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Investigating the inheritance of prolapsed nictitating membrane glands in a large canine pedigree

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Abstract

Objective—To investigate the inheritance of prolapsed nictitating membrane glands (PNMG) in a large pedigree of purpose-bred mongrel dogs.

Animals studied—Two lines of purpose-bred mongrel dogs kept at a research facility with controlled environment were analyzed for frequent occurrences of PNMG. The first line (GS line) consisted of 201 dogs, derived from one German shorthaired pointer and seven mongrel dogs. The second line (M line) was established from one mongrel dog and three miniature longhaired dachshund (MLHD) dogs followed by closed breeding practice ($n = 50$). The two canine lines were connected by a female dog, which contributed genetically to both lines.

Procedures—Medical records of all dogs were reviewed retrospectively for signalment, parental data, and the presence of PNMG. Pedigrees were constructed to facilitate assessment of inheritance.

Results—The overall prevalence of PNMG in the GS line was 4.0% (8/201) over a 12-year period. The prevalence in the M line was 10.0% (5/50) over 6 years, which increased to 23.1% (3/13) when only dogs aged 2 years or older were considered. Analysis of the pedigrees ruled out simple modes of Mendelian inheritance in both canine lines.

Conclusion—The high prevalence of PNMG in two canine lines bred and maintained under a strictly controlled environment supported the involvement of genetic risk factors. The mode of inheritance remains to be determined, but it appears to be complex and potentially multigenic.

Keywords

inheritance; nictitans; prolapsed nictitating membrane gland; third eyelid

INTRODUCTION

Prolapsed nictitating membrane gland (PNMG, also known as prolapse of the gland of the third eyelid or ‘cherry eye’) is the most common primary disorder of the canine nictitans.¹

While the pathogenesis of this condition is largely unknown, it is thought to be due to weakness in the connective tissue attachment of the nictitating membrane gland to periorbital tissues.^{1,2} It may also result from lymphoid hyperplasia of the gland in young animals exposed to environmental allergens.² The gland, normally not seen due to its deep embedding around the ventrum of the T-shaped cartilage within the nictitating membrane, displaces dorsally to protrude above the leading edge of the nictitating membrane.¹ Clinically, it is recognized as a round, smooth or follicular, pink swelling appearing from behind the nictitating membrane.

Prolapsed nictitating membrane glands tends to present as a unilateral condition in dogs <1–2 years of age; however, the fellow eye is often subsequently affected, especially in English and French bulldogs, shar peis, great Danes, and cane corsos.^{1,3–5} A recent retrospective study of 114 dogs with PNMG found a 3:2 ratio of unilateral vs. bilateral cases; 75.4% of the affected dogs were <1 year of age at the time of diagnosis.⁴ Most studies have not detected any sex predisposition for PNMG.^{4–7}

Due to chronic exposure, the prolapsed gland may enlarge, and inflammation and infection are frequent sequelae.^{1,2} Keratoconjunctivitis sicca (KCS) was found to occur in 42.8% of animals that had glands that remained prolapsed in a retrospective study.⁶ While surgical removal of the prolapsed portion of the gland was once considered to be a valid treatment for PNMG, Helper *et al.*⁸ demonstrated in 1974 that in dogs, the surgical removal of the nictitating membrane gland resulted in a 29–57% reduction of Schirmer tear test values. Other studies have also supported the importance of the nictitating membrane gland in maintaining normal tear production.^{6,7,9,10} Therefore, the current treatment of choice for PNMG is surgical repositioning of the gland either by an anchoring or pocket technique.^{1,5,6,11–13} Surgical replacement of the gland does not, however, guarantee that KCS will not develop, especially because many breeds that commonly develop PNMG are also predisposed to KCS.¹

While the etiology of PNMG has not been determined in dogs, genetic risk factors are suspected based on the increased prevalence of the condition in certain breeds, such as cocker spaniels, beagles, basset hounds, and brachycephalic dogs including Boston terriers, Lhasa apsos, shih tzus, Pekingese, and English bulldogs.^{1,4–7,14} We have recently identified two related lines of purpose-bred mongrel dogs with apparently increased prevalence of PNMG among an extended pedigree of research dogs. The purpose of this study was to investigate the inheritance of this condition by analyzing these pedigrees.

MATERIALS AND METHODS

Medical records were studied retrospectively of two canine lines within an extended pedigree of purpose-bred mongrel dogs. All of the dogs were either closely or remotely related and were housed in the same indoor research facility. In this extended pedigree, a number of dogs had been diagnosed with PNMG in recent years, while other canine lines in the same facility were generally not affected.

The first canine line (GS line, $n = 201$) was initially established to investigate autosomal recessive achromatopsia originally found in German shorthaired pointers (Fig. 1).¹⁵ The disease is also referred to as cone degeneration (cd) and is caused by a missense mutation in the *CNGB3* gene.¹⁵ The founders of the GS line consisted of a male German shorthaired pointer (0B) and seven mongrel dogs (Fig. 1). One of the mongrel contributors to the GS line (N212) was also used to establish the second research line to study cone-rod dystrophy 1 (cord1, Fig. 2).¹⁶ In this M line ($n = 50$), three other unrelated dogs (M0, M9, and M10) that were pure-bred miniature longhaired dachshunds (MLHDs), contributed as founders, followed by several generations of closed breeding practice (Fig. 2). As such, the genetic background of dogs in the M line was significantly influenced by the MLHD breed. The mongrel dogs housed at this particular research facility had a genetic background typically influenced by laboratory beagles along with other breeds that have been introduced over three decades, including Irish setters and miniature poodles.

With the exception of the German shorthaired pointer and MLHD founder dogs that had been introduced from external sources, all the dogs were bred, raised, and maintained under the same environment with a 12-h light, 12-h dark cycle, and were fed the same commercial diet. Retinal disease status of each animal was determined by *CNGB3* genotyping (GS line) and/or by the absence of cone function as assessed by photopic electroretinography under general anesthesia (GS and M lines).^{15,16} All dogs were routinely examined by veterinarians, including board-certified veterinary ophthalmologists, and clinical findings were recorded.

For the current study, the following information was extracted from all the dogs' records in the GS and M lines: parents, sex, date of birth, retinal disease status, the presence of PNMG, and, if applicable, the age at termination of the dog for the purpose of the primary investigation unrelated to PNMG. If a dog was affected by PNMG, the following information was extracted: the age at detection of the first PNMG and, if applicable, contralateral PNMG, the eye(s) affected, and involvement in any experimental procedures preceding the prolapse of the gland.

Using the parental data from each animal, pedigrees were drawn for GS and M lines (Figs 1 and 2). Animals that suffered pre-wean deaths were not included in the pedigree or calculations due to lack of phenotypic information. For one of the founders of the M line (M0), the PNMG phenotype was unknown as only semen was obtained for breeding. Additionally, the PNMG phenotype could not be determined for the male founder of the GS line (0B), which had been externally sourced for breeding. These two dogs (M0 and 0B) were therefore excluded from the analyses (GS line, $n = 201$; M line, $n = 50$).

The overall prevalence of PNMG over the period of 2000–2012 (GS line) or 2006–2012 (M line) was estimated as the total number of affected dogs divided by the total number of animals in each line. The median age at pro-lapsed gland diagnosis and the median age at euthanasia were determined for each line.

RESULTS

Clinical features

In the GS line, eight of 201 dogs (4.0%) were diagnosed with PNMG over a 12-year period. There were no affected dogs observed during the first 6 years, but new cases were detected during the latter years (Fig. 3). In the M line, five of 50 dogs (10.0%) developed PNMG during a 6-year period of observation. The first affected dog in the M line was identified among the progeny from the mating between two founder dogs: a female mongrel (N212) and a male MLHD (M0; Fig. 2).

Table 1 provides a summary of pertinent data for the affected animals. Six dogs in the GS line developed unilateral PNMG (OD, $n = 3$; OS, $n = 3$) at ages 1.8–5.2 months, while one outlier case developed the condition in OS at 32.9 months of age. Only one dog in the GS line was bilaterally affected; the first gland (OD) prolapsed at 1.8 months of age followed by the prolapse of the opposite gland (OS) 1.7 months later. Of the five dogs affected with PNMG in the M line, four were affected unilaterally (OD, $n = 1$; OS, $n = 3$) at ages 1–26.6 months, and one was affected bilaterally at 1.6 months of age. Overall, the median ages at PNMG detection in GS and M lines were 2.5 (range: 1.8–32.9) and 5.5 (range: 1.6–26.6) months, respectively (Fig. 4).

As the primary investigation unrelated to PNMG required early termination of some of the dogs, the median life expectancies in GS and M lines were 6.4 (range: 0.4–102.3) and 8.3 (range: 1.2–54.6) months, respectively. Hence, some dogs that did not live long enough for the disease phenotype to develop may have been falsely assessed as nonaffected. In an attempt to investigate the potential effect of early euthanasia or young age on the prevalence data, all M line dogs younger than or euthanized before 2 years of age were excluded for recalculation of prevalence. Thirteen M line dogs remained that were alive and examined at 2 years of age or elder; three of these 13 dogs were PNMG affected (23.1%; Fig. 2b).

Assessment of genetic and environmental risk factors

The relatively high prevalence of PNMG in two lines of mongrel dogs kept under the same controlled environment with frequent ophthalmologic examinations and attached with detailed medical records have provided a unique opportunity to investigate potential risk factors for this condition. Pedigree analysis demonstrated that simple modes of Mendelian inheritance would not describe PNMG in either GS (Fig. 1) or M lines (Fig. 2). In the GS line, the production of affected females from breedings between unaffected males and females, ruled out a simple X-linked trait. Given the equivalent numbers of affected males and females, sex-linked modes of inheritance did seem unlikely. An autosomal recessive inheritance with incomplete penetrance but not a simple autosomal dominance was possible as all affected dogs were produced from breedings between unaffected animals. No two affected dogs were bred to each other thus allelism of prolapsed glands in different individuals within the GS line was not confirmed.

In the M line, four of five affected dogs were female in this relatively limited pool of animals. A simple X-linked inheritance was therefore unlikely as males are more frequently affected with this inheritance pattern. Furthermore, two affected females (M4 and M5) were

produced from a normal sire. While production of four affected M dogs from affected-to-unaffected matings may indicate an autosomal dominant etiology, this mode of inheritance is unlikely because breeding between two affected dogs (M5 and M17) produced a litter of seven unaffected dogs (M52–M58), which were 8 months of age at the time of data collection. Given the young age of the progeny, the possibility of eventually developing the disease phenotype remains. Should none of the progeny go on to develop PNMG, this observation could indicate non-allelism in that PNMG etiologies are different in M5 and M17; this is possible as the age of onset is distant in M5 (1.6 months) and M17 (16.9 months). Alternatively, low penetrance as well as nongenetic etiology could be considered.

The GS and M lines were found to share an unaffected mongrel female (N212; Figs 1 and 2) as one of the genetic contributors. This dog was one of the four founders of the M line, as well as the dam of N237, a male dog in the GS line used for outcrossing. Three PNMG-affected dogs in the GS line (GS217, GS219, GS241) were direct progenies of N212, while the other five cases could not be traced back to N212. Yet in the M line, all five PNMG cases were traced back to N212, suggesting a genetic etiology.

Retrobulbar injections of saline are commonly performed in dogs at this research facility to facilitate the proper positioning of the globe under general anesthesia for electroretinography and subretinal injections for gene therapy. It is conceivable that the transient moderate increase in orbital content may facilitate the protrusion of the nictitating membrane gland. At 11, 22, and 455 days prior to the unilateral prolapsing of the glands, three of the eight affected dogs in the GS line received bilateral retrobulbar saline injections (Table 1). Two of the five affected dogs in the M line (M17, M46) received bilateral retrobulbar saline injections 63 and 272 days prior to unilateral gland prolapse. As the prolapses were always unilateral and did not occur until weeks or months after injection, a cause-and-effect relationship between bilateral retrobulbar injection and the development of PNMG seemed unlikely.

Additionally, no relationship between PNMG and retinal disease status was detected. Both retinal disease-affected and nonaffected dogs were diagnosed with prolapsed glands (Table 1).

DISCUSSION

Although the etiology of PNMG is unknown, a genetic predisposition is suspected in many dogs because of the increased prevalence in certain breeds.^{1,4–7,14} To the authors' knowledge, this study represents the first attempt to evaluate the inheritance of the condition by pedigree analysis. Although the number of dogs affected with PNMG is smaller compared with previous reports, the major advantage of the current study is the large number of related dogs with complete medical records and known familial background all sharing the same strictly controlled environment. Furthermore, this study followed a group of dogs over time, which increased the likelihood of accurately recording the presence of PNMG.

It is interesting to note the unique distributions of age at detection in the GS and M lines (Fig. 4, Table 1). All affected dogs in the GS line uniformly developed PNMG within 6 months of birth except for one outlier (GS140), which developed the condition at 32.9 months of age; this dog was the product of an outcrossing to a mongrel dog. In the M line, on the other hand, the age of PNMG detection showed a broader distribution (Fig. 4). The relatively narrowly defined window of the age of onset in the GS line and the apparent canine line-specific nature of disease onset indicates that the disease etiology is genetically determined and that the etiology between the two canine lines could be different. Experimental crossing between affected dogs from GS and M lines could help determine whether the PNMG conditions in these different lines are allelic.

The current investigation found PNMG to be unilateral in 84% (11/13) and bilateral in 16% (2/13) of cases. When considering the small number of affected dogs in our study, these results are comparable to the estimated 3:2 ratio of unilateral vs. bilateral PNMG in dogs.^{4,6,12} Neither of our bilateral PNMG-affected dogs were simultaneous; however, the opposite gland prolapse occurred within 0.4 and 1.7 months of the first. Mazzucchelli and colleagues recently reported that in 70.8% of nonsimultaneous bilateral cases, the prolapse of the second gland was noted within 3 months.⁴

The high prevalence of 4.0% and 10.0% in two lines of mongrel research dogs supports the involvement of genetic factors in PNMG. While a simple mode of Mendelian inheritance for PNMG was ruled out, the precise mode of inheritance remains elusive. An autosomal recessive (GS and M lines) or autosomal dominant (M line) inheritance each with incomplete penetrance is possible. Yet, in either of the lines, the condition may be multigenic with a complex mode of inheritance.

We recognize the complications regarding the prevalence reported in this study. Prevalence was determined as the proportion of PNMG cases in each canine line over a specific time period. As the lines were purpose-bred, offspring of certain dogs may have been over-represented (popular sire effect), and this may have affected the prevalence dramatically.

While PNMG is commonly detected before 2 years of age,^{1,4,5} the median ages of termination for the purpose of primary investigation were younger in the GS and M lines, at 6.4 (range 0.42–102.3) and 8.3 (range 1.2–54.6) months, respectively. The inclusion of very young PNMG-unaffected animals and the effect of early termination of dogs in the study pedigree could have led to the under-representation of affected cases and underestimation of prevalence. In an attempt to investigate the effect of early termination or young age on the prevalence data, all animals younger than or euthanized before 2 years of age were excluded from the M line for recalculation of data (Fig. 2b). This raised the M line PNMG prevalence from 10.0% (5/50) to 23.1% (3/13), indicating underestimation of the prevalence prior to removal of young dogs. However, the prevalence should still be interpreted with caution due to small sample size and the effect of purpose breeding.

Our initial hypothesis for the high incidence of PNMG in the GS line was increased homozygosity that was identical-by-descent due to inbreeding. However, pedigree analysis did not support this hypothesis, as two of the eight affected cases (GS121 and GS140) were

produced from direct outcrossings to mongrel dogs. This may indicate that PNMG in these dogs does not require two copies of the same genetic component responsible for the disease.

As both pure-bred and mongrel dogs contributed to the genetic background of the GS line and since the line evolved over several generations, no particular breed can be claimed responsible for the increased occurrence of PNMG in the GS line. The genetic background of the GS line may, in fact, be most influenced by beagles, which were considered to be the main genetic contributors to the mongrels used for outcrossing. Beagles are among the breeds more commonly affected with PNMG,^{1,7,14} and it is possible that the introduction of the beagle genetic background played a role in PNMG manifestation.

The genetic background of the M line is less complicated with three pure-bred MLHDs and a mongrel as founders and followed by several generations of closed breeding. Based on data issued by the Canine Eye Registration Foundation (CERF), miniature dachshunds may have a slightly increased prevalence of PNMG,¹⁴ although it is unknown whether the MLHD genetic background played a role in PNMG development in the M line.

To place the PNMG prevalence from this study in context, we reviewed the 2010 CERF database of ocular disorders presumed to be inherited in pure-bred dogs.¹⁴ The database was searched for every occurrence of PNMG. The following breeds were listed as presumably having a genetic predisposition to develop PNMG: beagles, bloodhounds, English and American bulldogs, Chinese shar peis, American cocker spaniels, Lhasa apsos, Neapolitan mastiffs, and Newfoundlands.¹⁴ The prevalence of PNMG in all affected breeds was calculated for the two time intervals 1991–1999 and 2000–2008.¹⁴ Breeds with >1% dogs affected examined in the years 1991–1999 included: English and American bulldogs (1.44%), miniature dachshunds (1.22%), Saint Bernard dogs (1.72%), and Neapolitan mastiffs (7.69%).¹⁴ Breeds with the highest percentages of prolapsed glands in the years 2000–2008 (>1%) included: American lamalese (5.26%), beagles (1.22%), bloodhounds (1.61%), bracco Italianos (2.44%), and English and American bulldogs (1.45%).¹⁴ Note that the particularly high percentages in some breeds may be associated with the small sample sizes; for example, Neapolitan mastiffs (1/13), American lamalese (1/19), and bracco Italianos (1/41).¹⁴

The overall occurrences of PNMG in canine GS and M lines in the current study were 4.0% (8/201) and 10.0% (5/50), respectively, over the period of observation. The frequency of the disease in these canine lines with reasonable sample sizes is much higher compared to that in the bulldog (1.44%), which is a ‘commonly’ affected breed according to the CERF database.¹⁴ However, the CERF database, a record of eye certification examinations, is not representative of all PNMG cases diagnosed in general clinical veterinary settings. Rather, it represents a single point-in-time assessment and does not follow the same animals over time. Some dogs that do not have PNMG at the time of the eye certification examination may develop it later in life, but this information would not be available to CERF. It is also possible that PNMG is being surgically repaired in some affected dogs prior to the eye certification examination. Because of these factors, it is likely that the relative prevalence of PNMG is higher than reported by CERF.

In addition to genetic risk factors, anatomic risk factors may also contribute to PNMG. Many of the breeds at risk are brachycephalic, such as the Boston terrier, Pekingese, Lhasa apso, shih tzu, cavalier King Charles spaniels, as well as French and English bulldogs.^{1,4-7} As all the dogs in the current study were dolichocephalic, anatomic risk factors were not examined.

The retrobulbar injection of saline for unrelated experimental purposes could not be identified as a risk factor for PNMG. It has been previously documented that volume-effect from a retrobulbar injection can transiently lead to an increase in intraocular pressure.^{17,18} It is conceivable that a retrobulbar injection could also lead to PNMG, especially in a dog that is predisposed due to weak nictitans connective tissue attachments. However, no association was found between the retrobulbar injection of saline and the occurrence of a prolapsed gland. In none of the affected animals was the bilateral retrobulbar saline injection performed <11 days prior to the gland prolapse. As none of the retrobulbar injections occurred within days prior to gland prolapse, and because the prolapsed glands were unilateral despite bilateral retrobulbar saline injections, it seemed unlikely that the injections were responsible for these cases of PNMG. Furthermore, while approximately 200 bilateral retrobulbar injections are performed annually at this research facility, PNMG has occurred only in the dogs reported in this study.

In conclusion, the results provide further support for the involvement of genetic risk factors in the development of PNMG in dogs. This study represents the first attempt of pedigree analysis of PNMG in a large extended canine pedigree in a strictly controlled environment. Future studies may include selective breeding of affected dogs within and across lines and gene expression studies of the weakened connective tissue bands that anchor the gland to other periorbital tissue.

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Figure 1.

Complete pedigree of the GS line. Dogs that are specifically discussed in the main text are marked with arrows and/or larger font labels.

Figure 2.

Pedigrees of the M line. (a) Complete M pedigree; (b) M line only including animals alive and examined at 2 years of age. Dogs that are specifically discussed in the main text are marked with larger font labels.

Figure 3.

New cases of prolapsed nictitating membrane gland over time in two lines of mongrel dogs (GS and M lines). The M line was not established until 2006.

Figure 4.

The distribution of age at detection of prolapsed nictitating membrane glands (PNMG) in two lines of mongrel dogs (GS and M lines). The bottom and top of boxes represent the lower and upper quartiles, respectively, and the horizontal line within the box shows the median. The ends of the whiskers represent the minimum and maximum values.

Table 1

List of all the dogs affected by PNMG

Line	Animal ID no.	Sex	Retinal disease status*	Age of detection (months)	Eye(s) affected	Retrolubar injection prior to detection?
GS	GS83	F	Affected	5.2	OS	No
	GS121	M	Unaffected	1.9	OS	No
	GS140	F	Unaffected	32.9	OS	Yes, 455 days prior
	GS166	F	Affected	2.1	OD	Yes, 11 days prior
	GS217	M	Unaffected	1.8	OS	No
	GS219	M	Unaffected	1.8	OD, OS [‡]	No
M	GS228	F	Affected	3.4	OD	No
	GS241	M	Affected	2.8	OD	Yes, 22 days prior
	M2	F	Unaffected	26.6	OS	No
	M4	F	Unaffected	1.6	OD	No
	M5	F	Affected	1.6	OD, OS [‡]	No
	M17	M	Unaffected	16.9	OS	Yes, 272 days prior
	M46	F	Affected	5.5	OS	Yes, 63 days prior

PNMG, prolapsed nictitating membrane glands.

* Based on the *CNGB3* genotyping (GS line) and/or absence of cone function as assessed by photopic electroretinography (GS and M lines).^{15,16}[‡] Right eye affected first at age of detection; left eye found affected 53 days (1.7 months) later.[‡] Right eye affected first at age of detection; left eye found affected 13 days (0.4 months) later.