



# Population genetic analysis and genome-wide association study of patellar luxation in a Thai population of Pomeranian dogs



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## ABSTRACT

The genetics of patellar luxation (PL) were investigated in Pomeranian dogs presented at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. A cohort of 339 Pomeranian dogs, part of a four-generation pedigree of 842 Pomeranians, was screened for PL from 2006 to 2013. PL was present in 77% of the screened dogs, with 84% having bilateral and 16% unilateral luxation. Medial PL was more common (95%) than lateral PL (2%) or bidirectional PL (3%). The risk of PL was similar in male and female dogs (female:male relative risk 1.11, 95% CI 0.98–1.25). The heritability of PL in the screened population was  $0.44 \pm 0.04$  using a threshold model. A genome-wide association study of PL (48 cases and 48 controls) using a high-density SNP array indicated the possible involvement of 15 chromosomal regions, of which CFA05 and CFA32 remained associated in a larger study involving an additional 128 cases and 7 controls. Candidate genes in these regions may be involved in the pathogenesis of PL in Pomeranian dogs.

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## 1. Introduction

Congenital patellar luxation (PL) in dogs, a developmental disorder, is thought to be a multifactorial inherited disease (LaFond et al., 2002). It is most common in small-breed dogs, and in Thailand the prevalence of medial patellar luxation (MPL) and lateral patellar luxation (LPL) in small-breed dogs is 87% and 13%, respectively (Wangdee et al., 2005; Wangdee and Kalpravidh, 2008). Pomeranian dogs are currently the highest-ranking breed for PL in the USA, with 37.2% of dogs reported to be affected (OFA, 2015). In Thailand, 75% of the Pomeranian dog population is affected (Soontornvipart et al., 2013). This breed predisposition suggests that the disease is inherited in Pomeranians (Priester, 1972; LaFond et al., 2002; Alam et al., 2007; OFA, 2015; Soontornvipart et al., 2013). In small-breed dogs, PL is more common in female dogs than in male dogs, with a female:male ratio of up to 1.9 (DeAngelis and Hohn, 1970; Priester, 1972; Hulse, 1993; Hayes et al., 1994; Alam et al., 2007; Linney et al., 2011; Soontornvipart et al., 2013). In contrast, in large-breed dogs, the female:male ratio of dogs with PL can be as low as 0.56 (Remedios et al., 1992; Gibbons et al., 2006; Arthurs and Langley-Hobbs, 2007). One exception was reported by Lavrijzen et al.

(2013), who showed that female Flat-Coated Retrievers were at higher risk of PL than male dogs (relative risk = 1.8). These sex differences suggest that PL might be influenced by hormone factors and/or X-linked factors (Priester, 1972). A preliminary genome-wide association study with Pomeranians suggested that a region on CFA07 is involved in MPL (Soontornvipart et al., 2013); however, the limited number of SNPs used meant that only loci with a large effect on the phenotype could be detected.

The aims of this study were to investigate the prevalence of PL in a cohort of Thai Pomeranians and to estimate its heritability. In addition, a genome-wide association analysis was performed to identify multiple chromosomal regions involved in the development and aetiology of MPL. Subsequently, selected SNPs were tested in a large population of Thai Pomeranians and in dogs of other breeds.

## 2. Materials and methods

### 2.1. Animals

Pomeranians referred to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand, were screened for PL from 2006 to 2013. The pedigrees of 842 Pomeranian dogs from four generations were recorded. Blood samples (4 ml) were collected from 339 of these Pomeranians for DNA analysis in the genetic study of PL;

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all dogs were investigated using a standard orthopaedic protocol (Piermattei et al., 2006). The dogs were included with informed consent of the owners. In dogs with medial and/or lateral PL in the right and/or left hind leg, respectively, PL was graded with the dogs standing and in lateral recumbency, using a PL grading system (grades I to IV) (Piermattei et al., 2006). In grade I PL, the patella can be manually luxated in full extension of the stifle joint, returning to the normal position when released. In grade II PL, the patella luxates more frequently than in grade I. The patella luxates easily, especially when the foot and tibia are rotated, while the patella is pushed in a medial or pulled in a lateral direction. Reduction occurs with the opposite manoeuvres. In grade III PL, the patella is permanently luxated, but can manually be reduced with the stifle in extension; however, flexion and extension of the stifle result in relaxation of the patella. In grade IV PL, the patella is permanently luxated and cannot be manually repositioned. Bidirectional patellar luxation (BPL) is defined when the patella of the same stifle luxates both medially and laterally.

The female-to-male ratio in the affected group was calculated. Since more female than male dogs 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 dogs with the disease,  $a_0$  is the number of exposed male dogs with the disease,  $n_1$  is the total number of exposed female dogs, and  $n_0$  is the total number of exposed male dogs. A RR of 1 indicates that the risk is the same in female and male dogs, a RR < 1 means that female dogs are at lower risk than male dogs, and a RR > 1 means that female dogs are at higher risk than male dogs (Priester, 1972; Dohoo et al., 2010).

## 2.2. Heritability of patellar luxation

Phenotypic score of PL was set to 0 for unaffected and 1 for affected. Variance components ( $\sigma^2$ ) and the resulting heritability of PL in the Pomeranian dogs were calculated with the program ASReml (Gilmour et al., 1995) using the following repeated measurement threshold model:

$$\text{Logit}(p_{ij}) = \mu + \text{animal}_i + \text{pe}_i$$

where  $p_{ij}$  is the probability that the stifle for dog  $i$  ( $i = 1, \dots, 339$ ) on side  $j$  ( $j = 1, 2$ ) has PL ( $PL = 1$ ) or not ( $PL = 0$ ),  $\mu$  is the overall mean. Fixed effects were tested with an  $F$ -statistic, with  $P < 0.05$  being considered significant. Preliminary analysis showed that animal sex and coat colour were not significant variables and they were excluded from the model. Random effects included polygenic animal ( $\text{animal}_i$ ) and permanent environment ( $\text{pe}_i$ ). Since each dog has two stifles and both scores were included in the analysis an additional factor ( $\text{pe}$ ) was included in the model to account for non-genetic effects that might affect both stifles on the same dog such as feeding, rearing etc. Normal distributions were assumed for the random effect models:  $\text{animal} \sim N(0, A\sigma_a^2)$ ,  $\text{pe} \sim N(0, I\sigma_{pe}^2)$ , where  $A$  contains the additive genetic relationship between animals and  $I$  is an identity matrix of appropriate size. The relationship matrix was constructed using 842 Pomeranians pedigree records. In the threshold model, the residual variance ( $\sigma_e^2$ ) was fixed at 3.289. Heritability on the underlying scale was calculated using the formula (Falconer, 1981):

$$h^2 = \text{additive genetic variation/phenotypic variation} \\ = \sigma_a^2 / (\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$$

## 2.3. Association study

The DNA samples of unrelated 96 Pomeranian selected from the cohort of 339 dogs were genotyped with Illumina CanineHD BeadChip, which contains 173,662 SNPs. Of these 96 dogs, 48 had MPL and 48

were unaffected controls. Genotype data were analysed for Hardy-Weinberg equilibrium with an inclusion threshold of  $P \geq 0.01$ . SNPs with a minor allele frequency below 5% and a genotyping success rate below 95% were excluded, as well as individual dogs with > 10% missing genotypes. Plink v1.07 software (Purcell et al., 2007) was used for allelic association testing. Population stratification was assessed by calculating the genomic inflation factor. None of the case and control dogs shared parents, thereby avoiding family stratification. Allele frequencies of SNPs were compared between cases and controls using a standard Chi-square based test. Results were corrected empirically by max(T) permutation with 1000 swaps of the phenotype (EMP1) and for multiple testing by comparing the permutation result of an individual SNP against that of all other SNPs (EMP2).

A competitive allele-specific PCR was used to genotype SNPs from regions of interest in a validation group of an additional 128 affected and 7 control Pomeranian dogs selected from unrelated cohort of 339 dogs. KASP™ chemistry (LGC genomics, Hoddesdon, UK) was used to generate allele-specific fluorescent signals as instructed by the manufacturer. The signals were recorded and scored using a BIORAD MYiQ2 iCycler (BIO-RAD, Herts, UK). A  $\chi^2$  test was used to compare the allele frequencies of these SNPs in cases and controls from the validation cohort and from the first group of 96 dogs included in the genome-wide association study. These SNPs were also genotyped additionally in 3 breeds from the Netherlands and in 2 breeds from Thailand including 32 PL case and 32 control Dutch Kooiker dogs, 32 case and 32 control Dutch Flat-Coated Retrievers, 16 case and 23 control Dutch Labrador Retrievers, 24 case and 8 control Thai Chihuahuas, and 23 case and 9 control Thai Miniature Poodles.

## 3. Results

### 3.1. Animals and phenotyping

Of the 202 female and 137 male Pomeranians investigated, 261 (77%) had PL (Table 1): 220 (84.3%) bilaterally and 41 (15.7%) unilaterally. Overall, 80% of the female dogs and 72% of the male dogs were affected, giving a RR of 1.11 (95% CI 0.98–1.25), which means that female dogs were not at significantly higher risk of PL than male dogs. Of 481 affected stifle joints, 94.8% displayed MPL, 2.3% LPL, and 2.9% BPL. The severity of PL varied from grade I to grade IV (Table 2).

### 3.2. Heritability

The heritability on the underlying scale of PL was  $0.44 \pm 0.04$  in this Pomeranian dog population.

### 3.3. Genome-wide association analysis

Of the 173,662 SNPs represented on the canine HD array, we excluded 45,366 non-informative SNPs, 6131 SNPs with a genotyping rate below 95%, and 1313 SNPs based on deviation from Hardy-Weinberg equilibrium. The remaining 123,456 SNPs were used to construct an identical-by-state (IBS) plot, based on the first two principal components of the multidimensional IBS matrix (Supplementary Fig. 1). The Pomeranian sample set was highly stratified with a genomic inflation

**Table 1**  
Prevalence of patellar luxation (PL) in Pomeranian dogs in Thailand from 2006 to 2013.

Status	Number (%)		
	Female	Male	Subtotal
Normal	40 (19.8%)	38 (27.7%)	78 (23%)
Patellar luxation	162 (80.2%)	99 (72.3%)	261 (77%)
- Bilateral PL	134	86	220 (84.3%)
- Unilateral PL	28	13	41 (15.7%)
Total	202 (100%)	137 (100%)	339 (100%)

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