary cut-off) without any identifiable cause of secondary glaucoma and the finding of severe PLA (>90% of the ICA affected) in the contralateral eye.

2.5 | *OLFML3* variant genotyping in Border Collie cases and controls

Primer pairs were designed to amplify the *OLFML3* candidate variant (c.590G>A) in all samples using Primer3 and sequence derived from CanFam3.1 (Table 1).[27] The variant was then genotyped using standard Sanger sequencing methodology, using BrilliantDye™ Terminator v3.1 chemistry (NimaGen). Sequencing products were separated on an ABI3130xl Genetic Analyzer and data analyzed using the Staden Gap4 software package (http://staden.sourceforge.net/). The genotype of each dog was ascertained on the basis of visualization of electropherogram traces and assessment of the nucleotide base at the known chromosomal location of the *OLFML3* variant (chr17:51,786,924, c.590G>A, CanFam3.1). Dogs were designated as homozygous for the wild-type allele (G/G) if only guanine was present at this site, homozygous for the variant allele if only adenine was present (A/A), and heterozygous if both guanine and adenine were present (G/A) (Figure 1).

2.6 | *OLFML3* variant genotyping in other dogs

For practical reasons, the remaining 440 dogs (103 additional Border Collies and 26 Leonbergers, 108 Basset Hounds, 50 Dandie Dinmont Terriers, 72 Flat Coated Retrievers, and 81 Welsh Springer Spaniels) were genotyped using allelic discrimination. A custom TaqMan™ SNP genotyping assay (Thermo Fisher Scientific, Table 2) was performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). This assay had been previously validated in the 106 Border Collies that had been already been genotyped by Sanger sequencing to ensure assay accuracy.

2.7 | Statistical analysis

Statistical difference in mean age between the two groups was assessed using the two-tailed t-test and difference in sex distribution between the two groups was assessed using the Fisher's exact test. The association of *OLFML3* variant frequency with case status was assessed using the Fisher's exact test. Statistical testing was performed using GraphPad online software (https://www.graphpad.com), and significance was set at $P < 0.05$.

TABLE 2 Details of the custom TaqMan™ allelic discrimination assay used

Assay mix concentration	Forward primer name	Forward primer sequence	Reverse primer name	Reverse primer sequence	Reporter 1 name	Reporter 1	Reporter 2 name	Reporter 2
40x	rs22561716_F	CATAAAGTCGGAACA TCACCTAAAGGA	rs22561716_R	TTCCGCAGATTT TGCCTTCTTTG	rs22561716_V	VIC-AGGAACGGT GAAATGT-NFQ	rs22561716_M	FAM-AGGAACG GTGCAATGT-NFQ
40x	OLFML3_c.590G_F	CAAGGTTGCGGGAC TTCAC	OLFML3_c.590G_R	GTCCTGTGCCC ACCCA	OLFML3_c.590G_V	VIC-CTGCCCGGAA AGC-NFQ	OLFML3_ c.590G_M	FAM-TGCCCAGA AAGC-NFQ

3 | RESULTS

3.1 | *OLFML3* variant genotyping in Border Collie cases and controls

Individual dog data including sex, age at examination, phenotype, case/control status, and genotype for the *OLFML3* variant are provided in Table 3. DNA samples were derived from a total of 106 dogs comprising 90 controls and 16 cases. The 16 cases comprised 11 dogs with PLA and five dogs with PCAG. The mean (±SEM) age of the controls was 55.0 (±4.66) months and that of the cases was 75.72 (±13.37) months. Difference in mean age between the groups was not significant ($P = 0.10$). There were 45 females and 45 males in the control group and nine females and seven males in the case group. Sex distribution between the control and case group was not significantly different ($P = 0.79$). Of the 90 controls, 71 were homozygous for the wild-type allele, two were homozygous for the variant, and 17 were heterozygous. Of the 16 cases, three were homozygous for the wild-type allele, 11 were homozygous for the variant, and two were heterozygous. The association of the variant allele with cases was significant ($P = 1.1 \times 10^{-9}$). Summarized results are presented in Table 4.

3.2 | *OLFML3* Variant Genotyping In Additional Border Collies

Of the 103 Border Collies that were collected for an unrelated study and had not undergone ophthalmic examination, 95 were homozygous for the wild-type allele, one was homozygous for the variant, and seven were heterozygous, indicating a variant frequency of 4.4% in this cohort.

3.3 | *OLFML3* variant genotyping in other breeds

All 337 dogs of the five other breeds were homozygous for the wild-type allele (ie, the *OLFML3* variant was not found in non-Border Collie dogs with PLA and/or PCAG).

4 | DISCUSSION

This study confirmed the association of a single variant in *OLFML3* with PLA and PCAG in Border Collies from the United Kingdom. This is a significant and interesting finding because PLA and PCAG have previously been considered to be complex diseases caused by multiple genetic and environmental factors.[18-21] The results of this study, however, are consistent with PLA and PCAG largely being a monogenic autosomal recessive trait in Border Collies from the United Kingdom. One possible explanation for this is that PLA and PCAG are relatively new diseases in this breed resulting from a recent

TABLE 3 Individual dog data including age at examination, gonioscopy findings, phenotype, case/control status, and *OLFML3* genotype

Case No.	Sex (M/F)	Age at examination (mo)	PLA left eye (%)	PLA right eye (%)	Phenotype	Case/control status	*OLFML3* variant genotype	
13994	M	89	100	NP	PCAG affected	Case	A/A	Homozygous variant
25166	M	51	100	NP	PCAG affected	Case	A/A	Homozygous variant
25428	F	8	100	NP	PCAG affected	Case	A/A	Homozygous variant
26488	M	32	90	90	PLA affected	Case	A/A	Homozygous variant
26820	F	16	30	40	PLA affected	Case	A/A	Homozygous variant
26823	M	37	90	90	PLA affected	Case	G/A	Heterozygous
26826	F	148	50	60	PLA affected	Case	A/A	Homozygous variant
26831	F	55	80	80	PLA affected	Case	G/G	Homozygous wild type
26848	F	86	90	90	PLA affected	Case	A/A	Homozygous variant
26849	F	115	80	80	PLA affected	Case	A/A	Homozygous variant
26856	F	86	90	90	PLA affected	Case	A/A	Homozygous variant
27581	M	153	90	90	PLA affected	Case	A/A	Homozygous variant
27775	M	173	50	50	PLA affected	Case	G/G	Homozygous wild type
29766	F	33	75	75	PLA affected	Case	G/A	Heterozygous
31174	M	12	100	NP	PCAG affected	Case	A/A	Homozygous variant
31618	F	120	100	NP	PCAG affected	Case	G/G	Homozygous wild type
26452	F	137	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26577	F	69	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26812	M	97	0	0	PLA unaffected	Control	G/A	Heterozygous
26813	M	36	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26814	M	12	0	0	PLA unaffected	Control	G/A	Heterozygous
26815	M	18	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26816	M	29	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26817	M	20	0	0	PLA unaffected	Control	G/A	Heterozygous

(Continues)

TABLE 3 (Continued)

Case No.	Sex (M/F)	Age at examination (mo)	PLA left eye (%)	PLA right eye (%)	Phenotype	Case/control status	*OLFML3* variant genotype	
26818	F	33	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26819	F	8	15	15	PLA unaffected	Control	A/A	Homozygous variant
26821	M	124	0	0	PLA unaffected	Control	G/A	Heterozygous
26822	M	48	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26824	F	12	10	10	PLA unaffected	Control	G/A	Heterozygous
26825	M	12	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26827	F	88	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26828	M	113	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26829	F	111	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26830	F	59	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26832	M	77	0	0	PLA unaffected	Control	G/A	Heterozygous
26833	M	16	5	5	PLA unaffected	Control	G/A	Heterozygous
26834	M	123	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26835	M	113	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26836	F	20	0	0	PLA unaffected	Control	G/A	Heterozygous
26837	F	12	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26839	F	34	0	0	PLA unaffected	Control	G/A	Heterozygous
26840	M	15	20	15	PLA unaffected	Control	G/A	Heterozygous
26841	M	38	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26842	M	53	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26843	F	8	0	0	PLA unaffected	Control	A/A	Homozygous variant

(Continues)

TABLE 3 (Continued)

Case No.	Sex (M/F)	Age at examination (mo)	PLA left eye (%)	PLA right eye (%)	Phenotype	Case/control status	*OLFML3* variant genotype	
26844	F	123	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26845	F	30	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26846	F	100	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26847	F	123	0	0	PLA unaffected	Control	G/A	Heterozygous
26850	F	6	0	10	PLA unaffected	Control	G/G	Homozygous wild type
26851	M	121	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26852	F	132	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26853	M	10	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26854	F	56	0	0	PLA unaffected	Control	G/G	Homozygous wild Type
26855	F	36	0	0	PLA unaffected	Control	G/G	Homozygous wild type
26857	F	11	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27535	F	85	5	10	PLA unaffected	Control	G/A	Heterozygous
27536	F	44	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27537	F	12	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27538	M	14	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27539	M	62	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27540	M	130	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27542	M	60	0	0	PLA unaffected	Control	G/G	Homozygous wild type

(Continues)

TABLE 3 (Continued)

Case No.	Sex (M/F)	Age at examination (mo)	PLA left eye (%)	PLA right eye (%)	Phenotype	Case/control status	*OLFML3* variant genotype	
27543	M	43	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27544	M	7	0	0	PLA unaffected	Control	G/A	Heterozygous
27545	F	39	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27547	F	8	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27548	F	81	0	0	PLA unaffected	Control	G/A	Heterozygous
27549	M	15	0	0	PLA unaffected	Control	G/G	Homozygous Wild Type
27550	F	25	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27551	F	2	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27552	M	48	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27553	F	54	0	0	PLA unaffected	Control	G/G	Homozygous wild Type
27554	F	30	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27555	M	22	0	0	PLA unaffected	Control	G/A	Heterozygous
27556	F	119	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27557	F	7	5	5	PLA unaffected	Control	G/G	Homozygous wild type
27558	F	10	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27561	F	37	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27562	F	14	0	0	PLA unaffected	Control	G/A	Heterozygous
27563	F	37	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27564	F	5	0	0	PLA unaffected	Control	G/G	Homozygous wild type

(Continues)

TABLE 3 (Continued)

Case No.	Sex (M/F)	Age at examination (mo)	PLA left eye (%)	PLA right eye (%)	Phenotype	Case/control status	*OLFML3* variant genotype	
27565	F	18	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27566	F	26	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27567	M	25	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27568	M	12	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27569	M	25	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27570	M	92	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27571	M	59	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27572	M	107	0	0	PLA unaffected	Control	G/A	Heterozygous
27573	F	143	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27574	F	88	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27575	M	92	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27577	M	82	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27578	M	39	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27579	M	66	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27580	M	25	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27582	M	15	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27583	M	7	0	0	PLA unaffected	Control	G/G	Homozygous wild type

(Continues)

TABLE 3 (Continued)

Case No.	Sex (M/F)	Age at examination (mo)	PLA left eye (%)	PLA right eye (%)	Phenotype	Case/control status	*OLFML3* variant genotype
27584	M	135	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27585	M	39	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27586	F	141	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27587	F	165	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27588	M	66	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27589	F	60	0	0	PLA unaffected	Control	G/G	Homozygous wild type
27590	M	133	0	0	PLA unaffected	Control	G/G	Homozygous wild type

F, female; M, male; No., number; NP, not performed (gonioscopy could not be performed in PCAG affected eyes)

and novel mutation. If a popular sire or dam harbored the novel causative mutation for PLA and PCAG, then this would explain why these diseases are now behaving as simple autosomal recessive traits in the majority of affected dogs in our study.

Although the association of the *OLFML3* variant with PLA and PCAG was significant, there were a few discordant results which should be discussed. If PLA and PCAG are truly autosomal recessive traits resulting from a single mutation, then it would be expected that unaffected dogs would either be homozygous for the wild-type allele or heterozygous. In our study, 88 of the 90 control dogs fulfilled this expectation but two dogs with normal eyes, which were eight months old at the time of examination, were homozygous for the *OLFML3* variant. Although a previous study which investigated PLA prevalence, using data derived from the same Border Collie dogs as the current study, failed to demonstrate an association of PLA with age,[22] such an age association has been shown in dogs of other breeds.[7,22,24-26] It has also been postulated that the previous Border Collie study was underpowered to detect any association because of the low prevalence of PLA in this breed.[22] Thus, it is still possible that PLA may be progressive in the Border Collie, and these two young dogs may develop PLA in later life. It will be important to monitor these dogs with serial gonioscopy examinations in the future.

Conversely, if PLA and PCAG were simple autosomal recessive traits, then it would be expected that both PLA and also PCAG cases would all be homozygous for the candidate variant. In fact, only 11 of the 16 cases were homozygous for the variant and the remaining five were either homozygous wild type or heterozygous. A possible explanation for this is that, although the majority of Border Collie PLA and PCAG cases may be explained by a single variant, other variants are likely to be involved in the etiopathogenesis of these diseases in a similar way as proposed in other breeds. It is also possible that, as yet unidentified, environmental factors are involved in the phenotypes of PLA and PCAG. Further investigations, however, are required to investigate this hypothesis.

Although there was significant association of PLA and PCAG with the c.590G>A *OLFML3* variant, it is not known whether this variant is the causative mutation or not. Pugh et al reported a genome-wide signal on chromosome 17 spanning 1.80 Mb to be associated with PLA and PCAG.[23] *OLFML3* resides within this locus, and a variant within this gene was shown to segregate with disease status. *OLFML3* has not been previously associated with glaucoma in any species; however, a mutation in a related gene, *OLFM2*, has been associated with open-angle glaucoma in humans making *OLFML3* a feasible candidate for primary glaucoma in the dog.[28] Functional evidence of the proposed *OLFML3* variant in the pathogenesis of PLA and PCAG is currently lacking, however, and, furthermore, 11 other genes reside within the identified chromosome 17 locus which are yet to be fully excluded as candidates for PLA and/or PCAG.

TABLE 4 Summarized *OLFML3* variant genotyping results in controls and cases

Case/control status	No. of dogs homozygous wild type (G/G)	No. of dogs heterozygous (G/A)	No. of dogs homozygous variant (A/A)	Total
Controls	71 (78.9%)	17 (18.9%)	2 (2.2%)	90 (100%)
Cases	3 (18.7%)	2 (12.5%)	11 (68.8%)	16 (100%)

Regardless of whether the *OLFML3* variant is causative of PLA and PCAG or not, a DNA test based on this variant can still be used effectively to reduce disease prevalence. This is because, even if the tested variant is not responsible for PLA and PCAG, the true causative variant is likely to be located very close to it on chromosome 17 and, therefore, will be genetically linked to it. In this situation, a DNA test based on the *OLFML3* variant would be acting as a linkage test which, although less reliable than a mutation detection test, would still have good sensitivity and specificity.

In the United Kingdom Border Collie population as a whole, we estimate 0.2% of Border Collies to be homozygous for the variant and thus affected by *OLFML3*-associated PLA and/or PCAG. We estimated a further 8.4% of Border Collies to be heterozygous (carriers of the variant). However, although we attempted to ensure our cohort of Border Collies was as representative of the UK population as possible, we were not able to formally assess relatedness of our samples and thus it is possible that these are either underestimates or overestimates.

Selective breeding based on DNA test results would allow the variant to be eliminated from the population easily and quickly. DNA testing should, however, be accompanied by breeding advice with the aim of reducing disease prevalence but not at the expense of reducing genetic diversity. Because PLA and PCAG in this breed appear to be mainly acting as monogenic autosomal recessive traits, dogs both heterozygous and homozygous for the variant can be used in breeding programs as long as mated with dogs homozygous for the wild-type allele. This approach will lead to a gradual reduction in the prevalence of the variant without the risk of creating dogs homozygous for the variant and thus being at risk of developing PLA and PCAG. It is important to emphasize, however, that, although PLA is a recognized risk factor for PCAG, only a minority of dogs with PLA develop PCAG. It is also very likely that other genetic and environmental factors are involved in PLA and PCAG pathogenesis, and thus, it is important that dogs continue to be clinically phenotyped by serial ophthalmic examinations including gonioscopy regardless of their genotype for the *OLFML3* variant.

In summary, our findings confirm the association of the c.590G>A *OLFML3* variant with PLA and PCAG in Border Collies from the United Kingdom. DNA testing for the variant and selective breeding can reasonably be expected to result in a reduction of PLA and PCAG prevalence in the breed. Our results suggest that DNA testing for this variant in dogs of other breeds is unlikely to be predictive for PLA or PCAG.

REFERENCES

1. Pizzirani S. Definition, classification, and pathophysiology of canine glaucoma. *Vet Clin North Am Small Anim Pract*. 2015;45(6):1127-1157.
2. Gelatt KN, Peiffer RL Jr, Gwin RM, et al. Glaucoma in the beagle. *Trans Sect Ophthalmol Am Acad Ophthalmol Otolaryngol*. 1976;81:Op 636-644.
3. Bedford P. A gonioscopic study of the iridocorneal angle in the English and Amercian breeds of Cocker Spaniel and Basset Hound. *J Small Anim Pract*. 1977;18:631-634.
4. Bjerkås E, Ekesten B, Farstad W. Pectinate ligament dysplasia and narrowing of the iridocorneal angle associated with glaucoma in the English Springer Spaniel. *Vet Ophthalmol*. 2002;5:49-54.
5. Ekesten B, Narfstrom K. Age-related changes in intraocular pressure and iridocorneal angle in Samoyeds. *Progress Vet Comp Ophthalmol*. 1992;2:37-40.
6. Ekesten B, Narfstrom K. Correlation of morphologic features of the iridocorneal angle to intraocular pressure in Samoyeds. *Am J Vet Res*. 1991;52:1875-1878.
7. Fricker GV, Smith K, Gould DJ. Survey of the incidence of pectinate ligament dysplasia and glaucoma in the UK Leonberger population. *Vet Ophthalmol*. 2016;19(5):379-385.
8. Read RA, Wood JL, Lakhani KH. Pectinate ligament dysplasia (PLD) and glaucoma in Flat Coated Retrievers. I. Objectives, technique and results of a PLD survey. *Vet Ophthalmol*. 1998;1:85-90.
9. Wood J, Lakhani KH, Mason IK, Barnett KC. Relationship of the degree of goniodysgenesis and other ocular measurements to glaucoma in Great Danes. *Am J Vet Res*. 2001;62:1493-1499.
10. Cottrell B, Barnett K. Primary glaucoma in the Welsh springer spaniel. *J Small Anim Pract*. 1988;29:185-189.
11. Wood JL, Lakhani KH, Read RA. Pectinate ligament dysplasia and glaucoma in Flat Coated Retrievers. II. Assessment of prevalence and heritability. *Vet Ophthalmol*. 1998;1:91-99.
12. Martin CL, Wyman M. Glaucoma in the Basset Hound. *J Am Vet Med Assoc*. 1968;153:1320-1327.
13. Oliver J, Rustidge S, Pettitt L, et al. Evaluation of ADAMTS17 in Chinese Shar-Pei with primary open-angle glaucoma, primary lens luxation, or both. *Am J Vet Res*. 2018;79:98-106.
14. Oliver J, Forman OP, Pettitt L, Mellersh CS. Two independent mutations in ADAMTS17 are associated with primary open angle glaucoma in the Basset Hound and Basset Fauve de Bretagne breeds of dog. *PLoS ONE*. 2015;10:e0140436.
15. Forman OP, Pettitt L, Komáromy AM, Bedford P, Mellersh C. A novel genome-wide association study approach using genotyping by exome sequencing leads to the identification of a primary

open angle glaucoma associated inversion disrupting ADAMTS17. *PLoS ONE*. 2015;10:e0143546.
16. Ahonen SJ, Kaukonen M, Nussdorfer FD, Harman CD, Komáromy AM, Lohi H. A novel missense mutation in ADAMTS10 in Norwegian Elkhound primary glaucoma. *PLoS ONE*. 2014;9:e111941.
17. Kuchtey J, Kunkel J, Esson D, et al. Screening ADAMTS10 in dog populations supports Gly661Arg as the glaucoma-causing variant in beagles. *Invest Ophthalmol Vis Sci*. 2013;54:1881-1886.
18. Komaromy AM, Petersen-Jones SM. Genetics of canine primary glaucomas. *Vet Clin North Am Small Anim Pract*. 2015;45(6):1159-1182.
19. Ahram DF, Grozdanic SD, Kecova H, Henkes A, Collin R, Kuehn MH. Variants in nebulin (NEB) are linked to the development of familial primary angle closure glaucoma in basset hounds. *PLoS ONE*. 2015;10:e0126660.
20. Ahram DF, Cook AC, Kecova H, et al. Identification of genetic loci associated with primary angle-closure glaucoma in the basset hound. *Mol Vis*. 2014;20:497-510.
21. Ahonen SJ, Pietilä E, Mellersh CS, et al. Genome-wide association study identifies a novel canine glaucoma locus. *PLoS ONE*. 2013;8:e70903.
22. Oliver JA, Ekiri AB, Mellersh CS. Pectinate ligament dysplasia in the Border Collie, Hungarian Vizsla and Golden Retriever. *Vet Rec*. 2017;180:279.
23. Pugh CA, Farrell LL, Carlisle AJ, et al. Arginine to glutamine variant in Olfactomedin Like 3 (OLFML3) is a candidate for severe goniodysgenesis and glaucoma in the border collie dog breed. *G3: Genes - Genomes - Genetics*. 2019;9:943–954.
24. Oliver JA, Ekiri A, Mellersh CS. Prevalence of pectinate ligament dysplasia and associations with age, sex and intraocular pressure in the Basset hound, Flatcoated retriever and Dandie Dinmont terrier. *Canine Genet Epidemiol*. 2016;3:1.
25. Oliver JA, Ekiri A, Mellersh CS. Prevalence and progression of pectinate ligament dysplasia in the Welsh springer spaniel. *J Small Anim Pract*. 2016;57(8):416-421.
26. Pearl R, Gould D, Spiess B. Progression of pectinate ligament dysplasia over time in two populations of Flat-Coated Retrievers. *Vet Ophthalmol*. 2015;18:6–12.
27. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol*. 2000;132:365–386.
28. Holt R, Ugur Iseri SA, Wyatt AW, et al. Identification and functional characterisation of genetic variants in OLFM2 in children with developmental eye disorders. *Hum Genet*. 2017;136:119–127.

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