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Progression of pectinate ligament dysplasia over time in two populations of Flat-Coated Retrievers

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Abstract

Objective Two of the authors (DG, BS) independently observed that a number of Flat-Coated Retrievers (FCRs) previously unaffected by pectinate ligament dysplasia (PLD) appeared to develop the condition later in life. This study was instigated to investigate progression of PLD within individual dogs over time.

Animals studied Flat-Coated Retrievers that had previously undergone gonioscopy under the UK/ECVO hereditary eye schemes were included in the study.

Procedure A second gonioscopic examination was performed 1.92–12.58 years later (mean 6, median 5.75 years) and the results compared. 39 FCR (17 males, 22 females) in the UK and 57 FCR (27 males, 30 females) in Switzerland were included. Slit-lamp biomicroscopy, indirect ophthalmoscopy, and gonioscopy were performed in all dogs. Gonioscopy allowed classification as either unaffected or affected; percentage of the iridocorneal drainage angle (ICA) affected by PLD was determined, before calculating progression observed as mild, moderate, or severe.

Results 39 of 96 (40.6%) dogs demonstrated progression of PLD ($P < 0.0001$). Of these, 13 of 96 (13.5%) were classified as mild progression (from either unaffected to 10–20% or 10–20% to 20–90% ICA affected). Progression was more extensive in 26 of 96 (27.1%) dogs ($P < 0.0001$), of which 12 of 96 (12.5%) went from unaffected to severe PLD of >90% ICA affected, consistent with a high risk of glaucoma.

Conclusions To the authors' knowledge, this is the first report describing progression of PLD in individual dogs over time, in a breed affected by primary, angle closure glaucoma.

Key Words: Flat-Coated Retriever, goniodysgenesis, gonioscopy, iridocorneal angle, pectinate ligament dysplasia, primary glaucoma

INTRODUCTION

Glaucoma describes a final common pathway of a group of diseases which cause retinal ganglion cell (RGC) impairment and death, optic nerve axonal loss, and concurrent optic nerve head cup enlargement with incremental reduction in visual fields and blindness.[1] The primary risk factor currently identified in the dog is an elevated intraocular pressure (IOP). Intraocular pressure is determined and maintained by the rate of aqueous humor formation, which equals rate of outflow.

Drainage of aqueous humor occurs at the iridocorneal angle (ICA), the anterior opening of the ciliary cleft, spanned by the comb-like pectinate ligament. Passage of aqueous humor between the intraligamentary spaces allows entry to uveal then corneoscleral trabecular meshworks before collection by the angular aqueous plexus, intrascleral plexus, and vortex venous drainage system.[1] Drainage via this conventional route accounts for 85% of aqueous outflow in the dog, and the venous resistance created by this contributes to approximately 50–75% of the resistance that determines IOP; the remaining 15% of aqueous outflow, draining via uveoscleral (or unconventional) outflow, is independent of IOP.[2]

Primary glaucomas represent progressive diseases of the aqueous humor outflow pathways and develop in the absence of antecedent intraocular disease, whereas secondary glaucomas occur when ocular disease obstructs aqueous outflow pathways.[1] Primary glaucomas in the dog have potential for bilateral development and are

considered hereditary in a number of breeds, including the Flat-Coated Retriever.[1] Classification is further determined according to an open or narrow ICA, either at gonioscopic examination or via imaging modalities such as high-resolution ultrasonography or ultrasound biomicroscopy.[1] Pectinate ligament dysplasia (PLD) describes the consolidation of adjacent pectinate ligaments into broad sheets and is often reported in association with many primary narrow and closed angle glaucomas. Read *et al.* (1998) demonstrated the presence of PLD to be significantly correlated with the risk of developing primary glaucoma in the Flat-Coated Retriever breed, and Wood *et al.* demonstrated a hereditary basis for the presence of PLD in that same body of research.[3,4]

Gonioscopy has been recognized as essential to the evaluation of ICA abnormalities in the dog since studies published by Martin in 1969 and Bedford in the 1970s.[5–8] Bedford examined a large number of dogs in the UK, including breeds considered at increased risk of primary glaucoma. He documented variations in the pectinate ligament structure and angle width in the English Cocker Spaniel and Basset Hound at an age of 4 to 5 months and concluded these changes were likely congenital and not related to disease or aging process.[6] Martin performed an early SEM study of the ICA morphology in puppies aged between 6 weeks prenatal and 4 weeks postnatal and noted ongoing development of the pectinate ligament morphology during those early postnatal weeks.[9] This established a process of rarefaction of an initial sheet of ICA tissue, with progressive opening of intraligamentary spaces. Samuelson and Gelatt further clarified the histological detail in a 1984 study of the ontogeny of the ICA in the normal Beagle.[10] Rarefaction of the initial fibrillar sheet was mostly complete by 2–4 weeks postnatal, leaving strands of intertwining collagen, progressively encased by attenuate trabecular cells, confluent with the anterior surface of the iris. Infrequent sheets, partially rarefied with holes, were, however, noted as late as 8 weeks postnatally, and it was from 8 weeks of age onwards that morphological development of the pectinate ligament (and deeper angle structures) was considered complete.

In breeds for which a hereditary basis has been established for PLD, gonioscopy forms a component of national hereditary eye disease screening programmes and allows the identification of PLD-affected individuals prior to breeding, as well as indicating risk of developing glaucoma in individual dogs. National hereditary eye disease screening programmes in Europe include the British Veterinary Association/Kennel Club/International Sheep Dog Society (BVA/KC/ISDS) scheme and the European College of Veterinary Ophthalmologists Hereditary Eye Disease (ECVO HED) scheme. Subsequent to the developmental studies by Samuelson and Gelatt and to facilitate ease of examination with regard to the size of the eye, the UK (BVA/KC/ISDS) and ECVO hereditary eye panel certification has considered the gonioscopic examination to be a 'once in a lifetime' test, performed from 6 months of age onwards.[11,12]

This study arose out of an observation that a number of individual adult Flat-Coated Retriever dogs appeared to demonstrate progression of PLD over a period of time. This was noted independently by authors DJG and BS, in the UK and Switzerland, respectively. To investigate this further, a joint study was undertaken to determine the incidence of PLD progression in representatives of the UK and Swiss populations of the Flat-Coated Retriever breed. Flat-Coated Retrievers that had previously undergone gonioscopy as part of the BVA/KC/ISDS or ECVO HED schemes were re-examined at a later age and gonioscopy repeated. The results of initial and later gonioscopic findings were compared to determine whether there had been progression of PLD over time.

MATERIALS AND METHOD

Two populations of FCR were examined, one cohort in the UK (FCR-UK) and the other in Switzerland (FCR-Swiss). The primary inclusion criteria were availability of gonioscopy data from a previous hereditary eye disease screening examination for each individual dog, under either the BVA/KC/ISDS eye panel in the UK or the ECVO HED scheme in Switzerland. Examinations of these dogs were organized after enlisting the help of the Flat-Coated Retriever breed club in each respective country. In the UK, dogs attended a single event at which these second eye examinations took place, whilst in Switzerland, dogs were invited to attend during regular clinic times and the second examinations took place over a longer time period. Data recorded included each individual dog's date of birth, sex, coat color, kennel club registration number, and permanent identification number (microchip or tattoo), and this was verified against previous examination identification.

Pre-existing, first gonio-examination data of FCR-UK dogs were performed by BVA/KC/ISDS eye panelists, following the protocol of the BVA/KC/ISDS eye scheme.[11] The second, prospective examinations were performed by three experienced BVA/KC/ISDS eye panelists, not stipulating the same examiners as those who performed the first examination, in case this limited the data available. Subsequent analysis of the first examination data did however reveal that these three examiners had performed two-thirds (26/39) of those first examinations. At second examination, it was ensured that at least two, if not all three of the examiners, looked at each FCR-UK dog with any PLD abnormality, thus ensuring consistency of examination technique and agreement of results. Results from the first examination were masked from the examiners until after performing and recording the second examination. Both initial and second gonioscopic examinations of FCR-Swiss were performed by

one author (BS). Data from the first examination were again masked from the examiner until after the second examination.

Prior to gonioscopy, ophthalmic examination protocol included slit-lamp biomicroscopy, indirect ophthalmoscopy, and direct ophthalmoscopy. Tonometry was not routinely performed unless indicated. The cornea was then anaesthetized with topical proxymetacaine (proparacaine) 0.5% (Bausch & Lomb, Chauvin Pharmaceuticals Ltd., Aubenas, France) prior to bilateral gonioscopic examination, performed as described by Read.[3] A Koeppe goniolens was used in all dogs, selecting sizes 17 or 19 mm in the UK dogs or 17, 18, or 19 mm in the Swiss dogs. Goniolens size was based on subjective assessment of corneal width and for the majority of these mature dogs, a size 19 mm was deemed appropriate. The selected goniolens was two-thirds filled with a coupling gel, typically either carbomer gel 2 mg/g (Viscotears; Alcon, Hemel Hempstead, UK) or hypromellose 1% (Isopto Alkaline; Alcon, Hemel Hempstead, UK), before placing in contact with the cornea, ensuring neither air bubble nor nictitating membrane entrapment. For FCR-UK dogs, the ICA was viewed using a handheld slit-lamp biomicroscope (Kowa SL-14 or SL-15). For FCR-Swiss dogs, the ICA was viewed using either a handheld slit-lamp biomicroscope (Clement Clarke BA904) or a Genesis-D fundus camera. All examinations were performed on conscious dogs, without sedation or pharmacological mydriasis and ensuring bilateral gonioscopic examination of the entire 360° of the ICA.

The ICA was primarily examined for the presence or absence of PLD, determined by abnormally broad and thickened pectinate ligament fibers or solid sheets of pectinate ligament tissue, with or without 'flow holes' but lacking in normal interfiber spaces. Where PLD was present, this was quantified by assigning a percentage of the 360° affected, determined after systematically viewing the entire circumference of the ICA. The examiners did not set out to score width of ICA, as quantified analysis could not be performed in relation to the formative examination, however, if significant narrowing or other abnormality was noted this was subjectively assessed.

Comparison of results between first and second gonioscopic examinations aimed to identify any progression of PLD, effectively testing a null hypothesis of 'no change in degree of PLD' for each dog. It was important to quantify the severity of any progression without overly interpreting a small change, which could be influenced by subjectivity. This was achieved by first simplifying the PLD percentage to a grade, according to an ordinal scale ranking as follows (see Figs 1a–e also for examples):

0 – Not affected
1 – Affected, mild PLD involving <20% of ICA
2 – Affected, moderate PLD involving 20–90% of ICA
3 – Affected, severe PLD involving more than 90% of ICA

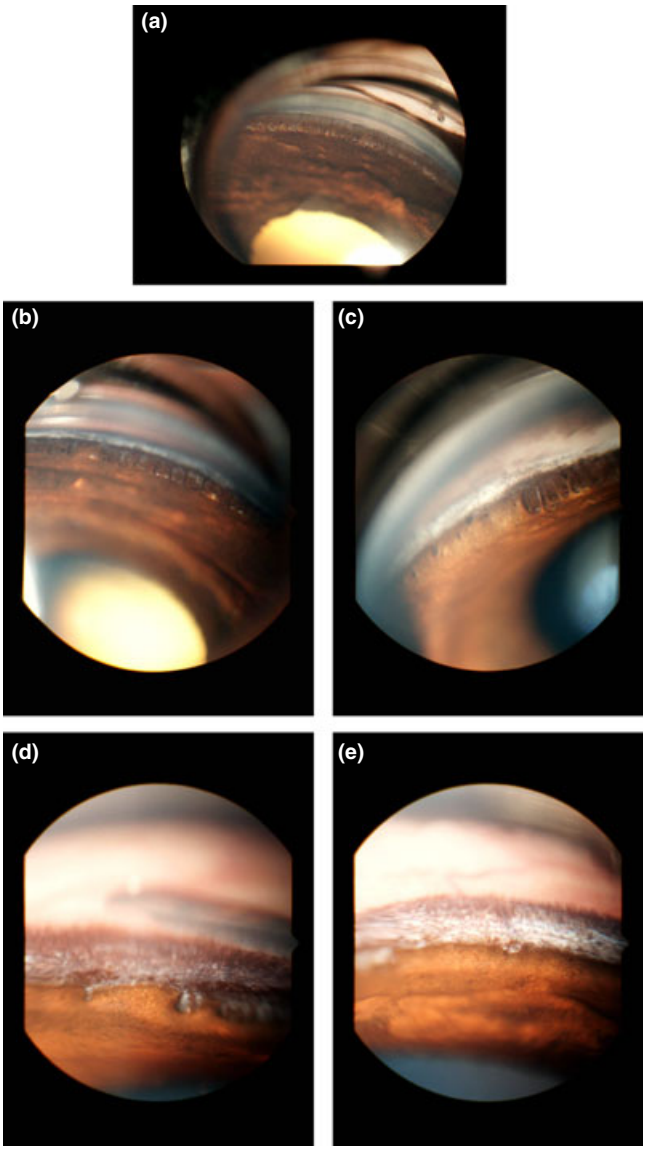


Figure 1. Gonioscopic photographs typically show a portion of the iridocorneal drainage angle (ICA) (between 15–25%) per slit-lamp biomicroscopy view and are included to indicate clinical examples of grading. It is important to note that the focus at the periphery is inadequate therefore legend descriptions and grading refers to that viewed in the mid-portion and grading applied as if the whole ICA is similarly affected. (a) Normal appearance of the pectinate ligaments, normal width of ICA. Unaffected or grade 0. (b) ICA of normal width with broad strands of pectinate ligament, one small area of lamina with flow hole. 10% affected or grade 1. (c) ICA occluded by a sheet of pigmented tissue spanning the angle from the base of the iris to the inner-pigmented band with several flow holes visible, alternating with sectors of normal appearing pectinate ligament. 50% affected or grade 2. (d) ICA occluded by a sheet of pigmented tissue spanning the angle from the base of the iris to the inner-pigmented band with several flow holes visible. 70% affected or grade 2. (e) ICA occluded by a sheet of pigmented tissue spanning the angle from the base of the iris to the inner-pigmented band with one flow hole visible. >90% affected or grade 3.

Any change in the grade of PLD between first and second gonioscopic examinations was then determined. Mild progression of PLD was determined as a one-step increase in the ordinal scale, that is, from grade 0 (unaffected) to 1, from 1 to 2, or from 2 to 3. Moderate progression of PLD was defined as either progression from grade 0–2 or 1–3. Severe progression of PLD was defined as an increase from grade 0–3.

Statistical analysis was performed using a Mann–Whitney–Wilcoxon test using commercial software (GraphPad Prism, version 5.01, GraphPad, CA, USA), to establish if change was significant, with an assigned P value of <0.005. The time period between first and second gonioscopic examinations for each dog was also evaluated.

RESULTS

Data for first and second gonioscopic examinations were recorded for 39 dogs in the FCR-UK group (17 males, 22 females) and 57 in the FCR-Swiss (27 males, 30 females). Examples of goniophotographs obtained from the FCR-Swiss are shown in Figs 1a–e.

In the FCR-UK group, the time interval between first and second gonioscopic examinations ranged from 1.92 to 12.58 years (mean of 6.65 years, median 6.58 years). In the FCR-Swiss group, the time interval between first and second gonioscopic examinations ranged from 3.25 to 11.17 years (mean of 5.68 years, median of 5.25 years). Date of the original certifying examination could not be verified for one FCR-Swiss dog. Examination interval mean for both populations was 6 years, median 5.75 years.

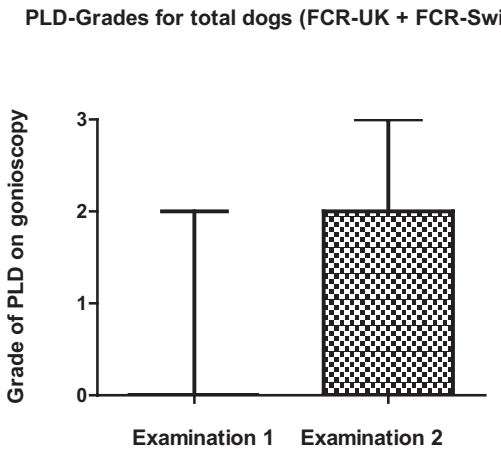
Age of the dogs at second examination ranged from 4.58 to 12.75 years in the FCR-UK (mean of 8.4 years and median of 8.08 years). In the FCR-Swiss, the age of examined dogs ranged from 2.25 to 13 years (mean of 7.04 years and median of 6.83 years).

The results of the first and second examinations from each group of dogs are summarized in Table 1. This demonstrates that 14 of 96 dogs were noted as PLD-affected at the first examination. In total, 11 of 96 were classified as grade 1 and 3 of 96 as grade 2, comprised of 3 of 39 FCR-UK dogs (all grade 1), and 11 of 57 FCR-Swiss dogs (8 of 57 grade 1 dogs and 3 of 57 grade 2). At the second examination, 45 of 96 dogs exhibited PLD. Of these affected dogs, 17 of 39 were from the FCR-UK group (8 of 39 were grade 1, 5 of 39 grade 2, and 4 of 39 grade 3) and 28 of 57 were FCR-Swiss (4 of 57 grade 1, 12 of 57 grade 2, and 12 of 57 grade 3). In total, 12 of 96 dogs were grade 1 PLD-affected, 17 of 96 were grade 2, and 16 of 96 were grade 3. These results are also depicted in Graph 1.

Comparison between first and second examinations revealed progression of PLD grade in 39 of 96 (40.6%) of the total dogs (summarized in Table 2), and this change was determined to be highly statistically significant ($P < 0.0001$). Of those dogs demonstrating progression, 13 of 96 (13.5%) exhibited mild progression, comprised of 6 of 39 of the FCR-UK dogs, and 7 of 57 of the FCR-Swiss. Moderate progression was observed in 14 of 96 (14.6%) dogs, including 6 of 39 FCR-UK and 8 of 57 FCR-Swiss. Severe progression was exhibited by 12 of 96 (12.5%) dogs, of which 3 of 39 were FCR-UK and 9 of 57 FCR-Swiss. As it could be argued that those which demonstrated only a mild degree of progression could have been influenced by examination subjectivity, the

Table 1. Summary of the results of the first [Flat-Coated Retrievers (FCR)-UK 1, FCR-Swiss 1] and second (FCR-UK 2, FCR-Swiss 2) eye examinations from each group of dogs

PLD classification	FCR-UK 1	FCR-UK 2	FCR-Swiss1	FCR-Swiss2
Unaffected	36	22	46	29
Grade 1	3	8	8	4
Grade 2	0	5	3	12
Grade 3	0	4	0	12
Total	39	39	57	57



Graph 1. Box and whisker plot to indicate pectinate ligament dysplasia (PLD) grade viewed on gonioscopy. Whiskers indicate full PLD range of the results on examination, including total of 96 dogs [Flat-Coated Retrievers (FCR)-UK + FCR-Swiss].

Table 2. Summary of progression of pectinate ligament dysplasia (PLD) over time in the UK and Swiss groups of Flat-Coated Retrievers. Progression of PLD was noted in 39 of 96 (40.6%) of dogs, of which this was moderate (two-step increase in PLD grade) in 14 of 96 (14.6%) of dogs and severe (three-step increase) in 12 of 96 (12.5%) of dogs

PLD progression	FCR-UK	FCR-Swiss	Total: (FCR-UK + FCR-Swiss)
None	24	33	57
Mild	6	7	13
Moderate	6	8	14
Severe	3	9	12
Total progressed	15	24	39
Total dogs	39	57	96

statistical analysis was repeated after eliminating 'mild' progression (changing these to 'no progression'), effectively including 'moderate' and 'severe' as the only relevant PLD progression. This revealed progression in 26 of 96, or 27.1% of dogs, and this change in PLD grade was still found to be highly statistically significant ($P < 0.0001$). Comparing the two populations, 9 of 39 (23.1%) of FCR-UK dogs demonstrated this moderate or severe progression and 17 of 57 (29.8%) of FCR-Swiss.

Fifty-seven dogs (59.4%) demonstrated no progression in PLD between first and second examinations. Of these, 24 dogs were from the FCR-UK group and 33 from the FCR-Swiss group (Table 2). Two of the 24 FCR-UK dogs were grade 1, the remainder unaffected (grade 0); 2 of 33 of the unchanged FCR-Swiss dogs were grade 1, and 2 of 33 were grade 2, with the remainder unaffected. All dog PLD grades either progressed or remained static, no reduction in PLD grade was observed.

In the FCR-UK group, it was noted at second examination that four dogs had 'narrow' ICA and one had 'closed' ICA. None of these had either PLD or abnormal angles described on first examination data.

Additionally, by the second examination, two of the dogs had developed clinical glaucoma, one from each of the FCR-UK and FCR-Swiss groups. Both exhibited a PLD grade of 3, that is, 90% or more of the ICA affected by PLD, at the second examination. The FCR-Swiss dog had undergone a moderate PLD progression, from grade 1 to grade 3. The UK dog had been described as 'unaffected OU' at first examination, and severe progression was noted OS at second examination. The contralateral eye could not be evaluated as it had previously been enucleated due to a clinical diagnosis of intractable primary glaucoma. Histopathology was not available for inclusion, but it was these reports of individual cases of primary glaucoma in certified 'unaffected' dogs that had prompted this study.

DISCUSSION

To the authors' knowledge, this is the first report describing progression of pectinate ligament dysplasia in individual dogs over time, in a breed affected by primary, angle closure glaucoma.

As already described, clinical reports of glaucoma in FCR dogs previously certified as 'unaffected' or <20% affected had incentivized the study. It was also ideal to look at a breed with an established link between PLD and glaucoma and in which glaucoma has been demonstrated to be hereditary. In the study by Read *et al* (1998)[3], examining a random population of 398 FCR in the UK, incidence of PLD was determined as 34.7%, compared with 6% in a control population of 100 dogs of various other breeds. In this study, only dogs which were 'unaffected' or grade 1 and therefore deemed to have 'passed for breeding' re-presented to us and could be included, thus these groups cannot be used to reflect on PLD prevalence in the breed. However, data were sought from the BVA/KC/ISDS Eye Scheme for this purpose and for the period 2007–2011 indicated prevalence of PLD (typically >20% ICA involvement) of 5.4%, in the examined UK FCR population, suggesting a significant reduction since initiation of gonioscopy screening for this breed (BVA, unpublished data, December 2012).

Read *et al.* also examined an additional target population of 48 FCR (either relatives of high-scoring PLD individuals or those presenting with PLD associated with glaucoma) and the relationship between age as a covariate and incidence of glaucoma was investigated. Age was found to be insufficient as an individual variable to account for glaucoma, in the face of the variable PLD, although mean PLD grade did increase with FCR age.[3] A survey of English springer spaniels in Norway, including 279 normotensive dogs of which five went on to develop glaucoma during the study plus nine additional glaucomatous dogs, established a clear relationship in that breed between both degree of PLD (prevalence of 25.5%) and narrowing of the relative width of the ciliary cleft (RWOCC) (prevalence of 17.9%) on glaucoma.[13] A positive relationship was also noted between both age and PLD grade on narrowing of the RWOCC. Both of these studies contrast to ours in that they were cross-sectional rather than longitudinal; however, the positive correlation noted between age and severity of PLD supports our findings, that PLD in some individuals advances with age.

The gonioscopy examination and interpretation method used here was based on that used by Read, which determined the ICA percentage affected by PLD before simplifying that percentage to an ordinal scale ranking.[3] The scale used by Read differed from ours in defining unaffected as <25% PLD, with a nominal value of 12.5%, so as not to overemphasise any potential normal ICA variation. In our study, it was preferable to use a value of <20% as grade 1 as although somewhat arbitrary this correlates with a level of ICA variation commonly considered acceptable for hereditary eye screen examinations in the UK.[11] Quantifying the higher percentages of PLD in to a meaningful ordinal scale ranking was important, but it was also necessary to not overly interpret percentages of PLD, considering the degree of subjectivity inherent to gonioscopy. This was particularly important considering comparisons were being made between a prospective or contemporary second examination and an historical first examination. The broad categorization of grade 2 as between 20% and 90% represented clinically relevant PLD. Grade 3, at >90%, was consistent with a near total ICA abnormality and a high risk of glaucoma.[3]

Consistency of examination technique between first and second examinations was imperative. All examiners were experienced eye panel ophthalmologists (BVA/KC/ISDS for the UK, ECVO for Switzerland). In the Swiss popula-

tion, consistency was aided by virtue of maintaining the same examiner. As already stated, this was not stipulated for the FCR-UK; however, the use of BVA/KC/ISDS eye panel ophthalmologists (all are experienced ophthalmologists who undergo a rigorous examination procedure prior to their acceptance on the panel) means variation in technique and expertise with regard to gonioscopy and quantifying PLD should be limited. As previously stated, it also transpired that two-thirds (26/39) of the examinations were performed by the same examiners plus at least two, if not all three examiners looked at affected dogs so as to ensure consistency of designated grade system.

Progression of pectinate ligament dysplasia was noted in 39 of 96 (40.6%) of the total population of FCR and statistical evaluation of this change determined it to be highly significant. Of these, 15 of 39 (38.5%) were FCR-UK and 24 of 57 (42.1%) were FCR-Swiss. So as to eliminate over-interpretation of results, the statistical analysis was repeated, with an assumption that those dogs, which demonstrated only 'mild' or 'one-step' progression, may not have demonstrated clinically relevant change or could have been influenced by examiner subjectivity. It was still shown that 26 of 96 (27.1%) dogs demonstrated progression of PLD, which was still highly statistically significant.

Further investigations are required both to further quantify these ICA changes and investigate the mechanism involved in this change in the pectinate ligament. Utilizing high-resolution ultrasound biomicroscopy and/or ultrasound biomicroscopy would allow assessment of the deeper recess of the ciliary cleft for each individual with digital recording for comparison of results, potentially reducing subjectivity and improving interexamination reproducibility.[14] However, these imaging modalities provide a cross-sectional view of the ICA, effectively perpendicular to the radial pectinate ligament itself, and it may be that this does not necessarily allow good quantification of circumferential PLD specifically.

As pectinate ligament structure is fully developed by 8 weeks postnatally [10], the progression of pectinate ligament 'dysplasia' over time must occur due to other factors. Possible mechanism of this change may be a unification between primary (anterior) and secondary ligaments or other altered structure due to degenerative process. Although Samuelson and Gelatt[10] considered the ICA morphology to be mature at 8 weeks of age, increased numbers of trabecular cells including melanocytes were noted later at 12 and 16 weeks and penetration of the pectinate ligament collagenous core deep into Descemet's membrane became progressively harder to identify due to increased envelopment by that thickening membrane. If progressive cellular deposition around the collagenous core of pectinate ligaments is continued throughout life, the ligament becomes increasingly thickened, forming wider sheets of tissue. Where there is an association with narrowing of the ICA, this could physical compress the pectinate ligament or contribute to reduced surface area available for cellular deposition. A relationship between PLD and narrowing of the ICA was indicated histologically in the Bouvier des Flandres breed by van der Linde-Sipman in 1987.[15] Deposition of periodic acid–Schiff (PAS) positive material, akin to thickened basal membrane, was also noted on the trabecular meshwork and behind the primary pectinate ligament, in both glaucomatous and severely PLD-affected eyes. Extensive basal lamina-like material including heparan sulfate-type proteoglycans has been noted to accumulate in the trabecular meshwork in human forms of glaucoma including goniodysgenetic glaucoma.[16]

Ekesten *et al.* scored the width of the ICA in his gonioscopic study of PLD in the Samoyed as well as the English springer spaniel and in both noted a progressive narrowing of the ICA with age.[13,17] The examiners in this study did not score angle width as it was considered too subjective to be evaluated particularly as it was not quantified in the first examination. Read *et al.*[3] had also not evaluated width of ICA in the 1998 study, considering it too highly variable, including between eyes in individual dogs and even within the same eye. In the Basset Hound, however, a relationship between progressive narrowing of the ICA and the development of glaucoma has been demonstrated.[18] In this study, it was stated at second examination that four dogs had 'narrow' ICA and one had 'closed' ICA. The ages of these 5 dogs ranged from 7.17 to 11.33 years with a mean of 9 years (1 year older than that for FCR-UK). None of these had either PLD or abnormal angles described on first examination data. Severity of PLD grade at second examination did not necessarily appear to correlate with the described width, although this could not be statistically evaluated due to the small numbers described affected. One was classified as PLD grade 1, two as grade 2, and one as grade 3 for the 'narrow' angles; the 'closed angle' dog was described as grade 2; these were all normotensive.

It is important to consider that gonioscopy also allows examination of peripheral anterior synechiae, and these may readily be misinterpreted as PLD by less-experienced examiners. These are typically broad-based at the iris and not within but anterior to and obscuring the ICA. The dogs in the current study had no history of antecedent ocular disease and were examined by experienced ophthalmologists, thus this is unlikely to be a confounding factor.

Bjerkas and Ekesten speculate that subclinical inflammatory events could have been responsible for the progression of PLD with age in their population of ESS dogs.[13] In the Basset Hound, uveitis frequently accompanies glaucoma and is suspected to contribute to deterioration of ICA conformation with time.[1] Bedford described abnormal pectinate ligament structure in the glaucomatous Basset, with a narrowed and darkly pigmented ICA.[6,7] Histopathologically, the closed ciliary cleft was covered by an extension and reflection of Descemet's membrane, and

he surmised that part of the amorphous material noted gonioscopically was aggregations of Descemet's membrane. Gonioscopy had revealed sheets of gray-white amorphous tissue with broad iris bases crossing the ICA. A process of descemetization of the ICA has also been suggested as the trigger that initiates glaucoma in PLD-affected individuals by Smith *et al.*[19].

The clinical relevance of this study ultimately arises as we consider the (12 of 96) dogs which demonstrated severe progression of PLD grade, from unaffected to grade 3. PLD of >90% is highly associated with a risk of glaucoma[3], and two of the dogs in the current study had already developed glaucoma. This indicates that performing gonioscopy once only and at a young age as part of hereditary eye screen examinations underestimates the number of dogs which will go on to be at high risk of glaucoma. Wood *et al.* demonstrated a hereditary basis for PLD and a hereditary basis for risk of primary glaucoma in the breed, in a population of FCR of varied ages.[4] Our study suggests when performing hereditary eye screening and certifying PLD grade of <20% this underestimates those numbers of dogs who will have a hereditary risk of PLD in their progeny. The relevance of ICA comparisons between different dogs needs therefore to take account of age. We also now aim to perform inheritance and genetic studies for these affected dogs, ideally with an expanded population of FCR.

The dogs in this study were effectively a random selection of normal FCR dogs. Despite aiming to represent populations in both the UK and Switzerland, only dogs determined unaffected or grade 1 affected at first examination re-presented as, inevitably selection pressure occurs due to the willingness of breeders and owners to participate. Further studies examining pectinate ligament change in a cohort of dogs with more severe PLD over a period of time would be useful, as the incidence of progression of PLD grade may differ.

In summary, this study indicates progression from normal ICA architecture to clinically significant PLD in individual dogs, over a period of time. Gonioscopic examination has become an essential component of hereditary eye screening within the UK BVA/KC/ISDS and ECVO hereditary screening, and potentially, this study suggests that this should not be a 'once in a lifetime' examination.

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