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Prevalence and genetics of patellar luxation in Kooiker dogs

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ABSTRACT

The prevalence of patellar luxation (PL) and genetic factors potentially involved in the disorder were investigated in Dutch Kooiker dogs. A cohort of 842 Kooiker dogs, the offspring of 195 sires and 318 dams, was screened for PL from 1994 to 2011. The cohort was included in a pedigree of 1737 Kooiker dogs comprising nine generations.

PL was present in 24% of screened dogs, with unilateral and bilateral luxation being observed equally frequently. Medial PL was more common (61%) than lateral PL (32%) or bidirectional PL (7%). The frequency of PL was similar in male and female dogs, with a female:male relative risk of 1.15 (95% confidence interval, CI, 0.90–1.48). The heritability of PL in the screened population was 0.27 ± 0.07. Since the start of the screening programme, the prevalence of PL decreased from 28% to 19%. A genome-wide association study of PL with 48 cases and 42 controls suggested the possible involvement of a region on chromosome 3 ($P_{raw} = 1.32 \times 10^{-5}$, $P_{genome} = 0.142$), but the involvement of this region could not be confirmed in a validation group.

Breeding programmes for complex diseases, such as PL, would benefit from combining pedigrees, phenotypes and genotypes, i.e. from genomic selection, as is currently the method of choice for breeding of production animals.

Introduction

The prevalence of patellar luxation (PL), one of the most common orthopaedic disorders of small breed dogs, appears to be increasing in small and large breed dogs (Remedios et al., 1992; Hayes et al., 1994; Johnson et al., 2001). PL can result in non-weight bearing lameness and can ultimately cause degenerative joint disease, with pain and chronic lameness (Hulse, 1981; Ness et al., 1996; Johnson et al., 2001; Gibbons et al., 2006). It has been suggested that the disease is inherited, which is supported by the predisposition of certain small breed dogs to PL (Priester, 1972; Hulse, 1981; LaFond et al., 2002). According to the Orthopedic Foundation for Animals (OFA), the six breeds with the highest prevalence of PL in the USA are the Pomeranian (39.5%), Yorkshire terrier (24.4%), Australian terrier (16.4%), Cocker spaniel (14.5%), Boykin spaniel (13.5%) and Tibetan spaniel (12.6%).[1]

The Dutch Kooiker dog, also called Dutch Kooikerhondje, Dutch decoy dog or Small Dutch waterfowl dog (weight 9–11 kg), is a working breed that was depicted in paintings of Dutch masters in the early 1600s and described in journals from the 16th century. The breed then disappeared but was re-established in 1942. The breed was recognised by the Dutch Kennel Club in 1971 and by the Fédération Cynologique Internationale (FCI) (group 8, section 2) in 2009[2] (FCI, 2009). About 467 Kooiker dogs (range 300–603 from 1991 to 2010) are registered annually with the Dutch Kennel Club.

PL is frequent in the Kooiker dog, as it is in the Cocker spaniel, Boykin spaniel and Tibetan spaniel, all of which are classified by the FCI in section 2 (Hazewinkel et al., 2013). A PL screening programme for Kooiker dogs based on orthopaedic examination was established in The Netherlands in 1994. At the same time, DNA samples of Kooiker dogs were routinely screened for a mutation in the von Willebrand factor gene (Van Oost et al., 2004). All DNA samples were stored in a database, which made it possible to investigate the genetics of PL in Dutch Kooiker dogs.

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[1] See: http://www.offa.org/stats_pl.html.

[2] See: http://www.dogdomain.com/fci-8.htm.

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Table 1
Categorical scores, quantitative values and grading of patellar luxation (PL) in dogs.

Description	Left stifle	Right stifle	Grade	Quantitative values
Normal	A	B	Free	97
Loose, lateral side	P	Q	Free	97
Loose, medial side	R	S	Free	97
Loose, lateral and medial side	T	U	Free	97
Grade 1 PL, lateral side	C	D	Grade 1	110
Grade 1 PL, lateral side with torsion of the tibia	G	H	Grade 1	120
Grade 1 PL, lateral and medial side	O	Z	Grade 1	110
Grade 1 PL, lateral and medial side, with torsion of the tibia	I	J	Grade 1	123
Grade 1 PL, medial side	E	F	Grade 1	110
Grade 1 PL, medial side, with torsion of the tibia	V	W	Grade 1	120
Grade 2 PL, lateral side	K	L	Grade 2	124
Grade 2 PL, medial side	M	N	Grade 2	124
Grade 3, 4 PL, or operated	X	Y	Grade 3 or 4	130

The aims of this study were to investigate the prevalence of PL in the Dutch Kooiker dog population and to analyse its heritability. In addition, a genome-wide association study was performed to identify chromosomal regions involved in the development and aetiology of PL. Putative single nucleotide polymorphisms (SNPs) were tested in a large group of phenotyped Kooiker dogs.

Materials and methods

Animals and phenotyping

A cohort of 842 Kooiker dogs, the offspring of 195 sires and 318 dams, was screened for PL from 1994 to 2011 in the framework of breeding regulations of the Dutch Kooikerhondje Association. The dogs were privately owned and included with informed consent of the owners. DNA was available from 182 of these dogs. The DNA was isolated from blood samples sent to the Department of Clinical Sciences of Companion Animals for testing for the gene that causes von Willebrand disease in the breed. The application form for the test informed the owners that, by signing the form, they consented to the use of the DNA for research purposes. As the dogs were handled by licensed veterinarians only, the study complied with the Dutch Law on the Practice of Veterinary Medicine of 21 March 1990 and approval of an ethics committee for the use of the samples was not necessary.

All dogs were at least 12 months old and were examined by members of a group of national board-certified veterinary specialists in orthopaedics using the same standardised protocol (Hazewinkel et al., 2009). The number of dogs investigated per year and the investigated dogs as a percentage of the total number of dogs registered with the Breed Society are detailed in Supplementary Table S1 (see Appendix A). In total, 1737 Kooiker dogs from nine generations were included with the cohort in a pedigree including 253 sires, originating from 96 grandsires and 107 grand-dams, and 449 dams, originating from 137 grandsires and 186 grand-dams. The average inbreeding coefficient for the 794 dogs with phenotypes is 0.0023 (0.23%), which is not high given the history of the breed, but our electronically available pedigree starts around 1990.

The grade and direction of the luxation (medial, lateral, or bidirectional) were classified as: (1) free of PL: normal or loose (i.e. the patella could be manually positioned on the ridges of the trochlear groove, but not completely out of the groove); (2) grade 1 PL: there were no clinical signs, but the patella could be manually luxated with full extension of the stifle joint, while returning to the normal position when released; (3) grade 2 PL: the patella could be luxated more easily than in grade 1, especially with rotation of the tibia, the patella luxated out of the trochlear groove and reduction was still accomplished with opposite manoeuvres; (4) grade 3 PL (the patella was permanently luxated, but could be manually returned with the stifle in extension; however, flexion and extension of stifle resulted in reluxation of the patella).

This information was scored for each joint on a categorical scale from A to Z. Scores A and B indicated 'normal'; scores; P, Q, R, S, T and U indicated 'loose'; scores C, D, E, F, G, H, I, J, O, V, W and Z indicated 'patellar luxation grade 1'; scores K, L, M and N indicated 'patellar luxation grade 2'; and scores X and Y indicated 'patellar luxation grade 3, 4 or operated' (Table 1). The categorical scores were transformed into quantitative values (Table 1). Yq is used as a name for the quantitative variable, assuming a continuous normally distributed liability, which is normal practice when analysing polygenic traits showing discrete phenotypic categories (Falconer, 1981; Van Grevenhof et al., 2009). Absence of PL was scored 97 points; PL grade 1 with medial, lateral or bidirectional luxation on the right and/or left side was scored 110 points (torsion of the tibia was scored 10 points); bidirectional luxation grade 1 with torsion of the tibia on the right and/or left side was scored 123 points; PL grade 2 was scored 124 points; and PL grade 3 and the dogs operated for PL were scored 130 points.

The female:male ratio in the affected group was calculated. However, since more females than males were tested, the relative risk (RR) was calculated according to $RR = (a_1/n_1)/(a_0/n_0)$, where a_1 is the number of exposed female animals with the disease, a_0 is the number of exposed male animals with the disease, n_1 is the total number of exposed female animals and n_0 is the total number of exposed male animals. A RR of 1 would indicate that the risk was the same in the test group of females as in the comparison group of males, a RR < 1 means the test group has a lower risk than the comparison group and a RR > 1 means the test group has a higher risk (Priester, 1972; Dohoo et al., 2010).

Heritability and genetic trend

Variance components (σ^2) and the resulting heritability for PL were calculated with the programme ASReml (Gilmour et al., 1995) using the following repeated measures model:

$$Yq = \mu + animal + dam + pe + e$$

where μ is the overall mean.

Fixed effects, including side (left and right), were tested with an *F* statistic, with $P < 0.05$ considered to be significant. Preliminary analysis showed that the sex of the animal did not have a significant effect, so this variable was excluded from the model. Random effects included animal, dam, permanent environment (pe), which refers to environmental influences with a permanent effect on the animal and which are therefore identical for both stifles of the same animal, but different between animals, and residual (e).

Normal distributions were assumed for the random effects: animal ~ $N(0, A\sigma_a^2)$, dam ~ $N(0, I\sigma_d^2)$, pe ~ $N(0, I\sigma_{pe}^2)$ and e ~ $N(0, I\sigma_e^2)$, where *A* contains the additive genetic relationship between animals and I is an identity matrix of appropriate size. The relationship matrix was constructed using pedigree records of 1737 Kooiker dogs.

Estimated breeding values (EBVs) were calculated according to the former model for all animals in the relationship matrix. Reliabilities of EBVs were calculated as:

$$1 - (\text{prediction error variance}/\text{additive genetic variance})$$

where the prediction error variance per individual is calculated using the model. Heritability (h^2, additive genetic variance/phenotypic variance) was calculated using the formula (Falconer, 1981):

$$h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_d^2 + \sigma_{pe}^2 + \sigma_e^2)$$

The prevalence of PL (grades 1 and higher were considered 'affected') was calculated. The EBVs of animals born from 1994 to 2009 were grouped by year of birth and an average EBV was calculated for each year to investigate the genetic trend and phenotypic trend.

Genome-wide association study

DNA samples of 90 dogs were genotyped with 174,450 SNPs using the CanineHD Bead chip (Illumina). Of these 90 dogs, 48 had PL and 42 were unaffected controls (see Appendix A: Supplementary Table S2). Genotype data analysed were in Hardy–Weinberg equilibrium ($P \geq 0.01$). SNPs with a minor allele frequency <5% and a genotyping success rate <95% were removed, as well as individual dogs with more than 10% missing genotypes, PLINK v1.07 software (Purcell et al., 2007) was used for allelic association testing. Population stratification was assessed using the genomic inflation factor. The case and control dogs did not share parents, in order to avoid family stratification. Allele frequencies of SNPs were compared between cases and controls using a standard χ^2 based test. Results were corrected empirically by max(T) permutation with 1000 swaps of the phenotype (EMP1) and for multiple testing by