Prevalence, genetics, and surgical treatment of patellar luxation in purebred dogs

Chalika Wangdee

Prevalence, genetics, and surgical treatment of patellar luxation in purebred dogs

Chalika Wangdee

PhD thesis, Utrecht University, The Netherlands

ISBN: 978-90-393-6168-9

Cover design: Anjolieke Dertien, Pajaree Sirotamarat and Supachai Pongsri
Layout: Chalika Wangdee
Printing: CPI - Koninklijke Wöhrmann

Correspondence and reprint requests: c.wangdee@hotmail.com

Prevalence, genetics, and surgical treatment of patellar luxation in purebred dogs

Prevalentie, genetica en chirurgische behandeling van patella luxatie bij rashonden

(met een samenvatting in het Nederlands)

การศึกษาด้านความชุก ลักษณะทางพันธุกรรมและการรักษาทางศัลยกรรม โรคสะบ้าเคลื่อนในสุนัขพันธุ์แท้

(และบทสรุปภาษาไทย)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op
donderdag 9 oktober 2014 des middags te 2.30 uur

door

Chalika Wangdee

geboren op 9 december 1977 te Bangkok, Thailand

Promotor: Prof.dr. H.A.W. Hazewinkel

Co-promotoren: Dr. P.A.J. Leegwater
Dr. L.F.H. Theyse

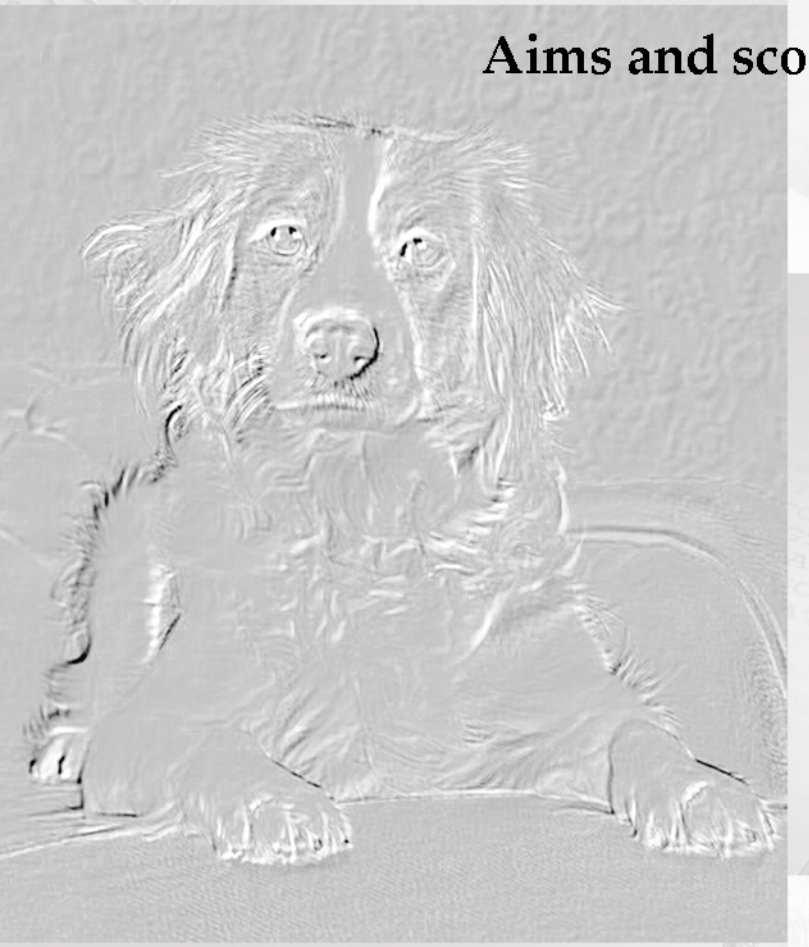
Publication of this thesis was financially supported by
Be Surgeon., Co. Ltd. (Thailand)
Elanco Animal Health
Scil animal care company BV
TROVET / Netlaa BV
Zoetis Nederlands
Zoetis (Thailand) Ltd.

Contents

The studies described in this thesis were conducted at and financially supported by Chulalongkorn University, Bangkok, Thailand, the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht The Netherlands and the American Kennel Club Canine Health Foundation.

Chapter 1

Aims and scope of the thesis



Introduction

Patellar luxation (PL) is one of the most common orthopaedic disorders of dogs, and especially of small-breed dogs. The disorder is considered developmental and can result from a variety of anatomical abnormalities of the pelvic limbs. Although different explanations have been given regarding the pathophysiology of PL, the primary cause of the disorder remains unclear (DeAngelis and Hohn, 1970; Hulse, 1993; Roush, 1993; Hayes et al., 1994). It has been suggested that the disease is inherited, which is supported by the predisposition of certain breeds to PL, such as Pomeranians, Yorkshire Terriers, Miniature and Toy Poodles, Chihuahuas, Boston Terriers, Pekingese, and Flat-Coated Retrievers (Priester, 1972; Hulse, 1981; LaFond et al., 2002; Alam et al., 2007; OFA, 2013; Lavrijsen et al., 2013).

Aims and scope of the thesis

The first aim of the studies described in this thesis was to investigate the outcome of surgical treatment of canine medial patellar luxation (MPL) using standard techniques, and to investigate the outcome of a novel surgical technique, i.e. proximal trochleoplasty, for treating bidirectional PL (BPL) in Pomeranian dogs.

A second aim was to study the genetic background of PL, including its prevalence and heritability, by investigating chromosome regions or genes that might predispose dogs to PL. These studies make use of data sets for three breeds of dog: Thai Pomeranians, Dutch Flat-Coated Retrievers, and Dutch Kooiker dogs. All Pomeranian dogs referred to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University from 2006 to 2011 had been screened for PL according to a standard orthopaedic protocol (Piermattei et al., 2006). Data for Flat-Coated Retrievers and Kooiker dogs are accessed from the 'Meutstege' database. These dogs had been investigated for PL, on a voluntary basis, from 1994 to 2011 by a listed, certified orthopaedic surgeon, using a designated protocol to examine and score PL in a uniform manner (Hazewinkel et al., 2013).

The general introduction of this thesis **(Chapter 2)** provides an overview of PL, including its background and prevalence, the anatomy and biomechanics of the stifle joint, the pathophysiology and clinical signs of the disease, diagnostic imaging findings, treatment modalities, and the genetics of PL. The study reported in **Chapter 3** evaluates prospectively the surgical treatment of MPL in 55 Pomeranian dogs. The choice of technique used depends on the depth of the trochlear sulcus and

the alignment of the patella, patellar ligament, and tibial tuberosity. Trochlear block recession (TBR) with or without tibial tuberosity transposition (TTT) is investigated in dogs with MPL, including dogs with grade 2, 3, or 4 PL. Treatment outcomes and lameness are evaluated for minimally 16 weeks post-operatively. The aim of the study reported in **Chapter 4** is to describe a novel surgical technique of extended proximal trochleoplasty for the treatment of BPL in Pomeranians and to evaluate the clinical and radiological outcomes. Patellar ligament length (PLL) and patellar bone length (PBL) are measured on radiographs, using a technique commonly used for large-breed dogs (Mostafa et al., 2008), to establish whether patella alta is a cause of BPL in Pomeranians.

Chapter 5 presents the results of an investigation into the incidence of PL in Pomeranian dogs in Thailand and of a genetic study into co-segregation of the phenotype, using five polymorphic microsatellites situated close to five collagen genes. In addition, an association study is performed with 1536 single nucleotide polymorphisms (SNPs) spread across the genome of Pomeranian dogs. The aims of the study described in **Chapter 6** are to investigate the prevalence of PL in Pomeranian dogs and to analyse its heritability. Genome-wide association analyses are performed to learn more about to identity of the chromosomal regions involved in the development and aetiology of PL. Subsequently, validated SNPs are tested in a large population of Pomeranian and other dog breeds.

In the study reported in **Chapter 7**, the prevalence and genetics of PL in the Dutch Kooiker dog population are investigated and the heritability of PL analysed. In a cohort study, a pedigree of 1737 Kooiker dogs comprising nine generations is screened for PL from 1994 to 2011. A genome-wide association study attempts to identify chromosomal regions involved in the development of PL and to elucidate the disease aetiology. The study described in **Chapter 8** concerns a genome-wide association analysis of PL in Flat-Coated Retrievers, followed by massive parallel DNA sequence analysis, in an attempt to describe the regions on the canine genome association with PL in a cohort of Flat-Coated Retrievers.

The findings of the studies involving these purebred dogs are discussed in **Chapter 9** from both surgical and genetic perspectives. A summary is presented in **Chapter 10**.

References

Alam, M.R., Lee, J.I., Kang, H.S., Kim, I.S., Park, S.Y., Lee, K.C., Kim, N.S., 2007. Frequency and distribution of patellar luxation in dogs: 134 cases (2000 to 2005). Veterinary and Comparative Orthopaedics and Traumatology 20, 59-64.

DeAngelis, M., Hohn, R.B., 1970. Evaluation of surgical correction of canine patellar luxation in 142 cases. Journal of the American Veterinary Medical Association 156, 587-594.

Hayes, A.G., Boudrieau, R.J., Hungerford, L.L., 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal of the American Veterinary Medical Association 205, 716-720.

Hazewinkel, H.A., Tellhelm, B., Leegwater, P.A., 2013. Screening for Orthopaedic Genetic defects: Part 1. Patella Luxation in Dogs. European Journal of Companion Animal Practice 23, 90-98.

Hulse, D.A., 1981. Pathophysiology and management of medial patellar luxation in the dog. Veterinary Medicine: Small Animal Clinician 76, 43-51.

Hulse, D.A., 1993. Medial patellar luxation in the dog. In: Bojrab, M.J. (Ed.), Disease Mechanisms in Small Animal Surgery. Lea&Febiger, Philadelphia, pp. 808-817.

LaFond, E., Breur, G.J., Austin, C.C., 2002. Breed susceptibility for developmental orthopedic diseases in dogs. Journal of the American Animal Hospital Association 38, 467-477.

Lavrijsen, I.C., Heuven, H.C., Breur, G.J., Leegwater, P.A., Meutstege, F.J., Hazewinkel, H.A., 2013. Phenotypic and genetic trends of patellar luxation in Dutch Flat-Coated Retrievers. Animal Genetics 44, 736-741.

Mostafa, A.A., Griffon, D.J., Thomas, M.W., Constable, P.D., 2008. Proximodistal alignment of the canine patella: radiographic evaluation and association with medial and lateral patellar luxation. Veterinary Surgery 37, 201-211.

OFA, 2013. Orthopedics Foundation for Animals: Patellar luxation statistics. http://www.offa.org/stats_pl.html (accessed 9 March 2014).

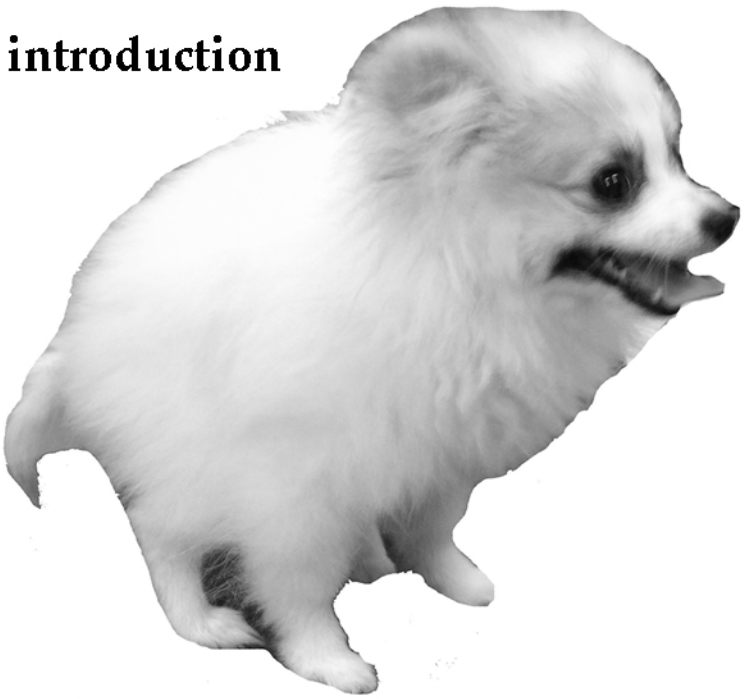
Piermattei, D.L., Flo, G.L., DeCamp, C.E., 2006. The stifle joint. In: Piermattei, D.L., Flo, G.L., DeCamp, C.E. (Eds.), Handbook of Small Animal Orthopedics and Fracture Repair. Fourth Ed. Saunders, St. Louis, pp. 562-632.

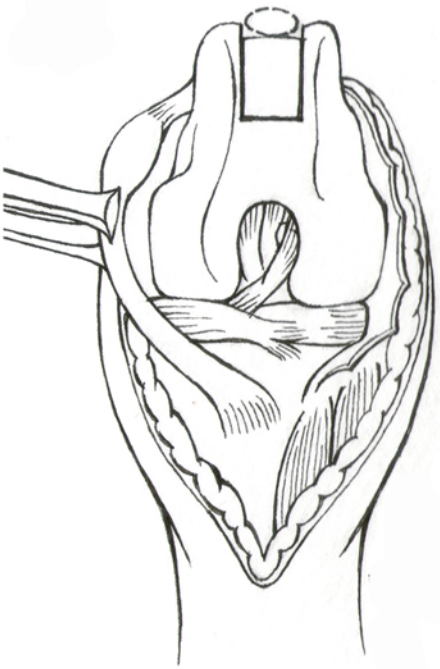
Priester, W.A., 1972. Sex, size, and breed as risk factors in canine patellar dislocation. Journal of the American Veterinary Medical Association 160, 740-742.

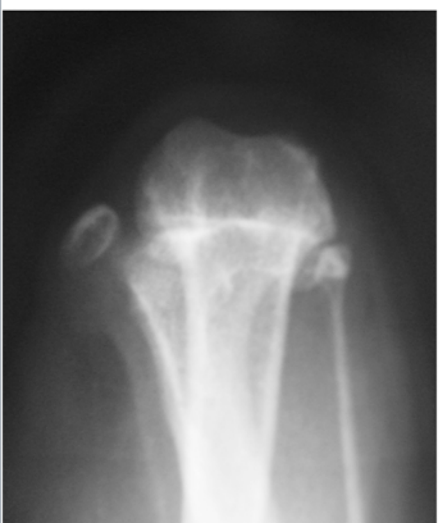
Roush, J.K., 1993. Canine patellar luxation. Veterinary Clinics of North America: Small Animal Practice 23, 855-868.

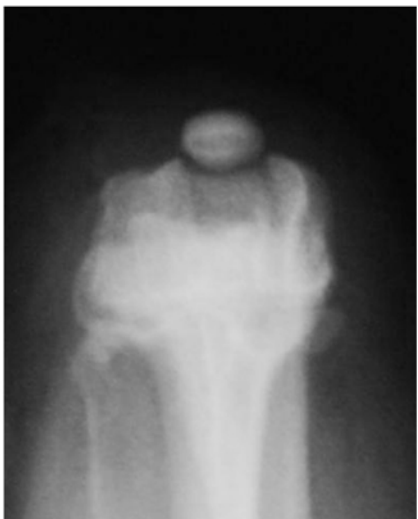
Chapter 2

General introduction









Patellar luxation (PL) is one of the most common orthopaedic disorders both found in small- and large-breed dogs. As a result of a developmental abnormality of the hind limb, the patella tends to become displaced from the groove in which it is normally positioned. PL is classified as congenital (a condition existing at birth or even before birth), developmental (encompass all orthopaedic problems seen in the growing animal with PL), traumatic or iatrogenic in origin (Hayashi et al., 2010). In order to present the current knowledge of PL in dogs, an overview is given in this chapter including prevalence of PL, anatomy and biomechanics of the stifle joint, the pathophysiology and clinical signs, diagnostic imaging and treatment modalities of PL.

2.1 Prevalence of patellar luxation

PL occurs frequently in dogs and is commonly seen in small animal practices. The patella can be displaced medially and/or laterally. Medial patellar luxation (MPL) is more common in all sizes and breeds of dogs than lateral patellar luxation (LPL). MPL occurs more often in small-breed dogs than LPL and small-breed dogs have a risk approximately 12 times that of large-breed dogs (DeAngelis and Hohn, 1970; Priester, 1972). LPL is mostly found in large-breed dogs although MPL is diagnosed more frequently (Robin, 1990; Hayes et al., 1994; Piermattei et al.,2006). Acquired traumatic PL is uncommon, but can be seen in any breed of dog subjected to trauma or iatrogenic after stifle surgery (Arthurs and Langley-Hobbs, 2006; Arthurs and Langley-Hobbs, 2007).

The prevalence of PL has been reported in different ways, including the percentages of registered dogs in particular breeds, percentages of dogs affected with PL, and relative risk (RR) and odds ratio (OR) of dog breeds with PL. The RR is the ratio of the risk of disease in the exposed group to the risk of disease in the non-exposed group. It can be computed in cohort studies, or in some cases cross-sectional studies. The OR is the ratio of the odds of having PL in breed 1 compared to the odds of having PL in a reference breed. The OR is approximately equal to RR if the disease occurs infrequently in the underling population (prevalence or incidence risk < 5%) (Dohoo et al., 2010).

The Orthopedic Foundation for Animals (OFA) scheme for registration of 57,779 animals, including both PL affected and unaffected dogs, from January 1974 to December 2013, lists Pomeranians as having the highest prevalence of PL with 39.5% affected in the USA (OFA, 2013). In Thailand, Pomeranians reveal a high

prevalence of PL with 75% affected during 2006 – 2008 (Soontornvipart et al., 2013). In a study that examined 69,245 dog patients from 10 colleges of Veterinary Medicine in the United States of America and Canada from March 1964 to January 1969, 542 dogs had PL with Pomeranian, Yorkshire Terrier, Chihuahua, Miniature and Toy Poodle, and Boston Terrier with a significantly increased risk of PL (DeAngelis and Hohn, 1970; Priester, 1972). The report of PL in different breeds in 4,419 cases and 4,419 controls from the Veterinary Medical Database (VMDB) at Purdue University from January 1986 to December 1995 (LaFond et al., 2002) and the investigation of the frequency and distribution of PL in 134 affected dogs from 2000 to 2005 (Alam et al., 2007) found PL in different breeds similarly as Priester reported (Priester, 1972). Additionally, a significant association of PL in Poodle and Yorkshire Terrier was found in a comparison between 124 PL cases and 248 controls (Hayes et al., 1994). In The Netherlands, the prevalence of PL was screened between 1994 and 2011 in 4288 screened dogs of four different breeds including Flat-Coated Retriever, Kooiker dogs, Jack Russell Terrier, and Chihuahua (Hazewinkel et al., 2013). The surgical studies of Linney et al. (2011) and Singleton (1969) also reported PL occurred in Yorkshire Terrier, Poodle, Chihuahua, and Boston Terrier. The relatively high prevalence of PL in specific breeds, and the high proportion of cases in which both stifles are affected, strongly suggests that it has a genetic basis (LaFond et al., 2002). The rank and percentages of dog breeds of OFA and the prevalence of PL as reported in other studies is shown in Table 1.

Table 1 A massive survey of particular breeds with patellar luxation in five reports.

The Orthopedic Foundation for Animals (OFA) scheme for registration of 57,779 animals both affected and unaffected dogs since January 1974 to December 2013 (OFA, 2013), the prevalence of PL between 1994 and 2011 in 4288 screened dogs of four different breeds (Hazewinkel et al., 2013), the data of 4,419 cases and 4,419 controls from the Veterinary Medical Database (VMDB) at Purdue University between January 1986 and December 1995 (LaFond et al., 2002). A case-control study used to compare 124 case dogs with PL and 248 control dogs with other orthopaedic problems (Hayes et al., 1994), and 542 of 69,245 patients with patellar luxation from 10 colleges of Veterinary medicine in the U.S.A and Canada from March 1964 to January 1969 (Priester, 1972).

Breed	**OFA, 2014**	**Hazewinkel et al., 2013**	**LaFond et al., 2002**	**Hays et al., 1994**	**Priester, 1972**
	% affected		Odds ratio		Relative risk
Pomeranian	39.5		18.6		8.1
Yorkshire Terrier	24.4		8.3	4.9	7.6
Australian Terrier	16.4		8		
Cocker Spaniel	14.5		2.1		0.6
Japanese Chin	9.6		4.8		
Chow Chow	9.2		6.1		
Lhasa Apso	7.2		3.4	0.7	
Chinese Shar-Pei	6.5		11.4		
Toy Fox Terrier	6.3		12.8		1.4
Boston Terrier	6		4.2		2.1
Chihuahua	5.8	45.2	8.9		5.1
Pug	5.6		3.3		
Labrador Retriever	5.2			1	0.2
Dachshund	5.2			0.1	0.2

continued

Breed	OFA, 2014	Hazewinkel et al., 2013	LaFond et al., 2002	Hays et al., 1994	Priester, 1972
	% affected		Odds ratio		Relative risk
Dachshund	5.2			0.1	0.2
Bichon Fries	4.6		4.8		
Bulldog	4.3		6.1		
Toy Poodle			9.7	9.1	4.2
Miniature Poodle	]4.2		4.1		
Standard Poodle			3.2		1.8
West Highland White Terrier	4.1		1.8		
Beagle	3.4				0.2
Papillon	3.3		8.4		
Cairn Terrier	3.2		1.9		
Maltese	2.8		6.5		
Keeshond	2.5		4.4		
Miniature Pinscher	2.2		14.4		
Cavalier King Charles Spaniel	2.1		9.1		
Silky Terrier	1.8		16		
Flat-Coated Retriever	1.5	18.3	2.9		
Shih Tzu	1.5		2.3		
Akita	1.3		6.7		
Great Pyrenees	1.2		64		
Pekingese			1.4		1.8
Kooiker dogs		20.6			
Jack Russell Terrier		10.5			

The male:female ratio in PL affected dogs ranged from 1:1.3 to 1:1.9 in screening of small-breed dogs (DeAngelis and Hohn, 1970; Priester, 1972; Denny and Minter, 1973; Willauer and Vasseur, 1987; Hulse, 1993; Hayes et al., 1994; Alam et al., 2007; Linney et al., 2011). In contrast in large-breed dogs, the ratio male:female dogs with PL varied from 1.3:1 to 1.8:1 (Remedios et al., 1992; Gibbons et al., 2006; Arthurs and Langley-Hobbs, 2007). However, the report of Lavrijsen et al. (2013) in Flat-Coated Retriever found the male:female ratio was 1:1.8. The ratio between male and female in small- to medium-breed dogs was almost the same in the studies of Singleton (1969) and Wangdee et al. (2014), i.e. 1:0.87, 1:1.15 as well as of Bound et al., (2009) with a ratio of 1:1.17 including large-breed dogs. The mean age at diagnosis of PL was less than 3 years of age (Remedios et al., 1992; Hayes et al., 1994; Alam et al., 2007) and luxations were equal in unilateral and bilateral in most reports (Gibbons et al., 2006; Alam et al., 2007; Linney et al., 2011; Lavrijsen et al., 2013). MPL was most common in both small- and large-breed dogs (Hayes et al., 1994; Gibbons et al., 2006; Alam et al., 2007; Bound et al., 2009; Wangdee et al., 2014). In contrast, LPL was more frequent in the Flat-Coated Retriever (Lavrijsen et al., 2013). We use the term bidirectional patellar luxation (BPL) when the patella of the same stifle luxates both medially and laterally (Kowaleski et al., 2012; Vidoni et al., 2006) which is reported in the Flat-Coated Retriever (Lavrijsen et al., 2013), Kooiker dog (Wangdee et al., 2014) and Pomeranian (Wangdee et al., 2014). The distributions of PL in both small- and large-breed dogs are presented in Table 2.

Table 2 Distributions of gender, age, luxation and direction of luxation of patellar luxation in different studies.

	Percentage or ratio	Reference
Male:female ratio	1:1.3 to 1:1.9	DeAngelis and Hohn, 1970; Priester, 1972; Denny and Minter, 1973; Willauer and Vasseur, 1987; Hulse, 1993; Hays et al., 1994; Alam et al., 2007; Linney et al., 2011; Lavrijsen et al., 2013
	1:1.1 to 1:1.2	Bound et al., 2009; Wangdee et al., 2014
	1.15:1	Singleton, 1969
	1.3:1 to 1.8:1	Remedios et al., 1992; Arthurs and Langley-Hobbs, 2006; Gibbon et al., 2006; Arthurs and Langley-Hobbs, 2007
Age	Less than 3 years old	Remedios et al., 1992; Hays et al., 1994; Alam et al., 2007
Luxation		
Unilateral	49.5-51%	Arthurs and Langley-Hobbs, 2006; Gibbon et al., 2006; Alam et al., 2007; Arthurs and Langley-Hobbs, 2007; Linney et al., 2011; Lavrijsen et al., 2013
	14%	Soontornvipart et al., 2013
Bilateral	49-50.5%	Arthurs and Langley-Hobbs, 2006; Gibbon et al., 2006; Alam et al., 2007; Arthurs and Langley-Hobbs, 2007; Linney et al., 2011; Lavrijsen et al., 2013
	86%	Soontornvipart et al., 2013
Direction*		
MPL	90-96.6%	DeAngelis and Hohn, 1970; Remedios et al., 1992; Alam et al., 2007; Bound et al., 2009
	78.2%	Vidoni et al., 2006
	61.1%	Wangdee et al., 2014
	31%	Lavrijsen et al., 2013
LPL	3.4-10%,	DeAngelis and Hohn, 1970; Remedios et al., 1992; Alam et al., 2007; Bound et al., 2009
	9.9%	Vidoni et al., 2006
	31.9%	Wangdee et al., 2014
	61%	Lavrijsen et al., 2013
BPL	11.9%	Vidoni et al., 2006
	8%	Lavrijsen et al., 2013
	7%	Wangdee et al., 2014

* MPL=medial patellar luxation, LPL=lateral patellar luxation, BPL=bidirectional patellar luxation

2.2 Anatomy and biomechanics of the stifle joint

To understand the underlying musculoskeletal abnormalities of PL, the normal anatomy, function, and interrelationship of the femur, patella and tibia must be understood.

The angle between the femoral neck and shaft axis (angle of inclination of femoral neck) in the anteroposterior projection is approximately 130° (Hulse, 1981a). Coxa valga is an increased femoral neck-shaft angle whereas coxa vara is a decreased femoral neck-shaft angle (Fig. 1A). Anteversion of the femoral head and neck is a forward inclination of the femoral head and neck. Retroversion is a caudal inclination of the femoral head and neck (a diminished anteversion angle) (Fig. 1B). The normal anteversion angle in puppies is near 0° and increases to approximately 27° in the adult. The transcondylar-femoral shaft axis or anatomical lateral distal femoral angle (aLDFA) in the anteroposterior projection is approximately 93° (Hulse, 1981a).

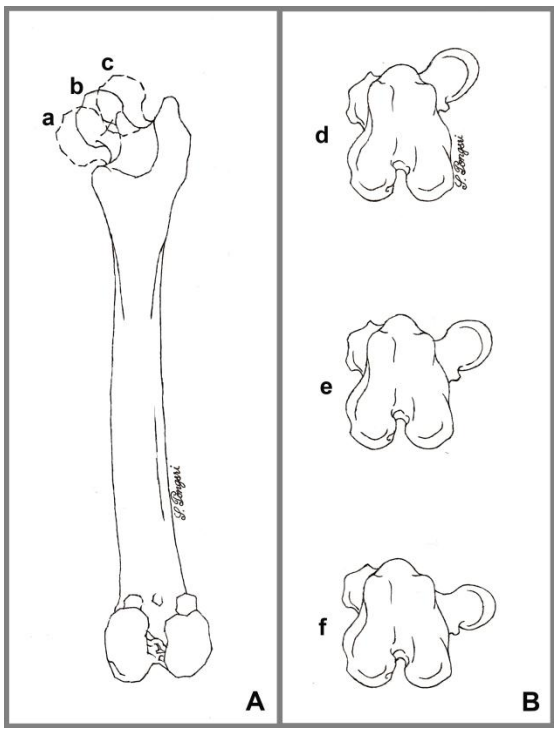


Figure 1 Diagram hip joint conformation (drawing modified from Hulse, 1981 by S.Pongsri).

(A) Angle between the femoral neck and shaft axis in the anteroposterior projection; normal femoral shaft-femoral neck axis **(b)**; coxa valga **(c)**; and coxa vara **(a)**.

(B) The relationship of the femoral neck axis to transcondylar axis; abnormal anteversion **(d)**; normal anteversion **(e)**; and retroversion **(f)**.

The stifle joint is a complex hinge joint involving two functionally discrete articulations. First, weight bearing occurs through the articulation between the femoral and tibial condyles including the menisci. Second, the femoropatellar articulation increases the mechanical efficiency of the quadriceps muscle group and promotes the extensor function (Vasseur, 2003). There are three distinct articulations including the femorotibial, femoropatellar and proximal tibiofibular joint. The patella is stabilised in the trochlear groove by two femoropatellar ligaments. Distal to the patella, the synovial and fibrous layers of the joint capsule are separated by the infrapatellar fat body (Vasseur, 2003). The extensor mechanism of the stifle consists of the quadriceps muscle group, patella, patellar ligament, tibial tuberosity, patellar retinaculum and adjacent soft tissues, and the femoral trochlea (Fig. 2) (Olmstead, 1981). The power of the extensor mechanism comes from the four heads of the quadriceps femoris muscle group. Vastus lateralis, vastus intermedius and vastus medialis originate from the proximal femur and rectus femoris originates from the ilium cranial to the acetabulum (Fig. 3A, B) (Olmstead, 1981). The patella rides in the trochlear groove of the femur which articular surface is concave and corresponds to the convex shape of the patella (Fig. 4A, B). The trochlear groove is formed by the lateral and medial trochlear ridges, with the medial ridge usually being larger than the lateral trochlear ridge. The trochlear ridges project from the cranial surface of the supracondylar region of the distal femur, and thus support the patella (Roush, 1993). The vastus lateralis and vastus medialis muscles insert onto the patella through the parapatellar fibrocartilages (Fig. 2) (Roush, 1993). The latter articulate with the trochlear ridges and increase the surface area of contact, thus spreading the force of the quadriceps muscle. The vastus medialis counteracts the lateral pull of the vastus intermedius and lateralis on the patella as the stifle is extends, so that the patella remains in its normal position (Olmstead, 1981). When the quadriceps muscle group contracts during weight bearing, it results in extension of the stifle joint. The hamstrings muscle group including biceps femoris, gracilis, semimembranosus and semitendinosus will contract during flexion of the stifle joint and act together with the cranial cruciate ligament (CCL) against cranial tibial translation. The normal range of motion of the stifle joint is 140° (Vasseur, 2003). The range of motion of the femorotibial angle during flexion is 41° and during extension is 162° in Labrador Retrievers (Jaegger et al., 2002).

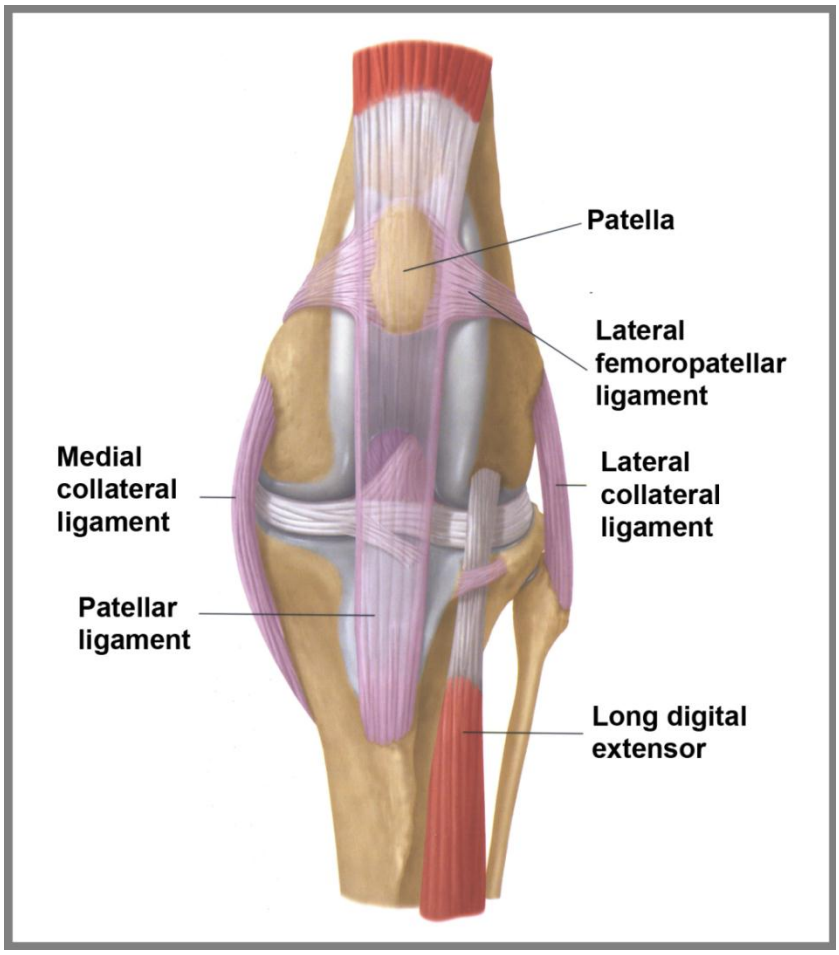


Figure 2 Anatomy of normal stifle joint (Wright et al., 2003).

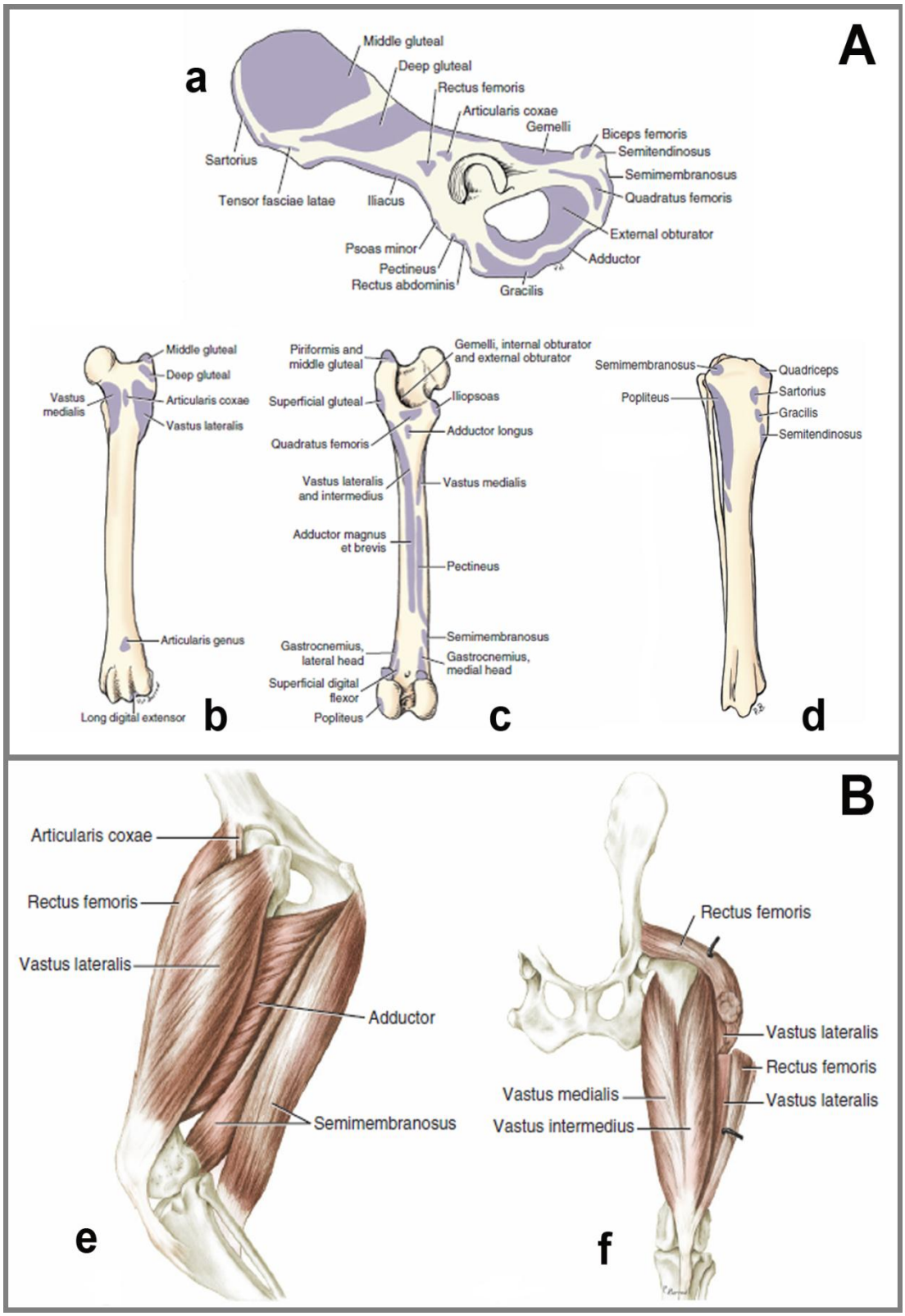


Figure 3 The anatomy of the pelvic limb (drawing from Hermanson, 2013).
(A) The areas of muscle attachment on the lateral aspect of ilium **(a)**, on cranial aspect **(b)** and on caudal aspect **(c)** of femur and on medial aspect of tibia **(d)**. **(B)** Deep muscles of thigh on lateral aspect **(e)** and on cranial aspect **(f)**.

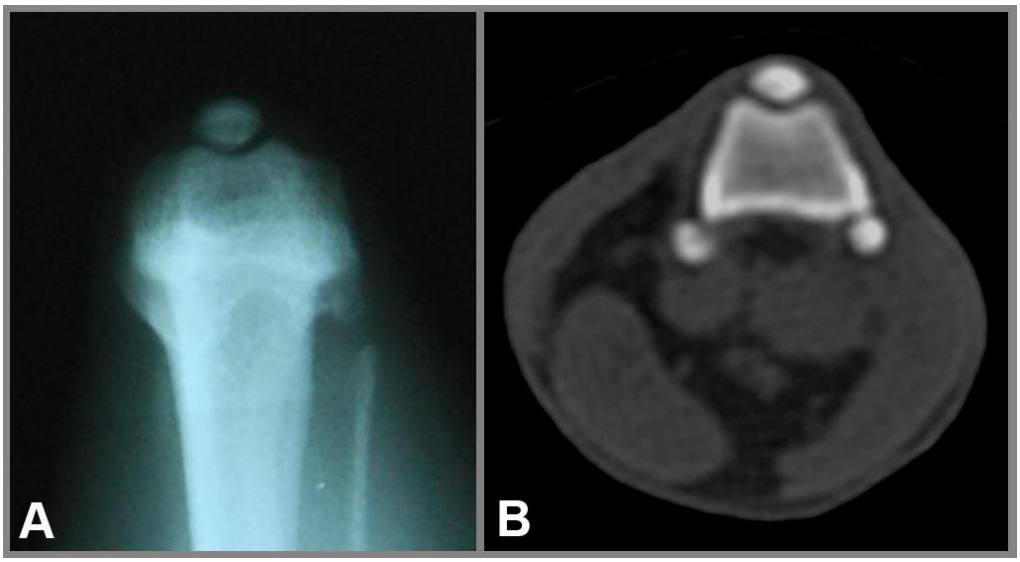


Figure 4 Skyline views show patella is position in the trochlear groove on a radiographic image (A) and on computed tomographic (CT) image (B).

The patella is the largest sesamoid bone, and is embedded in the tendon of the quadriceps muscle which converges on the patella and continues distally as the straight patellar tendon which inserts on the tibial tuberosity. The patella has a number of important functions including to maintain tension as the stifle is extended, to increase the mechanical leverage applied by the quadriceps group at the distal femur, and to decrease friction between the quadriceps and femoral condyles. In a normal stifle, the patella is localized and glides within the femoral trochlear groove. The alignment of the quadriceps, patella, trochlear sulcus, patellar tendon, and tibial tuberosity must be normal for the stability and proper function of the stifle. In particular, abnormal alignment of one or more of these structures results in abnormal mechanics and joint instability, which in turn places abnormal stresses on the ligaments of the stifle and may lead to luxation of the patella, cartilage erosion and subsequent osteoarthritis (OA) (Hulse, 1993). In the growing dog, the consequences of these abnormal forces are compounded by their effects on the growing cartilages of the distal femur and proximal tibia. A shallow trochlear sulcus and/or displacement of the quadriceps are usually seen in patients with permanent PL.

2.3 Pathophysiology and clinical signs

Medial patellar luxation

MPL is considered developmental disorder, in most cases developing after birth as a result of deformities that may be present at birth (Kowaleski et al., 2012). It is indicated that MPL is a multifactorial anatomic abnormality involving the entire pelvic limb (Hayashi et al., 2010). MPL results from malalignment and possibly progresses to the markedly functional deficit of the affected limbs. The pathology can vary from mild instability of the patella within the trochlear groove to severe permanent luxation with skeletal deformity (Fig. 6).

The pathogenesis of MPL has been extensively reviewed in different aspects; however, the underlying cause of PL is not entirely understood. Its cause suggested that coxa vara and relative retroversion were underlying skeletal abnormalities (Putnam, 1968). These disarrangements were consider to be the underlying cause of the complex sequence of skeletal changes in the pelvic limb that are typical of the small- and large-breed dogs with MPL. The typical deformities include decrease in femoral anteversion (relative retroversion), coxa vara (a decreased angle of inclination of the femoral neck), medial displacement of the quadriceps muscle group, femoral varus, genu varum, a shallow trochlear groove, dysplasia of the femoral condyle, medial displacement of the tibial tuberosity, internal rotation of the tibial relative to the femur, proximal tibia varus, rotational instability of the stifle joint, internal rotation of the foot despite distal external tibial torsion and degenerative joint disease (Hulse, 1981b; Kowaleski et al., 2012). Internal tibial rotation at the stifle joint in case of PL can be exacerbated by rupture of the cranial cruciate ligament in 19% and 50% in large-breed dogs with PL (Gibbon et al., 2006; Persuki et al., 2006). It has been hypothesized that the underlying disorders of coxa vara and diminished anterversion angle result in medial displacement of the quadriceps muscle group causing abnormal forces on the distal femoral physis retarding growth of the medial side, resulting in distal femoral varus and internal rotation of the tibia. This theory has been questioned because coxa valga was identified as a significant risk factor for MPL in small-breed dogs (Bound et al., 2009). It had been hypothesized according to a theory from human literature as the consequence of reduced anteversion angle causing external rotation of coxofemoral joint which needs compensatory internal rotation of the distal limb to place the foot properly. As a result, the lateral soft tissues supporting the stifle joint are stretched, and a lateral torsion force is exerted on the distal femur which displaces the femoral

trochlea lateral to the line of contraction of the quadriceps, causing medially displacement of the quadriceps muscle group (Hayashi et al., 2010). However, this is contrary to a study on the anteversion angle of the femoral neck using magnetic resonance image (MRI) which did not reveal any correlation between PL and the angle of anteversion (Kaiser et al., 2001b). A study on the inclination angle of Pomeranians using radiographic measurement also did not show significant differences between normal and MPL groups (Soparat et al., 2012).

The deformities vary from mild changes of the soft tissues to severe skeletal abnormalities. The severity is related to the age of dogs at the occurrence of PL, and the permanent nature of PL because the angular and torsional abnormalities occur secondary to abnormal forces directed to the physis of immature dogs (Hulse, 1981b). The Hueter-Volkmann principle states that the increased compressive force on an active physis retards growth, whereas decreased compressive force accelerates growth. PL and quadriceps displacement generate forces which act eccentrically on the active distal femoral physis and proximal tibial physis which results in skeletal bowing and torsion (Fig. 5A, B) (Hulse, 2011). The permanent luxation is important because the longer the abnormal forces are allowed to act on the physis, the greater the torsion and angular deformity. Medial displacement of the quadriceps muscle group in MPL, increases pressure at the medial side which results in a small hypoplastic medial femoral condyle and femoral varus, as well as the decreased lateral pressure results in an elongated lateral femoral condyle. Additionally the tibial growth plate reacts to abnormal forces. The result is a varus deformity of the proximal tibial and proximal tibial endotorsion (Fig. 5C and Fig. 6) (Hulse, 1981b). The Hueter-Volkmann principle is also responsible for a normal development of the trochlear groove. The normal physiologic pressure exerted by the patella on the trochlear groove is needed for proper trochlear development. Lacking of normal physiologic pressure results in a shallow or absent trochlear groove and malformation of the patella (Fig. 7 and Fig. 8A, B) (Hulse, 1981b).

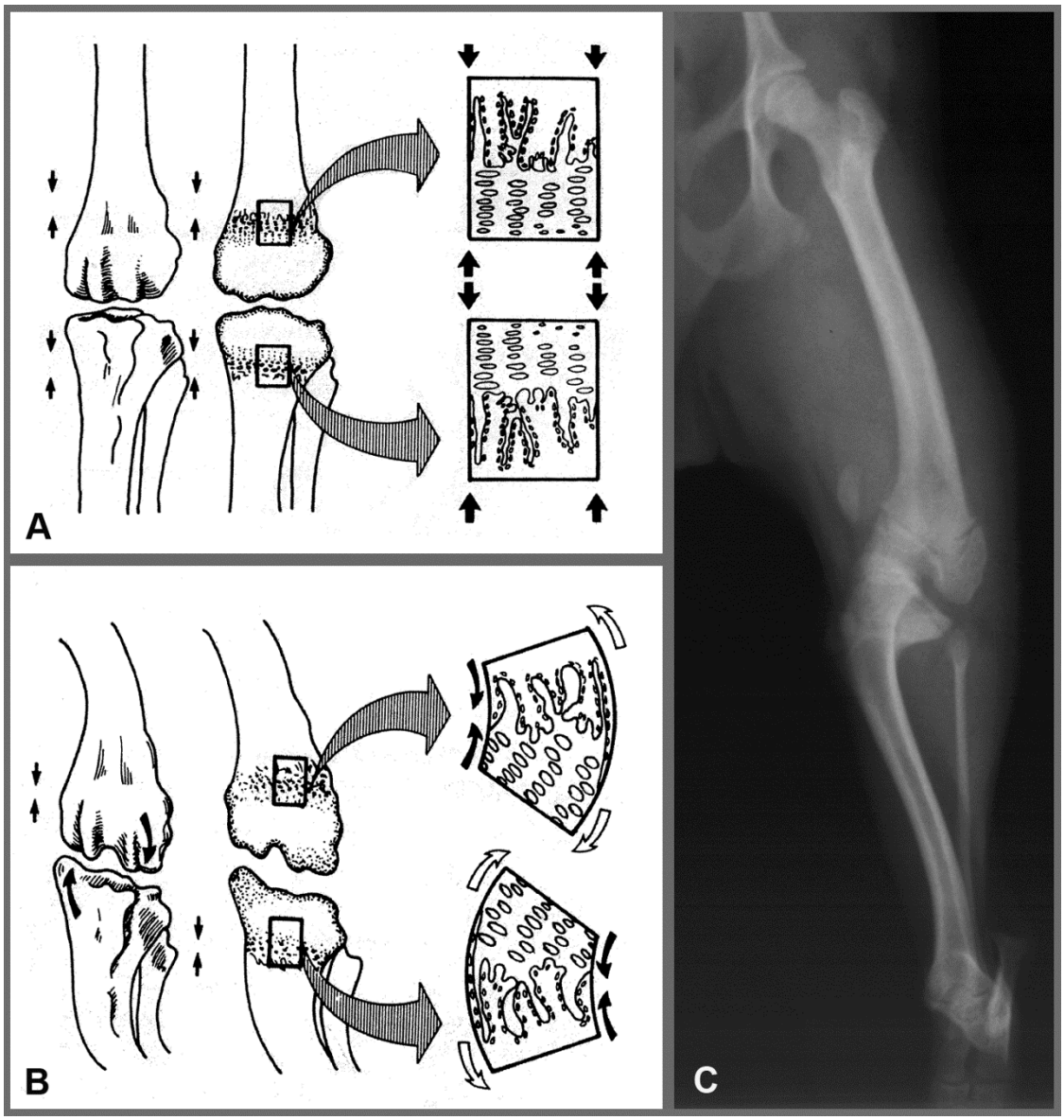


Figure 5 Diagrams of force excerted on the growth plate according to Hueter-Volkmann principle.

Normal forces excerted on the growth plate **(A)** and abnormal forces excerted on the growth plate by medial displacement of the quadriceps muscle group on medial patellar luxation (MPL) in growthing dog **(B)** (drawing from Hulse, 1981b).

Radiographic image (craniocaudal projections) of left hind limb of 6-month old female Pomeranian dog with MPL grade 4 shows medial bowing of the distal femur, medial displacement of tibial tuberosity, lateral bowing (valgus deformity) of the proximal tibial, and thus internal torsion of the proximal tibia **(C)**.

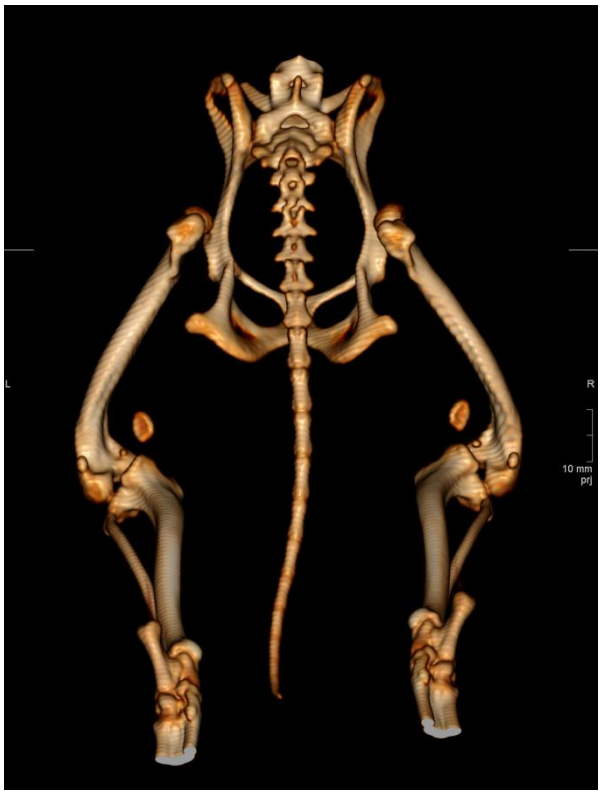


Figure 6 3-D reconstruction of 6-month old Chihuahua with bilateral medial patellar luxation grade 4.

The picture shows medial bowing of the distal femur, medial displacement of tibial tuberosity, lateral bowing (valgus deformity) of the proximal tibial, and thus internal torsion of the proximal tibia, based on the Hueter-Volkmann principle (acknowledgements of Prof.dr. G.Voorhout).

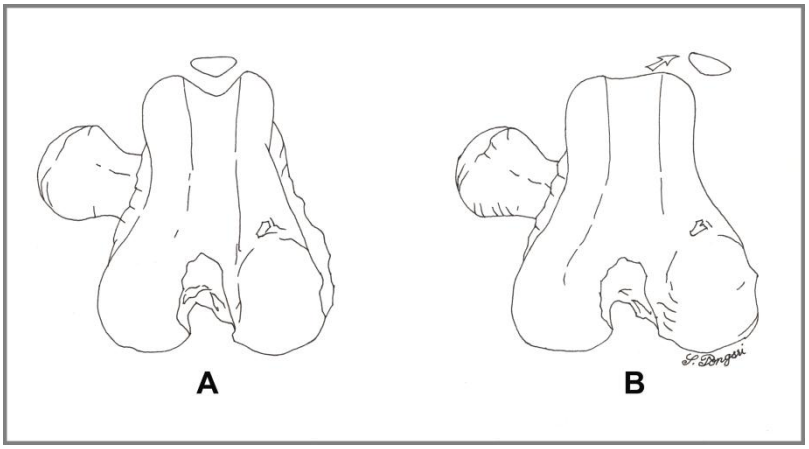


Figure 7 Development of the trochlear groove.

The normal physiological pressure exerted by the patella results in proper development of the trochlear groove **(A)**. In patellar luxation, loss of normal physiological pressure results in a shallow groove **(B)** (drawing modified from Hulse, 1981b by S.Pongsri).

Lateral patellar luxation

LPL can occur in both large- and small-breed dogs (Wangdee and Torwattanachai, 2010; Kalff et al., 2014) however it is more common in large-breed dogs (Lavrijsen et al., 2013). Similar to MPL, the normal development of the femoral condyle, trochlear groove and proximal tibia depends on the balance between gravity and muscle force during normal weight bearing (Hayashi et al., 2010). Coxa valga and excessive anteversion of the femoral neck may induce relative medialization and internal torsion of the distal femur, respectively, with regard to the line of action of the quadriceps muscle to lateral luxation of the patella. Subsequently, the increased compressive force on the lateral aspect of the distal femoral growth plate retards growth of the lateral femoral part of the growth plate compared with the medial one, causing lateral bowing of the distal third of the femur. The lateralization of the compressive loads across the joint may constrain the development of the tibia, leading to a lateral deviation of the limb at the stifle or genu valgum. This condition does not cause an abnormal medial condyle and tibial plateau development, however increased forces through the lateral aspect of the distal femoral physis can lead to lateral condylar dysplasia and lateral luxation of the patella. The femoral trochlea groove becomes shallow since the patellar pressure is absent during growth. In addition, the unconstrained patella is able to move laterally during contraction of the quadriceps muscle, leading to wear and erosion of the lateral trochlear ridge (Fig. 9B) and exacerbating the problem (Hayashi et al., 2010).

The severity affects the function of the limb and can cause pain due to stretching of the ligaments and inflammation of the joint capsule. Damaging of the articular cartilage of the patella and of the trochlear groove, leads to osteoarthritis of the stifle causing chronic pain and lameness (Hulse, 1981b).

Based on variable degrees of clinical and pathological changes, PL is classified into 4 grades according to the first classification system by Putnam (1968) and adapted by Singleton (1969). The latter system defines the varying degrees of the deviation of the tibial tuberosity and rotation of the tibial accompanied by a slight abduction of the hock joint as well as clinical signs and the morphological criteria of trochlear sulcus. Harrison (1975) emphysized that dogs with PL had a higher risk of cranial cruciate ligament rupture and considered additional criteria for classifying PL like retropatellar chondromalacia, peritrochlear osteophytes and osteoarthrosis of the stifle. In 1981, Hulse defined PL grade 1 to 4 as a function of the changes in angle and rotation observed in the femur. A patella riding high in the trochlea (incomplete

articulation of the patellar body with the trochlear groove without medial or lateral luxation) is classified by Koch et al. (1998) as grade 0; spontaneous luxation of the patella in the standing dog is classified as grade 3, even if spontaneous reduction on active flexion or extension in the knee joint with or without tibial rotation is possible (L'Eplattenier and Montavon, 2002). Meutstege defined the "loose patella", being the patella able to be moved at the ridge of the trochlea groove, but not over the ridge as in grade 1 (F.J. Meutstege-personal communication). We used the grading system as recently published (Hazewinkel et al., 2013) to classify our patients:

Grade 0: Patellae are moving inside trochlear groove and cannot be manually luxated.

Loose: patella can be manually positioned on the top of the trochlear ridge, but not over the top completely

Grade 1: The patella luxates manually at full extension of the stifle joint, but spontaneously returns to the trochlea when released.

Grade 2: The patella luxates spontaneously or on manipulation and remains luxated at a certain angle of the stifle joint, especially when the foot is rotated internally for MPL and externally for LPL. It is either spontaneously reduced on active flexion or extension or can be manually reduced by the examiner. Many cases with grade 2 MPL "live" with the condition reasonably well for many years, but the repeatedly luxation of the patella over the medial or lateral ridge of the trochlear can cause erosion of the articulating surface of the patella and the proximal area of the trochlear ridge (Fig. 9A, B). This results in crepitation which becomes apparent when the patella is luxated manually during clinical investigation.

Grade 3: The patella is luxated permanently most of the time, but can be manually reduced. However, reluxation occurs spontaneously. The trochlear groove is very shallow or even flattened (Fig. 8A).

Grade 4: The patella is permanently luxated and cannot be manually repositioned. The limb is carried or when bilateral the animal moves in a crouched position with the limbs partly flexed. The trochlear groove is absent or even convex (Fig. 8B).

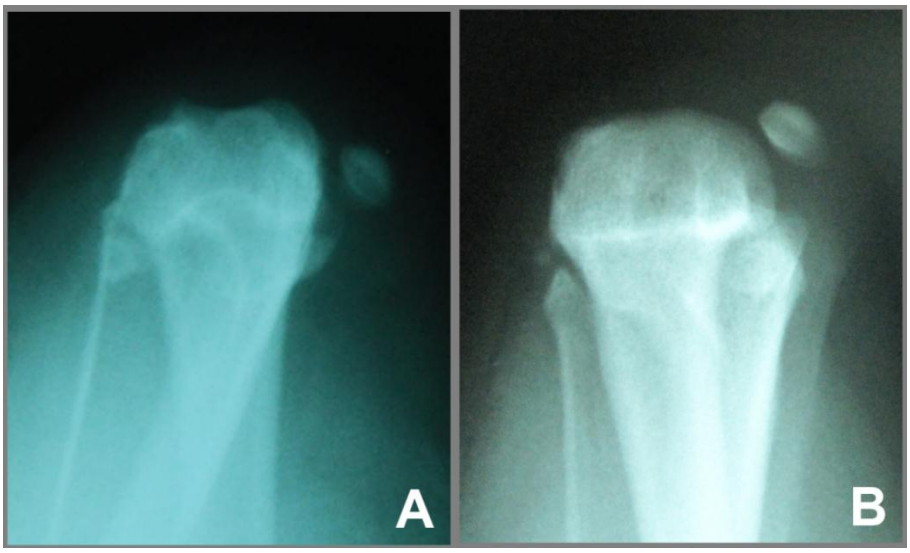


Figure 8 A shallow (A) or absent or even convex (B) trochlear groove, both with a grade 4 medial patellar luxation.

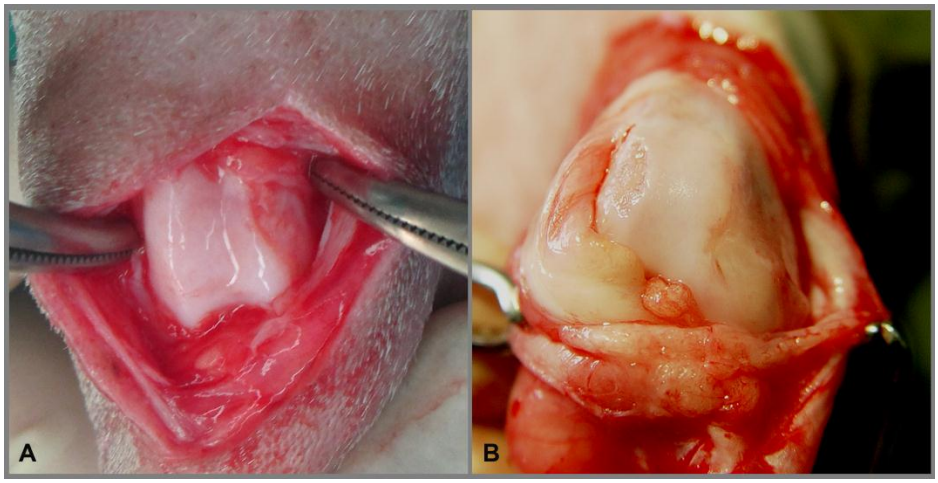


Figure 9 Erosion of the femoral trochlear ridge.
Erosion of the proximal area of the medial trochlear ridge in a Chihuahua with grade 2 medial patellar luxation (PL) **(A)**. Erosion of both medial and lateral trochlear ridges in a Pomeranian which had bidirectional PL at the early stage and the latter developed into grade 4 lateral PL **(B)**.

Clinical signs can vary from asymptomatic, mild or moderate weight-bearing with or without hindlimb abnormalities. When PL occurs in neonates or older puppies, they often show clinical signs of abnormal hindlimb carriage and function from the time they start walking with grade 3 and 4 PL. Young to mature dogs with grade 2 to 3 PL usually exhibit abnormal or intermittently abnormal gaits all their lives, but they are often presented when the symptoms worsen. In older dogs with grade 2 or

3 PL, they may exhibit sudden signs of lameness because of minor trauma or worsening of pain from degenerative joint disease (Piermattei et al., 2006).

Lameness may be intermittent or continuous. It varies from mild to moderate weight-bearing lameness with occasional carrying of the limb and some dogs may carry their leg most of the time. Dogs with LPL in general have more ambulation problems than those with MPL. Signs may worsen as the dog gains weight, articular cartilage erosion occurs, the luxation becomes permanent, or the cranial cruciate ligament ruptures (Piermattei et al., 2006).

Examination of the limb for PL screening may be performed in standing position and lateral recumbency (Hazewinkel et al., 2013).

1. Standing position

The examiner stands behind the animal and palpates both patellae simultaneously. The patella is located by following the patellar tendon proximally, starting at the tibial crest. Effusion of the stifle joint is mainly detectable medial and lateral to the patellar tendon. Buttress formation can be an indication of cruciate ligament rupture. The stability of the patella is checked by pushing the patella medially and pulling laterally with the thumb and forefinger, respectively, while the stifle is held extended. Finally each leg should be extended and flexed allowing the investigator to palpate whether or not the patella spontaneously luxates or repositions. In the normal dog it should not be possible to move the patella on or over the edge of the trochlea, or for spontaneous luxation of the patella to occur, and there should be no crepitation.

2. Lateral recumbency

This is the best position to evaluate PL. The animal is held in lateral recumbency, allowing the upper limb to be manipulated in full range of motion. The stifle should be extended and flexed and, in the context of screening for PL, note is taken of signs of crepitation and possible luxation or repositioning of the patella. Gentle palpation usually does not cause any pain. The examiner holds the metatarsus and uses it as a lever to rotate the tibia first internally, then externally, whilst flexing and extending the stifle and feels for the ability to luxate the patella with the other hand.

Observations should include the following (Piermattei et al., 2006).

1. Position of the patella
2. Instability in both medial and/or lateral direction
3. Presence of crepitus
4. Alignment of tibial tuberosity when the patella is in the trochlear groove and with the same alignment of foot
5. Limb torsion or angulation
6. Inability to reduce the patella, or place the patella on the trochlear ridge.
7. Location of the reduced patella within the trochlea. In straight-legged dogs such as the Akita or Shar-pei, the patella occasionally rides proximal in the trochlea "patella alta", whereas in chondrodystrophic dogs patella ride distal in the trochlea "patella baja".
8. Instability to extend the limb to a normal standing angle (in puppies with severe contracture with grade 4 PL).
9. Presence or absence of drawer movement.

2.4 Diagnostic imaging

Radiographic, computed tomographic (CT), and magnetic resonance imaging (MRI) studies are used to indicate pathophysiological changes of hip joint conformation, quadriceps alignment, patellar position, femoral and crural varus angle (FVA) (or valgus) and torsion both in normal dogs and dogs with PL (Kaiser et al., 2001a; Kaiser et al., 2001b; Johnson et al., 2002; Towle et al., 2005; Dudley et al., 2006; Johnson et al., 2006; Tomlinson et al., 2007; Mostafa et al., 2008; Swiderski et al., 2008; Mortari et al., 2009; Soparat et al., 2012). Most of the surgical failure or recurrent PL is focused on inadequate femoral and tibial alignment (Slocum and Slocum, 1998a). These radiographic studies may offer insight for a valuable improvement of the surgical treatment.

Abnormal hip joint conformation can coincide with MPL including abnormal angle of inclination, anteversion, decreased acetabular coverage and hip dysplasia (Fig. 6) (Hulse, 1981b; Hayes et al., 1994; Piermattei et al., 2006). However, the study on anteversion angle of the femoral neck using magnetic resonance image (MRI) did not reveal any correlation between PL and anteversion angle (Kaiser et al., 2001b). In addition, there are no significant differences in angle of inclination of the femoral neck between Pomeranians with normal stifle joint and Pomeranians with MPL using radiographic measurement (Soparat et al., 2012). Therefore there are no

indications for correcting MPL and hip problems in this situation. The studies of the inclination angle (IC), Norberg angle (NA), and anteversion angle (AA), between normal dogs and dogs with MPL are shown in Table 3 (Towle et al., 2005; Mortari et al., 2009). From this it is obvious that there are no any correlation among MPL grade 1, 2, 3 and 4 and IC and NA using radiographic measurement (Mortari et al., 2009) as well as there are no significant differences in IC and NA with the measurement methods between radiographic and CT.

The quadriceps alignment or Q-angle (QA) in dogs is defined as the angle between line from the origin of the rectus femoris muscle through the center of the trochlea and a line between the center of the trochlea and the tibial tuberosity. Deviation of the course of the patellar ligament in one direction generates a resultant force that pulls the patella in the same direction, resulting in PL (the greater the QA, the greater the risk of PL). The QA was measured by using images of radiography, CT, and MRI and shown in Table 3 (Kaiser et al., 2001a; Towle et al., 2005; Mortari et al., 2009). This indicates that there are significant differences in QA between dogs with MPL grade 1 and 3 and between dogs with MPL grade 2 and 3 on radiographic measurement (Mortari et al., 2009). There are significant differences in QA between normal dogs and dogs with MPL grade 2 and 3 as well as between dogs with MPL grade 1 and 3 on MRI measurement (Kaiser et al., 2001a).

Table 3 The studies of inclination angle (IC), Norberg angle (NA), anteversion angle (AA), and Q-angle (QA) (mean ± SD) in normal dogs and dogs with medial patellar luxation (MPL) in various breed sizes by using radiographic, computed tomographic (CT), and magnetic resonance imaging (MRI).

Reference	Breed	Method	Status	IC	NA	AA	QA
Tomlinson et al., 2007	Labrador Retrievers	radiographic	normal	134±5.3			
Kaiser et al., 2001a		MRI	normal				10.5±5.6 [a, c]
			grade 1				12.2±8.5 [c]
			grade 2				24.3±10.9 [b, c]
			grade 3				36.6±8.6 [b]
Kaiser et al., 2001b	Small-large	MRI	normal			7.6±5.5	
			grade 1			10.1±10.9	
			grade 2			8.6±12	
			grade 3			-0.4±11	
Towle et al., 2005	Small-medium	radiographic	≥ grade 2 MPL	128.5±5.3	93.0±11.3	35.1±9.8	16.4±4.9
		CT	≥ grade 2 MPL	130.7±7.6	90.6±9.0	20.9±3.8	11.0±6.0
Mortori et al., 2009	Small-medium	radiographic	grade 1 MPL	131.2±5.3	105.7±8.8		14.9±7 [a]
			grade 2 MPL	130.4±9.5	109.4±5.7		22.1±6.4 [a]
			grade 3 MPL	133.8±12	104.4±12.1		34.4±13.7 [b]
			grade 4 MPL	136.7±4.3	111.5±1.7		34.0±9.4
Soparat et al., 2012	Pomeranians	radiographic	normal	136.5±7.1			
			grades 1-2 MPL	136.8±6.0			
			grade 3 MPL	139.0±9.0			

Mean values in the same study and the same column that have different superscript lower case letter are significant different ($P < 0.05$)

Patella alta is defined as proximal displacement of the patella within the femoral trochlear groove. It has been association with recurrent patellar dislocation, subluxation, chondromalacia, and pain in the anterior aspect of the knee in humans (Insall et al., 1972; Kannus, 1992; Lancourt and Cristini, 1975; Walker et al., 1998). A radiographic study has been used to evaluate the patellar position in large-breed dogs (Kaiser et al., 2001a; Kaiser et al., 2001b; Johnson et al., 2002; Towle et al., 2005; Johnson et al., 2006; Mostafa et al., 2008). The mediolateral radiographs of stifle joints are used to measure the ratio of patellar ligament length and patellar bone length (PLL:PBL) (Fig. 10). This condition was studied first in large-breed dogs with a clinically normal stifle to define the vertical patellar position as the ratio of PLL:PBL, using the Insall-Salvati method (Fig. 10A) (Johnson et al., 2002). Then the ratio has been compared between large-breed dogs with clinically normal stifles and large-breed dogs with MPL (Johnson et al., 2006). The latter study showed that large-breed dogs with MPL had a significantly more proximal patellar position compared with that of large-breed dogs with a clinically normal stifle. In addition, it has been concluded that patella alta may play a role in MPL in large-breed dogs (Table 4).

Mostafa et al. (2008) evaluated the ratio of PLL:PBL using the modified PLL:PBL method (Fig. 10B). When the ratio is > 2.06, it is indicating the presence of patella alta in medium- to giant-breed dogs. The authors concluded that MPL is associated with a relatively long patellar ligament with patella alta; LPL however is associated with a relatively long proximal tibia and patella baja in medium- and giant-breed dogs. The ratio of PLL:PBL > 2.06 suggests that proximal displacement of the patella in dogs with patella alta may create a patella-femoral articulation that extends proximal to the femoral trochlear groove during extension of the stifle, resulting in a loss of the buttressing effects of the proximal trochlear ridges. This loss of patellar pressure at the femoral condyle facilitates MPL (Johnson et al., 2006; Mostafa et al., 2008). The evaluation of patella alta in large-breed dogs with MPL and concurrent proximal displacement of the patella within the femoral trochlear groove is advised to distally transpose the tibial tuberosity (Johnson et al., 2006). However, this is not a routine procedure for correcting MPL with patella alta in large-breed dogs. The results of the studies of PLL:PBL ratio are shown in Table 4.

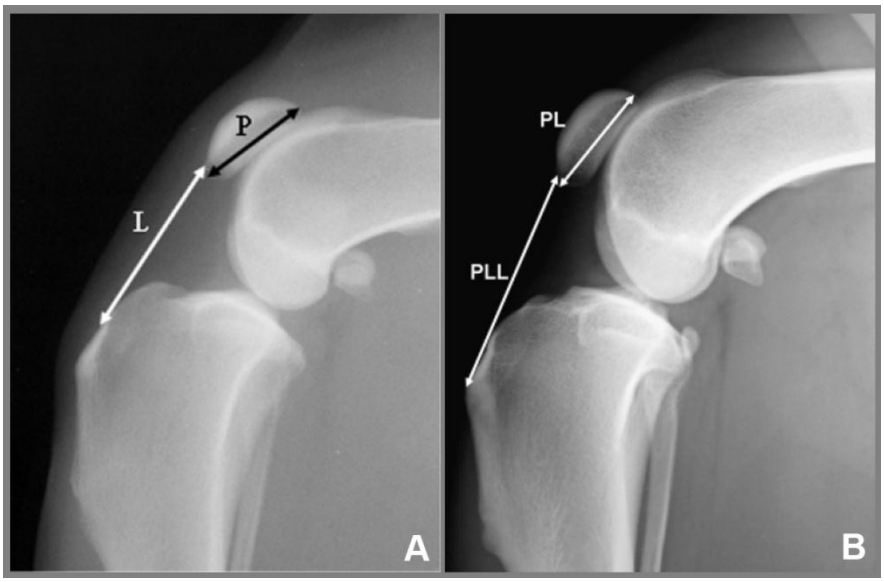


Figure 10 The landmark to measure the ratio of patellar ligament length and patella bone length (PLL:PBL) and modified PLL:PBL.

Both techniques use the same landmark for patellar bone length (longest dimension of the normal patella). Patellar ligament length (PLL) measured along the caudal aspect of the patellar ligament **(A)** (Johnson et al., 2006). Modified PLL measured the distance from point of origin of the ligament to its insertion at the proximal extent of the tibial creast **(B)** (Mostafa et al., 2008).

Table 4 The studies of the ratio of patellar ligament length and patellar bone length (PLL:PBL) in normal dogs and dogs with medial patellar luxation (MPL) in various breed sizes by using radiographic imaging.

Reference	Breed	Measurement method	Status	PLL:PBL
Johnson et al., 2002	Large breed	Insall-Salvati	normal	1.68±0.18
Towle et al., 2005	Small-medium	Insall-Salvati	MPL ≥ grade 2	1.8±0.3
Johnson et al., 2006	Large breed	Insall-Salvati	normal	1.71±0.020 [a]
			MPL	1.87±0.025 [b]
Mostafa et al., 2008	Medium-giant	modified PLL:PBL	normal	2.02±0.2 [a]
			MPL	2.23±0.23 [b]
			LPL	1.9±0.14
Mortori et al., 2009	Small-medium	modified PLL:PBL	MPL grade 1	2±0.2
			MPL grade 2	1.8±0.1
			MPL grade 3	1.9±0.2
			MPL grade 4	1.8±0.3

Mean values in the same study and the same column that have different superscript lower case letter are significant different ($P < 0.05$)

In a normal femur, femoral varus angle (FVA) or valgus is determined by measuring the anatomic lateral distal femoral angle (aLDFA) at the intersection of the distal femoral anatomic axis (dFAA) and transcondylar axis (TCA), using the radiographic method of Tomlinson et al. (2007) or CT method of Dudley et al. (2006) or three dimensional reconstruction (Kowaleski, 2011). Distal femoral limb deformity is a skeletal abnormality found in dogs with MPL (Fig. 11A, B) which is determined at the center of rotation of angulation (CORA) located at the intersection of the proximal and distal anatomical axes of the femur (Kowaleski, 2011). This may be the cause of failure of surgical treatment and recurrent PL in severe cases (Willauer and Vasseur, 1987; Remedios et al., 1992; Arthurs and Langley-Hobbs, 2006; Gibbons et al., 2006; Linney et al., 2011). Excessive distal femoral varus moves the alignment of quadriceps muscle to the medial side of the trochlea (Hulse, 1981b; Slocum and Slocum, 1998a; Slocum and Slocum, 2000; Dudley et al., 2006). The measurement of FVA in both normal dogs as reference values, and in dogs with MPL was performed in recent studies by using anatomic preparation, radiographic images, and CT and the average of FVA measurements as shown in Table 5 (Dudley et al., 2006; Tomlinson et al., 2007; Swiderski et al., 2008; Mortari et al., 2009; Soparat et al., 2012). FVA in Pomeranians with grade 3 MPL was greater than FVA in Pomeranians with grade 1-2 MPL and in normal Pomeranians (Soparat et al., 2012). However, in the study of Mortari, FVA was not significantly different among small-medium breed dogs with grade 1, 2, 3 or 4 MPL (Mortari et al., 2009) (Table 5).

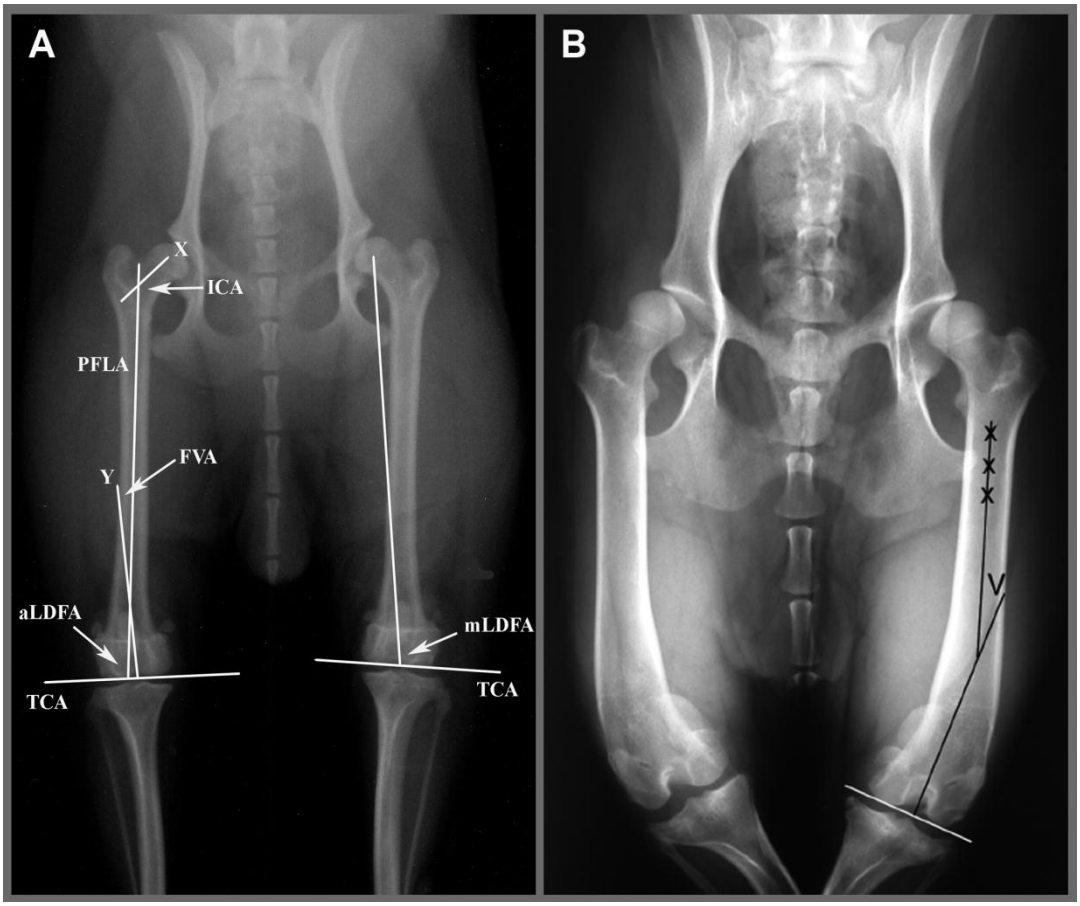


Figure 11 Craniocaudal radiographs of the hindlimbs.
Measurement of the inclination angle (ICA), femoral varus angle (FVA), anatomical lateral distal femoral angle (aLDFA), and mechanical lateral distal femoral angle (mLDFA); PFLA = the proximal femoral long axis; TCA = the transcondylar axis; X = the line connecting between the center of the femoral head and the bisection points of the femoral neck; Y = the distal femoral anatomic axis (dFAA) in Pomeranians **(A)** (drawing from Soparat, 2012). Measurement of FVA (V) in grade 4 MPL; xxx = middle of the proximal femoral axis **(B)** (Piras, 2011).

Table 5 The studies of femoral varus angle (FVA) in normal dogs and dogs with medial patellar luxation (MPL) in various breed sizes by using anatomic preparation, radiographic imaging and computed tomographic (CT).

Reference and Breed category	Method	Status	FVA
Dudley et al., 2006			
Medium-large	craniocaudal radiograph	normal	9.4±2.3
	horizontal beam		9.2±3.3
	CT		8.8±3.3
	anatomic preparation		7.4±3.9
Tomlinson et al, 2007			
Labrador Retriever	craniocaudal radiograph	normal	7±3.2
Golden Retriever			7±2.8
German Shepherd			4±3.3
Rottweiler			8±3.5
Swiderski et al., 2008			
Walker hound	craniocaudal radiograph		5.8±1.0
	anatomic preparation		5.2±2.1
Mortari et al., 2009			
Small-medium	craniocaudal radiograph	MPL grade 1	13.0±7.8
		MPL grade 2	10.3±5.2
		MPL grade 3	17.8±6.9
		MPL grade 4	18.2±2.8
Meggiolaro, 2009			
Medium-large	CT		6.81
Soparat et al., 2010			
Pomeranians	craniocaudal radiograph	normal	5.85±3.18 [a]
		MPL grade 1-2	9.38±3.73 [a]
		MPL grade 3	13.15±5.50 [b]

Mean values in the same study and the same column that have different superscript lower case letter are significant different ($P < 0.05$)

Treatment of patellar luxation

Luxation of the patella may be treated conservatively or surgically. Deciding which method is applicable for a patient depends on clinical history, physical findings, and age. Conservative treatment is recommended in asymptomatic PL or in some old dogs. Nevertheless these dogs still response well to late surgical repair (Piermattei et al., 2006). In young puppies, it is advisable to consider early repair (3 - 4 months) before irreversible quadriceps contracture occurs. In medium to large breeds, surgery is recommended before erosion and deformity to the trochlea occurs (Piermattei et al., 2006).

MPL is associated with secondary osteoarthritis (OA) both in case of surgical or non-surgical treatment (Roy et al., 1992) characterized by a progressive loss of articular cartilage and by reactive changes at the margins of the joints and bones (Edge-Hughes and Nicholson, 2007). Pain and loss of end-range extension is a frequent finding in early OA of the joints (Olmstead, 1995). Physiotherapy, aimed at building thigh muscle mass supporting the joint and to increase stability, is useful for animals with PL both in conservative and after surgical treatment. Regular exercise (walks or trotting) on a softer surface (grassy areas) should be encouraged. The use of an underwater treadmill or swim therapy can be very beneficial and utilized as a cross-training tool (Hamilton, 2002; Millis, 2004; Huang et al., 2005). Walking on hilly terrain could also alter joint loading and build different muscle groups (Edge-Hughes, 2002). Weight control should be considered in dogs with PL because excessive body weight can impact the stresses on articular cartilage (Edge-Hughes and Nicholson, 2007).

Surgery is indicated in any patient exhibiting lameness and is strongly advised in one with active growth plates, because skeletal deformity may worsen rapidly (Hulse, 1995). The objective of surgical treatment of PL is realignment of the extensor mechanism of the stifle joint and stabilization of the patella in the trochlear sulcus in order to reestablish normal anatomy and limb function. Surgical correction significantly improves lameness and limb use (DeAngelis and Hohn, 1970; Roy et al., 1992; Wangdee et al., 2008; Wangdee and Torwattanachai, 2010). Numerous surgical techniques have been proposed for the correction of this abnormality and are categorized as either soft tissue or bone reconstructions (Piermattei et al., 2006).

I) Soft tissue reconstruction

The aims are to relieve tension on the extensor mechanism and/or contracted fibrous joint capsule of the medial side and to tighten the extended tissue of the lateral side in case of MPL. There are many techniques including medial desmotomy, the overlapping and imbrication of the retinaculum and/or joint capsule, modified fascia transposition technique, proximal tube realignment, patellar and tibial antirotational suture ligaments, lateral reinforcement, tibial derotation and rectus femoris transposition.

II) Bone reconstruction

There are three principles of bone reconstruction including trochlear deepening, tibial tuberosity transposition and corrective osteotomies of the femur.

III) Miscellaneous

The decision as to which techniques to use is a judgment based primarily on the severity of the luxation. Generally, a combination of techniques is required, based on the depth of trochlear sulcus and alignment of the patella, patella ligament, tibial tuberosity and foot (Hulse, 1993). The surgical procedures that are recommended to correct MPL in dogs are a combination of soft tissue reconstruction, femoral trochlear deepening, and lateral transposition of the tibial tuberosity (Singleton, 1969; Slocum and Devine, 1985; Willauer and Vasseur, 1987; Hayes et al., 1994; Gibbons et al., 2006; Alam et al., 2007). Corrective osteotomy of limb deformity is also advocated in severe cases (Roush, 1993; Slocum and Slocum, 1998a; Slocum and Slocum, 2000; Vasseur, 2003; Piermattei et al., 2006; Hayashi et al., 2010).

Surgical procedures

I. Soft tissue reconstruction

In case of chronic luxation, the retinaculum and joint capsule of the opposite site becomes stretched. Soft tissue reconstruction is aimed to normalize the length of the distance between fabella and patella, thus shortening the stretched retinaculum. At the same time the distance between patella and fabella at the luxation site is shortened, not allowing the patella to place in the trochlea, especially in case of PL grade 3 and 4. A variety of techniques have been described to normalize this situation:

1. Medial desmotomy is performed by incising the medial retinaculum 3 to 5 mm from and parallel to the patella and undermining to relieve tension on the

medial site (Fig. 12A). Release of the medial joint capsule together with the quadriceps may be necessary in grade 3 or grade 4 MPL to allow for reduction of the patella (Slocum and Devine, 1985; Piermattei et al., 2006)

2. Overlapping and imbrication of the retinaculum and/or joint capsule can be used on either the lateral side for a MPL or on the medial side for a LPL. Overlapping technique is performed by incising of the retinacular fascia and joint capsule 3 to 5 mm lateral and parallel to the patellar ligament and patella. The incision extends from the proximal tibial to a point 1 to 2 cm proximal of the patella. The cut edge of the fascia attached to the patella is sutured beneath the more lateral fascia with several mattress sutures, placed through the fornix of the capsule with absorbable suture material. The superficial layers of fascia and capsule are next sutured to the fascia extending beyond the cranial midline of the joint sutured to the fascia on the opposite side of the patella.

Imbrication of the lateral retinaculum is performed by suturing the lateral retinaculum with absorbable suture and overlapping pattern to further tighten the lateral retinaculum (Fig. 12B) (Slocum and Slocum, 1998b).

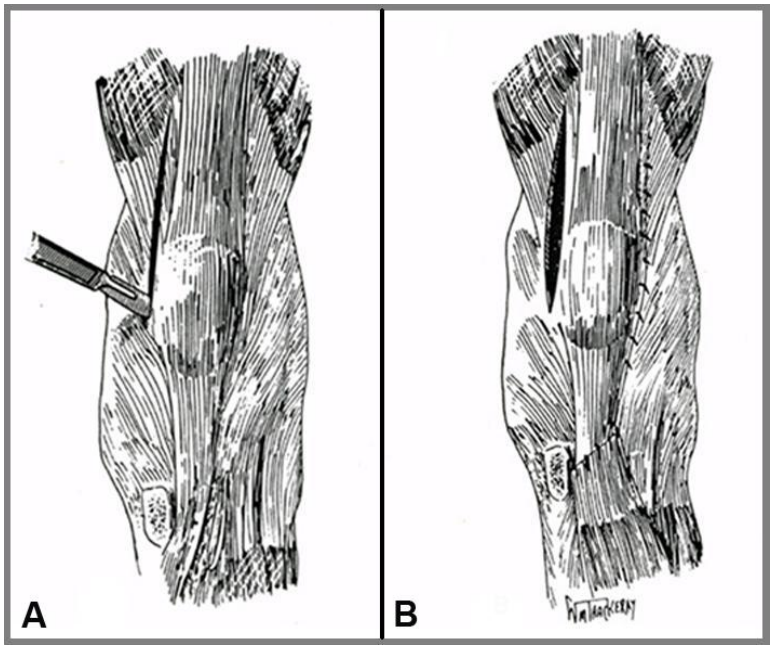


Figure 12 Medial desmotomy (A) and lateral imbrication (B) (drawing from Arnoczky and Tarvin in Slocum and Slocum, 1998b).

3. Modified fascia transplant technique is performed by a parapatellar incision in the fascia lata to form a strip of fascia, still attached both proximally and distally. The width of the strip should approximate that of the defect in the medial aspect of the joint capsule after desmotomy to correct a MPL (Fig. 13A). The isolated strip of fascia is lifted from the underlying redundant lateral joint capsule, pulled over the new properly aligned quadriceps group, and placed in the medial defect of the inadequate medial joint capsule (Fig. 13B). This method increases the width of the tight medial joint capsule and decreases the width of the loose lateral femoropatellar fascia (Trotter, 1980).

4. Proximal tube realignment is performed by incisions on the medial and lateral retinacula 3 to 5 mm from and parallel to the patella (Fig. 14A). Then, the caudal flap of the medial retinaculum is undermined to relieve tension on the medial side. The caudal flap of the lateral retinaculum is sutured, with absorbable suture and simple interrupted pattern, to the cranial flap of the medial retinaculum adjacent to the patella (Fig. 14B) (Wangdee and Kalpravidh, 2008).

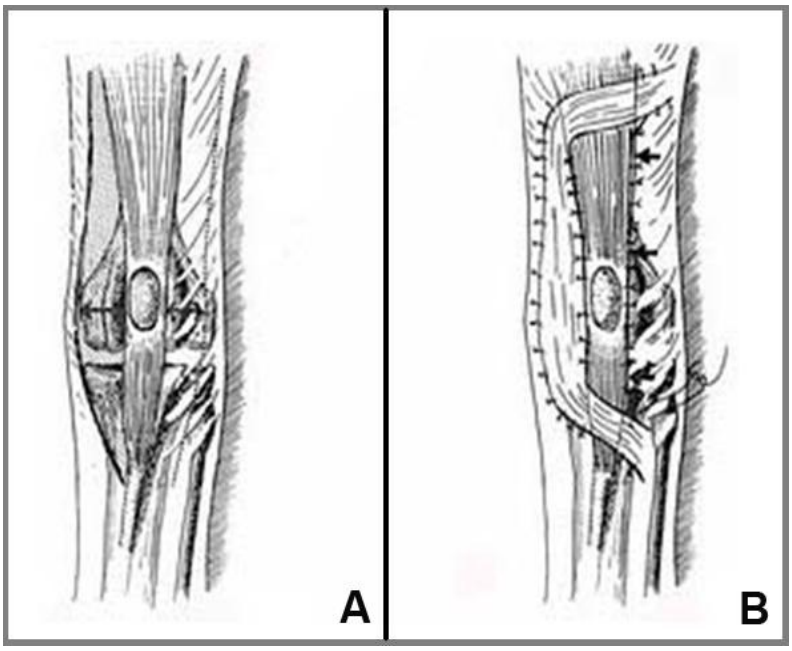


Figure 13 Modified fascia transplant technique (drawing from Palumbo in Trotter, 1980). **(A)** Defect of the medial joint capsule after desmotomy. **(B)** Fascia lata strip is placed and sutured in the medial defect.

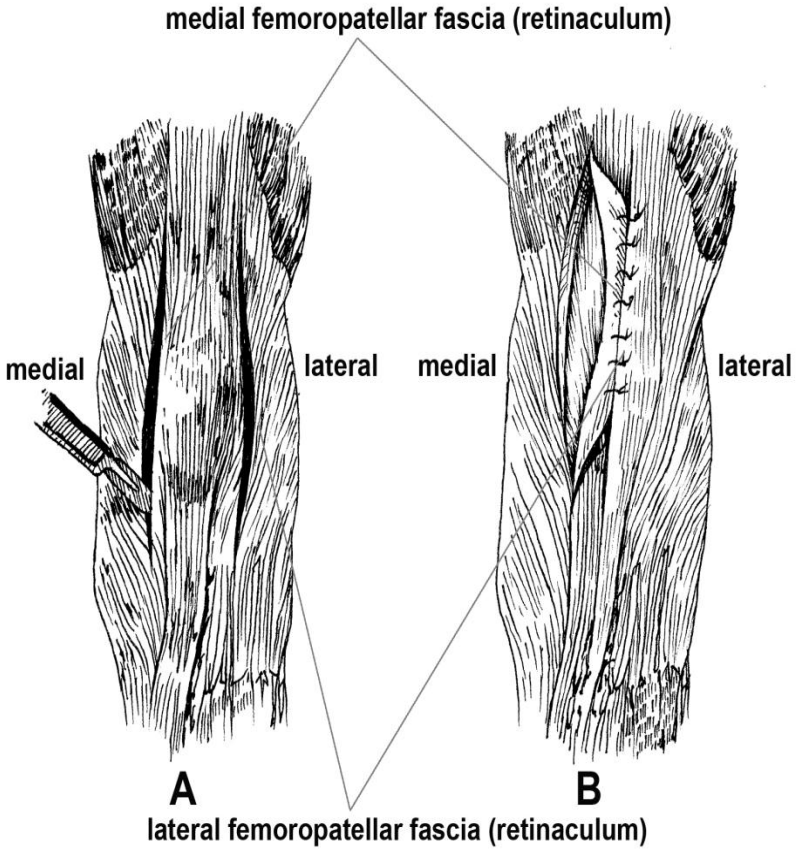


Figure 14 Tube realignment procedure for correction of MPL (drawing from Wangdee and Kalpravidh, 2008 by S.Pongsri).
(A) Incisions on the medial and lateral retinacula 3 to 5 mm from and parallel to the patella. **(B)** The caudal flap of the lateral retinaculum is sutured to the cranial flap of the medial retinaculum adjacent to the patella.

5. Patellar and tibial antirotational suture ligaments are performed by using non-absorbable suture placed between patella, patellar ligament or tibial tuberosity and fabella opposite to luxation side (Fig. 15). This technique can be used in case of PL grade 3 and 4 in very young puppies to pull the insertion of the patellar ligament back to the normal position and to prevent muscle contracture and fibrosis as temporary technique (Piermattei et al., 2006).

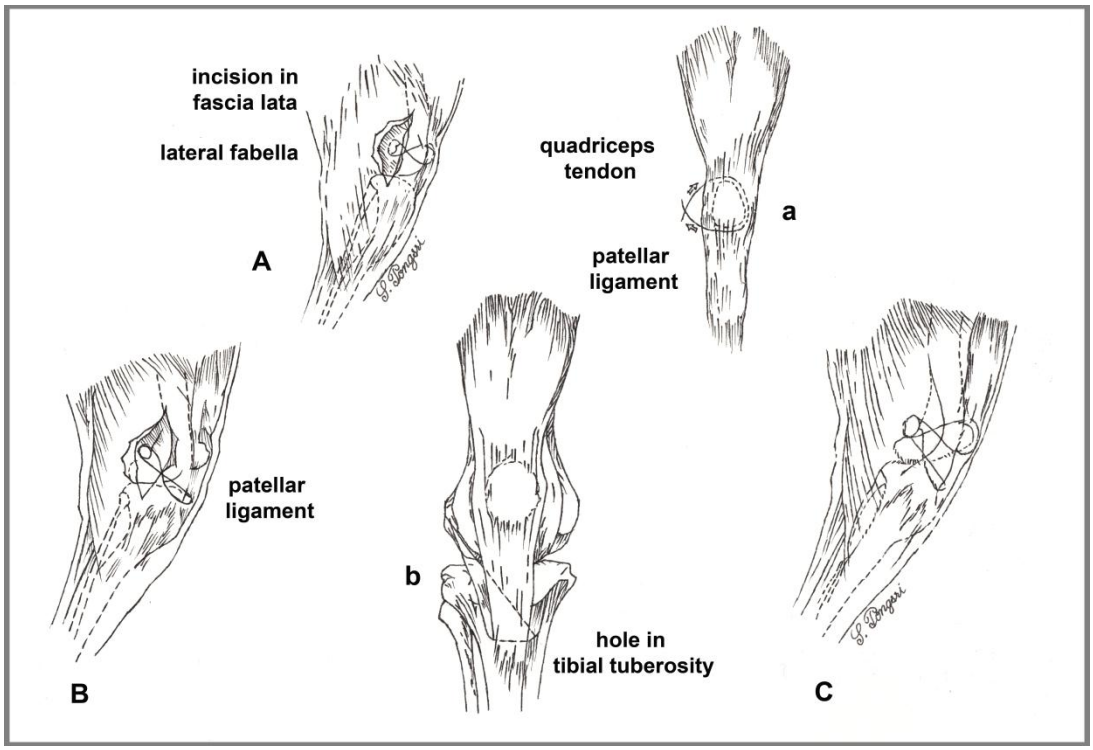


Figure 15 Patellar and tibial antirotational suture ligaments (drawing modified from Piermattei et al., 2006 by S.Pongsri).

The fascia lata is opened to expose the lateral fabella and braided polyester suture material is passed behind the lateral fabella and around the patella as shown in 'a'. The suture is tied to stabilise the patella **(A)**. A suture can be passed around the fabella, then placed either in the distal patellar ligament or in the tibial tuberosity 'b' to prevent medial tibial rotation. The suture is tied tightly enough to prevent rotation **(B)**. The two sutures can be combined. The caudal fascia lata has been overlapping in closing **(C)**.

6. Lateral reinforcement is performed by placement of non-absorbable suture from the lateral femoral-fabellar ligament to the lateral parapatellar fibrocartilage (Fig. 16A) or using a fascia lata graft passed beneath the femoral-fabellar ligament and sutured to the parapatella fibrocartilage (Fig. 16B). These techniques are used to reinforce the lateral retinaculum in order to help restrain the patella within the trochlear groove (Hulse, 1995).

7. Tibial derotation can limit internal rotational instability of the tibial tuberosity by using a lateral suture of heavy nonabsorbable material placed around the lateral fabella (Fig. 17A) or lateral collateral ligament (Fig. 17B) and tibial tuberosity (Trotter, 1980).

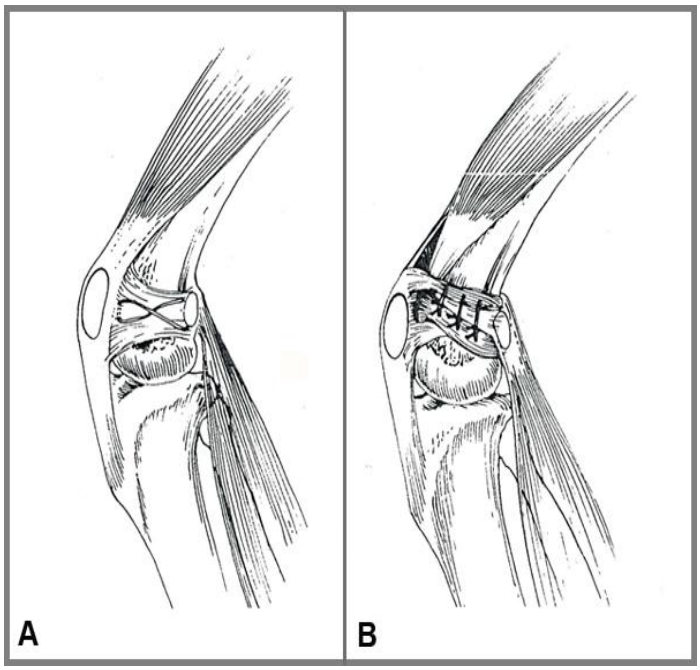


Figure 16 Lateral reinforcement (drawing from Hulse, 1995).
(A) Non-absorbable suture is placed from the lateral femoral-fabellar ligament to the lateral parapatellar fibrocartilage.
(B) A fascia lata graft passed beneath the femoral-fabellar ligament and sutured to the parapatella fibrocartilage.

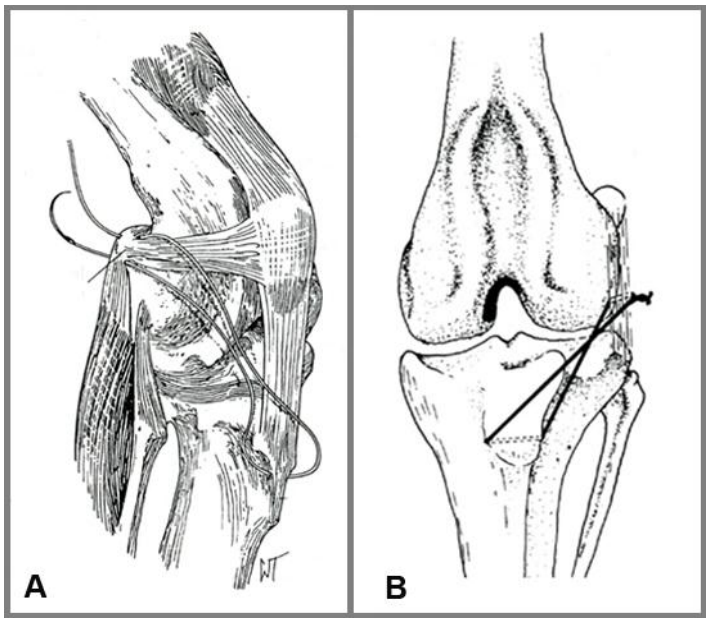


Figure 17 Tibial derotation suture.
(A) Suturing lateral fabella and tibial tuberosity (drawing from from Arnoczky and Tarvin, 1980 in Slocum and Slocum, 1998b).
(B) Suturing lateral collateral ligament and tibial tuberosity (drawing from Robins, 1990).

8. Rectus femoris transposition

The rectus femoris is a primary alignment muscle of the quadriceps muscle with its origin on the pelvis and the attachment on the tibial tuberosity which causes it to span two joints, the hip and the stifle (Fig. 3A, B). If the patient has external rotation at the hip, the line of force of the rectus femoris is between the pelvis, cranial to the acetabulum and the tibial tuberosity. This creates a medially directed pull on the patella and is one cause for MPL. The transfer of the rectus femoris origin to the third trochanter eliminates the effects of the hip rotation on the MPL, which reduces the luxation problem from a two joints to a one joint problem (Slocum and Slocum, 1998a).

In summary, the standard techniques of soft tissue reconstruction are medial desmotomy, the overlapping, imbrication of the retinaculum and/or joint capsule, patellar and tibial antirotational suture ligaments and lateral reinforcement (Alam et al., 2007; DeAngelis and Hohn, 1970; Gibbons et al., 2006; Hayes et al., 1994; Linney et al., 2011; Roy et al., 1992; Singleton, 1969; Slocum and Devine, 1985), modified fascia transplant technique, proximal tube realignment, tibial derotation, rectus femoris transposition do not routinely use in the surgical practice.

II. Bone reconstruction

Trochlear deepening

The techniques include trochlear sulcoplasty, trochlear chondroplasty, trochlear wedge recession, trochlear block recession and rotation of the femoral trochlear.

1. Trochlear sulcoplasty is performed by removing articular cartilage to the level of subchondral bone to create a sulcus deep enough to prevent patellar luxation (Fig. 18). This technique is used in case of severe cartilage erosion. Disadvantage of this technique is the lack of smooth articulation of the femoropatellar joint and induction of osteoarthritis and synovial inflammation (Moore et al., 1989; Roy et al., 1992). The trochlear sulcus may form granulation tissue after operation which will be replaced by fibrocartilage at the 4th week after surgery (Piermattei et al., 2006).

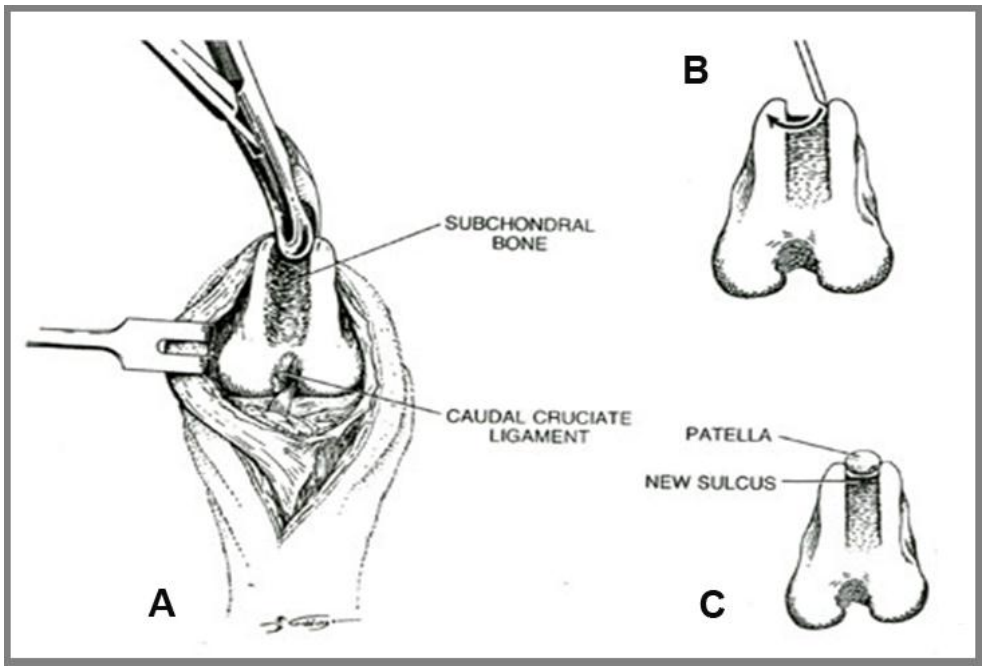


Figure 18 Trochlear sulcoplasty (drawing from Piermattei et al., 2006).
An outline of the proposed sulcus is made in the cartilage with a scalpel along the condyle ridges, and then articular cartilage and bone are removed within the outlined area to create a straight-sided, curved-bottomed trough, as shown in **"B" (A)**. The trough should be deep enough so that the patella does not touch bone in the bottom of the tough and wide enough so that the patella rides deeply in the new sulcus **(C)**.

2. Trochlear chondroplasty ("cartilage flap" technique)

A cartilage flap is created and elevated from the sulcus by using a periosteal elevator. Subchondral cancellous bone is removed from the groove and the flap is pressed back into the deepened sulcus (Fig. 19). This results in a deepened trochlea with preservation of articular cartilage in the sulcus and fibrocartilage or fibrous tissue at the incisional gaps. Hyaline cartilage of the trochlear sulcus survives under pressure of the patella. This technique is useful only in immature dogs up to 10 months of age. In mature dogs, the articular cartilage becomes thinner and more adherent to the subchondral bone, making flap dissection impossible.

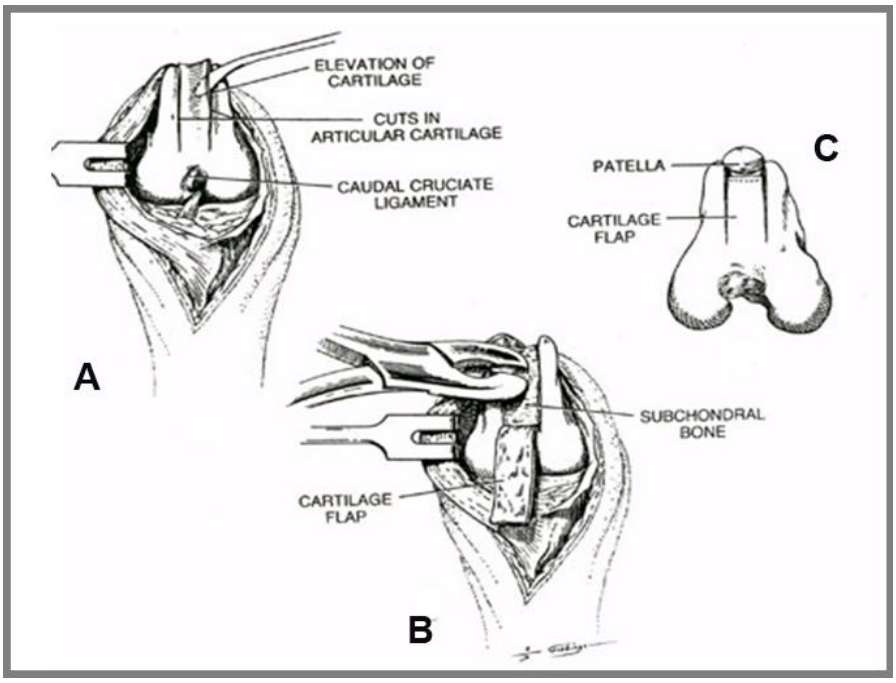


Figure 19 Trochlear chondroplasty (drawing from Piermattei et al., 2006).
The new sulcus is outlined by cuts through the thick adolescent cartilage. The proximal transverse cut is at the level of the proximal trochlear ridge, and then a sharp periosteal elevator is used to raise cartilage from subchondral bone with rongeurs **(A)**. The cartilage flap is hinged distally to allow removal of subchondral bone with rongeurs **(B)**. The sulcus is deep enough to retain the patella when the cartilage flap is replaced **(C)**.

3. Trochlear wedge recession is aimed at deepening the trochlear sulcus and to maintain both viable articular cartilage of the sulcus and the integrity of the trochlear ridges (Boone and Hohn, 1983). A wedge of the trochlear sulcus is performed by two intersecting saw or osteotome cuts that create an apex angle of 30 to 40°at the inner edges of medial and lateral trochlear ridge. Bone from the lateral wall of the wedge is removed to deepen the trochlea (Fig. 20A). More depth of the new trochlea can be created by cutting off a slightly amount of the wedge apex (Slocum and Slocum, 1993). The recession should allow the patella to be positioned 50% of its height into the depth of the new trochlea. The sufficient width of the groove should prevent the patella from riding on the trochlear ridge (Fig. 20B) (Slocum and Slocum, 1998b). The proximal trochlear sulcus should have enough width to prevent luxation during extension of stifle joint. The edges of the recessed throchlear segment will be replaced by fibrous to fibrocartilaginous tissue at the 12th week after surgery (Boone and Hohn, 1983).

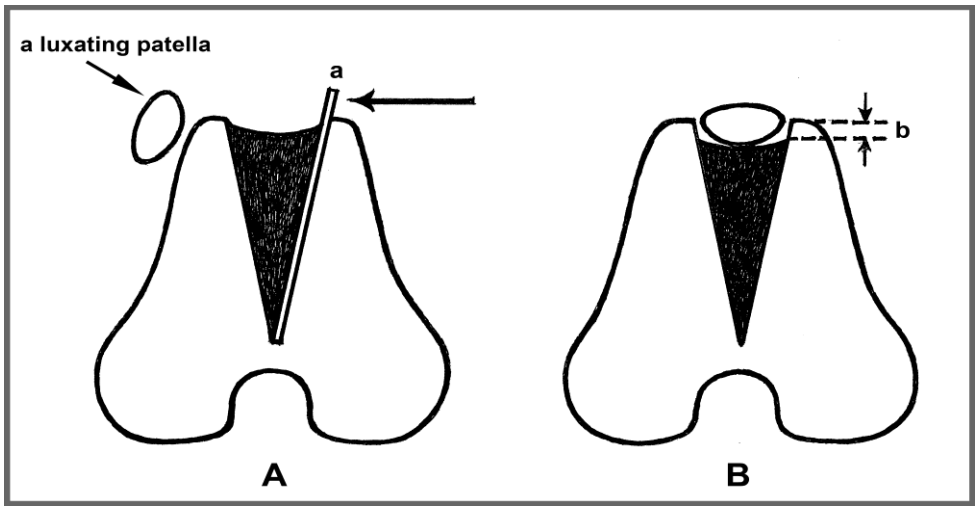


Figure 20 Trochlear wedge recession technique.
A V-shaped wedge is cut from the sulcus. The two cuts are slightly wider apart than the width of the patella. Then, a piece of bone **(a)** is cut and discarded from the trochlear ridge **(A)** to allow the trochlear sulcus to be recessed by half the thickness of the patella **(b)**. The original wedge is taken back to its place to form a new sulcus in which the patella is stabilised **(B)** (drawing modified from Slocum and Slocum, 1993 by S.Pongsri).

If the recession of trochlear sulcus is too narrow (Fig. 21A), increasing of the trochlear sulcus can be performed by removal of an additional piece of bone from the lateral trochlear ridge (Fig. 21B) (Slocum and Slocum, 1998b).

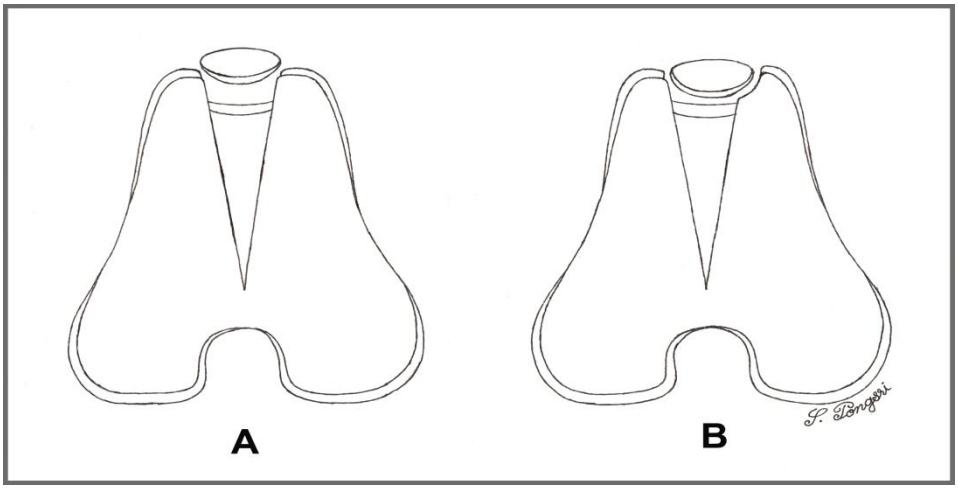


Figure 21 Modified trochlear wedge recession of narrowing trochlear sulcus.
The patella is on top of the medial and lateral trochlear ridges **(A)**. Removal of bone of the lateral trochlear ridge to reestablish an effective trochlear recession **(B)** (drawing modified from Slocum and Slocum, 1998 by S.Pongsri).

Patelloplasty is used to adjust the patellar shape to the trochlear groove to improve the femoro-patellar articulation within the new deepened trochlear groove following trochleoplasty technique. An abnormal patellar contour may occur in dogs because of abnormal wear against the condylar side or osteophyte formation along the borders of the patella and thickening of the joint capsule (Fig. 22A) which causes the patellar surface to become wider than the trochlear groove and consequently may lead to patellar reluxation. Patelloplasty is performed to adapt the patella to the newly formed trochlear groove (Fig. 22B, C) (Vezzoni, 2011b).

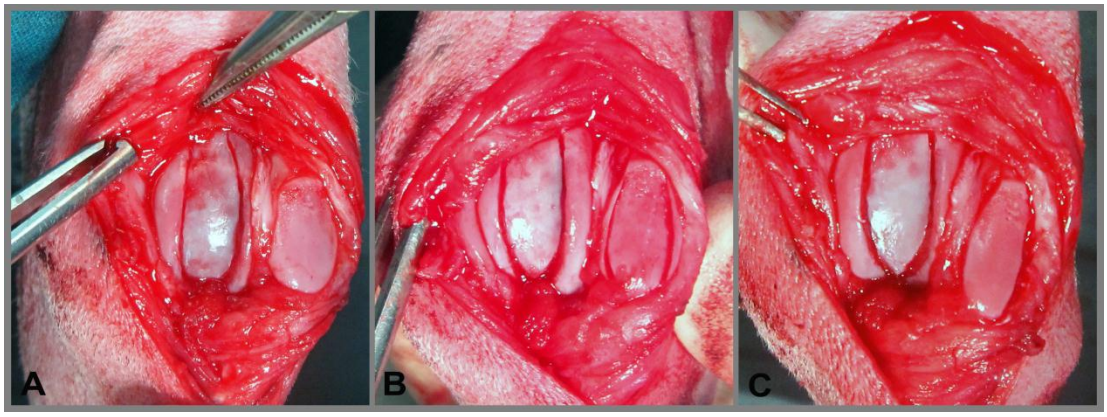


Figure 22 Combination of trochlear wedge recession and patelloplasty for correction of a LPL grade 3 in a 6-month old female poodle.
Erosion of articular cartilage presented on proximal trochlear ridge and proximal pole of patella **(A)**. The outline of patelloplasty was made by cuts through the cartilage of medial and lateral edges of the patella **(B)**. The patellar width fits to the trochlear sulcus **(C)** (Photo by Wangdee).

4. Trochlear block recession deepens the trochlear groove to restrain the patella and maintain the integrity of the patellofemoral articulation. The width of the cut is sufficient to accommodate the width of the patella, however the trochlear ridges should be preserved. A trochlear block in the femoral trochlea is performed by using the cutting edge of a No. 11 or 14 scalpel blade and osteotome to deepen the proximodistal cut 3 to 6 mm into the bone. An osteotome is inserted from both the proximal and distal extents of the osteotomy until the osteotomy meets in the middle. Then an appropriate thickness of bone is removed in order to avoid cracking or splitting the osteochondral segment. Additional subchondral bone is removed from the base of the groove by using a rongeur. Then the articular block is replaced into the defect in a recessed position when the depth is sufficient to accommodate 50% of the height of the patella (Fig. 23) (Talcott et al., 2000).

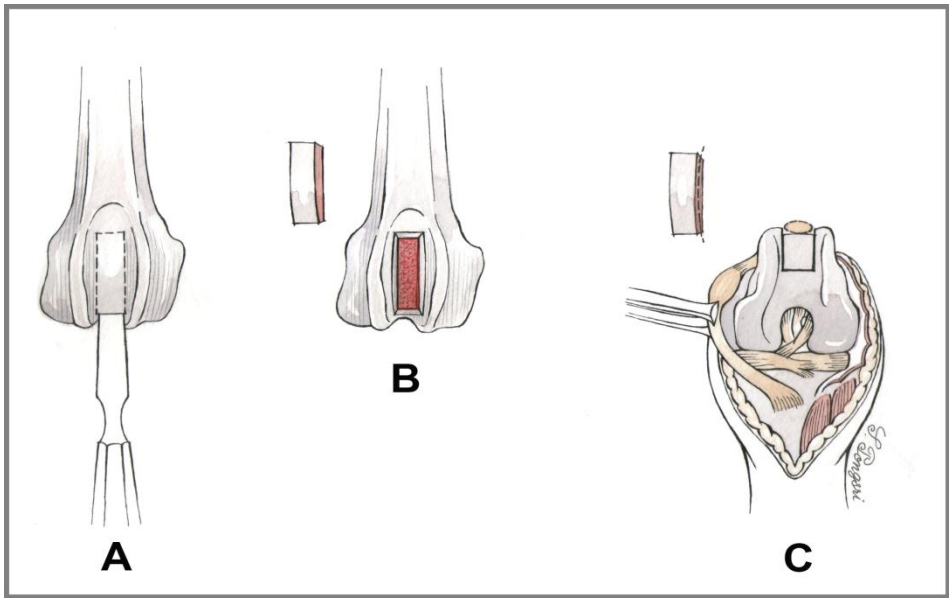


Figure 23 Trochlear block recession.
An osteotome is inserted from both proximal and distal extents of the osteotomy **(A)** until the osteotomy meets in the middle **(B)**. Additional subchondral bone is removed from the base of the groove after which the articular block is replaced into the defect in a recessed position **(C)** (drawing from S.Pongsri).

Transposition of the tibial tuberosity

1. Transposition of the tibial tuberosity (TTT) is performed with the aid of an osteotome or oscillating saw to remove the insertion site of the patellar tendon and its fixation after relocation to facilitate stable seating of the tuberosity in a lateral position. The cranialis tibialis muscle is elevated from the lateral surface of the proximal tibia (Fig. 24A) and the tibial tuberosity is cut with an osteotome beneath the patellar tendon 3 to 5 mm caudal to the cranial point of the tibial tuberosity, taking care to leave the distal periosteal attachment intact (Fig. 24B). The lateral edge of the cut surface is removed with a rongeur to facilitate stable seating of the tibial tuberosity in a lateral position and stabilise it with one to two small Kirschner wires (Fig. 24C) (Piermattei et al., 2006).

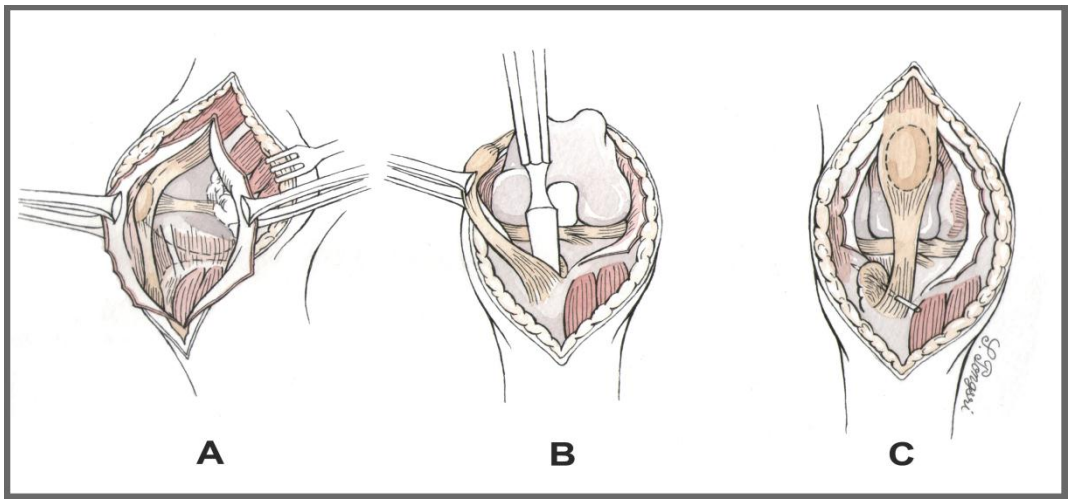


Figure 24 Transposition of the tibial tuberosity.
Parapatellar incision through the fascia and retinaculum craniolateral of the stifle joint and the cranialis tibialis muscle elevated from the lateral surface of the proximal tibia **(A)**. An osteotome is used to cut the tibial tuberosity beneath the patellar tendon 3 to 5 mm caudal to cranial point of the tibial tuberosity **(B)**. The tibial tuberosity is stablized at the lateral position with one to two small K-wires **(C)** (drawing from S.Pongsri).

2. A modified transposition of the tibial tuberosity was recently described. The standard TTT removes only a small portion of tibial tuberosity with patellar ligament and the fragment will be fixed at a caudal position compared with its original position (Fig. 25A). This causes an increase in retropatellar pressure. This could cause a detrimental change in the biomechanics of the patella and may result in a partial contact of the patella with the sulcus. This modified TTT was developed to transpose the tibial tuberosity to a more cranial position (Fig. 25B) which aims at decreasing the pressure of the patella on the femoral trochlea. The technique involves a more caudal osteotomy of the tibial tuberosity along an oblique plan, avoiding damage to the medial meniscus and to the tendon of the long digital extensor muscle (Fig. 25C, D). This caudal osteotomy results in a large surface of bone, allowing far transposition of the fragment without twisting the ligament or increasing retropatellar pressure (L'Eplattenier and Montavon, 2002).

3. Latero-distal transposition of the tibial tuberosity in case of MPL with patella alta was reported in 17 stifles (Segal et al., 2012). The outcome was good without recurrent PL. However latero-distal transposition of tibial tuberosity also increases the retropatellar pressure and changes in the biomechanics of the patella. Long-term outcomes have not been reported.

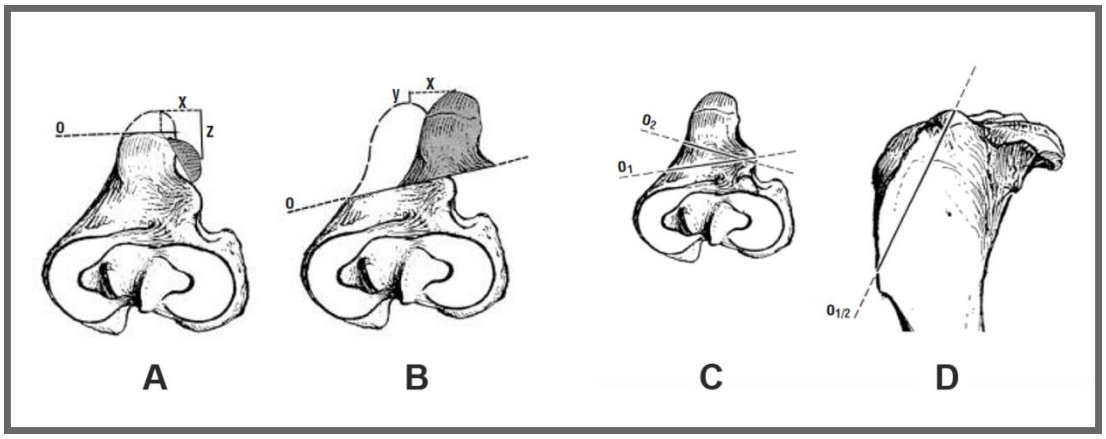


Figure 25 Transposition of tibial tuberosity.
Tibial tuberosity is fixed at the caudal position compared with its original position in standard technique **(A)**. The modified TTT was developed to transpose the tibial tuberosity to a more cranial position **(B)**. Proximodistal **(C)** and mediolateral **(D)** views of the proximal portion of the tibia indicate the position of the osteotomies for cranialization of the tibial tuberosity. (O_1 = plane of osteotomy for medial patellar luxation and O_2 = plane of osteotomy for lateral patellar luxation) (L'Eplattenier and Montavon, 2002).

4. Implantation of artificial ceramic graft to the medial side of the tibial tuberosity has been used to induce lateral transposition of tibial tuberosity (Fig. 26). The technique has been evaluated in grade 3 and 4 MPL dogs at 1 to 3½ months of age. This allows for normal alignment of the quadriceps muscle during growth of the dogs. The result was good and all 13 dogs had normal weight bearing at 1-3 weeks after surgery (Nagaoka et al., 1995). However, long term follow-up is lacking.

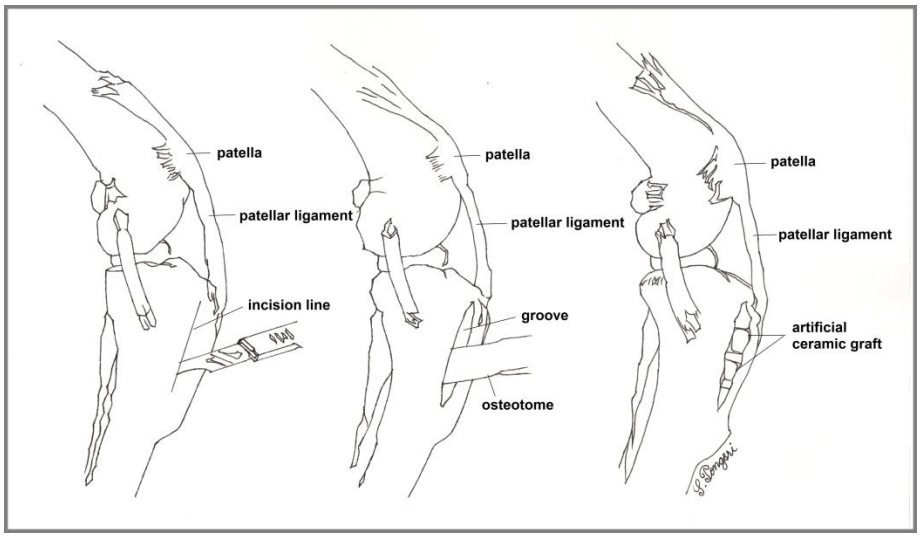


Figure 26 Implantation of artificial ceramic graft to the medial side of the tibial tuberosity (drawing modified from Nagaoka et al., 1995 by S.Pongsri).

Corrective osteotomies of the femur

Distal femoral ostectomy (DFO) is an effective treatment for MPL association with FVA, recommended when FVA ≥ 12 ° in large-breed dogs with ≥ grade 2 MPL (Swiderski and Palmer, 2007).

This technique has to assess the femoral varus deformity employing a well-positioned cranio-caudal radiographic view of the pelvic limb including both femora and proximal tibiae or CT method (Kowaleski, 2011). It is important for the determination of the FVA and for assessment of femoral angulation before distal femoral ostectomy (DFO) planning. The length of femur is determined and the line of anatomic axis of the femur or proximal femoral long axis (PFLA) which is drawn by connecting two points of the center of the femur at 33% and 50% of its length (Fig. 27). The distal joint reference line (DJRL) or trancondylar axis (TCA) is the line connecting the edge of the medial and lateral femoral condyles. The anatomic lateral distal femoral angle (aLDFA) is measured at the intersection of the PFLA and TCA. Comparison of the aLDFA to a breed specific reference range needs to be performed if a significant femoral varus deformity is present. The line of the distal anatomic axis that extends along the lateral aspect of the intercondylar notch is drawn. The center of rotation of angulation (CORA) located at the intersection of the proximal and distal anatomic connecting the center of the axes and its magnitude can be measured at this location (Fig. 27) (Kowaleski, 2011). If the specific normal value is not available, the opposite normal femoral can be measured as a reference (Kowaleski, 2011).

The corrective femoral osteotomy is performed by using a laterally-based, closing-wedge ostectomy with intraoperative aid of an alignment jig. The DFO is stabilised with a bone plate and screws placed on the lateral surface of the femur (Palmer, 2008). This technique is biomechanically more stable than an opening wedge, and access to the femoral shaft from lateral aspect provides ample exposure (Kowaleski et al., 2012).

In summary, the standard techniques of bone reconstruction are trochlear wedge or trochlear block recession and tibial tuberosity transposition (Singleton, 1969; DeAngelis and Hohn, 1970; Slocum and Devine, 1985; Roy et al., 1992; Hayes et al., 1994; Gibbons et al., 2006; Alam et al., 2007; Linney et al., 2011), and Corrective osteotomies of the femur is considered in dogs with femoral varus deformity (Palmer, 2008).

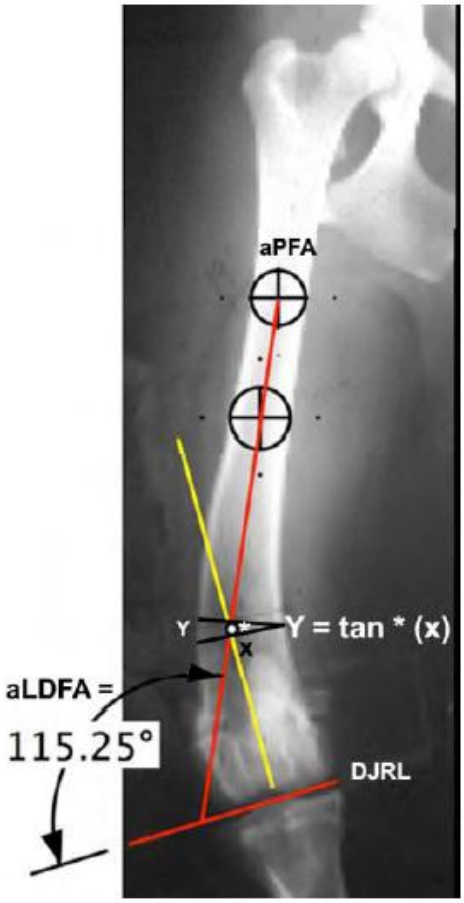


Figure 27 Preoperative planning for distal femoral ostectomy (DFO).
Radiograph shows aLDFA determined as the angle formed by the distal joint reference line (DJRL) and the anatomic proximal femoral axis (aPFA). The ostectomy is centered at the CORA and the base wedge (Y) is calculated from the angle of the desired wedge (*) and the measurement of the femoral diameter at the level of the ostectomy (X) using the formula Y = tan * (X) Palmer (2008).

III. Miscellaneous techniques

1. Fibular head transposition

This technique is commonly used for extracapsular repair of cranial cruciate ruptures however it may be used to decrease medial tibial rotation. Cranial movement of the fibular head tenses the lateral collateral ligament and externally rotates the tibia so the extensor mechanism is realigned (Roush, 1993).

2. Reconstruction of the medial femoral trochlear ridge is performed by using an U-shaped Kirschner wire for repairing grade 1 or 2 MPL in small breed dogs in order to increase height of the medial trochlear ridge in cases of a shallow femoral trochlear ridge (Fig. 28). Morphology and histology of patellar articular cartilage of this technique was observed in 6 normal laboratory dogs which showed normal articular surface of the patella, fibrocartilage and fibrous tissue growth at the excision site of the trochlear ridge. Outcome of the 10 patients (11 stifles) was good in 64% of the cases with normal weight bearing and patella in the good position (Srisuwatanasagul et al., 2003).

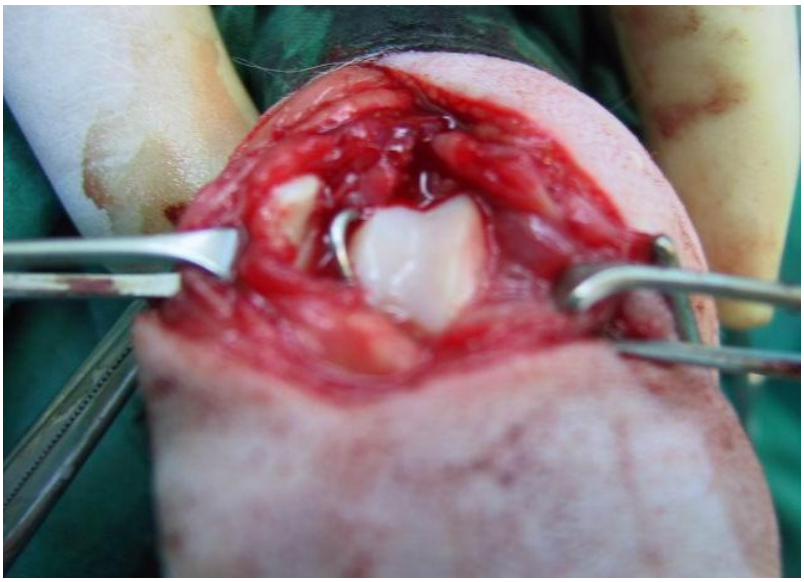


Figure 28 Reconstruction of femoral trochlear ridge by using U-shaped K-wire for medial patellar luxation (Photo of Srisuwatanasagul et al., 2003).

3. Patellar groove replacement (PGR) can be performed in chronic PL grade 4 or failed trochleoplasty leading to severe degenerative joint disease with chondromalacia, condyle deformation and massive osteophytosis. A trochlear prosthesis can be used to replace the damaged condylar trochlea with a less invasive surgical technique. A titanium alloy prosthesis (Kyon) shaped like the natural deep trochlear groove is fixed to the chondyle with a perforated grade 4 titanium base plate (Fig. 29A, B) (Vezzoni, 2011a).

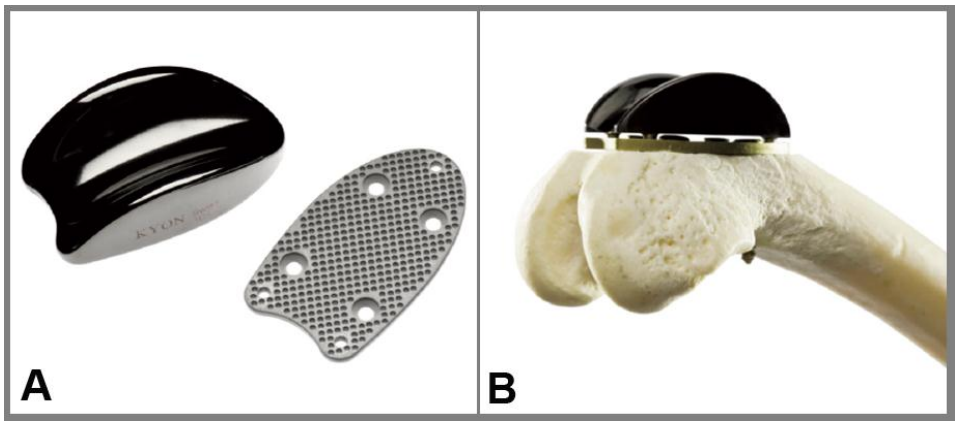


Figure 29 Patellar groove replacement (Vezzoni, 2011a).
The Patellar groove replacement (PGR) prosthesis is a two-component implant comprising: (i) the trochlear prosthesis, produced from a titanium alloy (Grade 5, TiAl6V4) and (ii) the base plate produced from grade 4 titanium **(A)**. The PGR prosthesis is fixed with screws to the femur at the planar osteotomy **(B)**.

4. RidgeStop technique (Orthomed)

A new technique of bone reconstruction using RidgeStop implants to increase a height of trochlear ridge was developed by Orthomed (Fig. 30). However, no long term results have been published so far.

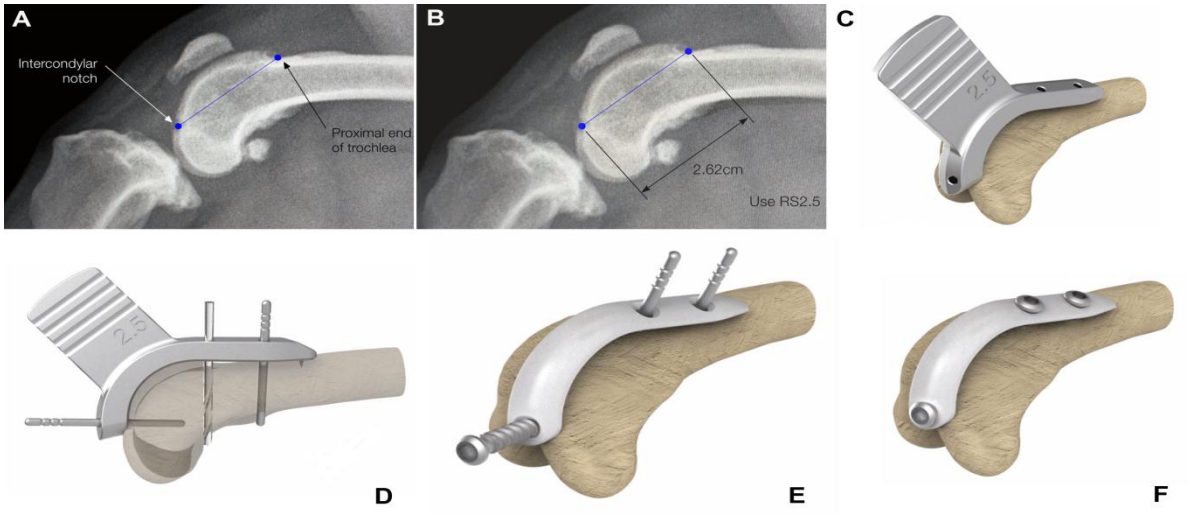


Figure 30 RigdeStop technique. The trochlear prosthesis is placed after a template has been used as a drill guide (C-E).

Selection of the surgical treatment of PL is based on age of the animal, grade of luxation, and duration of the luxation (Table 6) (L'Eplattenier and Montavon, 2002; Piermattei et al., 2006). In young dogs, bone is not fully developed and limb deformity is minimal. If the alignment of bone and patellar ligament is corrected, limb alignment will be normal. Muscular contracture in young dogs is so minimal that muscle and ligament can get their function back to normal faster and easier than older dogs. The outcome of surgical studies is shown in Table 7.

Post-operative care

The goals for post-operative care are to reduce pain, to promote healing, to maintain muscle mass and to promote muscular development and joint stability, to maintain joint flexibility, to return proprioception, balance and coordination, to facilitate early return to function, to prevent degenerative joint disease and rebuild cardiovascular endurance (Edge-Hughes and Nicholson, 2007). Immobilization is not necessary after surgery except for the active animals. Early ambulation is encouraged, but exercise should be limited to leash walks for the first 3 to 4 weeks especially in case of osteotomy, allowing the bone to heal. Active physiotherapy i.e. swimming or passive flexion-extension is recommended if the dog does not start to bear weight on the limb within 1 week after surgery.

Table 6 Treatment consideration of medial patellar luxation according to age of the animal, grade of luxation, and clinical signs

Grading	Range of age	Clinical signs	Conservative treatment	Surgical treatment				
				Soft tissue reconstruction		Bone tissue reconstruction		
				I	II	III	IV	V
1	immature dog	absent			✓			
	mature dog							
	1-3 years	absent	✓					
	> 3 years	absent	✓					
2	immature dog	absent			✓			
		present			✓			
	mature dog							
	1-3 years	absent	✓		✓	+/-		
		present	+/-		✓	+/-		
	> 3 years	absent	✓		✓	+/-		
		present	+/-		✓	+/-		
3	immature dog	present		✓	✓	✓	✓	×
	mature dog							
	1-3 years	present		✓	✓	✓	✓	+/-
	> 3 years	present		✓	✓	✓	✓	+/-
4	immature dog	present		✓	✓	✓	✓	×
	mature dog							
	1-3 years	present		✓	✓	✓	✓	+/-
	> 3 years	present		✓	✓	✓	✓	+/-

I = desmotomy/ joint capsule release, II = overlapping/imbrication/lateral suture, III = trochlear deepening, IV = tibial tuberosity transposition, V = osteomy, ✓= (clinical signs) present or (treatment) recommend, +/- = considering to abnormal finding and intra-operative decision.

Table 7 Outcome of surgical treatment of patellar luxation

Categories	I							II	III	
Reference	Arthurs and Langley-Hobbs. 2006	Willauer and Vasseur, 1987	Remedios et al., 1992	Hayes et al., 1994	Singleton, 1969	De Angelis and Hohn, 1970	Slocum and Devine, 1985	Linney et al., 2011	Gibbon et al., 2006	Alam et al., 2007
The number of dogs	109	34	16	124	56	125	28	91	59	
The number of stifles	131	52	21	209†	61	142	41		70	165
Follow up (%)			18							
Excellent			50				87.8			63
Good			44.4		90.2	90.1			94	31
Fair					4.9	9.9	7.3		6	3
Poor			5.6		4.9		4.9			3
Recurrent rate (%)	8	48					2.4	19.8	9	6
Concomitant CCLR (%)		4	13	15		15			19	12
Concomitant hip dysplasia (%)	6			11			22			
Concomitant OA						1.4				
Grade of luxation (%)										
1	3.2		4.5	10.5	32.8			1.4	7.1	18
2	48.4	53.8	50	44.8	24.6		22	24.3	51.4	27
3	30.7	40.4	41	32.2	21.3		52	68.9	27.1	47
4	17.7	5.8	4.5	12.6	21.3		26	5.4	14.3	8
Complication (%)	18								29	18
Postoperative CCLR	0.8	5.8				1.4		5.5		
Inflammation 2nd to licking								14.3		
Wound inflammation								8.8		
Seroma formation								11		2
Pin migration	2.3	3.8	6.3			2.1		7.7		
Suture reaction								3.3		
Infection	0.8‡					1.4‡		2.2		7
Dehiscence			6.3					2.2		
Cellulitis								1.1		
Overcorrection								1.1		
Trochlear wedge migration			6.3							
Swollen of patellar tendon										2
Hock hyperextension										1
Continued lameness		7.7	7.3					11		
OA/ DJD		78.8						No		

I = Multiple combinations and various surgical techniques; II = soft tissue reconstruction (ST) and tibial tuberosity transposition (TTT) without trochleoplasty; III = four group classification (1 = ST, 2 = ST + trochleoplasty, 3 = ST + TTT, 4 = ST + trochleoplasty + TTT.
CCLR = cranial cruciate ligament rupture; OA = osteoarthritis; DJD = degenerative joint disease;
† = surgery were performed in 126 stifles; ‡ = arthritis

2.7 Genetics of patellar luxation

Canine genetics is the study of canine biologic variation and inherited disease. Individual animals may inherit copies of genes that cause a disease or, more commonly, produce a susceptibility to a disease that becomes manifest with time and by environmental interaction.

The domestic dog (*Canis lupus familiaris*) originates from the gray wolf as shown by mitochondrial DNA sequence data (Vila et al., 1997). The American Kennel Club (AKC) started to register breeds in the 19th century, and categorized dog breeds into seven groups based on historical development, morphology and behavior (http://www.ack.org). In addition, the dog breeds can be divided into at least four hierarchical groups according to DNA marker datasets (Parker et al., 2004). The phenotypic characteristics of individual dog breeds including size (Sutter et al., 2007), coat color (Karlsson et al., 2007), coat texture (Cadieu et al., 2009), and body shape (Chase et al., 2002) involve a small number of gene variants to control each trait. Because of the strong selection for specific phenotypes, the fact that dog breeds are descended from small numbers of founders, and population bottlenecks, the genetic variation in a breed is limited (Ostrander and Kruglyak, 2000; Parker et al., 2010). Because the genetic architecture is simplified it is expected that a small number of genes have effect on complex traits (Parker et al., 2010).

The dog is a model for mapping genes relevant to human diseases because the physiology, disease presentation, and clinical response of dogs are closely related to human disease (Ostrander and Kruglyak, 2000). The study of the simpler aetiology in dogs may provide the requisite tools for understanding the complex disease in humans (Ostrander and Wayne, 2005).

The high susceptibility of patellar luxation (PL) in particular breeds (Priester, 1972; LaFond et al., 2002; Vidoni et al., 2006; OFA, 2010), together with an early age of onset, often with bilateral occurrence, give a strong premise that PL is an inherited entity with genetic predisposition (Ostrander and Kruglyak, 2000). The exact primary cause of the disease remains unclear (LaFond et al., 2002; Hayashi et al., 2010). The completion of the DNA sequence of the canine genome has resulted in a renewed interest in the genetics of canine disorders with orthopaedic manifestation including PL. PL probably has a multifactorial inheritance as most of the developmental orthopedic diseases that are attributable to both genetic and environmental factors (LaFond et al., 2002).

Mode of inheritance

Disease phenotypes can be divided into monogenic and polygenic traits. The monogenic "single gene" trait is controlled by a single highly penetrant locus and shows a Mendelian inheritance pattern of transmission of the trait from parents to offspring. The inheritance pattern of a monogenic trait can be either autosomal or X-linked, and recessive or dominant. The polygenic trait is controlled by multiple loci as well as environmental factors; therefore the inheritance pattern of polygenic traits does not follow the Mendelian pattern. The inheritance pattern of PL has been suggested as an autosomal recessive genetic trait by Kodituwakku (1962) (Singleton, 1969). An excess risk among female dogs, as revealed from studies in Jack Russell Terrier and Flat-Coated retriever (Hazewinkel et al., 2013) might be related to hormonal or to non-hormonal influences of a X-linked inheritance (Priester, 1972). However, it is clear that PL might be a polygenic trait which has been suggested by Loeffler and Lieger (1964) and Hutt (1968) (Singleton, 1969).

Genetic tools

The heritability (h^2) is the proportion of the phenotypic variance attributable to the additive genetic variance and is specific for the population and environment. The entity can be calculated from extensive phenotype and pedigree data and explains how much of the variation in a trait is due to variation in genetic factors. Heritability is used in breeding programmes to determine the genetic ability (estimated breeding value: EBV) of each dog within a breed. EBV is an estimate of the ability of an individual to pass susceptibility for a certain trait to its offspring, based on one or more measurement of phenotype, using values taken on the animal itself, and a large number of its relatives. DNA-information can be added for more reliable estimation of this genetic ability (Meuwissen and Goddard, 2001)

There are two approaches to identify and localize genes with a genetic disease including linkage analysis and genome-wide association studies (GWAS) (Hirschhorn and Daly, 2005; Kruglyak et al., 1996). Both methods have to take into account the occurrence of recombinations (meiotic cross-overs) between the searched for risk allele and the markers used for DNA mapping.

Linkage analysis

Linkage analysis is the strategy to genotype genetic markers across the chromosomes and to exam how those genetic markers segregate with the disease across families. This strategy can be applied to identify genetic variants that contribute to disorders that inherit according to Mendel's laws. It can be either parametric, involving a known mode of inheritance, or nonparametric (model-free linkage analysis). The methods test whether the inheritance of DNA marker alleles deviates from expectation under independent assortment (Kruglyak et al., 1996). The power of linkage analysis depends on the number of informative meioses. The logarithm of the odds ratio (LOD) score test is used to provide evidence for linkage and estimate of recombination fraction in linkage analysis (Mortor, 1955). A LOD score > 3 supports the linkage between the marker and the disease while a negative LOD score indicates that the linkage between the DNA marker and the trait is less likely.

The genetic mechanisms influencing common disorders are different from those that cause rare disorders. To identify the relevant genes has been difficult because each causal gene only makes a small contribution to the disease (Hirschhorn and Daly, 2005). The major limitations of linkage studies are relatively low power for complex disorders influenced by multiple genes, and the large size of chromosomal regions shared among family members (Pearson and Manolio, 2008). Linkage analysis has proven not to be appropriate for the study of common complex disorders, such as hip dysplasia (Janutta and Distl, 2006), fragmented coronoid process (Temwichitr et al., 2010), or cardiomyopathy (Distl et al., 2007).

Genome-wide association study

A genome-wide association study (GWAS) is an approach that involves scanning large numbers of DNA markers across the genome of many distantly related animals, to find genetic variations associated with a particular disease. GWAS is in advantage over family-based linkage studies for non-Mendelian traits. It is important to use unrelated individuals among both cases and controls to improve power and avoid family stratification. Allele frequencies are determined in cases and compared to the allele frequencies in controls. It is considered to be associated when the allele frequency in the cases is significantly higher than the allele frequency in the controls. The studies are particularly useful in finding genetic variations that contribute to common, complex diseases. Single nucleotide polymorphism (SNP)

arrays were developed by using the information of the canine genome. Nowadays, a high-density canine array with 170 000 SNPs is available. Many studies successfully identified associated loci and discovered the causative mutations contributing to disease, morphology and behavioral disorders (Candille et al., 2007; Karlsson et al., 2007; Sutter et al., 2007; Parker et al., 2009). The genome-wide association approach poses special problems because the massive number of statistical tests presents a potential for false-positive results. This led to new stringency requirements of levels of statistical significance and requirements for independent confirmation of the results (Hunter and Kraft, 2007).

A competitive allele-specific PCR, a genotyping SNP method, is used to confirm regions of interest found from GWAS in a replication group. KASP™ chemistry (LGC genomics, Hoddesdon, UK) is used to generate allele specific fluorescent signals as instructed by the manufacturer. The allele frequencies of these SNPs in the validation group are compared between cases and controls separately to confirm the level of statistical significance using a χ^2 test (Skol et al., 2006). Once new genetic associations are identified, researchers can use the information to develop better strategies to detect, treat and prevent the disease.

The genetic studies of PL in purebred dogs are aimed to gain more knowledge about the aetiology of the disease. When genes involved in PL are identified, their function might explain or connect to both the aetiology and the pathophysiology of PL. Moreover novel knowledge gathered from the genomic database together with the method to perform pathway analysis are available now which makes it possible to combine the results not only obtained in one breed but in several breeds (Arendt et al., 2013). Application of this knowledge in PL, can be an example for the study of other polygenetic diseases in dogs. In addition, the study of the population genetics of PL including results of screening programmes and estimated breeding values (EBVs) calculations, could help to reduce PL from the population of heavily affected breeds.

References

Alam, M.R., Lee, J.I., Kang, H.S., Kim, I.S., Park, S.Y., Lee, K.C., Kim, N.S., 2007. Frequency and distribution of patellar luxation in dogs: 134 cases (2000 to 2005). Veterinary and Comparative Orthopaedics and Traumatology 20, 59-64.

Arendt, M., Melin, M., Tonomura, N., Perloski, M., Courtay-Cahen, C., Ricketts, S., Fineberg, E., Boerkamp, K., Megquier, K., Jeffery, L., Murphy, S., McCarthy, C., London, C., Rutteman, G., Starkey, M., Lindblad-Toh, K., 2013. Genome-wide association of canine mast cell tumors in golden retrievers: two populations leading to a common pathway. Proceedings of the 7th International Conference on Advances in Canine and Feline Genomics and Inherited Diseases, Cambridge, Massachusetts, USA, p.59.

Arthurs, G.I., Langley-Hobbs, S.J., 2006. Complications associated with corrective surgery for patellar luxation in 109 dogs. Veterinary Surgery 35, 559-566.

Arthurs, G.I., Langley-Hobbs, S.J., 2007. Patellar luxation as a complication of surgical intervention for the management of cranial cruciate ligament rupture in dogs. A retrospective study of 32 cases. Veterinary and Comparative Orthopaedics and Traumatology 20, 204-210.

Boone, E.R., Hohn, R.B., 1983. Trochlear recession wedge technique for patellar luxation: An experimental study. Journal of the American Animal Hospital Association 19, 735-742.

Bound, N., Zakai, D., Butterworth, S.J., Pead, M., 2009. The prevalence of canine patellar luxation in three centres. Clinical features and radiographic evidence of limb deviation. Veterinary and Comparative Orthopaedics and Traumatology 22, 32-37.

Cadieu, E., Neff, M.W., Quignon, P., Walsh, K., Chase, K., Parker, H.G., Vonholdt, B.M., Rhue, A., Boyko, A., Byers, A. *and others*, 2009. Coat variation in the domestic dog is governed by variants in three genes. Science 326, 150-153.

Candille, S.I., Kaelin, C.B., Cattanach, B.M., Yu, B., Thompson, D.A., Nix, M.A., Kerns, J.A., Schmutz, S.M., Millhauser, G.L., Barsh, G.S., 2007. A-defensin mutation causes black coat color in domestic dogs. Science 318, 1418-1423.

Chase, K., Carrier, D.R., Adler, F.R., Jarvik, T., Ostrander, E.A., Lorentzen, T.D., Lark, K.G., 2002. Genetic basis for systems of skeletal quantitative traits: principal component analysis of the canid skeleton. Proceedings of the National Academy of Sciences of the United States of America 99, 9930-9935.

DeAngelis, M., Hohn, R.B., 1970. Evaluation of surgical correction of canine patellar luxation in 142 cases. Journal of the American Veterinary Medical Association 156, 587-594.

Denny, H.R., Minter, H.M., 1973. The long term results of surgery of canine stifle disorders. Journal of Small Animal Practice 14, 695-713.

Distl, O., Vollmar, A.C., Broschk, C., Hamann, H., Fox, P.R., 2007. Complex segregation analysis of dilated cardiomyopathy (DCM) in Irish wolfhounds. Heredity 99, 460-465.

Dohoo, I., Martin, W., Stryhn, H., 2010. Veterinary Epidemiologic Research. Atlantic Veterinary College, University of Prince Edward Island, Prince Edward Island, Canada, pp. 121-136.

Dudley, R.M., Kowaleski, M.P., Drost, W.T., Dyce, J., 2006. Radiographic and computed tomographic determination of femoral varus and torsion in the dog. Veterinary Radiology & Ultrasound 47, 546-552.

Edge-Hughes, L.M., 2002. Therapeutic exercise for the canine patient. In: Proceedings of the 2nd International Symposium on Rehabilitation and Physical Therapy in Veterinary Medicine, Knoxville, TN, USA, pp. 59–62.

Edge-Hughes, L., Nicholson, H., 2007. Canine treatment and rehabilitation. In: McGowan, C.M., Goff, L., Stubbs, N. (Eds.), Animal Physiotherapy: Assessment, Treatment and Rehabilitation of Animals. Blackwell, Singapore, pp. 207-237.

Gibbons, S.E., Macias, C., Tonzing, M.A., Pinchbeck, G.L., McKee, W.M., 2006. Patellar luxation in 70 large breed dogs. Journal of Small Animal Practice 47, 3-9.

Hamilton, S.A., 2002. Rehabilitation of osteoarthritis in a dog. In: In: Proceedings of the 2nd International Symposium on Rehabilitation and Physical Therapy in Veterinary Medicine, Knoxville, TN, p. 127.

Harrison, J.W., 1975. Patellar dislocation. In: Bojrab, M. J.(Ed.), Current techniques in small animal surgery. Lea & Febiger, Philadelphia, pp. 479-484.

Hayashi, K., Lansdowne, J., Déjardin, L., 2010. Patellar luxation in dogs. In: Bojrab, M.J., Monnet, E. (Eds.), Mechanisms of Disease in Small Animal Surgery, Third Ed. Teton NewMedia, USA, pp. 655-661.

Hayes, A.G., Boudrieau, R.J., Hungerford, L.L., 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal of the American Veterinary Medical Association 205, 716-720.

Hazewinkel, H.A., Tellhelm, B., Leegwater, P.A., 2013. Screening for Orthopaedic Genetic defects: Part 1. Patella Luxation in Dogs. European Journal of Companion Animal Practice 23, 90-98.

Hermanson, J.W., 2013. The muscle system. In: Evans, H.E., de Lahunta, A. (Eds.), Miller's Anatomy of the Dog, Fourth Ed. Elsevier Saunders, China, pp. 185-280.

Hirschhorn, J.N., Daly, M.J., 2005. Genome-wide association studies for common diseases and complex traits. Nature Reviews: Genetics 6, 95-108.

Hunter, D.J., Kraft, P., 2007. Drinking from the fire hose--statistical issues in genome wide association studies. The New England Journal of Medicine 357, 436-439.

Huang, M.H., Lin, Y.S., Lee, C.L., Yang, R.C., 2005. Use of ultrasound to increase effectiveness of isokinetic exercise for knee osteoarthritis. Archives of Physical Medicine and Rehabilitation 86, 1545-1551.

Hulse, D.A., 1981a. Medial patellar luxation in the dog. In: Bojrab, M.J., Smeak, D.D., Bloomberg, M.S. (Eds.), Disease Mechanisms in Small Animal Surgery, Second Ed. Lea & Febiger, Philadelphia, pp. 808-817.

Hulse, D.A., 1981b. Pathophysiology and management of medial patellar luxation in the dog. Veterinary Medicine: Small Animal Clinician 76, 43-51.

Hulse, D.A., 1993. Medial patellar luxation in the dog. In: Bojrab, M.J. (Ed.), Disease Mechanisms in Small Animal Surgery. Lea&Febiger, Philadelphia, pp. 808-817.

Hulse, D.A., 1995. The Stifle Joint. In: Olmstead, M.L. (Ed.), Small Animal Orthopedic. Mosby, St. Louis, pp. 395-404.

Hulse, D.A., 2011. Treatment of patellar luxation in immature dogs. In: Proceedings of the 15th Annual ESVOT Congress, Seminar on Patellar Luxation, Lyon, France, pp. 55-56.

Insall, J., Goldberg, V., Salvati, E., 1972. Recurrent dislocation and the high-riding patella. Clinical Orthopaedics and Related Research 88, 67-69.

Jaegger, G., Marcellin-Little, D.J., Levine, D., 2002. Reliability of goniometry in Labrador Retrievers. American Journal of Veterinary Research 63, 979-986.

Janutta, V., Distl, O., 2006. Inheritance of canine hip dysplasia: review of estimation methods and of heritability estimates and prospects on further developments. DTW. Deutsche tierarztliche Wochenschrift 113, 6-12.

Johnson, A.L., Broaddus, K.D., Hauptman, J.G., Marsh, S., Monsere, J., Sepulveda, G., 2006. Vertical patellar position in large-breed dogs with clinically normal stifles and large-breed dogs with medial patellar luxation. Veterinary Surgery 35, 78-81.

Johnson, A.L., Probst, C.W., DeCamp, C.E., Rosenstein, D.S., Hauptman, J.G., Kern, T.L., 2002. Vertical position of the patella in the stifle joint of clinically normal large-breed dogs. American Journal of Veterinary Research 63, 42-46.

Kaiser, S., Cornely, D., Golder, W., Garner, M., Waibl, H., Brunnberg, L., 2001a. Magnetic resonance measurements of the deviation of the angle of force generated by contraction of the quadriceps muscle in dogs with congenital patellar luxation. Veterinary Surgery 30, 552-558.

Kaiser, S., Cornely, D., Golder, W., Garner, M.T., Wolf, K.J., Waibl, H., Brunnberg, L., 2001b. The correlation of canine patellar luxation and the anteversion angle as measured using magnetic resonance images. Veterinary Radiology & Ultrasound 42, 113-118.

Kalff, S., Butterworth, S.J., Miller, A., Keeley, B., Baines, S., McKee, W.M., 2014. Lateral patellar luxation in dogs: a retrospective study of 65 dogs. Veterinary and Comparative Orthopaedics and Traumatology 27, 130-134.

Kannus, P.A., 1992. Long patellar tendon: radiographic sign of patellofemoral pain syndrome--a prospective study. Radiology 185, 859-863.

Karlsson, E.K., Baranowska, I., Wade, C.M., Salmon Hillbertz, N.H., Zody, M.C., Anderson, N., Biagi, T.M., Patterson, N., Pielberg, G.R., Kulbokas, E.J.,3rd *and others*, 2007. Efficient mapping of mendelian traits in dogs through genome-wide association. Nature Genetics 39, 1321-1328.

Koch, D.A., St, G., Savoldelli, D., L'Eplattenier, H., Montavon, P.M., 1998. The diagnosis of patellar luxation in small animals. Schweizer Archiv fur Tierheilkunde 140, 371-374.

Kowaleski, M.P., 2011. Assessment of femoral angulation/pre- DFO planning. Proceedings of the 15th Annual ESVOT Congress, Seminar on Patellar Luxation, Lyon, France, pp. 12-14.

Kowaleski, M.P., Boudrieau, R.J., Pozzi, A., 2012. Stifle joint. In: Tobias, K.M., Johnston, S.A. (Eds.), Veterinary Surgery: Small Animal. Elsevier Saunders, Canada, pp. 906-998.

Kruglyak, L., Daly, M.J., Reeve-Daly, M.P., Lander, E.S., 1996. Parametric and nonparametric linkage analysis: a unified multipoint approach. American Journal of Human Genetics 58, 1347-1363.

LaFond, E., Breur, G.J., Austin, C.C., 2002. Breed susceptibility for developmental orthopedic diseases in dogs. Journal of the American Animal Hospital Association 38, 467-477.

Lancourt, J.E., Cristini, J.A., 1975. Patella alta and patella infera. Their etiological role in patellar dislocation, chondromalacia, and apophysitis of the tibial tubercle. The Journal of Bone and Joint Surgery 57, 1112-1115.

Lavrijsen, I.C., Heuven, H.C., Breur, G.J., Leegwater, P.A., Meutstege, F.J., Hazewinkel, H.A., 2013. Phenotypic and genetic trends of patellar luxation in Dutch Flat-Coated Retrievers. Animal Genetics 44, 736-741.

L'Eplattenier H., Montavon P., 2002. Patellar luxation in dogs and cats: management and prevention. Compendium on Continuing Education for the Practicing Veterinarian 24, 292-300.

Linney, W.R., Hammer, D.L., Shott, S., 2011. Surgical treatment of medial patellar luxation without femoral trochlear groove deepening procedures in dogs: 91 cases (1998-2009). Journal of the American Veterinary Medical Association 238, 1168-1172.

Meggiolaro, S., 2009. Comparison of a 3-dimensional model and standard radiographic evaluation of femoral and tibial angles in the dog. Master thesis, University of Padova, Italy.

Meuwissen, T.H., Goddard, M.E., 2001. Prediction of identity by descent probabilities from marker-haplotypes. Genetics Selection Evolution 33, 605-634.

Millis, D.L., 2004. Managing chronic osteoarthritis using physical rehabilitation. Research. In: Proceedings of the 3rd International Symposium on Rehabilitation and Physical Therapy in Veterinary Medicine, Triangle Park, NC, pp. 127–130.

Moore, J.A., Banks, W.J., Blass, C.E., 1989. Repair of full-thickness defects in the femoral trochlea of dogs after trochlear arthroplasty. American Journal of Veterinary Research 50, 1406-1413.

Mortari, A.C., Rahal, S.C., Vulcano, L.C., da Silva, V.C., Volpi, R.S., 2009. Use of radiographic measurements in the evaluation of dogs with medial patellar luxation. The Canadian Veterinary Journal 50, 1064-1068.

Morton, N.E., 1955. Sequential tests for the detection of linkage. American Journal of Human Genetics 7, 277-318.

Mostafa, A.A., Griffon, D.J., Thomas, M.W., Constable, P.D., 2008. Proximodistal alignment of the canine patella: radiographic evaluation and association with medial and lateral patellar luxation. Veterinary Surgery 37, 201-211.

Nagaoka, K., Orima, H., Fujita, M., Ichiki, H., 1995. A new surgical method for canine congenital patellar luxation. The Journal of Veterinary Medical Science/the Japanese Society of Veterinary Science 57, 105-109.

Olmstead, M.L., 1981. Lateral luxation of the patella. In: Bojrab, M.J., Smeak, D.D., Bloomberg, M.S. (Eds.), Disease Mechanisms in Small Animal Surgery, Second Ed. Lea & Febiger, Philadelphia, pp. 818-820.

Olmstead, M.L., 1995. Small Animal Orthopaedics. Mosby, St Louis, MO.

OFA, 2013. Orthopedics Foundation for Animals: Patellar luxation statistics. http://www.offa.org/stats_pl.html (accessed March 9, 2014).

Ostrander, E.A., Kruglyak, L., 2000. Unleashing the canine genome. Genome Research 10, 1271-1274.

Ostrander, E.A., Wayne, R.K., 2005. The canine genome. Genome Research 15, 1706-1716.

Palmer, R.H., 2008. Controversies in the management of patellar luxation. In: Proceedings of the BVOA Spring Scientific Meeting, Birmingham, England, pp. 30-34.

Parker, H.G., Kim, L.V., Sutter, N.B., Carlson, S., Lorentzen, T.D., Malek, T.B., Johnson, G.S., DeFrance, H.B., Ostrander, E.A., Kruglyak, L., 2004. Genetic structure of the purebred domestic dog. Science 304, 1160-1164.

Parker, H.G., Shearin, A.L., Ostrander, E.A., 2010. Man's best friend becomes biology's best in show: genome analyses in the domestic dog. Annual Review of Genetics 44, 309-336.

Parker, H.G., VonHoldt, B.M., Quignon, P., Margulies, E.H., Shao, S., Mosher, D.S., Spady, T.C., Elkahloun, A., Cargill, M., Jones, P.G. *and others*, 2009. An expressed fgf4 retrogene is associated with breed-defining chondrodysplasia in domestic dogs. Science 325, 995-998.

Pearson, T.A., Manolio, T.A., 2008. How to interpret a genome-wide association study. The Journal of the American Medical Association 299, 1335-1344.

Persuki, A.M., Kowaleski, M.P., Pozzi, A., Dyce, J., Johnson, K.A., 2006. Treatment of medial patellar luxation and distal femoral varus by femoral wedge osteotomy in dogs: 30 cases (2000–2005). Proceedings of the 2nd World Veterinary Orthopaedic Congress & 33rd Annual Veterinary Orthopedic Society Conference, Keystone, CO, p. 240.

Piermattei, D.L., Flo, G.L., DeCamp, C.E., 2006. The stifle joint. In: Piermattei, D.L., Flo, G.L., DeCamp, C.E. (Eds.), Handbook of Small Animal Orthopedics and Fracture Repair. Fourth Ed. Saunders, St. Louis, pp. 562-632.

Piras, A., 2011. Radiographs: what projections are indicated. Proceedings of the 15th Annual ESVOT Congress, Seminar on Patellar Luxation, Lyon, France, pp. 8-11.

Priester, W.A., 1972. Sex, size, and breed as risk factors in canine patellar dislocation. Journal of the American Veterinary Medical Association 160, 740-742.

Putnam, R.W., 1968. Patellar luxation in the dog. Master thesis, University of Guelph, Canada.

Remedios, A.M., Basher, A.W., Runyon, C.L., Fries, C.L., 1992. Medial patellar luxation in 16 large dogs. A retrospective study. Veterinary Surgery 21, 5-9.

Robin, G.M., 1990. The canine stifle joint. In: Whittick, W.G. (Ed.), Canine Orthopedics, Second Ed. Lea & Febiger, Philadelphia, pp. 693-720.

Roush, J.K., 1993. Canine patellar luxation. Veterinary Clinics of North America: Small Animal Practice 23, 855-868.

Roy, R.G., Wallace, L.J., Johnston, G.R., Wickstrom, S.L., 1992. A retrospective evaluation of stifle osteoarthritis in dogs with bilateral medial patellar luxation and unilateral surgical repair. Veterinary Surgery 21, 475-479.

Segal, U., Or, M., Shani, J., 2012. Latero-distal transposition of the tibial crest in cases of medial patellar luxation with patella alta. Veterinary and Comparative Orthopaedics and Traumatology 25, 281-285.

Singleton, W.B., 1969. The surgical correction of stifle deformities in the dog. Journal of Small Animal Practice 10, 59-69.

Skol, A.D., Scott, L.J., Abecasis, G.R., Boehnke, M., 2006. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nature genetics 38, 209-213.

Slocum, B., Devine, T., 1985. Trochlear recession for correction of luxating patella in the dog. Journal of the American Veterinary Medical Association 186, 365-369.

Slocum, B., Slocum, T.D., 1993. Trochlear wedge recession for medial patellar luxation. An update. Veterinary Clinics of North America: Small Animal Practice 23, 869-875.

Slocum, B., Slocum, T.D., 1998a. Knee. In: Bojrab, M.J., Ellison, G.W., Slocum, B. (Eds.), Current Techniques in Small Animal Surgery, Fourth Ed. Williams & Wilkins, Baltimore, pp. 1187-1244.

Slocum, B., Slocum, T.D., 1998b. Knee. In: Bojrab, M.J. (Ed.), Current Techniques in Small Animal Surgery, Fourth Ed. Williams & Wilkins, Baltimore, pp. 1187-1244.

Slocum, B., Slocum, T.D., 2000. Alignment problems of the hindlimb. Proceedings of the 10th ESVOT Congress, Munich, Germany, pp. 60-62.

Soontornvipart, K., Wangdee, C., Kalpravidh, M., Brahmasa, A., Sarikaputi, M., Temwichitr, J., Lavrijsen, I.C., Theyse, L.F., Leegwater, P.A., Hazewinkel, H.A., 2013. Incidence and genetic aspects of patellar luxation in Pomeranian dogs in Thailand. The Veterinary Journal 196, 122-125.

Soparat, C., Wangdee, C., Chuthatep, S., Kalpravidh, M., 2012. Radiographic measurement for femoral varus in Pomeranian dogs with and without medial patellar luxation. Veterinary and Comparative Orthopaedics and Traumatology 25, 197-201.

Srisuwatanasagul, K., Tantilipikara, P., Komin, K., Kalpravidh, M., 2003. Canine patellar luxation repair by using a U-shaped K-wires as an artificial trochlear ridge repair in dogs. Proceeding of 28th World Congress of the World Small Animal Veterinary Association, Bangkok, Thailand, p. 743.

Sutter, N.B., Bustamante, C.D., Chase, K., Gray, M.M., Zhao, K., Zhu, L., Padhukasahasram, B., Karlins, E., Davis, S., Jones, P.G. *and others*, 2007. A single IGF1 allele is a major determinant of small size in dogs. Science 316, 112-115.

Swiderski, J.K., Palmer, R.H., 2007. Long-term outcome of distal femoral osteotomy for treatment of combined distal femoral varus and medial patellar luxation: 12 cases (1999-2004). Journal of the American Veterinary Medical Association 231, 1070-1075.

Swiderski, J.K., Radecki, S.V., Park, R.D., Palmer, R.H., 2008. Comparison of radiographic and anatomic femoral varus angle measurements in normal dogs. Veterinary Surgery 37, 43-48.

Talcott, K.W., Goring, R.L., De Haan, J.J., 2000. Rectangular recession trochleoplasty for treatment of patellar luxation in dogs and cats. Veterinary and Comparative Orthopaedics and Traumatology 13, 39-43.

Temwichitr, J., Leegwater, P.A., Hazewinkel, H.A., 2010. Fragmented coronoid process in the dog: a heritable disease. The Veterinary journal 185, 123-129.

Tomlinson, J., Fox, D., Cook, J.L., Keller, G.G., 2007. Measurement of femoral angles in four dog breeds. Veterinary Surgery 36, 593-598.

Towle, H.A., Griffon, D.J., Thomas, M.W., Siegel, A.M., Dunning, D., Johnson, A., 2005. Pre- and postoperative radiographic and computed tomographic evaluation of dogs with medial patellar luxation. Veterinary Surgery 34, 265-272.

Trotter, E.J., 1980. Medial patellar luxation in the dog. The Compendium on Continuing Education 2, 58-66.

Vasseur, P.B., 2003. Stifle joint. In: Slatter, D. (Ed.), Textbook of Small Animal Surgery, Second Ed. Saunders, Philadelphia, pp. 2090-2133.

Vezzoni, A., 2011a. Patellar groove prosthesis. Proceedings of the 15th Annual ESVOT Congress, Seminar on Patellar Luxation, Lyon, France, pp. 64-67.

Vezzoni, A., 2011b. Patelloplasty. Proceedings of the 15th Annual ESVOT Congress, Seminar on Patellar Luxation, Lyon, France, pp. 32-33.

Vidoni, B., Sommerfeld-Stur, I., Eisenmenger, E., 2006. Diagnostic and genetic aspects of patellar luxation in small and miniature breed dogs in Austria. The European Journal of Companion Animal Practice 16, 149-158.

Vila, C., Savolainen, P., Maldonado, J.E., Amorim, I.R., Rice, J.E., Honeycutt, R.L., Crandall, K.A., Lundeberg, J., Wayne, R.K., 1997. Multiple and ancient origins of the domestic dog. Science 276, 1687-1689.

Walker, P., Harris, I., Leicester, A., 1998. Patellar tendon-to-patella ratio in children. Journal of Pediatric Orthopedics 18, 129-131.

Wangdee, C., Kalpravidh, M., 2008. Tube realignment for patellar luxation repair in dogs. Thai Journal of Veterinary Medicine 38, 39-44.

Wangdee, C., Torwattanachai, P., 2010. Lateral patellar luxation in three Pomeranian dogs: a case report. Thai Journal of Veterinary Medicine 40, 227-231.

Wangdee, C., Leegwater, P.A., Heuven, H.C., van Steenbeek, F.G., Meutstege, F.J., Meij, B.P., Hazewinkel, H.A., 2014. Prevalence and genetics of patellar luxation in Kooiker dogs. The Veterinary Journal doi: 10.1016/j.tvjl.2014.05.036

Willauer, C.C., Vasseur, P.B., 1987. Clinical results of surgical correction of medial luxation of the patella in dogs. Veterinary Surgery 16, 31-36.

Wright, A.L., Wright, G.N., Biery, D.N., Johnston, S.A., 2003. The stifle. In: Anonymous Pfizer Atlas of Common Painful Conditions in Dogs and Cats, First Ed. The Gloyd Group, Inc., Wilmington, Delaware, pp. 12-17.

Chapter 3

Evaluation of surgical treatment of medial patellar luxation in Pomeranian dogs

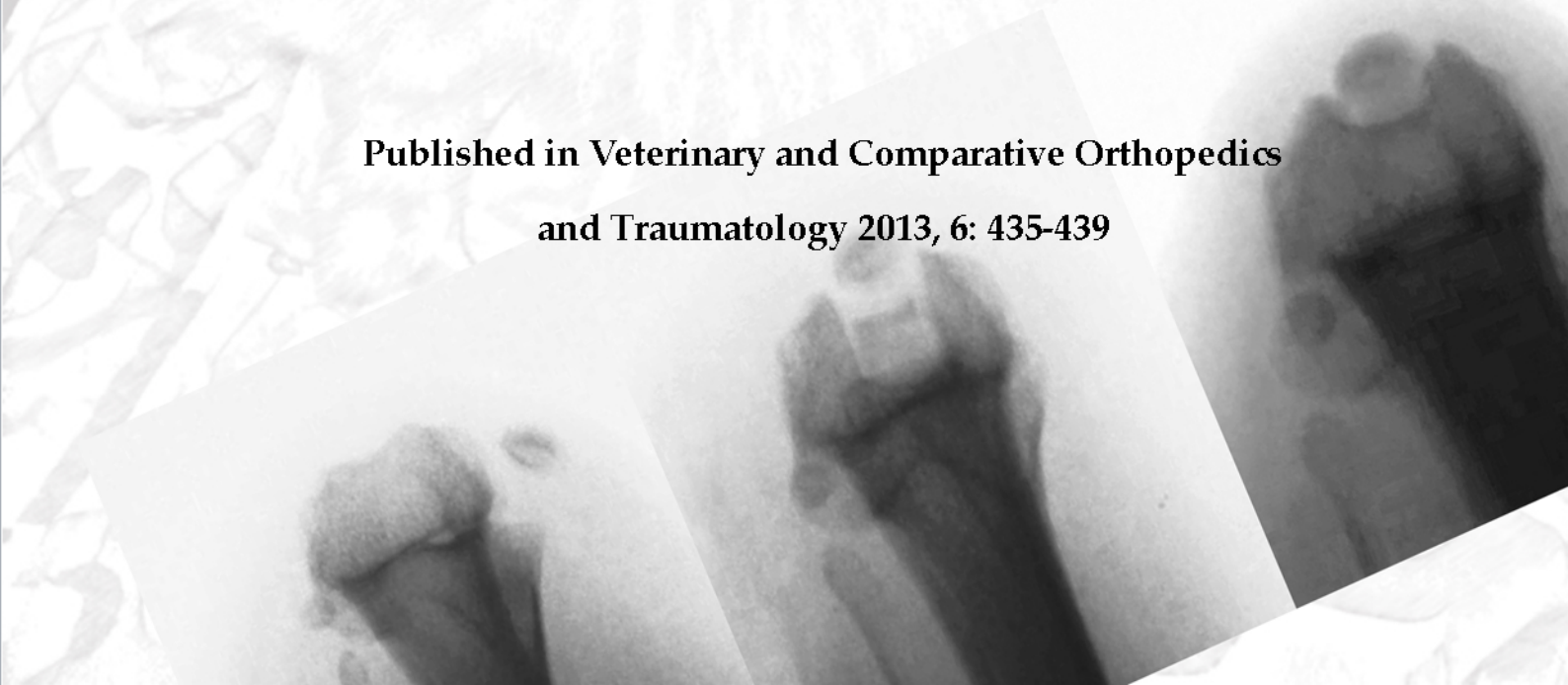
C. Wangdee[1,2], L.F.H. Theyse[2], M. Techakumphu[3],

K. Soontornvipart[1], H.A.W. Hazewinkel[2]

[1] Department of Veterinary Surgery, [3] Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

[2] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Published in Veterinary and Comparative Orthopedics and Traumatology 2013, 6: 435-439



Abstract

In a prospective study, the outcome of surgical correction of medial patellar luxation of 70 stifle joints in 55 Pomeranian dogs was evaluated. Trochlear block recession alone was performed in 46 stifle joints or in combination with tibial tuberosity transposition in 24 stifle joints, in cases with grade 2, 3 or 4 medial patellar luxation. Additional procedures were performed to restore lateral and medial retinacular function. The recurrence of patellar luxation and degree of lameness were evaluated up to at least 16 weeks after surgery. The overall recurrence rate was 10%. The outcome of surgery was considered good for grade 2 luxation with a 100% success rate. Recurrent medial patellar luxation was diagnosed in approximately 11% of dogs with grade 3 and in 36% of dogs with grade 4 luxation. The postoperative lameness score decreased significantly in comparison with the preoperative score at four weeks and thereafter until the end of the study.

Introduction

Medial patellar luxation (MPL) is one of the most common musculoskeletal disorders of small-breed dogs, especially Pomeranians (Soontornvipart et al., 2013). The Orthopedic Foundation for Animals has ranked Pomeranians as the breed with the highest incidence of patellar luxation (PL), with 41.2% of dogs being found to be affected during the evaluation period January 1974 to December 2012 (OFA, 2012). MPL results in a diminished power of stifle joint extensor muscles, which compromises weight bearing and eventually leads to osteoarthritis, pain, and lameness (Hulse, 1981). It is considered to be an inherited developmental disease and can result from a variety of anatomical abnormalities of the pelvic limbs (Piermattei et al., 2006).

Despite extensive study, the pathogenesis of PL remains unclear (DeAngelis, 1971; Hulse, 1993; Roush, 1993; Hayes et al., 1994). MPL can progress to severe loss of function of the affected limb. A compromised extensor mechanism may lead to medial displacement of the tendon of the quadriceps muscle group, and a shallow trochlear groove; in severe cases femoral torsion and femoral varus, contracture of the medial fascia, distraction of the lateral fascia, and internal torsion and angulation of the proximal tibia can also occur (Hulse, 1981). The objective of surgery for PL is to realign the extensor mechanism of the stifle joint in order to re-establish normal limb function. The surgical procedures intended to correct MPL in dogs include a combination of soft tissue reconstruction, femoral trochlear deepening, and lateral transposition of the tibial crest (Hulse, 1981; Roush, 1993; Slocum and Slocum, 1993; Hayes et al., 1994; Arthurs and Langley-Hobbs, 2007). In severe cases, corrective osteotomy to treat torsional and angular deformities of the femur and tibia may be indicated (Roch and Gemmill, 2008) .

The objective of this study was to evaluate the results of surgery for MPL in Pomeranian dogs by quantifying the recurrence rate for each grade of luxation and surgical procedure. Functional results were quantified by assessing the lameness scores before and after surgery.

Materials and Methods

Animals

Fifty-five Pomeranian dogs that were presented with the complaint of MPL at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, were evaluated according to a standardised orthopaedic protocol

(Piermattei et al., 2006). The stifle joints were examined for PL in medial and lateral directions in both hindlimbs, both in a standing position and in lateral recumbency. Dogs with other orthopaedic problems such as avascular necrosis of the femoral head, coxofemoral osteoarthritis, and cranial cruciate ligament disease were excluded from this study. PL was graded as described previously (Piermattei et al., 2006). In grade 1 PL, there were no clinical signs but the patella could be manually luxated in full extension of the stifle joint while returning to the normal position when released. In grade 2, intermittent lameness was encountered and the patella luxated more easily than in grade 1, especially with rotation of the tibia while the patella was pushed or pulled out of the trochlear groove. Reduction was still accomplished with opposite manoeuvres. In grade 3, the patella was permanently luxated, but could be manually reduced with the stifle in extension; however, flexion and extension of the stifle resulted in reluxation of the patella. In grade 4, the patella was permanently luxated and could not be manually reduced. Lameness was assessed preoperatively during locomotion on the leash according to the following grading scheme: 0 = no lameness; 1 = mild lameness, normal at walk with mild lameness at trot; 2 = moderate lameness, lameness at walk and increased lameness at trot; 3 = severe lameness; 4 = non-weight bearing lameness (Hazewinkel et al., 2008).

Anaesthesia

Acepromazine (0.02 mg/kg) and morphine (0.5 mg/kg) were administered as premedication. Fifteen to thirty minutes later, anaesthesia was induced with propofol and maintained with isoflurane in 100% oxygen. Prophylactic cefazolin (25 mg/kg) was administered intravenously. Epidural anaesthesia with 0.5% bupivacaine (1 mg/kg) combined with morphine (0.1 mg/kg) was performed in all dogs.

Surgical treatment

The two main surgical techniques used, were trochlear block recession (TBR) and tibial tuberosity transposition (TTT) depending on the depth of the trochlear sulcus (Fig. 1A) and the alignment of the patella, patellar ligament, and tibial tuberosity. Additional soft tissue reconstruction techniques were all aimed at restoring lateral and medial retinacular function including reconstruction with overlap of the lateral retinaculum, modified lateral reinforcement by using absorbable suture material, and proximal tube realignment (Flo and Brinker, 1970; Hulse, 1995; Wangdee and Kalpravidh, 2008).

A lateral parapatellar skin incision was made, starting from the tibial tuberosity lateral to the patellar ligament and continued proximally to the patella and then extending an equal distance along the cranial border of the femur. Arthrotomy was performed by incision through the fascia lateral to the patellar ligament and patella. Sufficient tissue was left on the lateral side of the patella to enable adequate closure of the joint and reconstruction of the lateral retinaculum. The cranial and caudal cruciate ligaments and the medial and lateral menisci were inspected to rule out additional pathology. Desmotomy of the medial retinacular structures and incision of the joint capsule 3 to 5 mm from and parallel to the patella was performed when there was excessive tension on the patella in the medial compartment, thereby enabling lateral realignment of the patella. In dogs with grade 3 or grade 4 of MPL, the medial joint capsule and medial retinaculum were always released.

With TBR, the width of the block was chosen to accommodate the width of the patella while preserving the trochlear ridges. A trochlear block technique was started by performing two longitudinal osteotomies in the medial and lateral ridges of the femoral trochlea. The trochlear block was released by executing two osteotomies from proximal and distal, respectively. A sufficient amount of subchondral bone was preserved, to avoid fracture of the osteochondral block. Additional subchondral bone was removed to lower the trochlear block by 3 to 6 mm, using a small bone rongeur. Then the trochlear block was replaced into the recessed defect. The depth of the trochlea was at least equal to 50% of the height of the patella (Fig. 1B) (Talcott et al., 2000). TTT was performed using osteotomy starting 3 to 5 mm caudal to the patellar tendon in a distal direction while preserving the distal periosteal attachment of the tibial crest. The tibial tuberosity was transposed laterally to realign the patellar tendon, and the tibial crest was stabilised using one or two 1 mm diameter Kirschner wires.

After surgery, cefazolin (25 mg/kg) was administered for seven days and carprofen (Rimadyl: Pfizer, New York, USA; 4 mg/kg per day) for 14 days. The dogs were restricted to short leash-walks for six weeks.

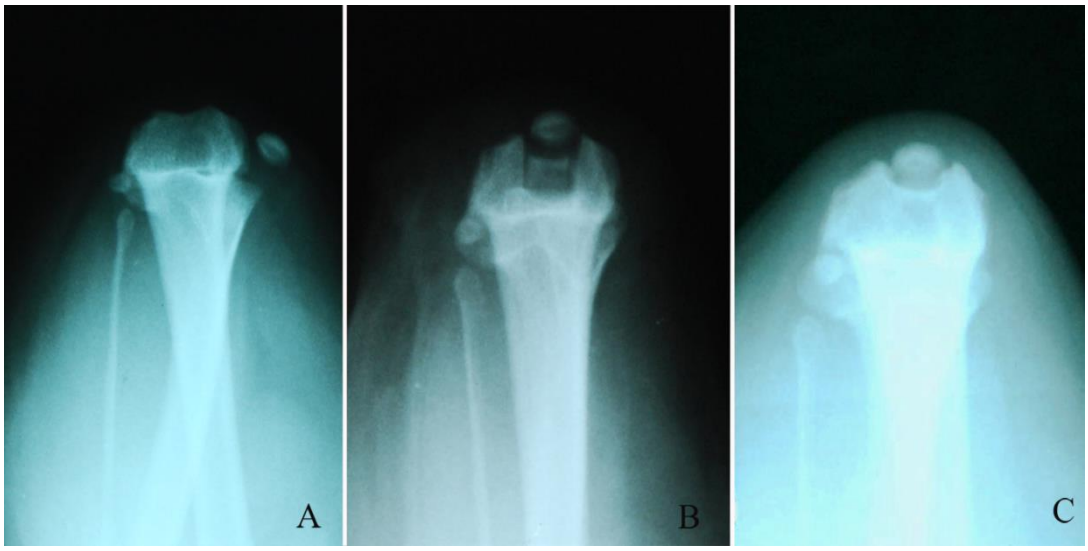


Figure 1 Skyline views of medial patellar luxation before and after surgery.
A 22-month-old female Pomeranian with grade 2 medial patellar luxation before surgery **(A)**. Trochlear block recession has been performed and patella is seated in the new groove **(B)**. 20 weeks postoperatively, the patella is in the trochlear groove with healed osteotomy **(C)**.

Evaluation

All dogs were evaluated at two, four, six, eight, 10, 12, and 16 weeks, with the same clinician (CW) scoring lameness during walking and trotting. The final outcome of treatment was established at least 16 weeks after surgery. The follow-up period was extended if a dog had a recurrent luxation or had persistent grade 3 or 4 lameness. The signalment, time after surgery of recurrence, outcome, and surgical techniques used for any dog with recurrent MPL were recorded. In dogs with recurrent PL, final lameness score after the revision surgery had been performed was assessed and used for statistical analysis.

Statistical analysis

Data for age and weight were summarized. The relative frequency of grade 2, 3 and 4 MPL in joints was calculated. The Wilcoxon signed rank test was used to compare differences in lameness score before and after surgery at two, four, six, eight, 10, 12, and 16 weeks for each grade of PL. Statistical significance was considered at a $P < 0.05$.

Results

There were 55 Pomeranians with MPL included in the study; 27 (49%) were female and 28 (51%) were male dogs. The age of the dogs was 26.9 ± 22.1 months (range 3–97 months), and their body weight was 3.1 ± 1.5 kg (range 0.8–8 kg). Luxation was bilateral in 51 dogs (93%) and unilateral in four dogs (7%). Surgery was performed unilaterally in 40 dogs and bilaterally in 15 dogs (70 stifles in total). The severity of MPL in these 70 stifle joints, was grade 2 (n = 33), grade 3 (n = 26) and grade 4 (n = 11). TBR was necessary in all 70 joints because the trochlear sulcus was shallow, flattened, or even convex (Fig. 1). TTT was performed as an additional procedure in two joints with grade 2, 11 joint with grade 3, and 11 joints with grade 4 MPL.

The outcome of surgical correction of medial patellar luxation

Corrective surgery for MPL in Pomeranians was successful in 90% (n = 63) of the stifles. Recurrent PL occurred in six dogs (7 stifle joints) that initially were diagnosed with a grade 3 (n = 3/26) or 4 (n = 4/11) MPL.

Management of recurrence

The signalment, time after surgery to recurrence, outcome, and type of surgery performed in the six dogs with recurrent MPL are shown in Table 1. Revision surgery was performed in five dogs (6 stifles). A second surgery was not considered necessary in one dog with a grade 1 MPL. Bilateral corrective osteotomy of the femur was performed in one dog with recurrence due to femoral and crural deformities. Arthrodesis was performed in one dog with preoperative grade 4 MPL (dog 5) to relieve pain and recurrent PL after the second surgical procedure.

Table 1 Detail of each dog with recurrent patellar luxation. Lameness scoring system of 1-4 as described in the text

Dog	Grade*	Surgical techniques	Weight (kg)	Age (months)	Recurrence time (weeks)	Lameness score† (preop.)	Revision surgery	Result
1	3	TBR	5.6	36	12	2	TBR (made it wider), lateral suture plus medial desmotomy and imbrication of lateral retinaculum	Walked well with lameness score 1 due to weight gain
2	3	TBR	3.2	20	10	1	None	Walked with lameness score 1
3	3	TBR plus TTT	1.7	23	8	3	Lateral suture, medial desmotomy and imbrication of lateral retinaculum	Walked with lameness score 0
4	4	TBR plus TTT	2.5	30	8	2	TBR (made it wider) and imbrication of lateral retinaculum	Walked with lameness score 0
5	4	TBR plus TTT	1	3	4	3	TBR, TTT, lateral suture plus medial desmotomy and imbrication of lateral retinaculum	Development of deformity after the 2nd surgery Lameness score 3 ‡
6 left	4	TBR plus TTT	1.3	5	4	4	Femoral osteotomy, TTT and medial desmotomy	Patella in the normal position Lameness score 1
6 right	4	TBR plus TTT	1.3	7	4	4	Femoral and tibial osteotomy, TTT, medial desmotomy and imbrication of lateral retinaculum	Good limb alignment but MPL grade 4 remained Lameness score 1

* Grade of medial patellar luxation (MPL) defined in the text; †Lameness scores as given in the text; ‡Third surgery with arthrodesis to correct limb deformity and to relief pain; TBR = trochlear block recession; TTT = tibial tuberosity transposition; preop. = preoperative.

Lameness score

Median lameness score of grade 4 MPL cases prior to surgery was higher than scores of grade 2 and 3 MPL. Lameness scores decreased at four weeks after surgery in all grades (Table 2 and Fig. 2). The lameness score was significantly improved from four weeks after surgery until the end of the follow-up period in comparison with preoperative lameness score in all grades of MPL ($P < 0.05$).

Table 2 Lameness scores of limbs after surgical correction of medial patellar luxation

MPL grade*	weeks after primary surgical correction or revision surgery†							
	0	2	4	6	8	10	12	16
2 (n=33)	2 (1-4)	2 (0-3)	1 (0-3)	1 0-3)	0 (0-3)	0 (0-3)	0 (0-3)	0 (0-4)
3 (n=26)	2 (2-4)	2 (1-3)	2 (0-3)	1 0-3)	0 (0-2)	0 (0-2)	0 (0-2)	0 (0-2)
4 (n=11)	3 (3-4)	2.5 (1-4)	2 (0-4)	1 (0-4)	1 (0-3)	1 (0-4)	0 (0-4)	0 (0-4)

*Grade of medial patellar luxation (MPL) as defined in the text. Values are shown as median (range).

†Details of revision surgery described in Table 1.

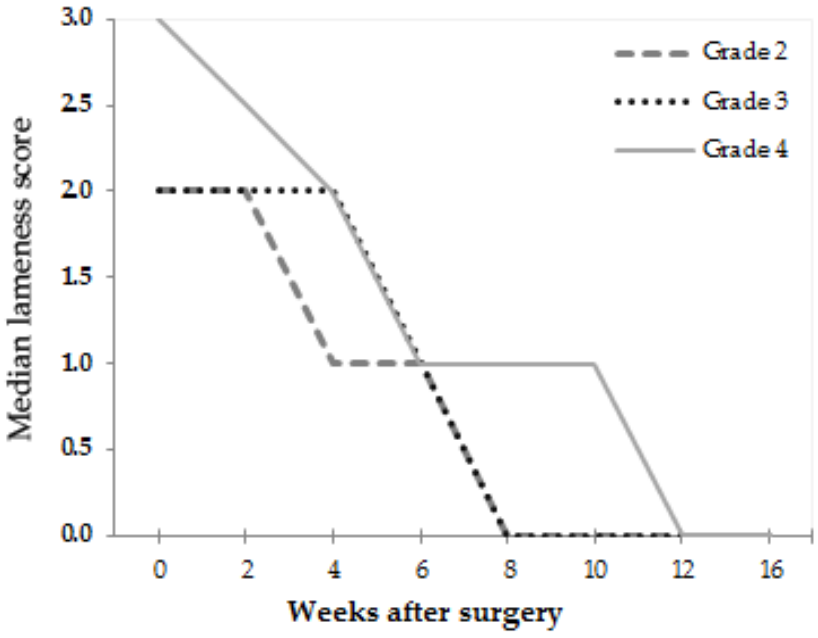


Figure 2 Median lameness score of dogs with grade 2, 3 and 4 medial patellar luxation before surgery and at 2, 4, 6, 8, 10, 12, and 16 weeks after surgery.

Discussion

MPL occurred bilaterally in 93% of our patient group, which is in line with other reports (Hayes et al., 1994; LaFond et al., 2002). Most of the dogs undergoing surgical correction were suffering from grade 2 or 3 MPL, which is the same as in other reports (Roy et al., 1992; Alam et al., 2007; Daems et al., 2009). The equal gender distribution is also similar to results of other studies (Daems et al., 2009). Surgery involved a combination of techniques to realign the displaced structures in the extensor mechanism and to deepen the trochlear sulcus, with the choice being dependent on preoperative and intra-operative evaluation. In this study, all Pomeranians with MPL had a very shallow trochlear sulcus, and therefore TBR deepening of the trochlear sulcus was conducted in all cases. Additional TTT was considered necessary to realign the extensor mechanism of the stifle in order to support the reduction of the patella within the trochlear sulcus, and was performed in all dogs with MPL grade 4. These techniques are advocated for retaining the patella within the trochlear sulcus and have a high success rate in Pomeranians (Roush et al., 1993; Slocum and Slocum, 1993).

In our study, PL recurred in 10% of the dogs, which is similar to the report of Alam et al. (6%), but lower than the 19.8% incidence reported by Linney et al. (2011) who treated MPL with a combination of lateral retinaculum imbrication and TTT techniques, but without TBR technique (Alam et al., 2007). We agree with the previous recommendations that at least one corrective osteotomy such as TTT, femoral trochleoplasty or tibial plateau levelling osteotomy with tibial axial re-alignment should be performed to reduce the patellar reluxation rate (Arthurs and Langley-Hobbs, 2007). In our study, the treatment outcome of grade 2 and 3 MPL after TBR was 100% and 88%, respectively, and weight bearing and lameness improved following surgery.

In this study, persistent lameness was seen in 6 dogs (8.6%) with a stable patella, which might be due to cartilage damage and osteoarthritis. The recurrence of luxation at four and 12 weeks after surgery in the dogs with grade 3 and 4 MPL might have been because tension on the side of luxation was inadequately relieved by surgery, TBR deepening of the trochlear sulcus was inadequately, or there was severe disturbance of the alignment of the extensor mechanism. In those dogs that appeared to have a limb deformity, the femoral varus angle has to be considered when performing corrective osteotomy (Arthurs and Langley-Hobbs, 2006; Roch et al., 2008; Soparat et al., 2012). However, corrective osteotomy is challenging in dogs

weighing less than 3 kg with a large femoral varus angle. Surgical correction of grade 3 and 4 MPL has been recommended at a young age in dogs, in order to realign the extensor mechanism (L´Eplattenier and Montavon, 2002). However in growing animals, corrective osteotomy carries the danger of compromising femoral and crural growth plate function.

Conclusion

MPL in Pomeranians was successfully managed with femoral trochleoplasty and soft tissue reconstruction, with additional tibial tuberosity transposition in cases of malalignment of the patellar tendon. The complications included reluxation of the patella in 10% of stifle joints and persistent lameness in 20% of stifle joints. The outcome of surgery was considered good for grade 2 luxation with a 100% success rate; recurrent PL occurred in about 11% of stifle joints with grade 3 MPL. The dogs with grade 4 PL had varying degrees of skeletal deformity, and PL recurred in 36% of these dogs. Alignment correction including corrective osteotomy should be considered in selected cases of grade 4 MPL (Soparat et al., 2012).

Acknowledgments

Authors would like to thank staff of the Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University, Thailand. We are grateful for Dr. Jane Sykes' help in correction of our English and for the statistical consultancies provided by Pichai Jirawattanapong.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

Alam, M.R., Lee, J.I., Kang, H.S., Kim, I.S., Park, S.Y., Lee, K.C., Kim, N.S., 2007. Frequency and distribution of patellar luxation in dogs: 134 cases (2000 to 2005). Veterinary and Comparative Orthopaedics and Traumatology 20, 59-64.

Arthurs, G.I., Langley-Hobbs, S.J., 2006. Complications associated with corrective surgery for patellar luxation in 109 dogs. Veterinary Surgery 35, 559-566.

Arthurs, G.I., Langley-Hobbs, S.J., 2007. Patellar luxation as a complication of surgical intervention for the management of cranial cruciate ligament rupture in dogs. A retrospective study of 32 cases. Veterinary and Comparative Orthopaedics and Traumatology 20, 204-210.

Daems, R., Janssens, L.A., Beosier, Y.M., 2009. Grossly apparent cartilage erosion of the patellar articular surface in dogs with congenital medial patellar luxation. Veterinary and Comparative Orthopaedics and Traumatology 22, 222-224.

DeAngelis, M., 1971. Patellar luxation in dogs. Veterinary Clinics of North America: Small Animal Practice 1, 403-415.

Flo, G.F., Brinker, W.O., 1970. Fascia lata overlap procedure for surgical correction of recurrent medial luxation of the patella in the dog. Journal of the American Veterinary Medical Association 156, 595-599.

Hayes, A.G., Boudrieau, R.J., Hungerford, L.L., 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal of the American Veterinary Medical Association 205, 716-720.

Hazewinkel, H.A., van den Brom, W.E., Theyse, L.F., Pollmeier, M., Hanson, P.D., 2008. Comparison of the effects of firocoxib, carprofen and vedaprofen in a sodium urate crystal induced synovitis model of arthritis in dogs. Research in Veterinary Science 84, 74-79.

Hulse, D.A., 1981. Pathophysiology and management of medial patellar luxation in the dog. Veterinary Medicine: Small Animal Clinician 76, 43-51.

Hulse, D.A., 1993. Medial patellar luxation in the dog. In: Bojrab, M.J. (Ed.), Disease Mechanisms in Small Animal Surgery. Lea&Febiger, Philadelphia, pp. 808-817.

Hulse, D.A., 1995. The Stifle Joint. In: Olmstead, M.L. (Ed.), Small Animal Orthopedic. Mosby, St. Louis, pp. 395-404.

LaFond, E., Breur, G.J., Austin, C.C., 2002. Breed susceptibility for developmental orthopedic diseases in dogs. Journal of the American Animal Hospital Association 38, 467-477.

L'Eplattenier H., Montavon P., 2002. Patellar luxation in dogs and cats: management and prevention. Compendium on Continuing Education for the Practicing Veterinarian 24, 292-300.

Linney, W.R., Hammer, D.L., Shott, S., 2011. Surgical treatment of medial patellar luxation without femoral trochlear groove deepening procedures in dogs: 91 cases (1998-2009). Journal of the American Veterinary Medical Association 238, 1168-1172.

OFA, 2012. Orthopedics Foundation for Animals: Patellar luxation statistics. http://www.offa.org/stats_pl.html (accessed March 9, 2013).

Piermattei, D.L., Flo, G.L., DeCamp, C.E., 2006. The stifle joint. In: Piermattei, D.L., Flo, G.L., DeCamp, C.E. (Eds.), Handbook of Small Animal Orthopedics and Fracture Repair. Fourth Ed. Saunders, St. Louis, pp. 562-632.

Roch, S.P., Gemmill, T.J., 2008. Treatment of medial patellar luxation by femoral closing wedge ostectomy using a distal femoral plate in four dogs. Journal of Small Animal Practice 49, 152-158.

Roush, J.K., 1993. Canine patellar luxation. Veterinary Clinics of North America: Small Animal Practice 23, 855-868.

Roy, R.G., Wallace, L.J., Johnston, G.R., Wickstrom, S.L., 1992. A retrospective evaluation of stifle osteoarthritis in dogs with bilateral medial patellar luxation and unilateral surgical repair. Veterinary Surgery 21, 475-479.

Slocum, B., Slocum, T.D., 1993. Trochlear wedge recession for medial patellar luxation. An update. Veterinary Clinics of North America: Small Animal Practice 23, 869-875.

Soontornvipart, K., Wangdee, C., Kalpravidh, M., Brahmasa, A., Sarikaputi, M., Temwichitr, J., Lavrijsen, I.C., Theyse, L.F., Leegwater, P.A., Hazewinkel, H.A., 2013. Incidence and genetic aspects of patellar luxation in Pomeranian dogs in Thailand. The Veterinary Journal 196, 122-125.

Soparat, C., Wangdee, C., Chuthatep, S., Kalpravidh, M., 2012. Radiographic measurement for femoral varus in Pomeranian dogs with and without medial patellar luxation. Veterinary and Comparative Orthopaedics and Traumatology 25, 197-201.

Talcott, K.W., Goring, R.L., De Haan, J.J., 2000. Rectangular recession trochleoplasty for treatment of patellar luxation in dogs and cats. Veterinary and Comparative Orthopaedics and Traumatology 13, 39-43.

Wangdee, C., Kalpravidh, M., 2008. Tube realignment for patellar luxation repair in dogs. Thai Journal of Veterinary Medicine 38, 39-44.

Chapter 4

Extended proximal trochleoplasty for the correction of bidirectional patellar luxation in Pomeranian dogs

C. Wangdee[1,2*], H.A.W. Hazewinkel[2], J. Temwichitr[3], L.F.H. Theyse[2]

[1] Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

[2] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

[3] Department of Companion Animals Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

Published in Journal of Small Animal Practice 2014

DOI 10.1111/jsap.12248

Abstract

Seven Pomeranians with bidirectional patellar luxation (BPL) were prospectively studied regarding aetiology and results of a new surgical technique. Radiographic evaluation of the ratio between patellar ligament length (PLL) and patellar bone length (PBL) revealed no differences between Pomeranians with BPL and healthy stifle joints. Functional rather than anatomic patella alta might be associated with BPL in Pomeranians. The surgical outcome of extended proximal trochleoplasty was good-to-excellent in 87.5% of the stifles and all dogs achieved functional recovery. There was only minimal radiographic progression of osteophyte formation at 48 weeks after surgery. To the authors' knowledge, this is the first report on BPL in small-breed dogs and its successful surgical treatment.

Introduction

Patellar luxation (PL) is one of the most common orthopaedic disorders of small-breed dogs (Hayes et al., 1994; OFA, 2013). Surgery is aimed at realigning and stabilising the extensor mechanism of the stifle joint and at re-establishing normal limb function. The patella can luxate medially, laterally, or both in a medial and lateral direction (Vidoni et al., 2006). The term bidirectional patellar luxation (BPL) is used when the patella luxates both medially and laterally. Dogs with BPL typically show hyperextension of the stifle in stance. In stifle extension, the patella is positioned proximal in the trochlear groove with decreased support of the medial and lateral trochlear ridges. Patella alta, characterized by proximal displacement of the patella within the femoral trochlear groove, can be described as the ratio of the patellar ligament length (PLL) to the patellar bone length (PBL). In large- breed dogs, an increased PLL:PBL ratio is associated with medial PL (Johnson et al., 2006; Mostafa et al., 2008).

Surgical procedures to correct unidirectional medial patellar luxation (UMPL) involve a combination of soft tissue reconstruction, femoral trochlear groove deepening, and lateral transposition of the tibial crest (Roush, 1993; Arthurs & Langley-Hobbs, 2007). Surgical procedures to treat BPL effectively have not yet been described.

This study investigated the involvement of patella alta in BPL and evaluated the results of a novel extended proximal trochleoplasty technique in combination with a lateral and medial retinacular imbrication in Pomeranians.

Materials and methods

Animals

Seven dogs with BPL were included in this prospective study, based on the results of a standardised orthopaedic protocol (Piermattei et al., 2006). PL was graded as described previously (Putnam, 1968). The gender distribution, age, and weight of the dogs are given in table 1. Lameness was assessed preoperatively and during clinical check-ups using a visual analogue scale (Hazewinkel et al., 2008).

Mediolateral radiographs were made at a stifle angle of 135° (range 130-145°) under general anaesthesia to measure the PLL and PBL digitally and assess the PLL:PBL ratio as described by Mostafa et al. (2008). Additionally, craniocaudal radiographs were performed to evaluate femoral and tibial alignment and all cases

had normal limb alignment. The PLL:PBL ratio was compared with the ratio in normal stifle joints in 34 Pomeranians.

Surgical treatment

For surgical treatment, a lateral parapatellar skin incision was started proximal to the patella and extending distal to the tibial tuberosity. Arthrotomy was performed through the fascia and retinacular structures lateral to the patellar ligament and patella.

An extended proximal trochleoplasty was performed using a modified trochlear wedge recession, with the view to deepening and extending the proximal part of the trochlear groove well into the distal femoral cortical bone. To achieve this, the periosteum of the distal femur was incised longitudinally, starting from the medial and lateral trochlear ridge in a proximal direction. The wedge resection was started proximally, leaving a wide base at the entrance of the trochlear groove, and lengthened distally while preserving the medial and lateral trochlear ridges (Fig 1A). The wedge resection was completed while preserving the proximal periosteal attachment of the wedge (Fig 2A). The periosteum at the proximal part of the wedge was elevated from lateral to medial from the cortical bone. The wedge was mobilised and rotated proximally to allow full access to the subchondral bone of the groove (Fig 1B).

Cancellous bone was removed from the underside of the wedge and from the groove to allow for adequate recession. Special care was taken to extend and deepen the trochlear groove proximally by removing cortical bone from the distal femur, using a dedicated small bone rongeur. Then the trochlear wedge was positioned into the recessed defect, making sure that the distal tip was flush with the articular cartilage of the trochlear groove (Fig 1C and 2B). The medial and lateral retinacular ligaments and joint capsule were imbricated using a standard technique.

All dogs received 25 mg/kg/day cephalosporin (Sporidin; Ranbaxy) and 4 mg/kg/day carprofen (Rimadyl; Pfizer) orally for 7 days. They were restricted to short leash walks for 6 weeks.

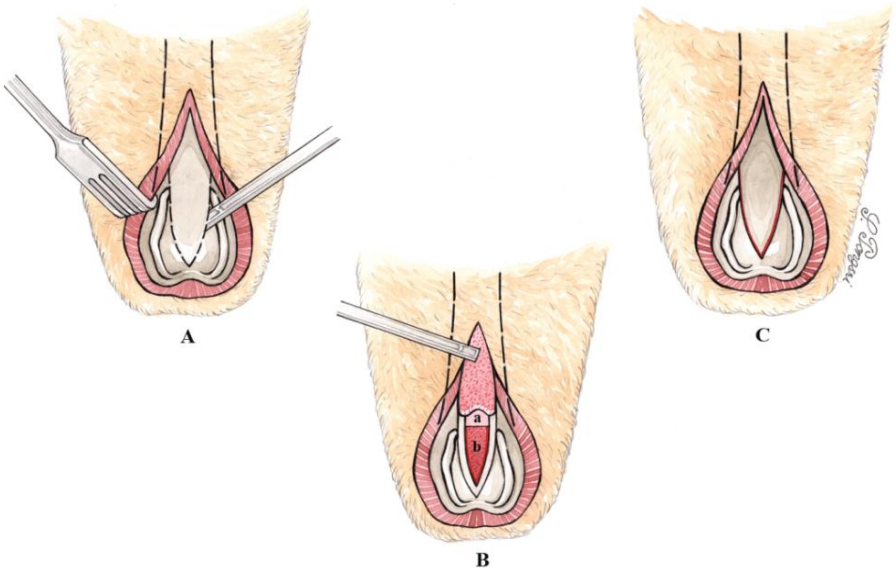


Figure 1 Extended proximal trochleoplasty technique (drawing from S.Pongsri).
An osteotome is inserted and the wedge is extended proximal to the normal position of the patella in the distal femur **(A)**. The periosteum at the proximal part of the wedge was elevated from the cortical bone while preserving its attachment to the cartilaginous part of the wedge **(B)**. Cortical bone from the distal femur (a) is removed by using a dedicated small bone rongeur, and additional cancellous bone (b) is removed from the underside of the wedge and from the groove to allow for adequate recession of the wedge. The trochlear wedge is positioned into the recessed defect **(C)**.

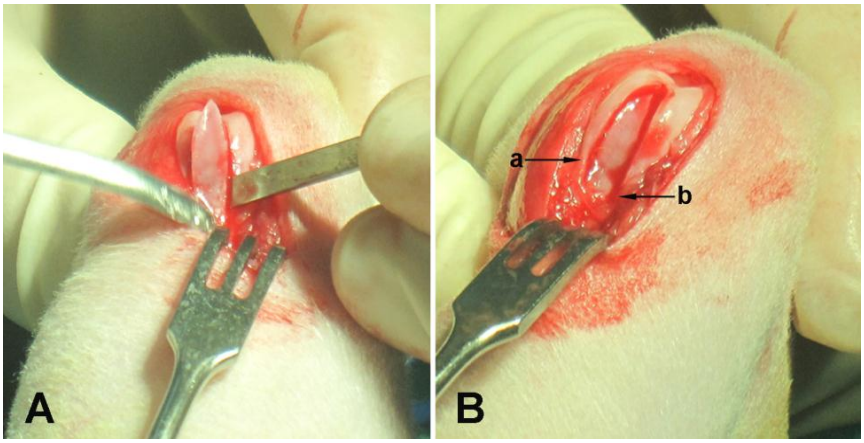


Figure 2 Extended proximal trochleoplasty performed in a 21-month-old male Pomeranian with bidirectional patellar luxation.
An osteotome is inserted and extended proximally into the distal femur **(A)**. The trochlear groove is deepened and the end of trochlear ridge and the end of the xtended proximal trochleoplasty are shown at arrow "a" and "b", respectively. The trochlear wedge, still attached to the periosteum, is positioned in the deepened groove **(B)**.

Evaluation

The dogs were assessed for lameness during locomotion and the stability of the patella was evaluated manually at 2, 4, 6, 8, 10, 12, and 48 weeks after surgery. Radiographic images were obtained before and at 48 weeks after surgery, with craniocaudal and mediolateral views in all dogs.

Osteophyte formation was evaluated on the radiographs (Fig 3A and 3B) at four points on craniocaudal views (medial and lateral femoral condyles, and medial and lateral edge of the tibial plateau) and at four points on mediolateral views (patella, proximal trochlear sulcus, cranial and caudal parts of the tibial plateau). Osteophyte formation was scored from absent (0) to severe (3) (Frost-Christensen et al., 2008). The total osteophyte score was used for statistical analysis (maximum score 24). One-way analysis of variance (ANOVA) was used to compare differences in the PLL:PBL ratio between the two groups. The Wilcoxon signed rank test was used to compare differences in osteophyte scoring before and 48 weeks after surgery. A P of < 0.05 was considered significant.

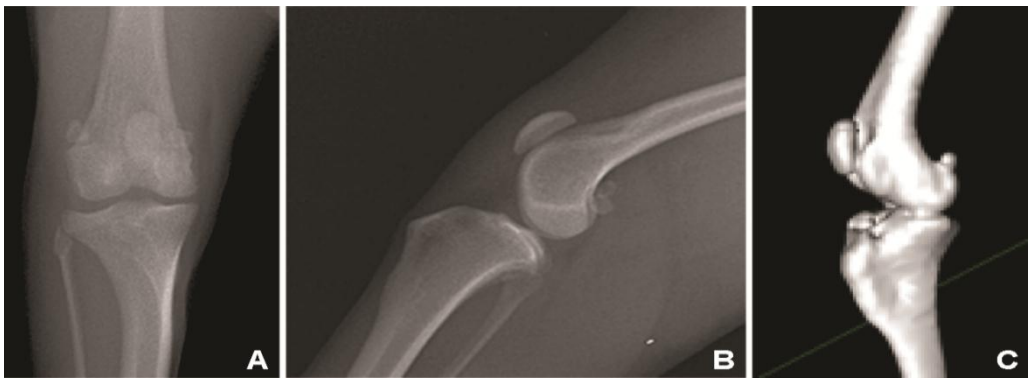


Figure 3 Radiographic image views and computed tomography (CT) image of a stifle joint with bidirectional patellar luxation at 1 month after surgery.

A 18-month-old male Pomeranian with bidirectional grade 2 patellar luxation. The patella can be seen lying in the new groove on craniocaudal view **(A)** elongated extended groove is seen on mediolateral view **(B)** and on CT image **(C)**.

Results

The mean age of the dogs was 35.4 ± 29.7 months and the mean weight was 2.8 ± 0.5 kg. All dogs had a grade 2 BPL. Six dogs had unilateral BPL and one dog had bilateral BPL. The PLL:PBL ratio of 1.87 ± 0.12 was not significantly different from that of healthy stifle joints (1.82 ± 0.14). Therefore there were no indications that anatomic patella alta was associated with BPL.

Surgical outcomes were good-to-excellent in seven of the eight stifle joints. Healing was uneventful and at 6 weeks after surgery all dogs showed full functional recovery without any signs of lameness. Dog number 5 had a recurrence of a grade 1 PL 12 weeks after surgery. This dog ultimately developed grade 2 UMPL with a lameness score of 1 at 48 weeks. Although the progression of osteophyte formation was minimal in all dogs at 48 weeks after surgery, the increase was statistically significant ($P < 0.05$) compared with preoperative scores (Table 1).

Table 1 Signalment, grade of bidirectional luxation, preoperative and postoperative lameness scores, and osteophyte sum score (before and after surgery).

No	Signalment *				Preoperative lameness score ***	Lameness score *** (weeks postoperative)							Sum of osteophyte score†	
	Sex	Age (month)	Weight (kg)	Grade **		2	4	6	8	10	12	48	Preoperative	48 weeks Postoperative
1	M	84	2.2	2	1	0	0	0	0	0	0	0	0	1
2	M	18	3	2	1	1	0	0	0	0	0	0	0	1
2	M	20	3	2	1	2	0	0	0	0	0	0	0	1
3	F	12	3.6	2	2	0	0	0	0	0	0	0	0	1
4	M	21	2.3	2	1	0	0	0	0	0	0	0	0	1
5	M	80	3.1	2	2	1	0	0	0	0	0	1	0	1
6	M	36	3	2	2	0	0	0	0	0	0	0	1	2
7	F	12	2.2	2	2	2	1	0	0	0	0	0	1	4

* Signalment: M=male, F=female

** Grade 2 of bidirectional patellar luxation defined as patella luxated easily both medially and laterally, especially when the foot and tibia are rotated, while the patella was pushed or pulled in medial or lateral direction. Reduction occurred with the opposite maneuvers

*** Lameness score 0 = no lameness; 1 = intermittent mild lameness; 2 = continuous moderate lameness (see text for reference)

† osteophyte scoring defined in the text

Discussion

The prevalence of BPL is also reported in other small- and miniature- breed dogs (Vidoni et al., 2006). It was unusual that two of the Pomeranians with BPL were older than 6 years at that time of referral, because clinical signs of UMPL usually develop at an earlier age (Wangdee et al., 2013). A thorough anamnesis revealed that these two dogs had signs of PL when younger, and that signs had gradually worsened over time.

An increased PLL:PBL ratio was not found as a causative factor in BPL. This is in contrast with the finding of an association between patella alta and UMPL in large-breed dogs (Johnson et al., 2006; Mostafa et al., 2008). As there were no differences between the PLL:PBL ratios in Pomeranians with BPL and normal stifle joints, an explanation for the bidirectional luxation could be a functional patella alta as a result of hyperextension of the stifle joint during locomotion in these dogs. Therefore, the aim of the surgical procedure was to deepen and to lengthen the proximal part of the trochlear groove (Fig 3B and 3C). In combination with reconstruction of the retinacular ligaments and joint capsule, extending the groove proximally into the distal femoral cortical bone appears to be effective in treating BPL in Pomeranians. Preservation of the periosteal attachment of the trochlear wedge was deemed essential to optimize blood supply to the wedge and to act as a cover of the exposed cancellous and cortical bone of the distal femur. Based on the clinical and radiographic data, there was no displacement of the trochlear wedges. There was no indication for patellar cartilage erosion nor pathology of the extended groove as supported by the absence of enthesophyte formation in the proximal part of the patella and of the osteophyte formation in the extended groove. This procedure led to a speedy recovery of normal limb function.

The outcome was favourable in the majority of cases. PL grade 2 recurred in one dog; however, the low number of cases in this study makes comparisons difficult. Osteophyte formation was seen in all dogs, as has been reported previously in dogs undergoing surgery for PL (Roy et al., 1992; Alam et al., 2011) because of damage to the joint cartilage (Frost-Christensen et al., 2008). The increase in osteophyte formation was radiologically significant but clinically insignificant in the dogs in this study. It can be concluded that deepening and extending the trochlear groove well into the distal femoral diaphysis in combination with reconstruction and imbrication of the medial and lateral retinacular structures and joint capsule, is a

favourable technique in treating BPL though some development of degenerative joint disease is expected.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Acknowledgments

This Study was supported by Chulalongkorn University, Bangkok, Thailand. Authors would like to thank staffs of the Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University, and staffs of the Department of Companion Animals Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Thailand. We are grateful for correcting the English by Dr. Jane Sykes, for drawing a diagram of the surgical technique by Supachai Pongsri and for the statistical consultancies of Pichai Jirawattanapong.

References

Alam, M.R., Lee, H.B., Kim, M.S., Kim, N.S., 2011. Surgical model of osteoarthritis secondary to medial patellar luxation in dogs. Veterinarni Medicina 3, 123-130.

Arthurs, G.I., Langley-Hobbs, S.J., 2007. Patellar luxation as a complication of surgical intervention for the management of cranial cruciate ligament rupture in dogs. A retrospective study of 32 cases. Veterinary and Comparative Orthopaedics and Traumatology 20, 204-210.

Frost-Christensen, L.N., Mastbergen, S.C., Vianen, M.E., Hartog, A., DeGroot, J., Voorhout, G., van Wees, A.M., Lafeber, F.B., Hazewinkel, H.A., 2008. Degeneration, inflammation, regeneration, and pain/disability in dogs following destabilization or articular cartilage grooving of the stifle joint. Osteoarthritis Cartilage 16, 1327-1335.

Hayes, A.G., Boudrieau, R.J., Hungerford, L.L., 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal of the American Veterinary Medical Association 205, 716-720.

Hazewinkel, H.A., van den Brom, W.E., Theyse, L.F., Pollmeier, M., Hanson, P.D., 2008. Comparison of the effects of firocoxib, carprofen and vedaprofen in a sodium urate crystal induced synovitis model of arthritis in dogs. Research in Veterinary Science 84, 74-79.

Johnson, A.L., Broaddus, K.D., Hauptman, J.G., Marsh, S., Monsere, J., Sepulveda, G., 2006. Vertical patellar position in large-breed dogs with clinically normal stifles and large-breed dogs with medial patellar luxation. Veterinary Surgery 35, 78-81.

Mostafa, A.A., Griffon, D.J., Thomas, M.W., Constable, P.D., 2008. Proximodistal alignment of the canine patella: radiographic evaluation and association with medial and lateral patellar luxation. Veterinary Surgery 37, 201-211.

OFA, 2013. Orthopedics Foundation for Animals: Patellar luxation statistics. http://www.offa.org/stats_pl.html (accessed March 9, 2014).

Piermattei, D.L., Flo, G.L., DeCamp, C.E., 2006. The stifle joint. In: Piermattei, D.L., Flo, G.L., DeCamp, C.E. (Eds.), Handbook of Small Animal Orthopedics and Fracture Repair. Fourth Ed. Saunders, St. Louis, pp. 562-632.

Putnam, R.W., 1968. Patellar luxation in the dog. Masters thesis, University of Guelph, Canada.

Roush, J.K., 1993. Canine patellar luxation. Veterinary Clinics of North America: Small Animal Practice 23, 855-868.

Roy, R.G., Wallace, L.J., Johnston, G.R., Wickstrom, S.L., 1992. A retrospective evaluation of stifle osteoarthritis in dogs with bilateral medial patellar luxation and unilateral surgical repair. Veterinary Surgery 21, 475-479.

Vidoni, B., Sommerfeld-Stur, I., Eisenmenger, E., 2006. Diagnostic and genetic aspects of patellar luxation in small and miniature breed dogs in Austria. The European Journal of Companion Animal Practice 16, 149-158.

Wangdee, C., Theyse, L.F., Techakumphu, M., Soontornvipart, K., Hazewinkel, H.A., 2013. Evaluation of surgical treatment of medial patellar luxation in Pomeranian dogs. Veterinary and Comparative Orthopaedics and Traumatology 26, 435-439.

Chapter 5

Incidence and genetic aspects of patellar luxation in Pomeranian dogs in Thailand

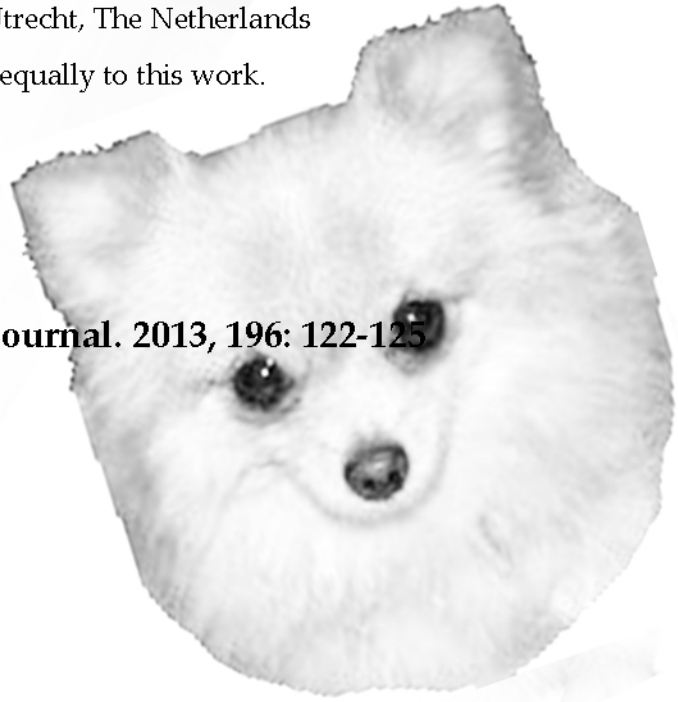
K. Soontornvipart[1,*], C. Wangdee[1,4,*], M. Kalpravidh[1], A. Brahmasa[1], M. Sarikaputi[2], J. Temwichitr[3], I.C.M. Lavrijsen[4], L.F.H. Theyse[4], P.A.J. Leegwater[4], H. A.W. Hazewinkel[4]

[1] Department of Veterinary Surgery, [2]Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

[3] Department of Companion Animals Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

[4] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

* These authors contributed equally to this work.



Adapted from The Veterinary Journal. 2013, 196: 122-125

Abstract

There is a high incidence of patellar luxation (PL) in Pomeranian dogs from Thailand. DNA samples were collected from 59 dogs originating from 15 families. PL was present in 75% of the dogs with a male:female ratio of 1:1.95. Polymorphic microsatellites situated close to the *COL6A1*, *COL6A3*, *COL9A1*, *COL9A2*, and *COL9A3* genes were analysed for linkage to the phenotype. Sibling-pair analysis revealed that none of the collagen markers analysed had a high non-parametric linkage score with the highest score, 1.56, for *COL9A2* ($P = 0.07$). The low LOD scores for these collagen genes indicated a non-involvement in the pathogenesis of PL in Pomeranians. An association study with a low density single nucleotide polymorphism (SNP) set indicated the possible involvement of a region on chromosome 7. The association of this region remained indicative when larger groups of 43 cases and 40 controls were compared (Chi square test $P = 0.01$).

Introduction

Patellar luxation (PL) is one of the most common orthopedic disorders found in small-breed dogs, especially Pomeranians. It has been suggested that the disease is inherited (Hayes et al., 1994). The mode of inheritance is unknown because the phenotype has not been analysed in large family groups. An insight into the genes involved could be used in breeding programs and aetiology studies of PL.

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). Pomeranians are currently also the highest ranking breed for PL in the USA, with 42.4% being affected (OFA, 2011). Defects in collagen formation underlie a number of orthopedic diseases in humans, and these disorders are often caused by inherited mutations in genes encoding collagen proteins (Salg et al., 2006). As collagen genes are suggested to be involved in PL and hyperextension syndrome in dogs (Temwichitr et al., 2007), we investigated the possible involvement of these candidate genes in PL in Pomeranian dogs.

Materials and Methods

A total of 238 Pomeranians presented at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University during 2006-2008 were screened for PL. All dogs were investigated according to a standard orthopedic protocol using a PL grading system (Piermattei et al., 2006). Blood (5 ml) was collected from 45 affected and 14 unaffected Pomeranians originating from 15 families (Fig. 1; selection A). DNA was isolated by the salt extraction method (Miller et al., 1988) and analysed for co-segregation of the phenotype with five polymorphic microsatellites situated close to five collagen genes: i.e., *COL6A1*, *COL6A3*, *COL9A1*, *COL9A2*, and *COL9A3* (Table 1). The development of these microsatellite markers and the method of genotyping have been described previously (Temwichitr et al., 2007).

Table 1 Genomic locations of candidate genes for patellar luxation with known phenotypes in man.

Gene	CFA[a]	Mb[b]	OMIM	Human phenotype of gene mutation
COL6A1	31	39.31	MIM ID 120220	Bethlem myopathy, Ullrich congenital muscular dystrophy
COL6A3	25	48.05	MIM ID 120250	Bethlem myopathy
COL9A1	12	32.81	MIM ID 120210	Multiple epiphyseal dysplasia
COL9A2	15	2.64	MIM ID 120260	Multiple epiphyseal dysplasia
COL9A3	24	46.65	MIM ID 120270	Multiple epiphyseal dysplasia

[a]CFA = *Canis familiaris* chromosome number; [b]Position in mega base pairs (Mb) of chromosome DNA sequence

Mlink software in Genehunter was used to calculate the logarithm of the odds (LOD) score for linkage of the phenotype with each of the markers in recessive and dominant inheritance models, assuming 90% penetrance of the genotype at risk and 10% phenocopies (Lathrop and Ott, 1990). With these parameters, we allowed for the observed deviations of Mendelian inheritance.

Forty-six DNA samples were genotyped with 1536 single nucleotide polymorphisms (SNPs) using the Illumina GoldenGate platform. PLINK software (Purcell et al., 2007) was used for association analysis. Allele frequencies of SNPs were compared between cases and controls using a standard Chi-square based test. Then, 10,000 permutations were performed, generating an empirical test statistic for the probability that the observed frequency differences between cases and controls would occur by chance. The case and control dogs did not share parents in order to avoid family stratification. Twenty-one Pomeranians were selected from the 15 families (Fig. 1; selection A) and an additional 25 Pomeranians from other families (Fig. 1; selection B) were genotyped with 1536 SNPs. Of these 46 dogs, 37 had MPL and 9 were healthy controls. The 1536 SNPs were distributed over the 38 autosomes with an average spacing of 1.5 Mb. Details of the SNP set are described by Leegwater et al. (2007). Larger groups including newly recruited dogs with 43 cases and 40 controls were analysed for the association of the SNP.

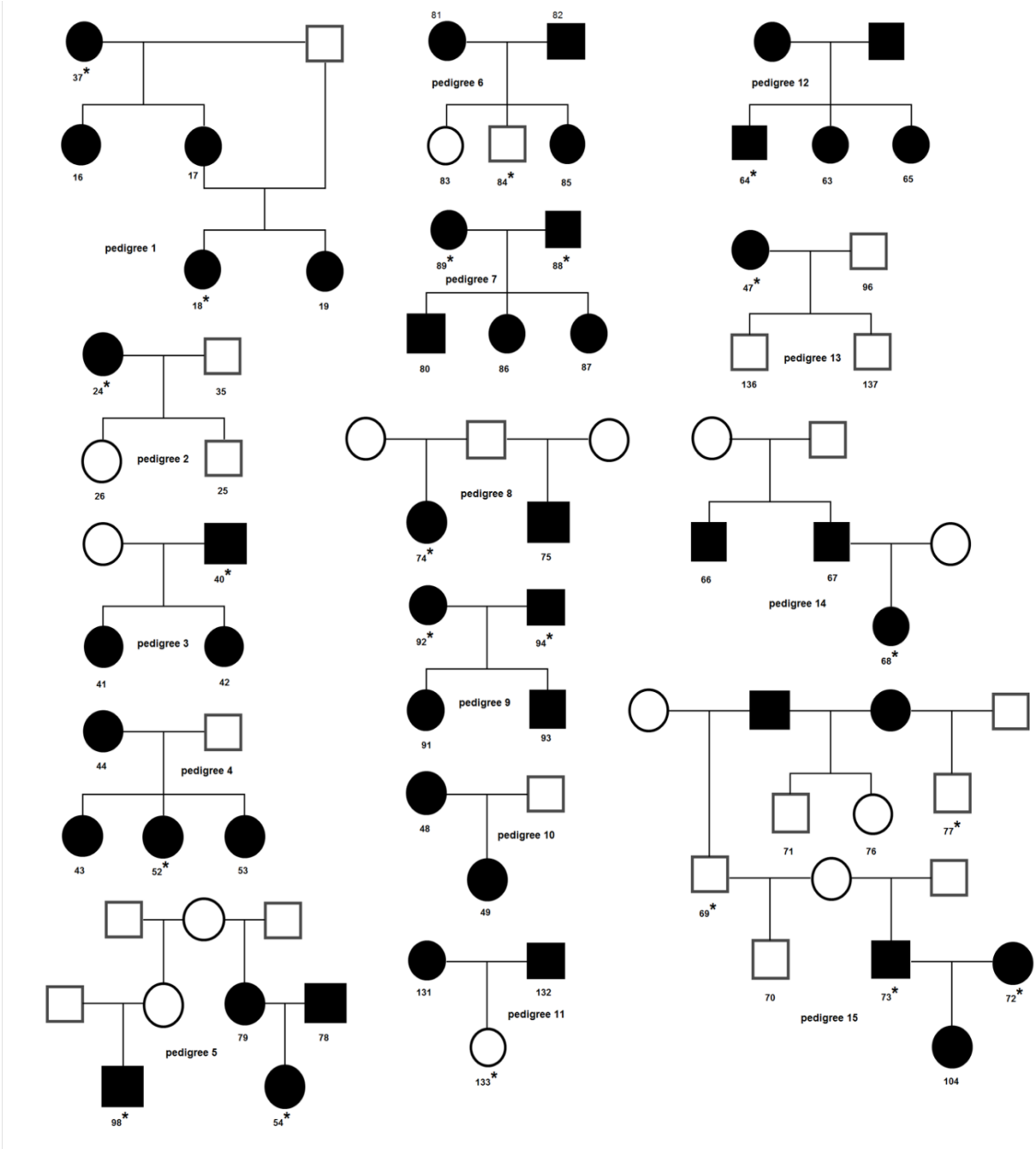


Figure 1 Pedigrees of Pomeranian dogs with bilateral medial patellar luxation.
Circles and squares represent female and male dogs, respectively. Filled symbols indicate dogs with patellar luxation, open symbols represent unaffected dogs. The numbers below the symbols indicate selection A of available DNA samples, asterisk denote selection B.

Results

The incidence of PL in Pomeranian dogs is shown in Table 2. Of the 238 Pomeranians investigated, 177 (75%) had PL. The ratio of male to female cases was 1:1.95. Of the 330 affected joints, 318 (96%) had MPL and only 12 (4%) LPL. Luxation was bilateral in 153 (86%) and unilateral in 24 (14%) of all PL affected dogs. The distribution of MPL affected dogs according to luxation was bilateral in 148 (87%) and unilateral in 22 (13%) and for LPL was bilateral in 5 (71%) and unilateral in 2 (29%). The pedigrees of the Pomeranian dogs with PL (Fig. 1) did not allow us to draw conclusions about the mode of inheritance. All affected dogs in the genetic study had bilateral MPL.

Table 2 Incidence of patellar luxation (PL) in Pomeranian dogs presented at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University (Bangkok, Thailand) during 2006, 2007, and 2008.

Pomeranian	Number of dogs			Number of joints affected with PL		
	Total	Female	Male	Total joints	Medial PL	Lateral PL
Status						
Normal	61	35	26			
Patellar luxation	177	117	60	330	318	12
Bilateral PL	153	99	54		148	5
Unilateral PL	24	18	6		22	2
Grading* (1 to 4)						
Grade 1				100	97	3
Grade 2				143	137	6
Grade 3				73	71	2
Grade 4				14	13	1

*Grading system according to Brinker et al. (1997); PL = patellar luxation.

The maximum obtainable LOD score for linkage of the phenotype from the 15 families was 0.85 for the recessive model and 1.27 for the dominant model. In a recessive inheritance model, the LOD scores for the *COL6A1*, *COL6A3*, *COL9A1*, *COL9A2*, and *COL9A3* genes were 0.19, 0.05, 0.29, 0.53, and 0.42, respectively. The LOD scores for a dominant model were -0.75, -0.99, -1.01, 0.24, and -0.61, respectively. Because of these low scores, model-free analysis was warranted. The allele sharing of affected sibling-pairs was statistically evaluated with Genehunter

software (versions 2.1_r6) (Kruglyak et al., 1996). The analysis showed that none of the markers had a significant non-parametric linkage (NPL) score (Table 3). The highest NPL score (1.56) was obtained for *COL9A2* (*P* = 0.07). We therefore switched to genome-wide association testing.

Table 3 Results of non-parametric linkage (NPL) score of collagen genes in 15 families of Pomeranian dogs using sibling-pair analysis.

Gene	Marker	Mb[a]	NPL score	*P*	Information content
COL6A1	CFA31_39.07	0.24	0.25	0.59	0.49
COL6A3	CFA25_48.50	0.45	1.09	0.89	0.58
COL9A1	CFA12_32.95	0.14	0.64	0.74	0.65
COL9A2	CFA15_2.65	0.01	1.56	0.07	0.60
COL9A3	CFA24_46.35	0.30	0.49	0.31	0.24

[a] Gene-marker distance (Canine genome assembly, *Canis Familiaris*, build 3.1).

The SNP BICF234J1226 on chromosome 7, which is located at base pair position 12241088 (Canine genome assembly, *Canis Familiaris*, build 3.1) between SNP BICF229J35202 and SNP BICF230J64732, was the most significant SNP in the association analysis. The frequency of allele A of this SNP was 0.42 in the cases and only 0.05 in the controls, which resulted in odds ratio of 13.6 for this SNP (Fig. 2). The associated SNP is situated in an intergenic region. The power of the SNP association study was limited because the control group was small and the number of SNPs was low.

Therefore, larger groups including newly recruited dogs with 43 cases and 40 controls were analysed for this SNP. The association of the region remained suggestive with a Chi-square *P* of 0.01 tested for difference of allele frequency. The frequency of the A allele was 0.32 in the cases and 0.48 in the controls.

Discussion

PL is one of the most common orthopedic problems found in Pomeranians. MPL was more common than LPL, and females were at great risk of PL than males given the ratio of male:female of 1:1.95. This is in accordance with Hayes et al. (1994) who found in dogs with PL a male:female ratio of 1:1.5. Bilateral luxation was common than unilateral luxation, which is in line with other report (Hayes et al., 1994).

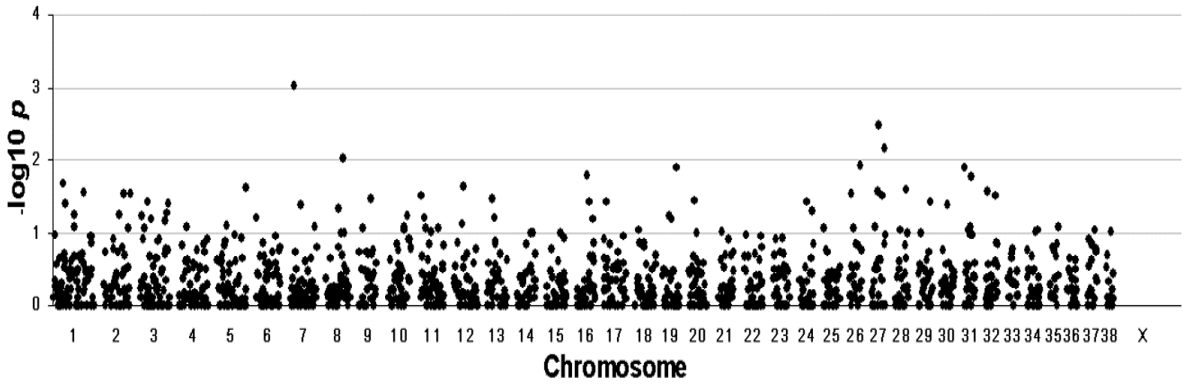


Figure 2 Association analysis of bilateral medial patellar luxation in Pomeranian dogs. The allele frequencies of 1536 SNPs in groups of 37 unrelated cases and 9 unrelated controls were compared by Chi-square tests. The raw pointwise permutated results from the PLINK analysis are shown. The most significant SNP (BICF234J1226), which is located at 12241088 bp between SNP BICF229J35202 and BICF230J64732, was located on chromosome 7.

The collagen genes were used to analyse PL in Pomeranians. The low LOD scores for the recessive and dominant inheritance models found in this study indicated that *COL6A1*, *COL6A3*, *COL9A1*, *COL9A2*, and *COL9A3* are not associated with PL in Pomeranian dogs. In addition, we did not find significance of these markers on non-parametric linkage analysis. It is indicated no linkage between these collagen genes and PL in Pomeranians. Then, the genome-wide association study was used to analyse PL in this study.

The SNP BICF234J1226 on chromosome 7, which is located at base pair position 12241088, was the most significant SNP in the association analysis. This SNP remained suggestive in the larger group. There are 43 genes located in the region of 4 Mb surrounding the associated SNP and none of these genes is known from other species to be involved in PL. Further studies with more markers in the region are required to establish the role and to identify the responsible gene.

Conclusion

We conclude that the *COL6A1*, *COL6A3*, *COL9A1*, *COL9A2*, and *COL9A3* collagen genes are not associated with MPL in Pomeranian dogs. We found a SNP on chromosome 7 potentially associated with MPL. The results of further studies focused on the genetic region involved might be used in breeding programs and may provide insight into the aetiology to PL.

References

Hayes, A.G., Boudrieau, R.J., Hungerford, L.L., 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal of the American Veterinary Medical Association 205, 716-720.

Kruglyak, L., Daly, M.J., Reeve-Daly, M.P., Lander, E.S., 1996. Parametric and nonparametric linkage analysis: a unified multipoint approach. American Journal of Human Genetics 58, 1347-1363.

Lathrop, G.M., Ott, J., 1990. Analysis of complex diseases under oligogenic models and infrafamilial heterogeneity by the LINKAGE programs. American Journal of Human Genetics.

Leegwater, P.A., van Hagen, M.A., van Oost, B.A., 2007. Localization of white spotting locus in Boxer dogs on CFA20 by genome-wide linkage analysis with 1500 SNPs. The Journal of Heredity 98, 549-552.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting-out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16, 1215.

OFA, 2011. Orthopedics Foundation for Animals: Patellar luxation statistics. http://www.offa.org/stats_pl.html (accessed March 9, 2012).

Piermattei, D.L., Flo, G.L., DeCamp, C.E., 2006. The stifle joint. In: Piermattei, D.L., Flo, G.L., DeCamp, C.E. (Eds.), Handbook of Small Animal Orthopedics and Fracture Repair. Fourth Ed. Saunders, St. Louis, pp. 562-632.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a toolset for whole-genome association and population-based linkage analysis. American Journal of Human Genetics 81, 559-575.

Salg, K.G., Temwitchitr, J., Imholz, S., Hazewinkel, H.A., Leegwater, P.A., 2006. Assessment of collagen genes involved in fragmented medial coronoid process development in Labrador Retrievers as determined by affected sibling-pair analysis. American Journal of Veterinary Research 67, 1713-1718.

Temwichitr, J., Hazewinkel, H.A., van Hagen, M.A., Leegwater, P.A., 2007. Polymorphic microsatellite markers for genetic analysis of collagen genes in suspected collagenopathies in dogs. Journal of Veterinary Medicine A 54, 522-526.

Wangdee, C., Soontornvipart, K., Chuthatep, S., 2005. Study of the canine patellar luxation surgical treatments. The Journal of Thai Veterinary Practitioners 17, 13-25.

Acknowledgments

This project was supported by the Thailand research fund (MRG5080124). Authors would like to thank staffs of the Biochemical unit, Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Thailand.

Chapter 6

Population genetic analysis and genome-wide association study of patellar luxation in Thai Pomeranians

C. Wangdee[1,3], P.A.J. Leegwater[3], H.C.M. Heuven[3,4], F.G. van Steenbeek[3], M. Techakumphu[2], H. A.W. Hazewinkel[3]

[1] Department of Veterinary Surgery, [2] Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

[3] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

[4] Animal Breeding and Genomics Centre, Wageningen University, Wageningen, The Netherlands

Submitted to Animal Genetics

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 2011. 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 ± 0.04. 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.

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 39.5% of dogs reported to be affected (OFA, 2013). 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, 2013; 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 Lavrijsen 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 analyse its heritability. In addition, 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.

Materials and methods

Animals

Pomeranians referred to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand, were screened for PL from 2006 to 2011. 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; 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 1 to 4) (Piermattei et al., 2006). In grade 1 PL, the patella can be manually luxated in full extension of the stifle joint, returning to the normal position when released. In grade 2 PL, the patella luxates more frequently than in grade 1. 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 3 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 reluxation of the patella. In grade 4 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).

Heritability of patellar luxation

Phenotypic score of PL was set to 0 for unaffected and 1 for affected. Variance components (σ^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:

$$\text{Logit (PL)} = \mu + \text{animal} + \text{pe} + \text{e}$$

where μ 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 animal, permanent environment (pe), which refers to environmental influences with a permanent effect on the animal and which are the same for both stifles of the same animal, but different between animals, and residual (e). Normal distributions were assumed for the random effect models: animal ~ N $(0,A\sigma^2_a)$, pe ~ N $(0,I\sigma^2_{pe})$, 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 was fixed at 3.289. Heritability was calculated using the formula (Falconer, 1981):

h^2 = additive genetic variation/phenotypic variation

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

Association study

The DNA samples of 96 Pomeranians 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 more than 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. KASPar 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 χ^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 in 32 PL case and

32 control Kooiker dogs, 32 case and 32 control Flat-Coated Retrievers, 16 case and 23 control Labrador Retrievers, 24 cases and 8 control Chihuahuas, and 23 case and 9 control Miniature Poodles.

Results

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 luxation varied from grade 1 to grade 4 (Table 2).

Table 1 Prevalence of patellar luxation (PL) in Pomeranians in Thailand from 1994 to 2011.

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%)

Table 2 Direction and gradation of patellar luxation (PL) in Pomeranians in Thailand from 1994 to 2011.

Direction of luxation	**Number (%) of stifle joints affected with PL**
Medial PL	456 (94.8%)
Lateral PL	11 (2.3%)
Bidirectional PL	14 (2.9%)
Total	481 (100%)
Grade of PL*	
Grade 1	136 (28.3%)
Grade 2	210 (43.6%)
Grade 3	98 (20.4%)
Grade 4	37 (7.7%)

*Grading system according to Piermattei *et al.* (2006)

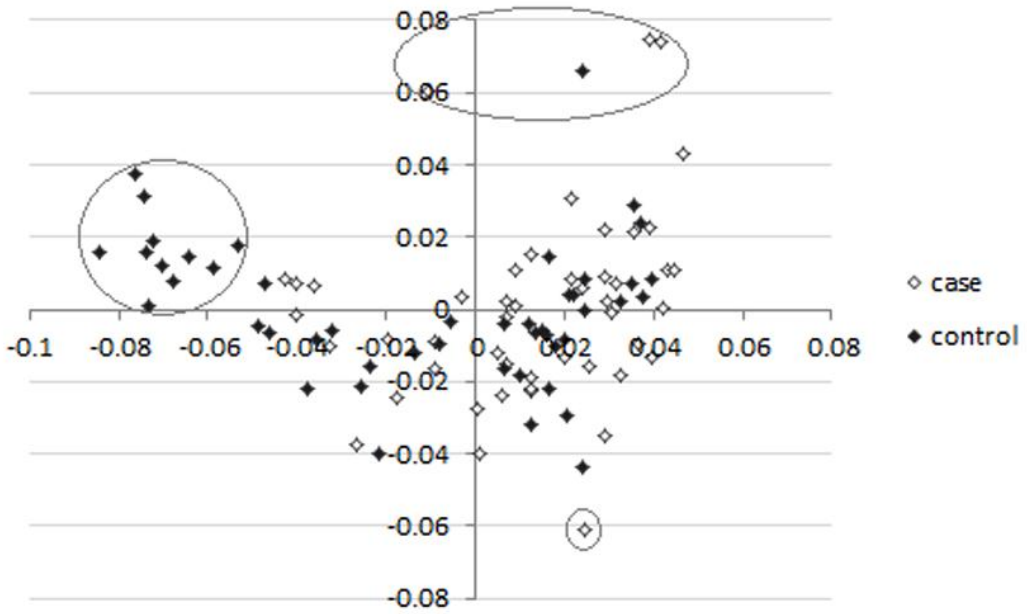
Heritability

The heritability of PL was 0.44 ± 0.04 in this Pomeranian dog population. The sex of the dog was not found to be a relevant factor.

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 (Fig. 1). The Pomeranian sample set was highly stratified with a genomic inflation factor of 1.46. Sixteen samples that deviated from the main population were excluded, leaving data for 44 cases and 36 controls for further analysis and reducing the genomic inflation factor to 1.09.

One single SNP on chromosome 13 displayed allelic association with MPL, using PLINK and the case/control phenotype, with $P = 4.32 \times 10^{-6}$ (Fig. 2). After correction using permutations of the phenotype, $P = 9.9 \times 10^{-4}$ and after max(T) correction for multiple testing $P = 0.3107$ (Table 3). The association of this SNP was then investigated in the validation cohort of 128 new cases and 7 new controls. In addition, another 21 SNPs on chromosome CFA02, CFA03, CFA05, CFA06, CFA07, CFA08, CFA09, CFA11, CFA17, CFA21, CFA24, CFA28, CFA32, and CFA37 with $P \leq 3.0 \times 10^{-3}$ were analysed in the validation cohort. In Pomeranians, the association signal of the SNP located at position CFA05:12113130 with PL improved from a Chi-square P of 1.09×10^{-4} to 1.39×10^{-5}. The association of another SNP (BICF2G630594583) located at position CFA32:17832518 with PL also increased slightly with a Chi-square P from 7.37×10^{-6} to 3.72×10^{-5} (Table 3). These SNPs were not associated with PL in the cohorts of Kooiker dogs, Flat-Coated Retrievers, Labrador Retrievers, Chihuahuas, and Miniature Poodles.



x-axis: principal component 1; y-axis: principal component 2

Figure 1 Multidimensional identical-by-state matrix

Two-dimensional representation of the first two principal components of a multidimensional identical-by-state matrix of 48 Pomeranians with patellar luxation (open dots) and 48 control dogs (filled dots). The sixteen circled samples were excluded from the subsequent association analysis.

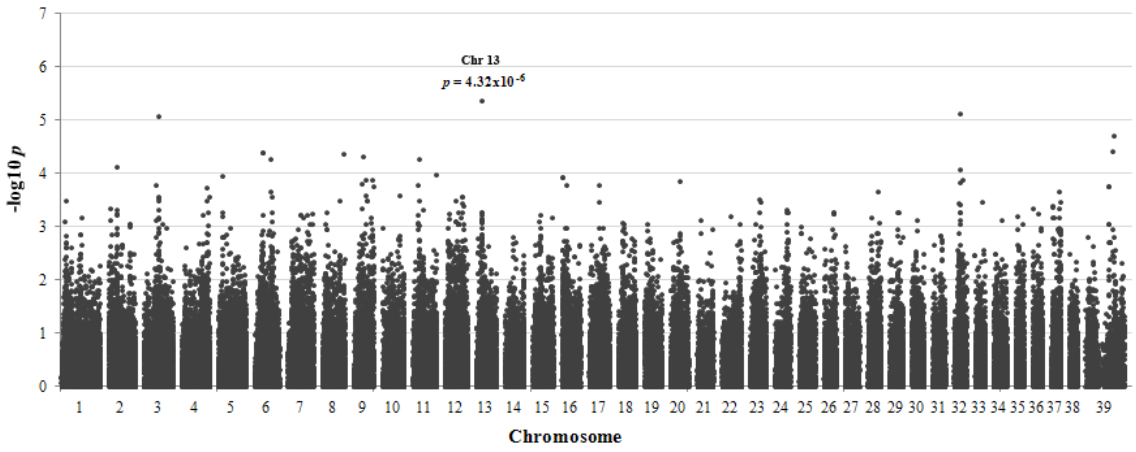


Figure 2 Manhattan plot from genome-wide association analysis of patellar luxation in Pomeranians shows the highest association SNP on chromosome 13.

Table 3 Association analysis of 22 single nucleotide polymorphisms (SNPs) with patellar luxation in Pomeranians shows chi-square *P* of GWAS, validation, and combined groups.

SNPs	CFA	Location	GWAS*	EMP2	Validation**	Combined***
BICF2S22915306	02	26427387	7.584E-05	0.994	0.581626	0.000228
BICF2S23636260	02	27963936	0.001307	1	0.069802	0.001718
BICF2P6182	03	46818844	8.153E-06	0.5015	0.466603	0.011986
BICF2P634673	05	12113130	0.000109	0.998	0.685786	1.39E-05
BICF2P168471	06	26696828	4.023E-05	0.967	0.126111	0.000577
BICF2P679664	06	51594569	5.417E-05	0.988	0.55635	0.002011
BICF2P16241	07	38453221	0.0005938	1	0.125579	0.007447
BICF2S23448189	08	68907027	4.165E-05	0.974	0.448636	0.002452
BICF2P1057164	09	26538011	4.862E-05	0.982	0.474363	0.002994
BICF2G630837296	09	36636684	0.0001277	1	0.405024	0.004332
BICF2S23450786	09	60353163	0.0001689	1	0.592331	0.012755
BICF2G630296947	11	22079033	0.001246	1	0.428399	0.109985
BICF2G630610959	13	17345582	4.319E-06	0.3197	0.213454	0.000355
BICF2S23628004	17	32936920	0.0001657	1	0.589799	0.002452
BICF2P578758	21	41405505	0.002995	1	0.583165	0.214599
BICF2G630495865	24	46793211	0.0000457	0.976	0.647035	0.015248
BICF2S23616264	28	33475362	0.0002198	1	0.520898	0.0114
BICF2G630594583	32	17832518	7.371E-06	0.4326	0.095588	3.72E-05
BICF2P955649	32	17258231	8.185E-05	0.995	0.088086	0.060258
BICF2S23116545	32	25356964	0.0001284	1	0.351672	0.082033
BICF2G630130877	37	25338016	0.0002111	1	0.401866	0.000731
BICF2G630132832	37	27512126	0.0003403	1	0.177016	0.00018

* The cohort of 44 cases and 36 controls of GWAS

** The cohort of 128 cases and 7 controls of validation group

*** The cohort of 172 cases and 43 controls of combined group

Discussion

PL is one of the most common orthopaedic problems found in Pomeranians, both in the USA (OFA, 2013) and in Thailand (Soontornvipart et al., 2013), where it has a prevalence of 39.5% and 77%, respectively. In this study of Pomeranians from Thailand, bilateral luxation (84%) was found to be five times more common than unilateral luxation (16%). This level of bilateral luxation is higher than that reported in other populations of small dogs, which had equal (50/50) levels of unilateral and bilateral PL (Remedios et al., 1992; Arthurs and Langley-Hobbs, 2006; Gibbons et al., 2006; Alam et al., 2007; Arthurs and Langley-Hobbs, 2007; Linney et al., 2011). PL was equally common in male and female Pomeranians, with a RR of 1.11 (95% CI 0.98 - 1.25), which is lower than that reported by Priester (1972), who investigated a heterogeneous cohort of 400 small-breed, 31 medium-breed, and 48 large-breed dogs of 33 different breeds including 37 Pomeranians (11 males and 26 females). MPL was more common than LPL, which is consistent with other reports in both small- and large-breed dogs (Remedios et al., 1992; Roush, 1993; Hayes et al., 1994; Gibbons et al., 2006; Alam et al., 2007). The only exception is a report by Lavijsen et al. (2013), who found LPL to be more common than MPL in Flat-Coated Retrievers. BPL was diagnosed in 2.9% of Pomeranians, a lower proportion than that reported earlier for small- and miniature-breed dogs (6.5%) (Vidoni et al., 2006), Kooiker dogs (7%) (Wangdee et al., 2014), and Flat-Coated Retrievers (8%) (Lavrijsen et al., 2013).
The predisposition to PL of certain breeds and the substantial proportion of dogs with bilateral PL suggests that the disorder is heritable, and the lack of sex predisposition and a Mendelian segregation pattern point towards it being a polygenic disorder. The heritability estimate was 0.44 ± 0.04 in this Pomeranian dog population, indicating that genetic factors are important, given the h^2 values in other breeds, i.e. Kooiker Dogs with a h^2 of 0.27 ± 0.07 (Wangdee et al., 2014) and in Flat-Coated Retrievers with a h^2 of 0.17 ± 0.03 (Lavrijsen et al., 2013). It also indicates that environmental and residual variances play a role in the phenotypic appearance of the trait. This means that unaffected dogs can transmit susceptibility to the disorder to their offspring, which might limit the success rate of a breeding programme based solely on the exclusion of clinically affected animals.

The most strongly associated SNPs in the genome-wide association study were located on chromosomes CFA02, CFA03, CFA05, CFA06, CFA07, CFA08, CFA09, CFA11, CFA 13, CFA17, CFA21, CFA24, CFA28, CFA32, and CFA37. The association

of the regions on CFA05 and CFA32 remained suggestive when a larger group of dogs was investigated (Table 3). That the validation cohort of Pomeranians was not a statistically independent group was because of the small number of controls.

The genes of interest that may be involved in MPL in Pomeranians situated are *SORL1* and *SC5D* in the associated region on CFA05 and *BMPR1B* and *UNC5C* in the region on CFA32. The *SC5D* gene on chromosome 5 encodes an enzyme of cholesterol biosynthesis. Mutations in this gene have been associated with lathosterolosis in humans, a disorder associated with multiple congenital anomalies including abnormal bone calcification, limb malformation, and liver disease (Kelley and Herman, 2001; Rossi et al., 2005; Rossi et al., 2007). The *BMPR1B* gene located on chromosome 32 encodes a member of the bone morphogenetic protein (BMP) receptor family of transmembrane serine/threonine kinases. BMPs are members of the TGF-beta superfamiliy, which are involved in endochondral bone formation and embryogenesis. Mutations in *BMPR1B* are associated with chrondrodysplasia (Demirhan et al., 2005). Further studies investigating the DNA sequence of this gene and *SC5D* in affected dogs are required to establish their involvement in the disease. An earlier study of PL in Pomeranians from Thailand suggested a SNP on chromosome 7 to be associated with MPL (Soontornvipart et al., 2013), but we could not confirm this association. The earlier study was probably underpowered because of the limited number of only 9 controls.

Analysis of the validation cohorts did not reveal the loci identified in Pomeranians to be associated with PL in Kooiker dogs, Flat-Coated Retrievers, Labrador Retrievers, Chihuahuas, and Miniature Poodles. The loci involved in PL may be different in these breeds.

Conclusions

PL, and particularly MPL, is a widespread hereditary disease in Pomeranians. The heritability of PL in this population was 0.44, and regions on chromosome 5 and chromosome 32 were associated with the occurrence of MPL in these dogs, although genome-wide significance was not reached. The *SC5D* gene located on CFA05 and the *BMPR1B* gene located on CFA32 have been implicated in bone malformation and cartilage formation, and therefore are good candidate genes for a role in the pathogenesis of MPL in Pomeranians. Further research to confirm the role of either of the genes in PL is required.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Acknowledgments

We acknowledge Mrs. M. Vos-Loohuis for her analytical work.

References

Alam, M.R., Lee, J.I., Kang, H.S., Kim, I.S., Park, S.Y., Lee, K.C., Kim, N.S., 2007. Frequency and distribution of patellar luxation in dogs: 134 cases (2000 to 2005). Veterinary and Comparative Orthopaedics and Traumatology 20, 59-64.

Arthurs, G.I., Langley-Hobbs, S.J., 2007. Patellar luxation as a complication of surgical intervention for the management of cranial cruciate ligament rupture in dogs. A retrospective study of 32 cases. Veterinary and Comparative Orthopaedics and Traumatology 20, 204-210.

Arthurs, G.I., Langley-Hobbs, S.J., 2006. Complications associated with corrective surgery for patellar luxation in 109 dogs. Veterinary Surgery 35, 559-566.

Barth, P.G., Valianpour, F., Bowen, V.M., Lam, J., Duran, M., Vaz, F.M., Wanders, R.J., 2004. X-linked cardioskeletal myopathy and neutropenia (Barth syndrome): an update. American Journal of Medical Genetics.Part A 126A, 349-354.

DeAngelis, M., Hohn, R.B., 1970. Evaluation of surgical correction of canine patellar luxation in 142 cases. Journal of the American Veterinary Medical Association 156, 587-594.

Demirhan, O., Turkmen, S., Schwabe, G.C., Soyupak, S., Akgul, E., Tastemir, D., Karahan, D., Mundlos, S., Lehmann, K., 2005. A homozygous BMPR1B mutation causes a new subtype of acromesomelic chondrodysplasia with genital anomalies. Journal of Medical Genetics 42, 314-317.

Dohoo, I., Martin, W., Stryhn, H., 2010. Measures of association. In: Veterinary Epidemiologic Research. Atlantic Veterinary College, University of Prince Edward Island, Prince Edward Island, Canada, pp. 121-136.

Dreyer, S.D., Zhou, G., Baldini, A., Winterpacht, A., Zabel, B., Cole, W., Johnson, R.L., Lee, B., 1998. Mutations in LMX1B cause abnormal skeletal patterning and renal dysplasia in nail patella syndrome. Nature Genetics 19, 47-50.

Falconer, D.S., 1981 Heritability. In: Introduction to Quantitative Genetics. Longman Group Limited, Essex, UK, pp. 148-169.

Gibbons, S.E., Macias, C., Tonzing, M.A., Pinchbeck, G.L., McKee, W.M., 2006. Patellar luxation in 70 large breed dogs. Journal of Small Animal Practice 47, 3-9.

Gilmour, A.R., Thompson, R., Cullis, B.R. 1995. Average Information REML: An Efficient Algorithm for Variance Parameter Estimation in Linear Mixed Models. Biometrics 51, 1440-1450.

Hayes, A.G., Boudrieau, R.J., Hungerford, L.L., 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal of the American Veterinary Medical Association 205, 716-720.

Hulse, D.A., 1993. Medial patellar luxation in the dog. In: Bojrab, M.J., (Ed.), Disease Mechanisms in Small Animal Surgery. Second Ed. Lea&Febiger, Philadelphia, pp. 808-817.

Kelley, R.I., Herman, G.E. 2001. Inborn errors of sterol biosynthesis. Annual Review of Genomics and Human Genetics 2, 299-341.

LaFond, E., Breur, G.J., Austin, C.C., 2002. Breed susceptibility for developmental orthopedic diseases in dogs. Journal of the American Animal Hospital Association 38, 467-477.

Lavrijsen, I.C., Heuven, H.C., Breur, G.J., Leegwater, P.A., Meutstege, F.J., Hazewinkel, H.A. 2013. Phenotypic and genetic trends of patellar luxation in Dutch Flat-Coated Retrievers. Animal Genetics 44, 736-741.

Linney, W.R., Hammer, D.L., Shott, S. 2011. Surgical treatment of medial patellar luxation without femoral trochlear groove deepening procedures in dogs: 91 cases (1998-2009). Journal of the American Veterinary Medical Association 238, 1168-1172.

OFA, 2013. Orthopedics Foundation for Animals: Patellar luxation statistics. http://www.offa.org/stats_pl.html (accessed March 9, 2014).

Piermattei, D.L., Flo, G.L., DeCamp, C.E., 2006. The stifle joint. In: Piermattei, D.L., Flo, G.L., DeCamp, C.E. (Eds.), Handbook of Small Animal Orthopedics and Fracture Repair. Fourth Ed. Saunders, St. Louis, pp. 562-632.

Priester, W.A., 1972. Sex, size, and breed as risk factors in canine patellar dislocation. Journal of the American Veterinary Medical Association 160, 740-742.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a toolset for whole-genome association and population-based linkage analysis. American Journal of Human Genetics 81, 559-575.

Remedios, A.M., Basher, A.W., Runyon, C.L., Fries, C.L. 1992. Medial patellar luxation in 16 large dogs. A retrospective study. Veterinary Surgery 21, 5-9.

Romero, P., Sanhueza, F., Lopez, P., Reyes, L., Herrera, L. 2011. c.194 A>C (Q65P) mutation in the LMX1B gene in patients with nail-patella syndrome associated with glaucoma. Molecular Vision 17, 1929-1939.

Rossi, M., D'Armiento, M., Parisi, I., Ferrari, P., Hall, C.M., Cervasio, M., Rivasi, F., Balli, F., Vecchione, R., Corso, G., Andria, G., Parenti, G. 2007. Clinical phenotype of lathosterolosis. American Journal of Medical Genetics.Part A 143A, 2371-2381.

Rossi, M., Vajro, P., Iorio, R., Battagliese, A., Brunetti-Pierri, N., Corso, G., Di Rocco, M., Ferrari, P., Rivasi, F., Vecchione, R., Andria, G., Parenti, G. 2005. Characterization of liver involvement in defects of cholesterol biosynthesis: long-term follow-up and review. American Journal of Medical Genetics.Part A 132A, 144-151.

Roush, J.K., 1993 Canine patellar luxation. Veterinary Clinics of North America: Small animal practice 23, 855-868.

Soontornvipart, K., Wangdee, C., Kalpravidh, M., Brahmasa, A., Sarikaputi, M., Temwichitr, J., Lavrijsen, I.C., Theyse, L.F., Leegwater, P.A., Hazewinkel, H.A. 2013. Incidence and genetic aspects of patellar luxation in Pomeranian dogs in Thailand. The Veterinary Journal 196, 122-125.

Vidoni, B., Sommerfeld-Stur, I., Eisenmenger, E., 2006. Diagnostic and genetic aspects of patellar luxation in small and miniature breed dogs in Austria. The European Journal of Companion Animal Practice 16, 149-158.

Wangdee, C., Kalpravidh, M., 2008. Tube realignment for patellar luxation repair in dogs. The Thai Journal of Veterinary Medicine 38, 39-44.

Wangdee, C., Soontornvipart, K., Chuthatep, S., 2005. Study of the canine patellar luxation surgical treatments. The Journal of Thai Veterinary Practitioners 17, 13-25.

Wangdee, C., Leegwater, P.A., Heuven, H.C., van Steenbeek, F.G., Meutstege, F.J., Meij, B.P., Hazewinkel, H.A., 2014. Prevalence and genetics of patellar luxation in Kooiker dogs. Veterinary Journal doi: 10.1016/j.tvjl.2014.05.036

Chapter 7

Prevalence and genetics of patellar luxation in Kooiker dogs

C. Wangdee[1,2], P.A.J. Leegwater[2], H.C.M. Heuven[2,3], F.G. van Steenbeek[2], F.J. Meutstege[4], B.P. Meij[2], H.A.W. Hazewinkel[2]

[1] Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

[2] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

[3] Animal Breeding and Genomics Centre, Wageningen University, Wageningen, The Netherlands

[4] Steenen Camer 78, 3721ND Bilthoven, The Netherlands

Published in The Veterinary Journal 2014

DOI 10.1016/j.tvjl.2014.05.036

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 the 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 (Praw = 1.32 x 10^{-5}, Pgenome = 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%) (OFA, 2014).

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 (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 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 by F.J. Meutstege. 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.

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 of 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 Appendix A: Table 1. 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 by F.J. Meutstege. 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).

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

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 $(0,A\sigma^2_a)$, dam ~ N $(0,I\sigma^2_d)$, pe ~ N $(0,I\sigma^2_{pe})$ and e ~ N $(0,I\sigma^2_e)$, 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-(prediction error variance/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 of the CanineHD Bead chip (Illumina). Of these 90 dogs, 48 had PL and 42 were unaffected controls (Appendix A: Table 2). 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 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 40 affected and 52 control Kooiker dogs (Appendix A: Table 2). KASPar chemistry (LGC genomics, Hoddesdon, UK)

was used to generate allele specific fluorescent signals. The signals were recorded and scored using a BIORAD MYiQ2 iCycler. The sequences of the PCR primers are listed in Appendix A: Table 3. The allele frequencies of these SNPs in the validation group were compared between cases and controls separately and together with the first group of 90 dogs included in the genome-wide association study, using a χ^2 test.

Results

Animals and phenotyping

Of the 842 Kooiker dogs investigated, 199 (23.6%) had PL (Table 2). Unilateral and bilateral PL were equally common. The ratio of female:male dogs with PL was 1.62; 123/493 (24.9%) female dogs and 76/349 (21.8%) male dogs were affected, giving a RR of 1.15 (95% CI 0.90 – 1.48), which means that female dogs did not have a significantly higher risk of PL than male dogs. Of 298 affected stifle joints, 182/298 (61.1%) had medial PL, 95/298 (31.9%) had lateral PL, and 21/298 (7.0%) had bidirectional PL, especially grades 1 and 2 (Table 3).

Heritability and genetic trend

The heritability of PL (Yq) was 0.27 ± 0.07 in this Kooiker dog population. The prevalence of PL in Kooiker dogs decreased from 28% to 19% between 1994 and 2009 (Fig. 1A), as did the average breeding value per birth year (Fig. 1B). The reliabilities of EBVs ranged from 0 to 0.71, with a mean value of 0.26.

Table 2 Prevalence of patellar luxation in Kooiker dogs in The Netherlands screened from 1994 to 2011.

Status	Number (%)		
	Female	Male	Subtotal
Normal	370 (75.1%)	273 (78.2%)	643 (76.4%)
Patellar luxation	123 (24.9%)	76 (21.8%)	199 (23.6%)
Bilateral patellar luxation	59	40	99
Unilateral patellar luxation	64	36	100
Total	493 (100%)	349 (100%)	842 (100%)

Table 3 Direction and gradation of patellar luxation (PL) in Kooiker dogs screened in The Netherlands from 1994 to 2011.

	Number (%) of stifle joints affected with PL
Direction of luxation	
Medial PL	182 (61.1%)
Lateral PL	95 (31.9%)
Bidirectional PL	21 (7.0%)
Total	298 (100%)
Grade of PL [a]	
Grade 1	253 (84.9%)
Grade 2	40 (13.4%)
Grade 3	5 (1.7%)

[a] Grading system according to Hazewinkel et al. (2009).

Genome-wide association study

The genome-wide association study of PL in the Kooiker dogs pointed towards a complex inheritance pattern. From the 174,450 SNPs represented on the array, we excluded 69,790 non-informative SNPs (minor allele frequency < 5%), 10,374 SNPs with a genotyping rate < 95% and 1379 SNPs based on deviation from Hardy-Weinberg equilibrium ($P < 0.01$). The remaining 98,754 SNPs were used to construct an identical-by-state (IBS) plot, based on the first two principal components of the multidimensional IBS matrix (Appendix A: Fig. 1). The genomic inflation factor of 1.04 indicated that population stratification had minimal effect on the results and so no adjustments were made for relatedness.

One region on chromosome 3 displayed association with PL, using PLINK and the case/control phenotype, with $P = 1.32 \times 10^{-5}$ (Fig. 2). Correction using permutations of the phenotype resulted in $P = 9.9 \times 10^{-4}$ and, after max(T) correction for multiple testing in $P = 0.142$. This SNP (BICF2G630361390) was located at base pair position CFA03:90901801 (Ensembl Canine Genome Assembly, *Canis lupus familiaris*, build 3.1) (Ensembl, 2012). The allele frequency of this SNP was 0.38 in the cases but only 0.09 in the controls, which resulted in an odds ratio of 5.7 for this SNP.

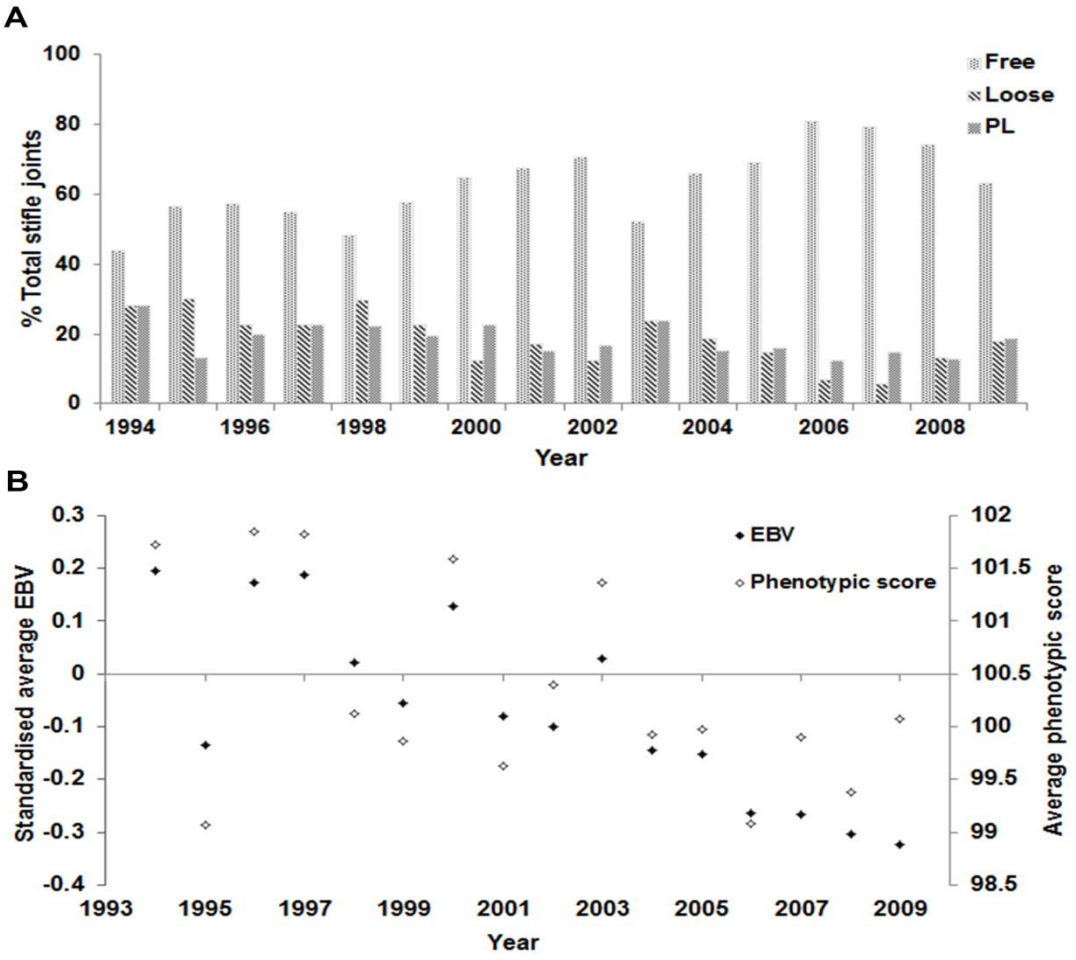


Figure 1 The prevalence of patellar luxation in Kooiker dogs.
Prevalence of patellar luxation in Kooiker dogs by year from 1994 to 2009 shown as percentage affected stifle joints **(A)**. Phenotypic and genetic trend of patellar luxation from 1994 to 2009 in Kooiker dogs **(B)**. Free = free of patella; Loose = positional on the ridge of the trochlea, but not luxable; PL = patellar luxation grades 1–3; Year = year of birth; EBV = estimated breeding value; Average phenotypic score, average quantitative values according to Table 1.

The association of this SNP was then investigated in 40 other dogs with PL and 52 controls. In addition, three SNPs situated near this location were analysed (Table 4). The association signal could not be confirmed in the validation group. However, combination of all available cases and controls did not lead to deterioration of the *P* value obtained with the genome-wide association study (Table 4).

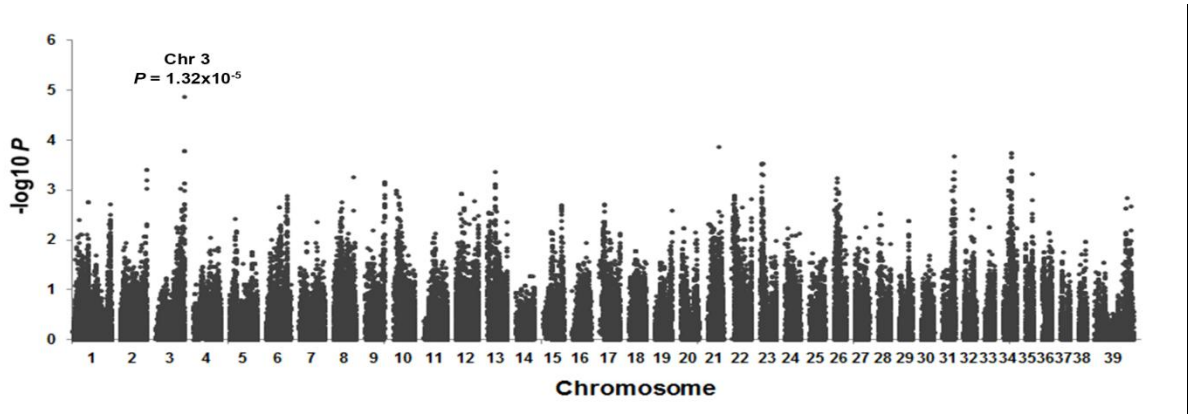


Figure 2 Manhattan plot of genome-wide association analysis of patellar luxation in Kooiker dogs, demonstrating the highest association with chromosome 3.

Table 4 Allelic association analysis of single nucleotide polymorphisms on chromosome 3 with patellar luxation in genome-wide association study (GWAS) group, validation group and combined groups of Kooiker dogs.

SNP	Position	*P*			allele frequency					
		GWAS[a]	Validation[b]	Combined	GWAS[a]		Validation[b]		Combined	
					case	control	case	control	case	control
1	86,797,855	0.004	0.204	0.005	0.2	0.39	0.25	0.34	0.22	0.36
2	87,276,208	0.017	0.123	0.006	0.26	0.43	0.29	0.40	0.28	0.41
3	88,383,054	0.006	0.420	0.005	0.19	0.37	0.80	0.85	0.47	0.63
4	90,901,801	1.32×10^{-5}	0.731	0.003	0.38	0.09	0.25	0.34	0.32	0.22

SNP1 = BICF2G630356455, SNP2 = BICF2G630357248, SNP3 = BICF2P192508, SNP4 = BICF2G630361390

[a] GWAS analysis in 48 dogs with patellar luxation and 42 control dogs; [b] Validation in 42 dogs with patellar luxation and 50 control dogs

Discussion

Although the prevalence of PL in Kooiker dogs decreased from 28% in 1994 to 19% in 2009, based on calculation of the annual breeding value, it was still slightly higher than the prevalence of PL in other dogs in the 'flushing dogs' group, such as Cocker spaniel, Boykin spaniel and Tibetan spaniel, as reported by the OFA (OFA, 2013). This suggests that measures other than selection of breeding animals based on body screening are required to further reduce the prevalence of PL. The limited number of Kooiker dogs used to re-establish the breed might be the cause of the relatively widespread distribution of undesirable inherited traits (Mandigers et al., 1994).

PL was equally common in male and female Kooiker dogs, with an RR of 1.15 (95% CI 0.90 - 1.48), which is lower than that reported by Priester (1972) who investigated a heterogeneous group of 431 small breed and 48 large breed dogs. Medial PL was more common than lateral PL, but similar to that reported in other studies of PL (Remedios et al., 1992; Roush, 1993; Hayes et al., 1994; Gibbons et al., 2006; Alam et al., 2007). Bidirectional PL was diagnosed in 7% of Kooiker dogs, similar to values reported in small and miniature breed dogs (6.5%) (Vidoni et al., 2006) and in large breed dogs (8%) (Lavrijsen et al., 2013).

The predisposition to PL in certain breeds and the substantial proportion of dogs with bilateral PL suggest that the disorder is heritable, which the lack of a Mendelian segregation pattern points towards PL being a disorder with a complex inheritance pattern. The estimated heritability of 0.27 ± 0.07 for PL in Kooiker dogs means that there is low genetic variation compared to the phenotypic variation in the population analysed and indicates that environmental and genetic factors, as well as residual variances, play a role in the phenotypic expression of the trait. Unaffected dogs can transmit susceptibility to the disorder to their offspring, which might limit the success of a breeding programme based on the exclusion of affected animals. Calculation of the EBV, which includes phenotypic information of all screened relatives, could reduce this problem, especially when dogs without PL are considered for breeding. This approach puts more emphasis on family information and therefore is superior to phenotypic selection.

Our study showed that PL should be regarded as a complex trait and genotypic information can be included in selecting the superior dogs for breeding using the method described as 'genomic selection' (Meuwissen et al., 2001). We therefore suggest that information regarding pedigree, phenotypes and genotypes should be combined in a selection programme against PL.

In the genome-wide association study of PL, the most highly associated SNP was located on chromosome 3 at position 90.9 Mb. The association signal was not significant after correction for multiple testing by the max(T) permutation test. The lack of a significant association signal suggests that the number of genes that contribute to the PL phenotype is larger than anticipated and is not determined by a few genes with a major effect. The association of the CFA03 region with PL was not confirmed nor excluded when a validation group of Kooiker dogs was investigated. Genes located in the region could be included in future studies to the genetic background of PL in Kooiker dogs.

Conclusions

PL, particularly medial PL, is a common inherited disease in the Dutch Kooiker dog, being present in 24% of dogs of this breed in the Netherlands. The heritability of PL in the screened population was 0.27. Although the Dutch screening programme, based on orthopaedic examination, has reduced the prevalence of PL from 1994 to 2001, the prevalence of the disorder is currently stable. Combining pedigree, phenotypes and genotypes in the breeding programme could improve the effectiveness of breeding to reduce the prevalence of PL. A SNP on chromosome 3 was not significantly associated with the occurrence of PL in Kooiker dogs. However, genes located in the region should be investigated for PL, since genes with a small effect may play a role in the complex inheritance of PL.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was supported by the European Commission FP7 project LUPA-GA201370. The authors acknowledge T.E.S. Bruinen for assistance with data analysis.

References

Alam, M.R., Lee, J.I., Kang, H.S., Kim, I.S., Park, S.Y., Lee, K.C., Kim, N.S., 2007. Frequency and distribution of patellar luxation in dogs: 134 cases (2000 to 2005). Veterinary and Comparative Orthopaedics and Traumatology 20, 59-64.

Dohoo, I., Martin, W., Stryhn, H., 2010. Measures of association. In: Veterinary Epidemiologic Research. Atlantic Veterinary College, University of Prince Edward Island, Prince Edward Island, Canada, pp. 121-136.

Ensembl, 2012. Dog (CanFam 3.1): Region overview. http://www.ensembl.org/Canis_familiaris/Location/Overview?r=3:90200000-91889043 (accessed November 13, 2013).

Falconer, D.S., 1981 Heritability. In: Introduction to Quantitative Genetics, Second Edn. Longman, Essex, UK, pp. 148-169. .

FCI, 2009. Fédération Cynologique Internationale-FCI-2: Breed Groups. http://www.dogdomain.com/fci-8.htm (accessed November 11, 2013).

Gibbons, S.E., Macias, C., Tonzing, M.A., Pinchbeck, G.L., McKee, W.M., 2006. Patellar luxation in 70 large breed dogs. Journal of Small Animal Practice 47, 3-9.

Gilmour, A.R., Thompson, R., Cullis, B.R., 1995. Average information REML: An efficient algorithm for variance parameter estimation in linear mixed models. Biometrics 51, 1440-1450.

Hayes, A.G., Boudrieau, R.J., Hungerford, L.L., 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal of the American Veterinary Medical Association 205, 716-720.

Hazewinkel, H.A., Meij, B.P., Theyse, L.F., Van Rijssen, B., 2009. Locomotor system. In: Rijnberk, A., van Sluijs, F.J. (Eds.), Medical History and Physical Examination in Companion Animals, 2nd Edn. Saunders, Toronto, Canada, pp. 135-159.

Hazewinkel, H.A., Tellhelm, B., Leegwater, P.A., 2013. Screening for orthopaedic genetic defects: Part 1. Patella luxation in dogs. European Journal of Companion Animal Practice 23, 90-98.

Hulse, D.A., 1981. Pathophysiology and management of medial patellar luxation in the dog. Veterinary Medicine, Small Animal Clinician 76, 43-51.

Johnson, A.L., Probst, C.W., DeCamp, C.E., Rosenstein, D.S., Hauptman, J.G., Weaver, B.T., Kern, T.L., 2001. Comparison of trochlear block recession and trochlear wedge recession for canine patellar luxation using a cadaver model. Veterinary Surgery 30, 140-150.

LaFond, E., Breur, G.J., Austin, C.C., 2002. Breed susceptibility for developmental orthopedic diseases in dogs. Journal of the American Animal Hospital Association 38, 467-477.

Lavrijsen, I.C., Heuven, H.C., Breur, G.J., Leegwater, P.A., Meutstege, F.J., Hazewinkel, H.A., 2013. Phenotypic and genetic trends of patellar luxation in Dutch Flat-Coated Retrievers. Animal Genetics 44, 736-741.

Mandigers, P.J., Ubbink, G.J., van den Broek, J., Bouw, J., 1994. Relationship between litter size and other reproductive traits in the Dutch Kooiker dog. The Veterinary Quarterly 16, 229-232.

Meuwissen, T.H., Hayes, B.J., Goddard, M.E., 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819-1829.

Ness, M.G., Abercromby, R.H., May, C., Turner, B.M., Carmichael, S., 1996. A survey of orthopedic conditions in small animal veterinary practice in Britain. Veterinary and Comparative Orthopaedics and Traumatology 9, 43-52.

OFA, 2013. Orthopedics Foundation for Animals: Patellar luxation statistics. http://www.offa.org/stats_pl.html (accessed March 9, 2014).

Priester, W.A., 1972. Sex, size, and breed as risk factors in canine patellar dislocation. Journal of the American Veterinary Medical Association 160, 740-742.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: A toolset for whole-genome association and population-based linkage analysis. American Journal of Human Genetics 81, 559-575.

Remedios, A.M., Basher, A.W., Runyon, C.L., Fries, C.L., 1992. Medial patellar luxation in 16 large dogs. A retrospective study. Veterinary Surgery 21, 5-9.

Roush, J.K., 1993. Canine patellar luxation. Veterinary Clinics of North America: Small Animal Practice 23, 855-868.

Van Grevenhof, E.M., Ducro, B.J., van Weeren, P.R., van Tartwijk, J.M., van den Belt, A.J., Bijma, P., 2009. Prevalence of various radiographic manifestations of osteochondrosis and their correlations between and within joints in Dutch warmblood horses. Equine Veterinary Journal 41, 11-16.

Van Oost, B.A., Versteeg, S.A., Slappendel, R.J., 2004. DNA testing for type III von Willebrand disease in Dutch Kooiker dogs. Journal of Veterinary Internal Medicine 18, 282-288.

Vidoni, B., Sommerfeld-Stur, I., Eisenmenger, E., 2006. Diagnostic and genetic aspects of patellar luxation in small and miniature breed dogs in Austria. European Journal of Companion Animal Practice 16, 149-158.

Appendix A:

Table 1

Number of investigated dogs per year and the investigated dogs as a percentage of the total number of Kooiker dogs registered at the Dutch Kennel Club.

		Kennel Club registered Kooiker dogs			
Year	Investigated dogs	Total	Male	Female	Percentage
1994	10	492	244	248	2.03
1995	8	467	218	249	1.71
1996	15	541	314	227	2.77
1997	32	496	276	220	6.45
1998	29	459	240	219	6.32
1999	37	451	238	213	8.20
2000	45	492	271	221	9.15
2001	49	603	330	273	8.13
2002	57	517	281	236	11.03
2003	50	518	265	253	9.65
2004	61	480	232	248	12.71
2005	89	434	224	210	20.51
2006	76	431	220	211	17.63
2007	61	462	247	215	13.20
2008	75	461	235	226	16.27
2009	54	354	181	173	15.25
2010	72	309	154	155	23.30

Table 2

Distribution of sex and status of Kooiker dogs for GWAS and KASPar analysis.

Analysis method	status	Male	Female	Binomial phenotype [a]
GWAS	normal	15	27	0
	PL grade 1	12	25	1
	grade 2	2	8	
	grade 3	1		
KASPar	normal	19	32	0
	PL grade 1	9	31	1
	grade 2		1	
	grade 3			

PL = patellar luxation

[a] Used in GWAS and validation analysis, 0 = unaffected, 1 = affected.

Table 3

Sequencing of primer for KASPar assay.

Position (CanFam 2)	Primer	Sequence
CFA3:89623082	A1	GAAGGTGACCAAGTTCATGCTGACTTCTGTCTATATTTCTTCTCTTTCAAT
	A2	GAAGGTCGGAGTCAACGGATTACTTCTGTCTATATTTCTTCTCTTTCAAC
	C1	GCACTAATTTCTGCCTTGGACCCTA
CFA3:90101416	A1	GAAGGTGACCAAGTTCATGCTCACACAGCCTAGAAGAAAGAATGC
	A2	GAAGGTCGGAGTCAACGGATTGCACACAGCCTAGAAGAAAGAATGT
	C1	GCTATGTCAACATGCAGGTCTTTGAAATT
CFA3:91209284	A1	GAAGGTGACCAAGTTCATGCTCCTAGGAGATAGATTTCCATTTAAGCAT
	A2	GAAGGTCGGAGTCAACGGATTCTAGGAGATAGATTTCCATTTAAGCAC
	C1	CCTCTACTGCTCTTTCCCACCATA
CFA3:93731523	A1	GAAGGTGACCAAGTTCATGCTCACAGATTTCAAAGAACTTGACCCG
	A2	GAAGGTCGGAGTCAACGGATTGCACAGATTTCAAAGAACTTGACCCA
	C1	GGACCTGGGGCAGACCCCA

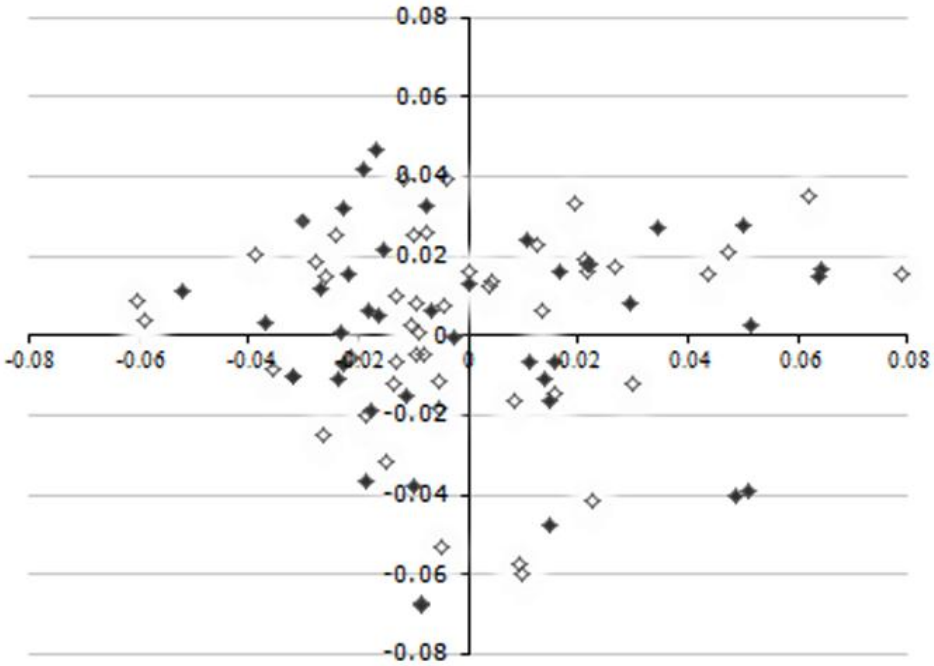


Figure 1

Two-dimensional representation of the first two principal components of a multi-dimensional identical-by-state matrix of 48 Kooiker dogs with patellar luxation (open dots) and 42 control dogs (filled dots).

x-axis, principal component 1; y-axis, principal component 2.

Chapter 8

Genome-wide survey indicates involvement of loci on canine chromosomes 7 and 31 in patellar luxation in Flat-Coated Retrievers

I.C.M. Lavrijsen[1], P.A.J. Leegwater[1], C. Wangdee[1,2], F.G. van Steenbeek[1], M. Schwencke[3], G.J. Breur[4], F.J. Meutstege[5], I.J. Nijman[6], E. Cuppen[6], H.C.M. Heuven[1,7] and H.A.W. Hazewinkel[1]

[1] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

[2] Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

[3] Dierenkliniek Putten, Roosendaalseweg 162c, Putten, The Netherlands.

[4] Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Purdue University, West Lafayette, USA

[5] Steenen Camer 78, Bilthoven, The Netherlands.

[6] Hubrecht Institute, The Royal Dutch Academy of Arts and Sciences, University Medical Center Utrecht, Utrecht, The Netherlands.

[7]Animal Breeding and Genomics Centre, Wageningen University, Wageningen, The Netherlands.

Published in BMC genetics 2014, 15: 64

Abstract

Patellar luxation is an orthopedic disorder in which the patella moves out of its normal location within the femoral trochlea of the knee and it can lead to osteoarthritis, lameness, and pain. In dogs it is a heritable trait, with both environmental and genetic factors contributing to the phenotype. The prevalence of patellar luxation in the Dutch Flat-Coated Retriever population is 24%. In this study, we investigated the molecular genetics of the disorder in this population.

Genome-wide association analysis of 15,823 single nucleotide polymorphisms (SNPs) in 45 cases and 40 controls revealed that patellar luxation was significantly associated with a region on chromosome CFA07, and possibly with regions on CFA03, CFA31, and CFA36. The exons of the genes in these regions, 0,5 Mb combined, were analysed further. These exons from 15 cases and a pooled sample from 15 controls were enriched using custom genomic hybridization arrays and analysed by massive parallel DNA sequencing. In total 7,257 variations were detected. Subsequently, a selection of 144 of these SNPs were genotyped in 95 Flat-Coated Retrievers. Nine SNPs, in eight genes on CFA07 and CFA31, were associated with patellar luxation ($P < 10^{-4}$). Genotyping of these SNPs in samples from a variety of breeds revealed that the disease-associated allele of one synonymous SNP in a pseudogene of *FMO6* was unique to Flat-Coated Retrievers. One of the other genes, *TNR* coding for tenascin R, is a candidate gene for PL because mutations in the related gene *TNXB* are the cause of Ehlers-Danlos syndrome type III in humans.

Genome-wide association analysis followed by targeted DNA sequencing identified loci on chromosomes 7 and 31 as being involved in patellar luxation in the Flat-Coated Retriever breed.

Introduction

Patellar luxation (PL) is a common developmental orthopedic disorder in dogs (Johnson et al., 1994; Ness et al., 1996; LaFond et al., 2002). Normally, the patella is located within the trochlea of the femur at a fixed distance from the tibial crest and has only limited sideways movement within this groove (Evans and Miller, 1993). PL occurs when the patella moves out of the trochlear groove and it can lead to degenerative joint disease. Although surgical correction can be performed in most cases, joint cartilage damage may lead to osteoarthritis and consequently to permanent pain and lameness.

Developmental PL is most often seen in small breed dogs (Priester, 1972; Hayes et al., 1994), with the prevalence of PL in breeds appearing to decrease with increasing body size (Hayes et al., 1994; Chase et al., 2009). Both medial and lateral PL (inward and outward movability, respectively) occur in dogs, and medial PL is more common than lateral PL in all breeds but one. Lateral PL is less uncommon in large breed dogs than in small breed dogs (Priester, 1972; Remedios et al., 1992; Hayes et al., 1994; Gibbons et al., 2006). The one breed population where lateral PL is more common than medial PL is the Dutch Flat-Coated Retriever (Lavrijsen et al., 2013). In this population, involvement of both hind legs of a dog occurred in half the cases and PL was more common in female than male dogs, as has been reported in other breeds (Priester, 1972; Hayes et al., 1994; Alam et al., 2007). The prevalence of PL in Dutch Flat-Coated Retriever was 24%, much higher than the prevalence reported for presumably mostly American Flat-Coated Retriever (1.6%) by the Orthopedic Foundation for Animals [www.offa.org]. In the Dutch population, lateral PL was diagnosed in 61% of the cases, medial PL in 31% and both lateral and medial in the remaining 8% (Lavrijsen et al., 2013).

The predisposition of certain breeds to PL and the high proportion of dogs with bilateral PL strongly suggests that PL is a heritable trait. The sex predisposition and lack of a Mendelian segregation pattern points to PL being a polygenic disorder (Priester, 1972; Hayes et al., 1994). The previously calculated heritability of 0.17 in the Dutch Flat-Coated Retriever population indicated that both environmental factors and genetic factors play a role in the phenotypic appearance of the trait (Lavrijsen et al., 2013). Chromosomal regions or genes that predispose to PL have not yet been identified.

We report on the genome-wide association analysis of PL in the Dutch Flat-Coated Retriever population followed by massive parallel DNA sequence analysis

and identified loci on CFA07 and CFA31 as having a major influence on the phenotype. This is the first step toward identifying genes that are involved in the development of PL and may help us gain insight into the aetiology of this crippling disorder.

Material and Methods

Animals

The animals used in this study were part of a Flat-Coated Retriever cohort (n = 3835) that had been screened for PL as adults between 1990 and 2007. The dogs were investigated in standing position and in lateral recumbency to control the location of the patella and the possibility to luxate or reposition the patella to grade the movability as introduced by Putnam (1968), Grade 0: patella is moving inside the trochlear groove and cannot be manually luxated; grade 'loose': patella can be manually positioned on the ridge of the trochlear groove but cannot be positioned out of the groove; grade 1: manually luxatable patella with spontaneous repositioning; grade 2: spontaneous luxation with repositioning upon active extension of the stifle; grade 3: constant spontaneous PL which can be manually reduced; grade 4: constant PL which cannot be manually reduced. All dogs included in this study have been graded by a single board certified veterinary orthopedic specialist (FJM) who made use of the above mentioned grading system and included grade 'loose' in the group of grade 0, both referred to as 'PL-negative'.

Pedigree records were available of 3324 of the phenotyped dogs. These were sired by 398 sires and 678 dams. There were 283 grandfathers and 416 grandmothers of the phenotyped animals. An estimated breeding value (EBV) was calculated for those dogs for which pedigree information was available as described previously (Lavrijsen et al., 2013). The average EBV for 723 cases was 1.71 (ranging from −2.0 to 6.9) and the average EBV in 2600 controls was −0.45 (ranging from −2.7 to 3.5). We calculated EBVs using all dogs and then chose the dogs to be included based on a high or low EBV and their relationship with other dogs in the sample. Selected dogs with a high EBV did not share parents as did the dogs with a low EBV. In the 93 dogs used for genotyping, the average EBV in the 45 cases was 1.96 (ranging from 0.2 to 4.6) and the average EBV in the 48 controls was −1.37 (ranging from −2.7 to 3.5). The dogs used for genotyping were selected on the basis of their PL status, relatedness to other affected dogs, and their EBV. Of the 45 cases, 40 had PL grade 1

(manually luxable patella with spontaneous repositioning), and 5 had PL grade 2 (spontaneous luxation with repositioning upon active extension).

The Dutch Flat-Coated Retriever Breeders Club provided the addresses of the dog owners, who were contacted by letter, which was also written on behalf of the Breeders Club, to inform them about the project and with the request that they ask their licensed veterinarian to take a 4 ml blood sample from their dog for DNA isolation. The samples were forwarded by the veterinarians. All dogs were privately owned and owneres provided informed consent. The study complied with the conditions of the Dutch 'Wet op de Uitoefening van de Diergeneeskunde' (Law on the Practice of Veterinary Medicine) of March 21, 1990. Approval by an ethics committee for the use of the blood samples was not necessary.

Genotyping and data analysis

DNA was isolated from the samples collected from the 45 PL-positive and 48 PL-negative dogs, using a standard salt extraction method (Miller et al., 1988). The Illumina CanineSNP20 BeadChip with approximately 22,000 single nucleotide polymorphisms (SNPs) was used to genotype the 93 dogs. Only SNPs that had a minor allele frequency of more than 1% and that were genotyped in more than 90% of samples were included in the further analysis. PLINK software (Purcell et al., 2007) was used to calculate an identical-by-state matrix between all 93 samples.

Single SNP association analyses were conducted using both the PL status of the animals as a binary trait and the EBV of the animals as a quantitative trait. A χ^2 based allelic association analysis with 45 cases and 40 controls was performed, as well as linear regression modeling using the EBVs of the cases and controls. The sex of the animal was included as a covariate in the linear regression. Both analyses were carried out using PLINK v1.07 software (Purcell et al., 2007). A Bonferroni correction was applied to correct for multiple testing (with 15,823 tests), using $\alpha = 0.05$ as the threshold for significance ($P < 1 \times 10^{-5.5}$). Permutations were also performed (EMP2, n = 1000) as a less stringent method than the Bonferroni correction to correct for multiple testing.

The multiple SNP association analysis was based on a Bayesian variable selection method using iBay software (George and McCulloch, 1993; Heuven and Janss, 2010). To detect associated regions, we used a model that included a polygenic effect as well as all SNPs simultaneously. A priori a mixture model was used, which assumed that all SNPs belonged to one of two normal distributions: the first distribution contained SNPs with little to no effect on the phenotype (most the SNPs

were included in this category), and the second distribution contained the few SNPs that did affect the phenotype. Underlying assumptions about the properties of the two distributions were: (1) the first distribution contained 95% or more of all SNPs and the second contained less than 5%; and (2) the first distribution explained 0.5% of the phenotypic variation observed while the second explained 99.5%. Analogous to the computation and use of the Bayes Factor between two models, we used a 'parameter-wise Bayes Factor' (BF) as the odds ratio between posterior and prior probabilities for an individual marker to be in either of the two distributions. According to Kass and Raftery (1995), a BF value higher than 3.2 is considered 'substantial', a BF value higher than 10 as 'strong', and a BF value higher than 100 as 'decisive'.

Targeted enrichment of genomic DNA fragments and massive parallel sequencing

DNA samples from 15 cases and 15 controls were purified using phenol/chloroform extraction and ethanol precipitation by standard techniques, and the DNA concentration was measured using Qubit® (Invitrogen). DNA from the 15 cases was individually sequenced (1 μg/sample). Equal amounts of DNA from the control dogs were pooled and 1 μg of pooled DNA was used. The 15 individual samples and the pooled sample were sheared by sonication, underwent end-repair and phosphorylation steps, and then adaptors containing barcode addresses were ligated to the resulting fragments and nick translated as described (Harakalova et al., 2011). Fragments were purified, amplified, and hybridized to a custom designed Agilent® Comparative Genomic Hybridisation Microarray. This array was designed to capture all coding exons including 20 bp flanks in the four candidate regions (CanFam2, ensemble57) as well as in the genes *COL15A1*, *THRB1*, *COL6A3*, *FGF6*, *FGF23*, and *WNT5B*. The captured DNA fragments were sequenced using the SOLiD version 4 system. Array design, library preparation, enrichment hybridization and elution, SOLiD sequencing, and mapping of the DNA sequence data were performed at the Hubrecht Institute (Utrecht, the Netherlands) as described (Mokry et al., 2010; Harakalova et al., 2011).

Genotyping of DNA sequence variations

We genotyped selected variations detected by DNA sequencing in 95 Flat-Coated Retrievers, using the Komparative Allele Specific PCR (KASPar) assay (LGC genomics, Hoddesdon, UK). Two differently labeled allele-specific primers and a common reverse primer were designed for eachvariable position. Oligo extension

PCR in the presence of universal fluorescent reporting dyes, in combination with the fluorescence resonance energy transfer (FRET) technique, makes it possible to determine the distribution of the two alleles in the PCR product. Kluster Caller software (LGC genomics, Hoddesdon, UK) was used to determine the genotypes.

The multi-breed sample set contained material from 24 different breeds, 4 samples from English Cocker Spaniels, American Cocker Spaniels, Cavalier King Charles Spaniels, West Highland White Terriers, Cairn Terriers, Border Terrier, Airedale Terriers, Welsh Corgis, Kooikerhondjes (Small Dutch Waterfowl Dog), Basset Hounds, Miniature Schnauzer, Giant Schnauzers, Wetterhouns (Frisian Water Dog), Irish Setters, German Short-haired Pointing Dogs, Dobermanns, Boerboels, Bloodhounds, German Shepherd Dogs, Dutch Shepherd Dogs, Golden Retrievers, Labrador Retrievers, Bernese Mountain Dogs, and 3 samples from Chesapeake Bay Retrievers. The previously described KASPar assay was used to determine genotypes.

Results

Genome-wide association analysis

More than 22,000 SNPs were genotyped in 45 Flat-Coated Retrievers with signs of PL and 48 control dogs of the same breed. In total 15,823 SNPs passed quality control, and these were used to construct an identical-by-state (IBS) plot, based on the first two principal components of the multidimensional IBS matrix (Fig. 1). Eight control samples that deviated from the main population were excluded from further analysis.

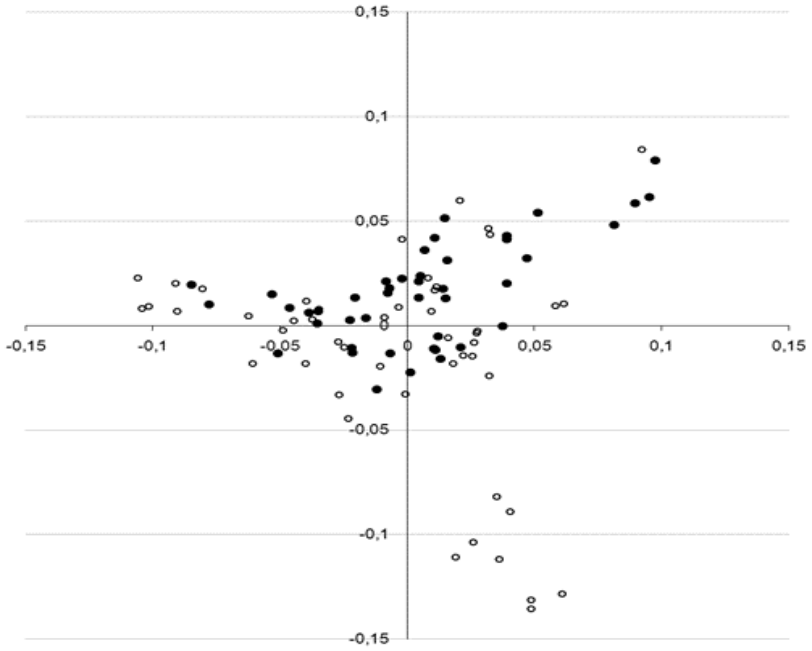


Figure 1 Identical-by-state plot of Flat-Coated Retrievers.
The 93 dogs were genotyped using arrays for 22,000 SNPs. The first two principal components of a multidimensional identical-by-state matrix of 45 dogs with patellar luxation (filled symbols) and 48 control dogs that were negative for patellar luxation (open symbols) were calculated with PLINK software. The cluster of 8 controls at the bottom right part of the plot was excluded from further analysis.

The SNP data were analysed using two phenotypes and two statistical approaches. A discrete case–control phenotype was alternated with estimated breeding values (EBVs) for individual animals. A χ^2 analysis of the allele distribution of individual SNPs in PL-positive and PL-negative dogs indicated that PL is associated with a region on chromosome CFA07 (Fig. 2A, Table 1). The uncorrected *P* was 6.9×10^{-7} and the empirical *P* after correction for multiple testing using permutations of the genotype data over the groups of dogs was 1.0×10^{-3}; the Bonferroni corrected *P* was 1.1×10^{-2}.

Quantitative trait association analysis of individual SNPs with PLINK software and using the EBVs of the 85 dogs instead of their PL status again indicated PL to be associated with CFA07 (uncorrected $P = 2.2 \times 10^{-8}$) and CFA36 (uncorrected $P = 1.9 \times 10^{-6}$). Both associations were significant after correction for multiple testing using the Bonferroni correction (Figure 2B).

Table 1 Comparison of top SNPs associated with patellar luxation defined as binary trait or by estimated breeding value

			MAF*		Single-SNP		Multi-SNP	
CFA	BP	SNP	cases	controls	PL status	EBV	PL status	EBV
01	99888625	BICF2S2314252	0.42	0.23	2.20	2.94	0.42	1.18
03	67056782	BICF2P309055	0.16	0.43	4.01	2.90	3.97	0.22
04	16996349	BICF2S23034244	0.17	0.45	4.24	4.67	0.41	0.16
07	17648777	BICF2S2293048	0.18	0.46	4.20	2.77	0.65	0.04
07	18970233	BICF2G630553500	0.12	0.41	4.79	4.54	0.57	0.21
07	19071723	BICF2P1448362	0.14	0.44	4.64	4.34	0.39	0.26
07	19746349	BICF2G630553889	0.12	0.46	6.06	4.83	1.30	0.69
07	20109002	BICF2P1333659	0.13	0.46	5.65	4.52	1.07	0.31
07	20145907	BICF2P1335550	0.13	0.46	5.65	4.52	1.22	0.40
07	21065761	BICF2S23030368	0.13	0.45	5.33	4.87	0.81	0.31
07	22157845	BICF2P233561	0.19	0.51	5.05	4.11	0.73	0.20
07	23113211	BICF2P1060266	0.12	0.41	4.79	5.06	0.26	0.37
07	24186445	BICF2P424667	0.12	0.41	4.79	5.06	0.33	0.24
07	25490867	BICF2P205579	0.23	0.53	4.07	3.88	0.46	0.15
07	27099172	BICF2S2457585	0.11	0.45	6.16	7.34	1.62	8.11
07	28293222	BICF2P1386712	0.12	0.46	6.06	7.67	1.23	5.95
07	32710038	BICF2G630555333	0.26	0.49	2.73	5.33	0.09	0.14
25	49858895	BICF2P1461096	0.24	0.49	3.01	3.49	1.08	0.19
27	43484050	BICF2G630153501	0.58	0.33	3.02	1.98	1.16	0.17
27	46605159	BICF2G630154851	0.58	0.34	2.77	2.74	1.17	0.33
31	15166531	BICF2S23135348	0.36	0.09	4.48	3.35	1.29	0.12
36	29549762	BICF2S22944651	0.51	0.21	4.22	5.73	1.22	0.07
36	29608881	BICF2G630757990	0.64	0.29	5.22	5.17	1.45	0.09

***** MAF: minor allele frequency; Single-SNP associations are presented as –log *P*; Multi-SNP associations as Bayesian factors.

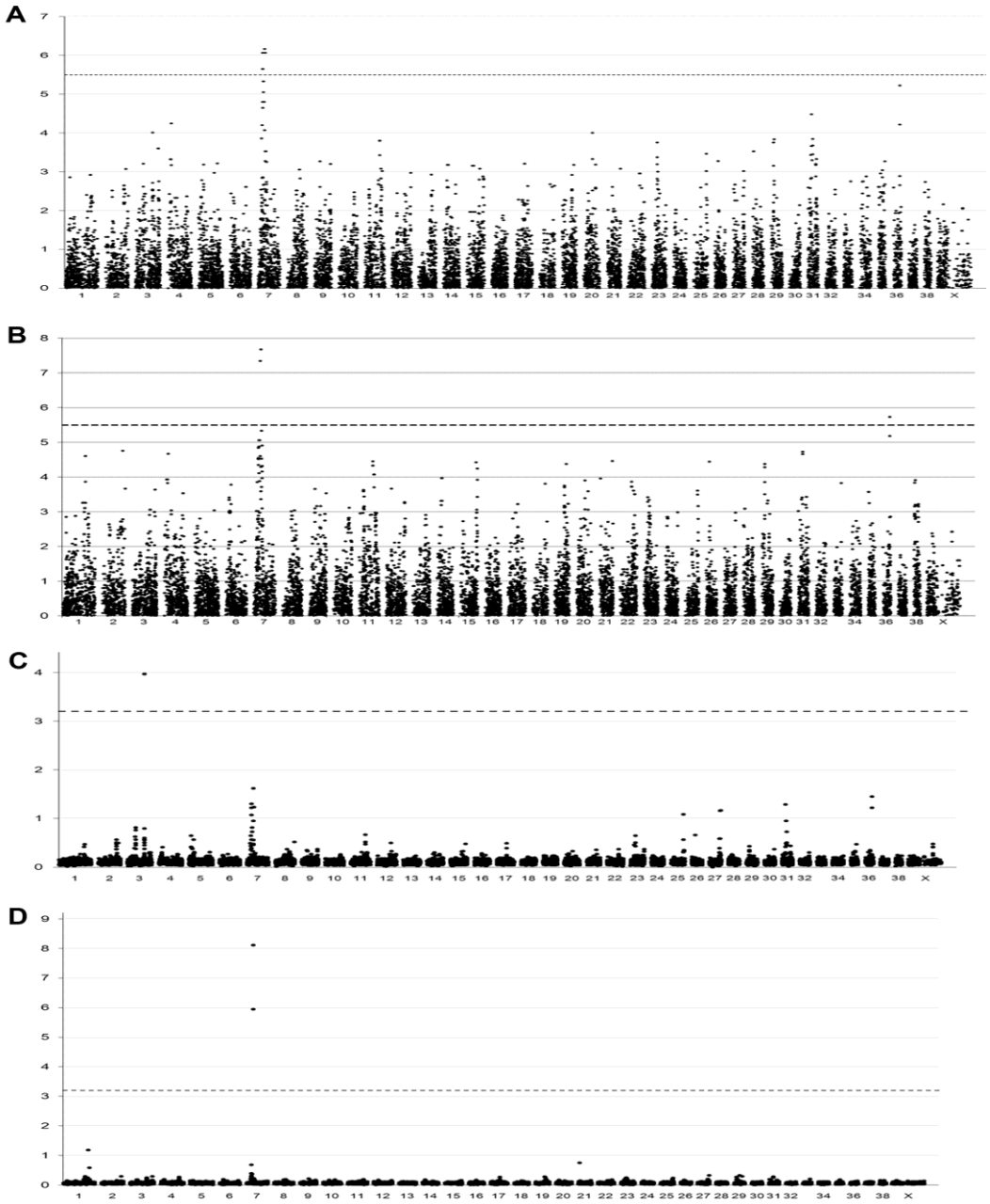


Figure 2 Genome-wide association analysis of patellar luxation in Flat-Coated Retrievers. **(A)** Association of individual SNPs with patellar luxation was analysed with PLINK software by comparing allele frequencies in the cases (n = 45) and controls (n = 40). **(B)** Association analysis of individual SNPs using the Estimated Breeding Values (EBV) as phenotype using PLINK. **(C)** Multi-SNP association analysis was performed using the case/control patellar luxation status and iBAY software. **(D)** same as **(C)** using EBV as phenotype. The –10log of the *P* obtained of individual SNPs are plotted in **(A)** and **(B)**, with the dotted lines indicating the Bonferroni threshold over 15,823 SNPs (α = 0.05). Multi-SNP association values are presented as Bayes factors in (**C**) and **(D)**, with the dotted lines indicating the 'substantial' effect threshold according to guidelines by Kass and Raftery (1995).

Multiple SNP analysis using a Bayesian variable selection method (George and McCulloch, 1993), as implemented in iBay software, revealed regions associated with PL (Bayesian factor > 1) on chromosomes 3, 7, 25, 27, 31, and 36 when PL was considered as a binary trait (Figure 2C, Table 1). The region on CFA03 explained the largest part of the phenotypic variation (Bayesian factor = 3.97). When the EBV was used as phenotypic score, chromosomes 1 and 7 were associated with the PL phenotype (Bayesian factor >1, Figure 2D, Table 1).

Four regions of interest were selected on the basis of the results from the individual and multiple SNP association studies for both phenotypes. These regions included CFA03 (Canfam2 position 64-69 Mb), CFA07 (15–29.5 Mb), CFA31 (13-21 Mb), and CFA36 (27.5-32 Mb).

Targeted massive parallel DNA sequencing

The exons of all genes in the four candidate regions were selected for microarray-based enrichment and DNA sequence analysis. The total size of the candidate regions was approximately 32 Mb, and we designed enrichment arrays that covered about 0.5 Mb. The selected regions were sequenced in 15 individual dogs with PL and in a pooled sample from 15 controls.

Enrichment probes could be designed for 93% of the target DNA, so that 476,935 base pairs were represented. Approximately 30% of the generated reads could be mapped to the targeted regions. The average coverage in the target regions was about 80 fold. In all, 7257 variations were observed in fragments that were covered at least 10 times in one or more of the cases and at least 10 times in the control pool. The frequency of the reads of the alternate alleles was used as an indication of the allele frequency in the control pool. In total, 407 variations were detected with a coverage of more than 25 reads in at least 10 cases and in the control pool. The difference in the average allele frequency based on the number of reads per allele between the cases and the control pool was more than 10%. The 40 SNPs with the largest difference in frequency between the cases and the pool of controls are depicted in Table 2.

Genotyping of candidate SNPs in a large cohort

A set of 124 SNPs was selected for further analysis on the basis of the differences in allele frequency between cases and controls. Because the SNPs with the greatest difference in frequency were mainly in regions on CFA07, 20 SNPs were added from

regions on CFA03, CFA31, and CFA36. The complete set of 144 selected SNPs is listed in Appendix A (Table 1). These SNPs were genotyped in a group of 95 Flat-Coated Retrievers. This was done to expand the dataset and to ascertain the genotype deduced from the read coverage of each allele. This group of dogs largely overlapped, but was not identical to, the group used in the genome wide SNP analysis. This was because only a limited amount of DNA was available for 6 control dogs. These were replaced by other controls and 10 more controls were added. In total, 127 SNPs were reliably genotyped, 30 of which were monomorphic. Single SNP χ^2 based analysis of the remaining 97 SNPs identified 8 SNPs on CFA07 and 1 on CFA31 that were associated with PL ($P < 1.0 \times 10^{-4}$, Table 3); the SNPs associated with PL located on CFA03 and CFA36 were less significant ($P > 1.0 \times 10^{-4}$).

Table 2 Top 40 variations associated with patellar luxation derived from DNA sequence data

CFA	Position	Alleles	Associated allele	Frequency cases	Frequency controls	Frequency difference
03	67172456	[A/G]	G	0.54	0.33	0.21
07	15554687	[G/A]	A	0.45	0.23	0.22
07	15995236	[T/C]	C	0.55	0.27	0.28
07	17387000	[A/G]	G	0.53	0.31	0.22
07	19204281	[C/G]	G	0.72	0.50	0.22
07	19301203	[T/C]	C	0.82	0.53	0.29
07	19865384	[A/G]	G	0.65	0.36	0.29
07	20790820	[T/C]	C	0.42	0.16	0.26
07	21406148	[C/T]	C	0.26	0.57	0.31
07	22035860	[A/G]	G	0.72	0.44	0.28
07	22172500	[G/A]	A	0.81	0.57	0.24
07	22173886	[G/A]	A	0.84	0.49	0.35
07	22420986	[C/A]	A	0.51	0.26	0.25
07	23548193	[A/G]	A	0.09	0.33	0.24
07	24673491	[G/T]	T	0.27	0.06	0.21
07	24704299	[C/T]	C	0.12	0.46	0.34
07	25534837	[G/A]	A	0.61	0.37	0.24
07	27238943	[G/A]	G	0.37	0.58	0.21
07	28291838	[C/T]	T	0.84	0.47	0.37
07	28294930	[T/C]	C	0.66	0.31	0.35
07	29308525	[C/T]	T	0.75	0.37	0.38

continued

07	29526712	[G/T]	T	0.38	0.17	0.21
07	30605944	[C/T]	T	0.43	0.21	0.22
07	30605965	[G/T]	T	0.39	0.15	0.24
07	31235880	[T/C]	C	0.67	0.29	0.38
07	31855627	[T/C]	C	0.71	0.46	0.25
07	31856484	[T/C]	C	0.65	0.41	0.24
07	31859005	[T/A]	T	0.16	0.44	0.28
07	32013108	[A/G]	A	0.19	0.47	0.28
07	32149996	[T/C]	C	0.63	0.15	0.48
07	32161825	[G/A]	A	0.71	0.35	0.36
07	32162626	[T/C]	C	0.62	0.27	0.35
23	22621361	[G/A]	A	0.28	0.03	0.25
23	22687566	[G/A]	A	0.40	0.16	0.24
31	14341470	[G/A]	A	0.47	0.26	0.21
31	17088962	[G/T]	T	0.71	0.50	0.21
36	27757717	[C/A]	A	0.54	0.28	0.26
36	27770444	[C/T]	T	0.34	0.13	0.21
36	28095751	[A/T]	A	0.13	0.35	0.22
36	29133623	[T/C]	C	0.54	0.33	0.21

Table 3 Intragenic SNPs associated with patellar luxation

CFA	Position	alleles	-logp	Gene	Gene ID	Effect	Gene Description
07	27010438	G/A	5.44	TNR	490334	Synonymous	tenascin R
07	28294930	T/C	5.08	SERPINC1	480066	Synonymous	serpin peptidase inhibitor
07	28329409	T/C	4.21	KLHL20	480067	Intronic	kelch-like 20
07	30605944	G/A	4.21	FMO2	480076	Synonymous	flavin containing monooxygenase 2
07	30669327	C/T	4.32	FMO6P	490346	Synonymous	flavin containing monooxygenase 6
07	31856484	G/C	4.02	SELE	403999	Non_Synonymous	selectin E
07	32149996	C/T	5.35	BLZF1	490354	Synonymous	basic leucine zipper nuclear factor 1
07	32162626	T/C	5.09	BLZF1	490354	Intronic	basic leucine zipper nuclear factor 1
31	14864500	T/C	5.35	NRIP1	478385	Synonymous	nuclear receptor interacting protein 1

We then investigated whether the 8 SNPs on CFA07 that were associated with PL were also polymorphic in other breeds (24 breeds, with 3–4 dogs per breed). Most alleles associated with PL in the Flat-Coated Retriever breed were also detected in

other breeds, with the exception of the synonymous SNP in the *FMO6* pseudogene at position 30669327, which was not common in the other breeds.

Discussion

In this study, we analysed the susceptibility of Flat-Coated Retrievers to PL in two ways: we used the PL status of the animals as a binary trait (PL present or absent) and we used the EBV of all dogs as a quantitative trait. The breeding value takes into account all available phenotypic data from relatives and the animal itself and is a better indicator of genetic susceptibility than an animal's disease status alone. The observation that the EBV approach resulted in more significant *P* than the binary trait approach illustrates the usefulness of the EBV approach, indicating that a region on CFA07 is involved in the development of PL. The level of significance obtained for this complex disorder using a relatively low number of cases and controls suggests that this region is a major determinant of PL. The choice of DNA sequencing strategy was influenced by two considerations. First, there was the large size (9 Mb) of the region on CFA07 associated with PL. By choosing an exon sequencing strategy, not only could the entire associated region on CFA07 be included, but also additional regions. Second, the number of DNA samples that could be sequenced was limited by the small number of DNA barcode addresses available when the study was performed. Only 30% of the reads mapped to the targeted chromosomal regions instead of the minimally expected 60% (Kass and Raftery, 1995). We have no explanation for this low yield of the used enrichment procedure with genomic hybridization arrays. In unrelated projects, we obtained higher yields with in solution enrichment protocols.

We pooled control samples because we thought that the allele frequency of DNA sequence variants could be established on the basis of their representation in the reads. However, analysis of individually sequenced DNA of the cases indicated that with an average coverage of 80 reads the allele representation was highly variable and therefore an unreliable indicator of the underlying genotype. The availability of more barcodes since then means that the use of pooled DNA samples can be avoided in future studies.

Approximately 25% of the SNPs genotyped using the KASPar assay were monomorphic, which highlights the importance of confirming Next Generation Sequencing results using independent methods.

The function of the extensor mechanism of the stifle joint depends on the proper alignment of the skeletal and soft tissue elements involved, and different anatomical abnormalities that cause malalignment of these elements have been suggested to be the basis of PL. Ventro-dorsal radiographs of the hip and knees of eight Dutch Flat-Coated Retrievers with PL did not show signs of bony malalignment [Lavrijsen, unpublished data], and therefore the involvement of muscles or ligaments in PL seems more likely, as suggested by others (Mostafa et al., 2008).

We identified nine DNA sequence variants in eight positional candidate genes for PL in affected dogs. One of these, *TNR* coding for tenascin R, is a candidate gene for PL, because mutations in one of its paralogues, *TNXB*, are known to cause Ehlers-Danlos syndrome type III in humans (omim:130020). Ehlers-Danlos syndrome is a connective tissue disorder that is characterized by skin hyperextensibility, articular hypermobility, and tissue fragility. In humans, several disease-causing mutations have been identified in genes involved in the development and maintenance of connective tissue. Ehlers-Danlos syndrome type III is associated with recurrent dislocation of the shoulder joint, the temporomandibular joint, and the patella, without any skeletal deformity. The associated synonymous variant in the tenascin R gene on dog CFA07 could affect the expression of the gene by disturbing the splicing machinery or decreasing the stability of the mRNA. In combination with other genetic risk factors, this variant might predispose Flat-Coated Retrievers to PL. However, studies indicate that human *TNR* is expressed solely in the brain (Carnemolla et al., 1996), which is not compatible with its involvement in PL.

It should be noted that although we achieved an average coverage of between 80–90 DNA sequence reads per location, not all target regions were covered sufficiently. It is therefore possible that we missed relevant mutations. In addition, as we only analysed exons and intron/exon boundaries, we cannot rule out that variants in promoter regions or introns contribute to the phenotype. To confirm the involvement of tenascin R in PL in Flat-Coated Retrievers, the *TNR* gene needs to be analysed in a replication cohort of cases and controls. Investigation of the gene in other breeds predisposed to PL may also lead to confirmation of its role. Additional fine-mapping of the other regions on chromosomes 3, 31, and 36 associated with PL may identify more genetic factors involved in the disorder.

Conclusions

We identified regions on chromosomes 3, 7, 31, and 36 that are associated with PL in the Dutch Flat-Coated Retrievers. Fine-mapping of the region on CFA07 that showed the strongest association led to the identification of a synonymous variant of *TNR* coding for tenascin R. Mutations in the related protein tenascin XB are the cause of joint dislocations in humans. Follow-up is needed to confirm the involvement of the CFA07 region in PL in the Flat-Coated Retriever and possibly other breeds.

Availability of supporting data

SNP data obtained with microarrays are available in the ArrayExpress database under accession number E-MTAB-2040 (ArrayExpress).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ICML participated in the design of the study, performed the statistical analysis and the molecular genetic studies, and drafted the manuscript. PAJL participated in the design and coordination of the study and helped to draft the manuscript. CW participated in the molecular genetic studies and in the statistical analysis. FGvS participated in the DNA sequence analysis. MS participated in the sample collection. GJB participated in the design of the study. FJM participated in the phenotyping and the design of the study. IJN designed the DNA sequence analysis and aligned the sequence. EC participated in the design of the study. HCMH participated in the design of the study, the statistical analysis and helped to draft the manuscript. HAWH conceived of the study, participated in the design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was funded in part by the Canine Health Foundation of the American Kennel Club, grant 00580. We gratefully acknowledge Mrs. Elly van Gent of the Dutch Flat-Coated Retriever Breed Club for assistance with sample collection. The authors would like to thank Manon Vos-Loohuis for assistance with laboratory experiments.

References

Alam, M.R., Lee, J.I., Kang, H.S., Kim, I.S., Park, S.Y., Lee, K.C., Kim, N.S., 2007. Frequency and distribution of patellar luxation in dogs: 134 cases (2000 to 2005). Veterinary and Comparative Orthopaedics and Traumatology 20, 59-64.

Carnemolla, B., Leprini, A., Borsi, L., Querzé ,G., Urbini, S., Zardi, L., 1996. Human tenascin-R. Complete primary structure, pre-mRNA alternative splicing and gene localization on chromosome 1q23-q24. Journal of Biological Chemistry 271, 8157–8160.

Chase, K., Jones, P., Martin, A., Ostrander, E.A., Lark, K.G., 2009. Genetic mapping of fixed phenotypes: Disease frequency as a breed characteristic. Journal of Heredity 100 (Suppl 1), S37-41.

Evans, H.E., Miller, M.E., 1993. Miller's anatomy of the dog. Philadelphia, WB Saunders.

George, E.I., McCulloch, R.E., 1993. Variable selection via Gibbs sampling. Journal of the American Statistical Association 88, 881-889.

Gibbons, S.E., Macias, C., Tonzing, M.A., Pinchbeck, G.L., McKee, W.M., 2006. Patellar luxation in 70 large breed dogs. The Journal of Small Animal Practice 47, 3-9.

Harakalova, M., Mokry, M., Hrdlickova, B., Renkens, I., Duran, K., van Roekel, H., Lansu, N., van Roosmalen, M., de Bruijn, E., Nijman, I.J., Kloosterman, W.P., Cuppen, E., 2011. Multiplexed array-based and insolution genomic enrichment for flexible and cost-effective targeted next-generation sequencing. Nature Protocols 6, 1870–1886.

Hayes, A.G., Boudrieau, R.J., Hungerford, L.L., 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal of the American Veterinary Medical Association 205, 716-720.

Heuven, H.C., Janss, L.L., 2010. Bayesian multi-QTL mapping for growth curve parameters. BMC Proceedings 4(Suppl 1), S12.

Johnson, J.A., Austin, C.C., Breur, G.J., 1994. Incidence of canine appendicular musculoskeletal disorders in 16 veterinary teaching hospitals from 1980 through 1989. Veterinary and Comparative Orthopaedics and Traumatology 7, 56-69.

Kass, R.E., Raftery, A.E., 1995. Bayes factors. Journal of the American Statistical Association 90, 773-795.

LaFond, E., Breur, G.J., Austin, C.C., 2002. Breed susceptibility for developmental orthopedic diseases in dogs. Journal of the American Animal Hospital Association 38, 467-477.

Lavrijsen, I.C., Heuven, H.C., Breur, G.J., Leegwater, P.A., Meutstege, F.J., Hazewinkel, H.A., 2013. Phenotypic and genetic trends of patellar luxation in Dutch Flat-Coated Retrievers. Animal Genetics 44, 736–741.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16, 1215.

Mokry, M., Feitsma, H., Nijman, I.J., de Bruijn, E., van der Zaag, P.J., Guryev, V., Cuppen, E., 2010. Accurate SNP and mutation detection by targeted custom microarray-based genomic enrichment of short-fragment sequencing libraries. Nucleic Acids Research 38, e116.

Mostafa, A.A., Griffon, D.J., Thomas, M.W., Constable, P.D., 2008. Proximodistal alignment of the canine patella: radiographic evaluation and association with medial and lateral patellar luxation. Veterinary Surgery 37, 201-211.

Ness, M.G., Abercromby, R.H., May, C., Turner, B.M., Carmichael, S., 1996. A survey of orthopedic conditions in small animal veterinary practice in Britain. Veterinary and Comparative Orthopaedics and Traumatology 9, 43-52.

Priester, W.A., 1972. Sex, size, and breed as risk factors in canine patellar dislocation. Journal of the American Veterinary Medical Association 160, 740-742.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: A toolset for whole-genome association and population-based linkage analysis. American Journal of Human Genetics 81, 559-575.

Putnam, R.W., 1968. Patellar luxation in the dog**.** In: Master thesis. University of Guelph, Veterinary College.

Remedios, A.M., Basher, A.W., Runyon, C.L., Fries, C.L., 1992. Medial patellar luxation in 16 large dogs. A retrospective study. Veterinary Surgery 21, 5-9.

ArrayExpress. www.ebi.ac.uk/arrayexpress

Appendix A:

Table 1

Evaluation of DNA sequence variations in Flat-Coated Retrievers.

CFA	Position	alleles	Result	F_U CONTROL	F_A CASE	P1	associated allele
03	66990848	[C/A]	Failed				
03	67144566	[C/G]	Dimorph	0.829787234	0.72826087	0.094918795	C
03	67170761	[A/G]	Monomorph				
03	67172456	[A/G]	Dimorph	0.510638298	0.366666667	0.049232551	G
03	67371947	[C/T]	Dimorph	0.75	0.522222222	0.001366976	T
03	67596606	[G/A]	Failed				
03	68080542	[G/C]	Dimorph	0.393617021	0.173913043	0.00090389	G
03	69317383	[C/T]	Dimorph	0.895833333	0.804347826	0.078190691	C
07	14979277	[C/T]	Dimorph	0.382978723	0.308510638	0.260742443	C
07	15554687	[G/A]	Failed				
07	15556395	[C/T]	Failed				
07	15995236	[T/C]	Monomorph				
07	16213031	[T/C]	Dimorph	0.760416667	0.542553191	0.001578001	C
07	16699668	[G/T]	Failed				
07	17387000	[A/G]	Dimorph	0.395833333	0.180851064	0.001087468	G
07	18222175	[C/G]	Dimorph	0.364583333	0.276595745	0.190809256	G
07	18753742	[G/T]	Dimorph	0.447916667	0.319148936	0.066525826	G
07	19173815	[A/G]	Failed				
07	19204281	[C/G]	Dimorph	0.554347826	0.391304348	0.026444339	G
07	19301203	[T/C]	Dimorph	0.329787234	0.445652174	0.103273695	T
07	19443809	[G/A]	Dimorph	0.614583333	0.553191489	0.37903652	A
07	19865384	[A/G]	Failed				
07	20112041	[T/G]	Dimorph	0.489583333	0.265957447	0.001489048	G
07	20213077	[T/A]	Dimorph	0.395833333	0.315217391	0.248604341	T
07	20359591	[G/A]	Dimorph	0.4375	0.319148936	0.090546601	A
07	20520927	[T/A]	Failed				
07	20790820	[T/C]	Monomorph				
07	21257913	[A/G]	Dimorph	0.75	0.904255319	0.004940244	A
07	21406148	[C/T]	Dimorph	0.614583333	0.554347826	0.401415649	C
07	21719011	[T/A]	Failed				
07	22034869	[G/A]	Dimorph	0.489583333	0.260869565	0.001210692	G
07	22035658	[A/G]	Dimorph	0.574468085	0.554347826	0.752970305	A
07	22035860	[A/G]	Dimorph	0.095744681	0.063829787	0.38198121	A
07	22172500	[G/A]	Failed				
07	22173886	[G/A]	Monomorph				
07	22420986	[C/A]	Dimorph	0.458333333	0.329787234	0.068863389	C
07	22427094	[A/G]	Dimorph	0.614583333	0.553191489	0.37903652	A
07	22848669	[G/T]	Failed				

continued

CFA	position	alleles	result	F_U CONTROL	F_A CASE	P1	associated allele
07	23115505	[C/T]	Dimorph	0.489583333	0.265957447	0.001489048	C
07	23548193	[A/G]	Dimorph	0.659574468	0.595744681	0.340473729	A
07	23712843	[C/T]	Dimorph	0.59375	0.563829787	0.64594795	C
07	24234983	[C/G]	Failed				
07	24673491	[G/T]	Monomorph				
07	24704299	[C/T]	Dimorph	0.904255319	0.776595745	0.015999021	C
07	25312623	[T/C]	Dimorph	0.447916667	0.638297872	0.008266238	T
07	25534837	[G/A]	Failed				
07	25971448	[A/G]	Dimorph	0.791666667	0.630434783	0.014429485	G
07	26217651	[G/A]	Dimorph	0.638297872	0.722222222	0.222740476	A
07	26608089	[C/T]	Monomorph				
07	26608395	[C/G]	Monomorph				
07	27010438	[G/A]	Dimorph	0.608695652	0.902173913	0.00000364	A
07	27015728	[A/G]	Dimorph	0.927083333	0.833333333	0.047864473	A
07	27015758	[C/T]	Dimorph	0.445652173	0.391304348	0.4549	T
07	27016243	[C/T]	Monomorph				
07	27019828	[A/G]	Monomorph				
07	27052030	[A/T]	Dimorph	0.135416667	0.217391304	0.139689635	A
07	27052313	[G/A]	Failed				
07	27231747	[G/A]	Dimorph	0.604166667	0.819148936	0.001075849	G
07	27238943	[G/A]	Dimorph	0.595744681	0.436170213	0.028222759	A
07	27240481	[G/A]	Dimorph	0.353658537	0.568965517	0.002826955	G
07	27241446	[C/G]	Dimorph	0.604166667	0.819148936	0.001072512	C
07	27247148	[C/T]	Dimorph	0.604166667	0.815217391	0.0015	T
07	27344022	[T/A]	Dimorph	0.717391304	0.478723404	0.000867828	T
07	28027739	[A/T]	Dimorph	0.395833333	0.445652174	0.48904563	T
07	28291838	[C/T]	Dimorph	0.041666667	0.031914894	0.68560359	T
07	28294930	[T/C]	Dimorph	0.385416667	0.106382979	0.00000832	T
07	28324433	[G/A]	Monomorph				
07	28329409	[G/C]	Dimorph	0.552083333	0.265957447	0.0000611	C
07	28329867	[T/G]	Dimorph	0.854166667	0.72826087	0.03282399	T
07	28331626	[T/C]	Dimorph	0.541666667	0.787234043	0.000337447	C
07	28332305	[A/C]	Dimorph	0.848837209	0.744186047	0.084052731	A
07	28346057	[A/T]	Dimorph	0.854166667	0.734042553	0.0404	T
07	28349301	[C/A]	Dimorph	0.854166667	0.739130435	0.047199978	A
07	28369475	[T/A]	Dimorph	0.854166667	0.720930233	0.025959094	A
07	28382193	[T/C]	Failed				
07	28382201	[T/C]	Dimorph	0.861702128	0.755319149	0.0636	C
07	28396836	[T/C]	Dimorph	0.802325581	0.720930233	0.210327917	C
07	28399631	[G/A]	Dimorph	0.861702128	0.75	0.051061508	A
07	28519405	[A/T]	Monomorph				
07	28574303	[A/G]	Dimorph	0.916666667	0.863636364		A
07	28574327	[G/T]	Monomorph				

Continued

CFA	position	alleles	Result	F_U CONTROL	F_A CASE	P1	associated allele
07	28728522	[G/C]	Dimorph	0.59375	0.436170213	0.029775923	C
07	29308525	[C/T]	Dimorph	0.666666667	0.574468085	0.190275397	T
07	29526712	[G/T]	Dimorph	0.3125	0.293478261	0.776620689	T
07	30000680	[C/A]	Dimorph	0.454545455	0.3	0.029201506	C
07	30376835	[G/A]	Dimorph	0.208333333	0.304347826	0.12955025	A
07	30544302	[A/G]	Failed				
07	30556515	[C/T]	Dimorph	0.59375	0.423913043	0.019583234	T
07	30577625	[C/T]	Dimorph	0.40625	0.563829787	0.029371031	T
07	30605944	[C/T]	Dimorph	0.552083333	0.265957447	0.0000611	T
07	30605965	[G/T]	Monomorph				
07	30653958	[C/T]	Dimorph	0.604166667	0.829787234		T
07	30662143	[A/G]	Dimorph	0.595744681	0.436170213	0.028590298	G
07	30669327	[G/A]	Dimorph	0.385416667	0.127659574	0.0000478	A
07	30688350	[T/A]	Failed				
07	30769324	[A/T]	Failed				
07	30792495	[T/A]	Monomorph				
07	30797162	[G/A]	Dimorph	0.90625	0.880434783	0.519802222	A
07	31196733	[A/T]	Dimorph	0.59375	0.434782609	0.028912913	A
07	31235880	[T/C]	Monomorph				
07	31581091	[G/T]	Monomorph				
07	31646733	[G/C]	Monomorph				
07	31804840	[G/T]	Dimorph	0.606382979	0.826086957	0.000892086	G
07	31855627	[T/C]	Failed				
07	31856484	[T/C]	Dimorph	0.552083333	0.27173913	0.0000954	T
07	31859005	[T/A]	Dimorph	0.606382979	0.815217391	0.0017	T
07	31882998	[T/G]	Failed				
07	31958856	[T/C]	Dimorph	0.604166667	0.826086957	0.000762555	C
07	31962216	[G/A]	Monomorph				
07	32011935	[C/A]	Dimorph	0.40625	0.563829787	0.0298	A
07	32011967	[C/T]	Dimorph	0.604166667	0.819148936	0.001075849	C
07	32012387	[C/T]	Dimorph	0.353658537	0.55	0.006364054	T
07	32013108	[A/G]	Dimorph	0.604166667	0.819148936	0.001072512	G
07	32024695	[T/C]	Dimorph	0.604166667	0.815217391	0.0015	T
07	32074836	[C/T]	Monomorph				
07	32075037	[G/C]	Monomorph				
07	32140258	[A/G]	Monomorph				
07	32149996	[T/C]	Dimorph	0.395833333	0.106382979	0.00000442	C
07	32161825	[G/A]	Dimorph	0.583333333	0.404255319	0.013372111	G
07	32162626	[T/C]	Dimorph	0.385416667	0.106382979	0.00000812	C
07	32245967	[C/T]	Monomorph				
07	32431354	[T/C]	Monomorph				
07	32435359	[A/G]	Dimorph	0.09375	0.130434783	0.416476402	A

continued

CFA	position	alleles	result	F_U CONTROL	F_A CASE	P1	associated allele
07	32436300	[T/C]	Dimorph	0.90625	0.872340426	0.447228114	C
07	32436308	[A/G]	Dimorph	0.914893617	0.872340426		G
23	22621361	[G/A]	Monomorph				
23	22687566	[G/A]	Dimorph	0.583333333	0.457446809	0.082445441	A
31	14133669	[G/T]	Monomorph				
31	14305403	[A/C]	Dimorph	0.510416667	0.436170213	0.297086907	C
31	14341470	[G/A]	Monomorph				
31	14864500	[T/C]	Dimorph	0.395833333	0.106382979	0.00000442	C
31	15669122	[A/G]	Dimorph	0.760416667	0.531914894	0.000966386	A
31	16137704	[T/C]	Dimorph	0.854166667	0.755319149	0.083403976	T
31	17088962	[G/T]	Dimorph	0.5	0.308510638	0.007187009	G
31	17636529	[C/T]	Dimorph	0.627659574	0.489361702	0.055503136	T
36	27757717	[C/A]	Dimorph	0.614583333	0.521276596	0.19097792	C
36	27770444	[C/T]	Dimorph	0.479166667	0.260869565	0.001964445	T
36	28095751	[A/T]	Monomorph				
36	28779044	[C/T]	Failed				
36	29133623	[T/C]	Dimorph	0.583333333	0.554347826	0.68823708	C
36	29169954	[T/G]	Dimorph	0.822916667	0.712765957	0.070281021	T
36	29521125	[C/A]	Failed				
36	30551798	[A/G]	Monomorph				
36	31294058	[A/C]	Dimorph	0.819148936	0.739130435	0.185184612	A

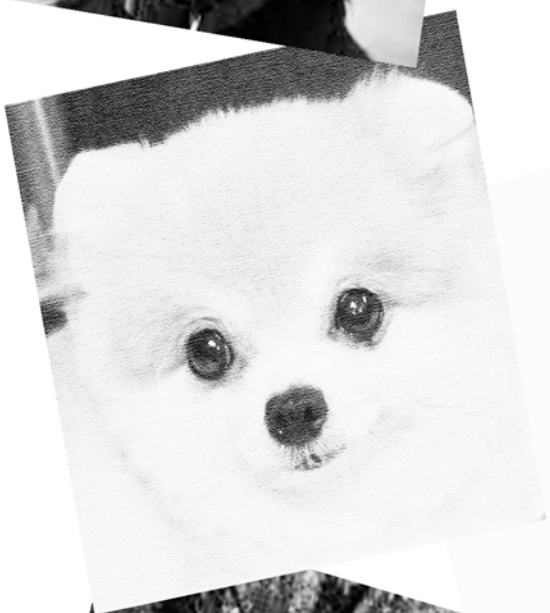
F_U: frequency of associated allele in unaffecteds

F_A: frequency of associated allele in affecteds

P1: X2 p-value

Chapter 9

General discussion



The objective of this chapter is to discuss the surgical treatment, prevalence, pathophysiology, and genetics of patellar luxation (PL) in Pomeranians, Kooiker dogs, Flat-Coated Retrievers.

Surgical treatment of PL

Before 1900, PL was treated by bandaging, exercise, and manual reduction (DeAngelis and Hohn, 1970). Medial desmotomy of the femoropatellar ligament was the first surgical techniques described by Quitman in 1927. Later, it was used in combination with other technique and it is still applied as part of the treatment for PL grade 3 and 4 (Piermattei et al., 2006; Slocum and Devine, 1985). However, this technique results in a high frequency of patellar reluxation (Arthurs and Langley-Hobbs, 2006). Partial or total patellectomy has been mentioned in severe arthritis or as a salvage operation when previous procedures have failed. Stabilization of the patella with suture materials or with a fascia strip has been in use between 1935 and 1970 (DeAngelis and Hohn, 1970). Nowadays, stabilization, i.e. antirotational suture or lateral reinforcement, is still in use alone and in combination with other techniques (Hulse, 1995; Piermattei et al., 2006).

Decreasing depth of the trochlear groove characterized by flattening of the groove or even convex appearance is a pathophysiological condition that occurs in the developmental PL. The first described procedure to deepen the trochlear groove is trochlear sulcoplasty; however, this technique results in removal of hyaline articular cartilage and exposure of the subchondral bone. The surface of the sulcus may be irregular and roughened which causes obvious patella cartilage pathology (Hulse et al., 1986). Therefore trochleoplasty techniques including trochlear wedge (TWR) and trochlear block recession (TBR) are recommended to deepen the trochlear groove. TWR preserves the articular cartilage; however, exposure of subchondral bone occurs along the proximal, distal, and abaxial margins of the wedge (Slocum et al., 1982). It is remarkable that only from a very few surgical techniques longitudinal follow-up studies are present in literature as described in **chapter 2**.

In **chapter 3**, TBR was chosen to accommodate the width of the patella while preserving the medial and lateral trochlear ridges because the advantages above TWR are increased proximal trochlear depth and more patellar articular contact in the extended stifle position (Johnson et al., 2001). All Pomeranians with medial PL (MPL) had very shallow trochlear sulcus which supports the concept that abnormalities are occurring during the development of this condition. Tibial

tuberosity transposition (TTT) is a main technique to correct the developmental condition of PL in case of quadriceps malalignment (Singleton, 1957; Alam et al., 2007). It is an effective technique to realign the extensor mechanism of the stifle with low frequency of complication and recurrence of PL (Arthurs and Langley-Hobbs, 2006). TTT was performed in this study in all Pomeranians with grade 3 and 4 MPL. Recurrent luxation occurred in 13% and in 9% of grade 3 PL in Pomeranians treated with TBR or with TBR combined with TTT, respectively. However, only in 42% of the Pomeranians with grade 3 MPL, TTT was necessary in addition to the surgical release of the soft tissues medial to the patella and TBR. Medial release alone was sufficient to realign quadriceps muscle group in 58% of the cases with grade 3 MPL, suggesting that soft tissue tightness/laxity plays a significant role as the primary signs of MPL in this population of Pomeranians, and may possibly eventually lead to skeletal deformity. However, more research should be performed to find out if and why grade 2 MPL will develop into grade 3 MPL in certain dogs. We found a high recurrence rate of luxation in grade 4 MPL after TTT with TBR, therefore surgical correction should be considered in case of femur and/or tibia deformity.

Varus deformity is associated with PL in immature dogs due to the abnormal compressive force on an active physis according to the Hueter-Volkmann principle. Corrective osteotomy was extensively debated in 2007 (Swiderski and Palmer, 2007) after the radiographic studies of femoral varus had been described in the normal stifle and in MPL, both in small- and in large-breed dogs (Dudley et al., 2006; Mortari et al., 2009; Soparat et al., 2012; Swiderski et al., 2008). However, a reference value of femoral varus in a specific breed is necessary for planning the corrective osteotomy. These reference values are only available in Labrador Retriever, Golden Retriever, German Shepherd, Rottweiller (Tomlinson et al., 2007), and Pomeranians (Soparat et al., 2012).

A low recurrence rate of PL after surgical treatment in Pomeranians with MPL was found in our study described in **chapter 3**, which is similar to other reports (Arthurs and Langley-Hobbs, 2006; Gibbon et al., 2006; Alam et al., 2007) but lower than the recurrence rate reported by Linney et al., (2011). However, the follow-up period of our study was 16 weeks, which is longer than the follow-up period used in the other studies (Alam et al., 2007; Linney et al., 2011). Based on the outcome of our study, TBR alone or combined with TTT is the preferable surgical technique with good outcome in Pomeranians with grades 2 and 3 MPL. Persistent lameness including patellar reluxation in this study was seen in 8.6% of the operated dogs, which is similar to other reports who investigate percentages of lameness although

these authors excluded reluxation in dogs from the analysis (Willauer and Vasseur, 1987; Remidios et al., 1992; Linney et al., 2011). Persistent lameness might be due to cartilage damage and osteoarthritis (OA) both prior to surgery due to cartilage erosion, or due to exposure of the subchondral bone as consequence of the surgical correction. Patellar instability and abnormal contact due to PL can cause cartilage erosion of the articulation of the femoral trochlear ridges and of the patellar surface. There were only two studies evaluating OA following trochlear sulcoplasty using a radiographic score for long-term follow-up to 12 months. The first study found mild OA in 78.8 % of the surgical cases (Willauer and Vasseur, 1987). The other study compared OA between non-surgical and surgical treatments, which showed no significant difference in radiographic scores between non-surgical and surgical treatments both determined at the starting time and at the end of follow-up evaluation (average follow-up period 33 months). However OA had progressed significantly when compared at the starting time and at the end of follow-up evaluation both in non-surgical and in surgical treatment groups (Roy et al., 1992). These studies illustrate that OA progresses slowly in case of PL. Evaluation of the correlation among PL grading, lameness, and OA in even more long-term studies following the use of a standard technique e.g. TBR, or TWR, may provide more evidence-based treatment options.

In **chapter 4**, a novel technique, i.e. extended proximal trochleoplasty, was performed in Pomeranians with Bidirectional PL (BPL), which show hyperextension of the stifle in stance. Hyperextension of the stifle causes proximal displacement of patella within the femoral trochlear groove and thus a dynamic patella alta. The aim of the extended proximal trochleoplasty was to deepen and to lengthen the proximal part of the trochlear groove. The advantage of this technique is to perform a TBR-like technique at the proximal end of the trochlea allowing to adapt the depth and length of the trochlea with attachment to the periosteum at the proximal part in cases with proximal displacement of the patella. Long-term (48 weeks) follow-up with radiographic evaluation was performed, which showed a favourable outcome. With this technique minimal exposure of the subchondral bone was accomplished. There was minimal radiographic progression of osteophyte formation (OA) during the 48 week follow-up period, and all dogs achieved functional recovery. This technique is a primary study in Pomeranians that showed a good outcome and rapid recovery, therefore this technique might also be an alternative option in large-breed dogs suffering from BPL with proximal displacement of patella or with patella alta.

Prevalence and pathophysiology of PL

MPL occurs more common in small- and large-breed dogs than lateral patellar luxation (LPL) (DeAngelis and Hohn, 1970; Remeios et al., 1992; Hayes et al., 1994; Vidoni et al., 2006; Alam et al., 2007; Bound et al., 2009; Wangdee et al, 2014) which is consistent with our screening population of Pomeranian and of Kooiker dogs. However, LPL is more common in the Flat-Coated Retrievers in our screened population of 3834 dogs. There are only three reports of PL in Flat-Coated Retrievers (Table 1 in Chapter 2) and only one found MPL in 1 dog (Alam et al., 2007) whereas the others do not mention the direction of the luxation (LaFond et al., 2002; OFA, 2013). According to the review given in chapter 2, there are several studies mentioning the pathophysiology of PL, however the underlying cause is still unclear. Genetic studies might find a clue to the underlying cause of PL.

There are no large data sets available on the prevalence of PL in a particular breed except from our studies and the archive of the Orthopedic Foundation for Animals (OFA) in the USA (OFA, 2013). The grading distribution in the OFA data is not available so a detailed comparison of the Dutch or Thai and the American populations is not possible. The prevalence of PL in 3 purebred dogs, i.e. Dutch Flat-Coated Retrievers, Dutch Kooiker dogs, and Thai Pomeranians, was 24%, 24%, and 77%, respectively. The prevalences of PL in the current study are higher than those reported in the USA (OFA, 2013). This difference may be attributed to the lack of a PL screening program in the USA thereby overlooking the non-clinical cases (like PL grade 1) and resulting in an underestimation of the PL prevalence. In addition, the different prevalence of PL in the European and American populations of the specific dog breeds could be the result of genetic drift. Principle component analysis of DNA marker data demonstrated a marked divergence between Dutch and American Golden Retrievers and between Dutch and American Labrador Retrievers (Karlsson et al., 2007; Lavrijsen, 2014). Interestingly, despite the absence of a systematic screening program for PL of Pomeranians in Thailand, the observed prevalence in this population is distinctly higher than in the Dutch Flat-Coated Retrievers and the Dutch Kooiker dogs. Possibly Thai breeders select affected dogs for breeding in order to keep some preferable morphological characteristics and do not recognize PL as a severe clinical problem.

Most of the affected Pomeranians had grade 2 PL which means that the dogs had clinical signs. This is in line with the report of Hayes et al. (1994) who collected data on the frequency and distribution of PL in small-, medium-, and large-breed

dogs. The percentage of grade 1 PL is 28% (Table 2 in Chapter 6) which differs from the 10% as mentioned by Hayes et al. (1994) (Table 7 in Chapter 2). The higher prevalence of PL in our study can be attributed to the fact that Pomeranians were included which were presented for routine procedures such as vaccinations at 3 months of age or because the owner was concerned about PL. Of all investigated Pomeranians, 20% and 8% of the affected Pomeranians had grade 3 and 4, respectively, which indicates the severity of PL in this breed. We speculate that PL grade 1 or 2 diagnosed at the age of 3 months progresses to a PL grade 3 or 4 at 6 to 12 months of age. Unfortunately, information on PL grade progression in time was not available. In terms of a better understanding of the progression of PL, prospective studies with breeds susceptible to PL should be the focus of future research.

The sex distribution of dogs with PL has been reported in many studies. The notion is supported that PL may be more common in female small-breed dogs and male large-breed dogs (Priester, 1972; Denny and Minter, 1973; Remedios et al., 1992; Hulse, 1993; Hayes et al., 1994; Gibbons et al., 2006). Since the amount of investigated dogs is not equally divided between male and female dogs, a relative risk ratio is a more suitable method to evaluate the female:male ratio. The female:male relative risks of our studies are 1.11, 1.15, and 1.8 in Pomeranians, Kooiker dogs, and Flat-Coated Retrievers, respectively (Lavrijsen et al., 2013; Wangdee et al., 2014; Chapter 6). There are no significant sex differences of PL in Thai Pomeranians and Dutch Kooiker dogs. This is also consistent with the female:male ratio of 1.15:1 in Dutch Chihuahuas (Bruinen, 2013) and in a study in small-, medium-, and large-breed dogs of Bound et al. (2009) and the female:male ratio of 1:1.15 in small to medium breeds of Singleton (1969). Interestingly, PL was twice more common in female than in male Flat-Coated Retrievers, which is in contrast with the reports in other large-breed dogs of Remedios et al. (1992) and Gibbon et al. (2006) who evaluated heterogeneous groups from various breeds. Although both Kooiker dog and Flat-Coated Retriever breeds are quite small populations in The Netherlands, an average of 10.8% and 23.2% of registered dogs of these breeds, respectively, was included in the screening programmes. This makes the study of these breeds both valuable and representative. Bilateral patellar luxation revealed to be more common than unilateral luxation in Pomeranians (Table 1 in Chapter 5) compared with Kooiker dogs (Table 2 in Chapter 7) and Flat-Coated Retrievers. Bilateral occurrence gives a strong premise that PL is an inherited entity with genetic predisposition (Ostrander and Kruglyak, 2000).

The heritability of PL in Flat-Coated Retrievers and Kooiker dogs was 0.17 and 0.27, respectively (Lavrijsen et al., 2013; Wangdee et al., 2014), indicating a low additive genetic variation compared to the phenotypic variation in the population analysed. The environmental factors, genetic factors as well as residual variance play a role in PL in these breeds. The h^2 of 0.44 in Pomeranians indicates that the influence of genetic factors is remarkably high in comparison to that in Flat-Coated Retrievers and Kooiker dogs (Chapter 6). However, lack of complete pedigree information and long-term family registration of Pomeranians could have led to over-estimation of the heritability in this Pomeranian population.

Based on our molecular genetic studies, it is most likely that PL is a multifactorial inherited disease. Screening programmes in Dutch Kooiker dogs and Dutch Flat-Coated Retrievers have been active for 16 years and supplied extensive information on the phenotype and genetic trends, and on the effects of breeding selection on the phenotype. Combining pedigree and phenotype data in the breeding programmes could improve the effectiveness of selective breeding to reduce the prevalence of PL. This is shown in the phenotypic trend of PL in Kooiker dogs indicating decreasing PL prevalence over the period of 16 years of selective breeding (Fig. 1) and in Flat-Coated Retrievers which decrease from 28% to 19% between 1994 and 2009, and from 28% to 20% between 1992 and 2007, respectively. Therefore, we strongly recommend starting registration and screening of Thai Pomeranians to improve the effectiveness of breeding in reducing the prevalence of PL and other traits.

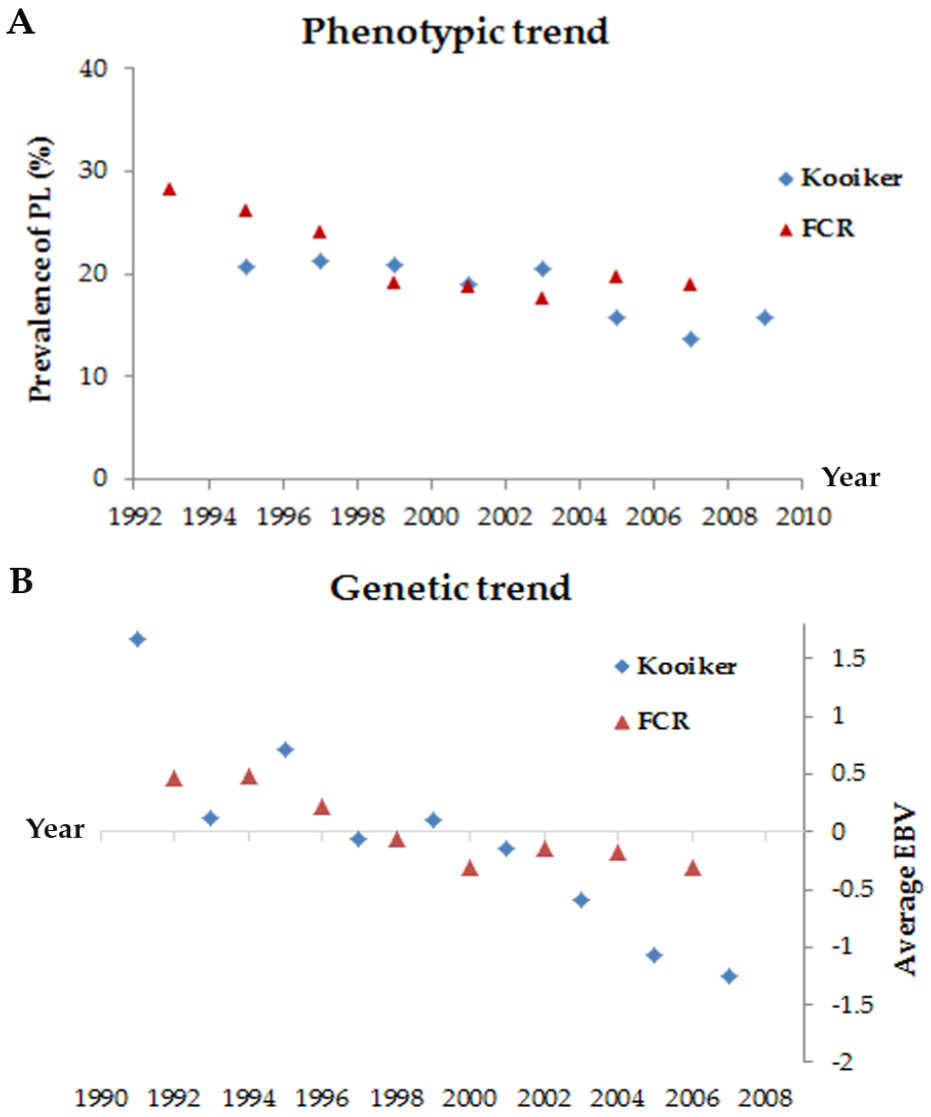


Figure 1 Prevalence and average estimated breeding value (EBV) of patellar luxation (PL) in Kooiker dogs from 1994 to 2009 and in Flat-Coated Retrievers (FCR) from 1992 to 2006. Two-year average of prevalence of PL showed decrease of the prevalence both in Kooiker dogs and in Flat-Coated Retrievers (A). Two-year average of genetic trend showed decrease of average EBV in both Kooiker dogs and Flat-Coated Retrievers (B).

There has been much speculation on the pathophysiology of PL but the underlying cause of PL is still not entirely understood. It has been suggested that coxa vara and a diminished anteversion angle can result in medial displacement of the quadriceps muscle in small- and large-breed dogs (Hulse, 1981). This muscular displacement results in abnormal forces on the distal femoral physis, resulting into retarded growth of the medial side of the growth plate and hence distal femoral varus. Furthermore, medial displacement of the quadriceps and consequent MPL results in a shallow or absent trochlear groove, internal rotation of tibia relative to the femur

and displacement of the tibial tuberosity medially (Fig. 6 in Chapter 2). However, coxa valga has also been associated with MPL in small-breed dogs (Bound et al., 2009) and the inclination angle did not show significant differences between normal and MPL groups of Pomeranians (Soparat et al., 2012). Furthermore, the anteversion angle and PL are not correlated (Kaiser et al., 2001). Even more so, large-breed dogs with MPL have an increased risk of developing cranial cruciate ligament disease (Gibbon et al., 2006; Persuki et al., 2006). In contrast, we encountered cranial cruciate ligament rupture in only 3.9% of Pomeranian with MPL (Wangdee, unpublished data). Altogether, these aspects question the current concept of PL pathophysiology.

During the last decade, a new hypothesis emerged as the primary cause of PL. MPL has been associated with a relatively long patellar ligament resulting into patella alta in medium- to giant-breed dogs (Johnson et al., 2006 and Mostafa et al., 2008). Researchers speculate that proximal displacement of the patella or patella alta in medium-, large-, and giant-breed dogs may create a patellofemoral articulation that extends proximal to the femoral trochlear groove during extension of the stifle (Johnson et al., 2006). The latter may result in a loss of the buttressing effects of the proximal end of the trochlear ridge, and thus in PL. The loss of patellar pressure on the femoral condyle can facilitate medial luxation of the patella (Johnson et al., 2006; Mostafa et al., 2008). In line with this theory, in humans, patella alta associated with patellofemoral instability is one of the causes of lateral patellar dislocation (Khan et al., 2011). Of patients with recurrent patellar dislocation 72% had patella alta (Thestrup, 1936). In conclusion, patella alta seems to be a plausible initiator of PL and it is tempting to hypothesize that this may also be the case in Flat-Coated Retrievers. Unravelling the genetic background of patella alta in Flat-Coated Retrievers will not only assist in improving breeding policy, but may also contribute as an animal model to identify the genetic background of PL in humans.

Patella alta seems to be implicated in the pathophysiology of PL only in large-breed dogs and not in small breed dogs. The vertical position of the patella did not differ among 4 grades of MPL in a variety of small-breed dogs (Towle et al., 2005; Mortari et al., 2009). However breed matched controls without PL were lacking in both studies. Interestingly, BPL occurs in 2.9% of the Pomeranians with PL (Table 2 in Chapter 6), possibly due to the patella positioned proximal in the trochlear groove in the extended stifle joint. Based on this it was hypothesized that BPL might be

associated with patella alta (Johnson et al., 2006; Mostafa et al., 2008). However, we investigated BPL affected and control Pomeranian and concluded that there is no correlation between the vertical patellar position and BPL in Pomeranians.

Altogether, there are a few differences in the pathophysiology of PL in small- and large-breed dogs. These need to be confirmed by investigating larger populations, with more focus on the distinct differences of skeletal anatomy of the specific breeds.

Genetics of patellar luxation

Collagen is a main component of ligaments, connective tissue, cartilage, and bone; collagen defects have been implicated in a variety of human disorders (Myllyharju and Kivirikko, 2001; Eyre, 2002). Type VI collagen genes have been suggested to be candidate genes for involvement in PL and hyperextension syndrome in dogs (Temwichitr et al., 2007). Therefore, the involvement of collagen genes in PL was studied by genetic linkage analysis of Pomeranian families with PL.

In **chapter 5**, co-segregation of the PL phenotype with five DNA markers situated close to *COL6A1*, *COL6A3*, *COL9A1*, *COL9A2*, and *COL9A3* indicated that these collagen genes were not involved in the pathogenesis of PL in Pomeranians. It turned out that linkage analysis was not an appropriate technique; it had a relatively low power for a complex disease like PL which is influenced by multiple genes. In follow up studies, we employed a genome-wide association study (GWAS). DNA samples of Pomeranians were genotyped with 1536 single nucleotide polymorphisms (SNPs), which indicated the involvement of a region on chromosome 7 in MPL. However, the low density of SNPs used in the first analysis was not suitable to detect loci with a small effect on the phenotype. Therefore, a high density SNP chip array was used to genotype DNA samples of Pomeranians and Kooiker dogs as described in **chapter 6 and 7**. DNA samples of 48 cases and 48 controls of Pomeranians were genotyped with 173,662 SNPs. GWAS suggested the possible involvement of a region on chromosome 5 and 32 in Pomeranians. *SC5D* encoding for sterol-C5-desaturase located on chromosome 5 and *BMPR1B* encoding for bone morphogenetic protein receptor, type 1B located on chromosome 32 implicated in bone formation and chondrogenic differentiation associated with chondrodysplasia are good candidate genes for a role in the pathogenesis of MPL in Pomeranians (Demirhan et al., 2005). DNA samples of 48 cases and 42 controls of Kooiker dogs were genotyped with 174,450 SNPs. A GWAS suggested the possible involvement of a region on chromosome 3 in Kooiker dogs although genome wide

significance was not reached. However, *FGFRL1*, *IDUA*, and *MYL5* encoding for fibroblast growth factor receptor-like 1, iduronidase, and myosin light chain 5, respectively, located in the region on chromosome 3 should be investigated in the future for PL in Kooiker dogs.

Several genes with a small effect may play a role in the complex inheritance of PL in these breeds. In **chapter 8**, a GWAS of PL in Flat-Coated Retrievers revealed significant association of regions on chromosome 7, as well as suggestive association on chromosome 3, 31, and 36. The exons of all genes in these candidate regions were selected for DNA sequence analysis in limited numbers of cases and controls. Nine of the detected DNA sequence variations in 8 genes displayed association to PL in the complete cohort of Flat-Coated Retrievers. The most strongly associated SNP was located in *TNR* on chromosome 7 coding for tenascin R. In humans, mutations in *TNXB*, a paralog of *TNR*, have been described in Ehlers-Danlos syndrome type III, which is characterized by joint hyper extensibility (Burch et al., 1997). We consider *TNR* as an excellent candidate gene for PL in the Flat-Coated Retrievers.

We studied the genetic background of PL by GWAS in three different breeds: Pomeranians, Kooiker dogs, and Flat-Coated Retrievers. Unfortunately, GWAS reached genome significance only in the Flat-Coated Retrievers. However, considering the fact that there may be genes with a small effect that play a role in the complex inheritence of PL, several regions on various chromosomes that displayed suggestive association with the phenotype should be investigated for their role in PL. Assuming that the PL phenotype is similar between the three breeds, we hypothesized that there may be genes from different chromosomal regions that may influence a common pathway implicated in the pathophysiology of PL. Therefore an integrative analysis was performed based on the line of thought that disease causing mutations in different genes in different breeds originating from one and the same pathway might result in the same phenotype. This integrative concept has successfully been employed and identified a common pathway associated with mast cell tumours in the Golden Retriever from two populations (Arendt et al., 2013). The results from the three GWAS were integrated as follows: relevant chromosomal regions with PL were selected for pathway analysis by MetaCore. Selection criteria for chromosomal regions were the presence of at least two associated SNPs within a 1 Mb window. These included 66, 33, and 67 associated SNPs in Pomeranians, Kooiker dogs, and Flat-Coated Retrievers, respectively. Genes for the analysis were included when they were located in these chromosome regions or within 50 kb from one of these regions. We selected 572 canine genes of which 435 had a human

orthologue, comprising 173 genes originating from Pomeranians, 106 genes from Kooiker dogs, and 156 genes from Flat-Coated Retrievers. To narrow down the number of genes of interest, we analysed which of these genes or their products have a direct interaction with other genes or gene products from the selection (Fig. 2).

There are some specific transcription factors, like c-Myc, RING2, Dicer, MYOD which are involved in many pathways (Fig. 2). The latter does not give leads towards specific pathways related to PL; however, we cannot exclude involvement of those genes at this point.

Remarkable is the possible involvement of several microRNAs (miRNAs) suggested by this network. MicroRNAs are important regulators of gene expression and contribute to the regulation of a variety of biological functions (Guo et al., 2010). A single miRNA can regulate the expression of many target genes and a target gene can also be regulated by several miRNA (Li and Ruan, 2009). Their main function is to decrease target mRNA levels (Guo et al., 2010). In order to link their presence in the network of interactions to a function, we evaluated the predicted target genes of the specific miRNAs. This resulted in 177 predicted target genes, which are regulated by at least three miRNAs. Gene Ontology (GO) analysis was performed to describe genes of interest in the biological process by using Gene Ontology Enrichment Analysis Software Toolkit (GOEAST) (Zheng and Wang, 2008).

This pathway analysis was done on the list of interacting genes and separately on the predicted target genes of the miRNAs encoded in or near chromosome regions that resulted from the three association studies. The 25 most significant terms of the analysis of predicted target genes of the miRNAs are shown in Fig. 3. A wide variety of processes is included; some are for instance related to kidney function and others to organ morphogenesis. Interestingly, 7 of the 25 processes could be relevant for the aetiology of PL. These involve chondrocyte development, fibroblast growth factor regulation, and bone mineralization. In future research the DNA sequences of the miRNA genes in affected dogs should be analysed for mutations that are possibly causal. The 19 most significant terms of the analysis of the interacting genes excluding the miRNAs are listed in Fig. 4. None of these seems to be relevant for any of the proposed aetiologies of PL. We therefore think that analysis of the miRNA genes is the most promising line of further investigation.

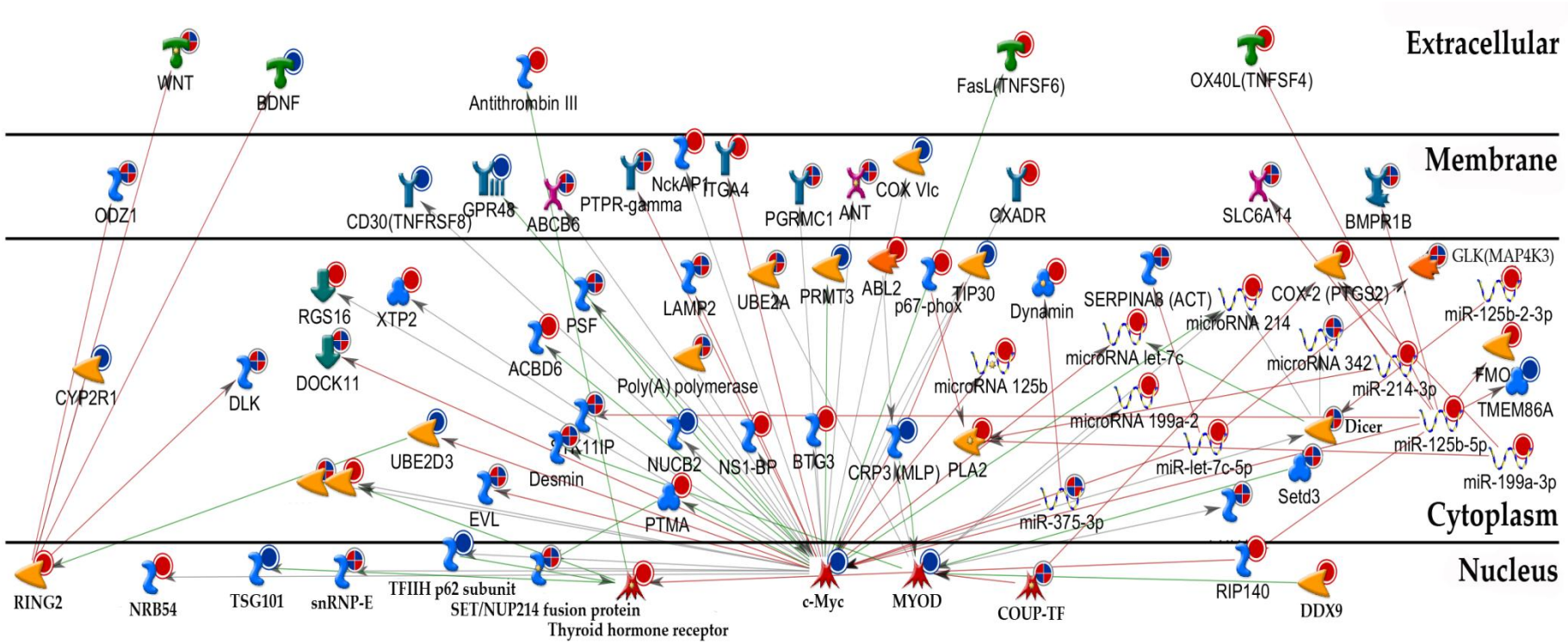


Figure 2 Direct interaction of genes or their protein products associated with patellar luxation in either of three breeds.
This figure illustrates genes that might be involved in PL in three breeds. The colour of the filled circle indicates the breed; the explanation of each symbol is given below; the gene involved is given underneath each symbol. The genes are organized based on their cellular component location. The figure was generated with MetaCore™ (Thomson Reuters) by Dr. Frank van Steenbeek.

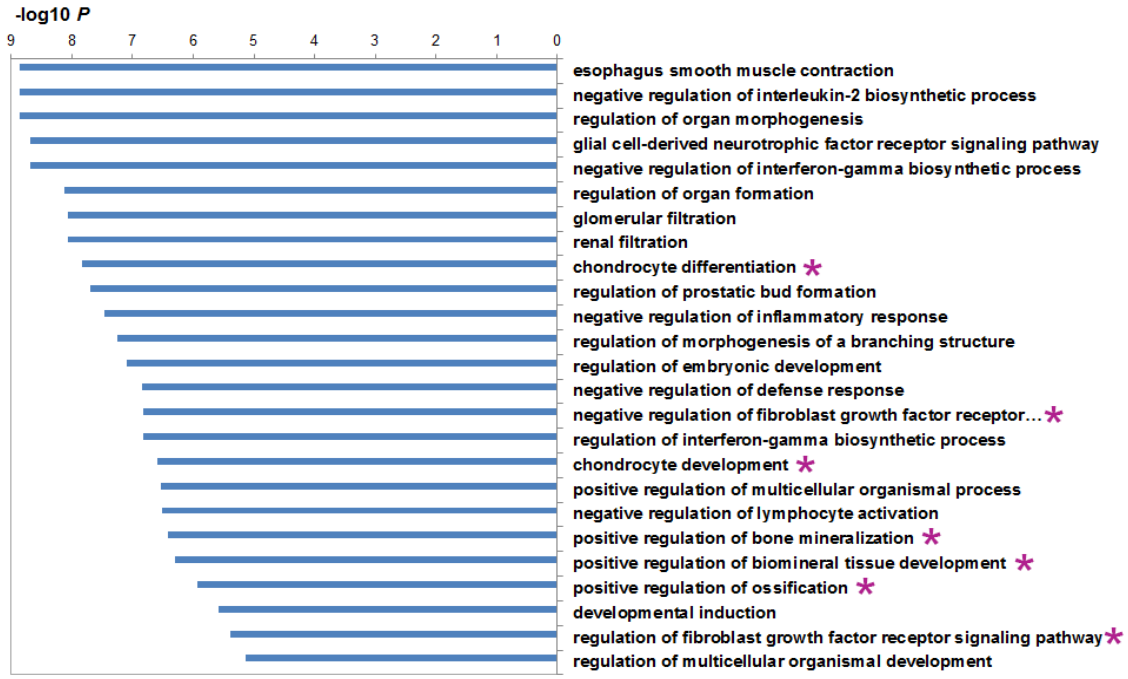


Figure 3 An analysis on biological process of interacting target genes of microRNAs within PL associated chromosome regions.

The most 25 significant terms with *P* of < 1 x 10^{-5} on biological processes of pathway analysis based on the predicted targets of miRNAs which may contribute to the pathophysiology of PL in Pomeranians, Kooiker dogs, and Flat-Coated Retrievers.

* Indicated 7 of the 25 relevant processes for the aetiology of PL. These involve chondrocyte development, fibroblast growth factor regulation, and bone mineralization.

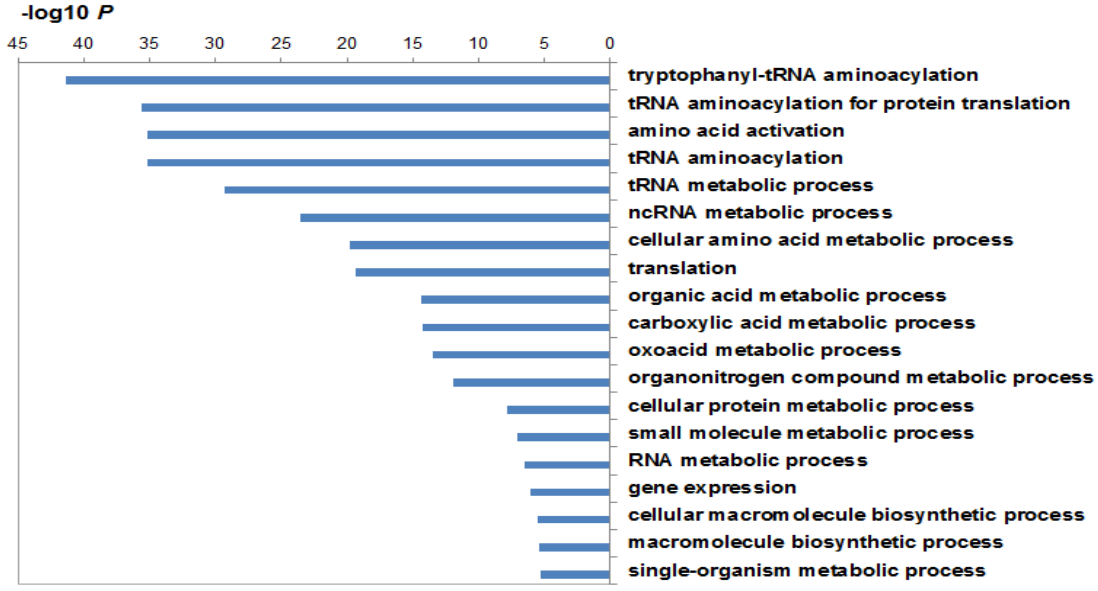


Figure 4 An analysis on biological process of interacting genes from chromosome regions associated with PL.

The most 19 significant terms with *P* of < 1 x 10^{-5} on biological processes of pathway analysis based on genes in the direct interaction which may contribute to the pathophysiology of PL in Pomeranians, Kooiker dogs, and Flat-Coated Retrievers.

In conclusion, Pomeranians, Kooiker dogs and Flat-Coated Retrievers have a predisposition to develop PL. Combining pedigrees, phenotypes, and genotypes in the breeding programme could improve the effectiveness of breeding to reduce the prevalence of PL (Meuwissen and Goddard, 2001). Standard surgical techniques e.g. TBR and soft tissue reconstruction with/without TTT are favorable for treating PL in Pomeranians given the good outcome of the follow-up study. However the success depends on the preoperative evaluation and intraoperative findings. Extended proximal trochleoplasty, a novel technique, showed a good outcome in Pomeranians with BPL. The prevalence of PL and the heritabilities of the disorder support that PL is an inherited disease in which both genetic and environment factors play a role in the occurrence of the disease. We did not identify the causative genes of PL, most probably due to small effect of many genes in the complex inheritance of PL as demonstrated in the breeds of this study. Integrating the associated chromosomal regions of three different breeds further confirmed the complex nature of PL pathophysiology. Interestingly, miRNAs were identified in all three breeds and a pathway analysis on their predicted targets indicated that bone, cartilage and/or fibroblast growth factors could be involved in the pathogenesis of PL in dogs. Future studies should concentrate on larger numbers of affected individuals and controls and the spin off results will require confirmation with independent groups.

References

Alam, M.R., Lee, J.I., Kang, H.S., Kim, I.S., Park, S.Y., Lee, K.C., Kim, N.S., 2007. Frequency and distribution of patellar luxation in dogs: 134 cases (2000 to 2005). Veterinary and Comparative Orthopaedics and Traumatology 20, 59-64.

Anderson, P.T., 1964. Congenital deformities of the knee joint in dislocation of the patella and achondroplasia. Acta Orthopaedica 28, 31-50.

Arendt, M., Melin, M., Tonomura, N., Perloski, M., Courtay-Cahen, C., Ricketts, S., Fineberg, E., Boerkamp, K., Megquier, K., Jeffery, L., Murphy, S., McCarthy, C., London, C., Rutteman, G., Starkey, M., Lindblad-Toh, K., 2013. Genome-wide association of canine mast cell tumors in Golden Retrievers: two populations leading to a common pathway. Proceedings of the 7th International Conference on Advances in Canine and Feline Genomics and Inherited Diseases, Cambridge, Massachusetts, USA, p.59.

Arthurs, G.I., Langley-Hobbs, S.J., 2006. Complications associated with corrective surgery for patellar luxation in 109 dogs. Veterinary Surgery 35, 559-566.

Bound, N., Zakai, D., Butterworth, S.J., Pead, M., 2009. The prevalence of canine patellar luxation in three centres. Clinical features and radiographic evidence of limb deviation. Veterinary and Comparative Orthopaedics and Traumatology 22, 32-37.

Bruinen, T.E.S., 2013. Patellar luxation in four dog breeds in the Netherlands: Trends in incidence and gradation between 1994 and 2011. Student thesis, Utrecht University, Utrecht, The Netherlands.

Burch, G.H., Gong, Y., Liu, W., Dettman, R.W., Curry, C.J., Smith, L., Miller, W.L., Bristow, J., 1997. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nature Genetics 17, 104-108.

DeAngelis, M., Hohn, R.B., 1970. Evaluation of surgical correction of canine patellar luxation in 142 cases. Journal of the American Veterinary Medical Association 156, 587-594.

Demirhan, O., Turkmen, S., Schwabe, G.C., Soyupak, S., Akgul, E., Tastemir, D., Karahan, D., Mundlos, S., Lehmann, K., 2005. A homozygous BMPR1B mutation causes a new subtype of acromesomelic chondrodysplasia with genital anomalies. Journal of Medical Genetics 42, 314-317.

Denny, H.R., Minter, H.M., 1973. The long term results of surgery of canine stifle disorders. Journal of Small Animal Practice 14, 695-713.

Dudley, R.M., Kowaleski, M.P., Drost, W.T., Dyce, J., 2006. Radiographic and computed tomographic determination of femoral varus and torsion in the dog. Veterinary Radiology & Ultrasound 47, 546-552.

Eyre, D.R., 2002. Review: collagen of articular cartilage. Arthritis Research 4, 30-35.

Gibbons, S.E., Macias, C., Tonzing, M.A., Pinchbeck, G.L., McKee, W.M., 2006. Patellar luxation in 70 large breed dogs. Journal of Small Animal Practice 47, 3-9.

Guo, H., Ingolia, N.T., Weissman, J.S., Bartel, D.P., 2010. Mammalian microRNA predominantly act to decrease target mRNA levels. Nature 466, 835-840.

Hayes, A.G., Boudrieau, R.J., Hungerford, L.L., 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal of the American Veterinary Medical Association 205, 716-720.

Hulse, D.A., 1981. Pathophysiology and management of medial patellar luxation in the dog. Veterinary Medicine: Small Animal Clinician 76, 43-51.

Hulse, D.A., Miller, D., Roberts, D., 1986. Resurfacing canine femoral trochleoplasties with free autogenous periosteal grafts. Veterinary Surgery 15, 284-288.

Hulse, D.A., 1993. Medial patellar luxation in the dog. In: Bojrab, M.J. (Ed.), Disease Mechanisms in Small Animal Surgery. Lea&Febiger, Philadelphia, pp. 808-817.

Hulse, D.A., 1995. The Stifle Joint. In: Olmstead, M.L. (Ed.), Small Animal Orthopedic. Mosby, St. Louis, pp. 395-404.

Johnson, A.L., Probst, C.W., DeCamp, C.E., Rosenstein, D.S., Hauptman, J.G., Weaver, B.T., Kern, T.L., 2001. Comparison of trochlear block recession and trochlear wedge recession for canine patellar luxation using a cadaver model. Veterinary Surgery 30, 140-150.

Johnson, A.L., Broaddus, K.D., Hauptman, J.G., Marsh, S., Monsere, J., Sepulveda, G., 2006. Vertical patellar position in large-breed dogs with clinically normal stifles and large-breed dogs with medial patellar luxation. Veterinary Surgery 35, 78-81.

Kaiser, S., Cornely, D., Golder, W., Garner, M.T., Wolf, K.J., Waibl, H., Brunnberg, L., 2001. The correlation of canine patellar luxation and the anteversion angle as measured using magnetic resonance images. Veterinary Radiology & Ultrasound 42, 113-118.

Karlsson, E.K., Baranowska, I., Wade, C.M., Salmon Hillbertz, N.H., Zody, M.C., Anderson, N., Biagi, T.M., Patterson, N., Pielberg, G.R., Kulbokas, E.J., 3rd *and others*, 2007. Efficient mapping of mendelian traits in dogs through genome-wide association. Nature Genetics 39, 1321-1328.

Khan, N., Fithian, D.C., Nomura, E., 2011. Anatomy of patellar dislocation. In: Sanchis-Alfonso, V. (Ed.), Anterior knee pain and patellar instability. Second Ed. Springer, New York, pp. 89-103.

LaFond, E., Breur, G.J., Austin, C.C., 2002. Breed susceptibility for developmental orthopedic diseases in dogs. Journal of the American Animal Hospital Association 38, 467-477.

Lavrijsen, I.C., Heuven, H.C., Breur, G.J., Leegwater, P.A., Meutstege, F.J., Hazewinkel, H.A., 2013. Phenotypic and genetic trends of patellar luxation in Dutch Flat-Coated Retrievers. Animal Genetics 44, 736-741.

Lavrijsen, I.C., 2014. Genetics of hip dysplasia, elbow dysplasia and patellar luxation in purebred dogs. In: PhD thesis, Utrecht University, Utrecht, The Netherlands, ISBN 978-94-91602-21-4

Li, W., Ruan, K., 2009. MicroRNA detection by microarray. Analytical and Bioanalytical Chemistry 394, 1117-1124.

Linney, W.R., Hammer, D.L., Shott, S., 2011. Surgical treatment of medial patellar luxation without femoral trochlear groove deepening procedures in dogs: 91 cases (1998-2009). Journal of the American Veterinary Medical Association 238, 1168-1172.

Meuwissen, T.H., Goddard, M.E., 2001. Prediction of identity by descent probabilities from marker-haplotypes. Genetics Selection Evolution 33, 605-634.

Mortari, A.C., Rahal, S.C., Vulcano, L.C., da Silva, V.C., Volpi, R.S., 2009. Use of radiographic measurements in the evaluation of dogs with medial patellar luxation. The Canadian Veterinary Journal 50, 1064-1068.

Mostafa, A.A., Griffon, D.J., Thomas, M.W., Constable, P.D., 2008. Proximodistal alignment of the canine patella: radiographic evaluation and association with medial and lateral patellar luxation. Veterinary Surgery 37, 201-211.

Myllyharju, J., Kivirikko, K.I., 2001. Collagen and collagen-related diseases. Annals of Medicine 33, 7-21.

OFA, 2013. Orthopedics Foundation for Animals: Patellar luxation statistics. http://www.offa.org/stats_pl.html (accessed March 9, 2014).

Ostrander, E.A., Kruglyak, L., 2000. Unleashing the canine genome. Genome Research 10, 1271-1274.

Piermattei, D.L., Flo, G.L., DeCamp, C.E., 2006. The stifle joint. In: Piermattei, D.L., Flo, G.L., DeCamp, C.E. (Eds.), Handbook of Small Animal Orthopedics and Fracture Repair. Fourth Ed. Saunders, St. Louis, pp. 562-632.

Persuki, A.M., Kowaleski, M.P., Pozzi, A., Dyce, J., Johnson, K.A., 2006. Treatment of medial patellar luxation and distal femoral varus by femoral wedge osteotomy in dogs: 30 cases (2000–2005). Proceedings of the 2nd World Veterinary Orthopaedic Congress & 33rd Annual Veterinary Orthopedic Society Conference, Keystone, CO, p. 240.

Priester, W.A., 1972. Sex, size, and breed as risk factors in canine patellar dislocation. Journal of the American Veterinary Medical Association 160, 740-742.

Remedios, A.M., Basher, A.W., Runyon, C.L., Fries, C.L., 1992. Medial patellar luxation in 16 large dogs. A retrospective study. Veterinary Surgery 21, 5-9.

Roy, R.G., Wallace, L.J., Johnston, G.R., Wickstrom, S.L., 1992. A retrospective evaluation of stifle osteoarthritis in dogs with bilateral medial patellar luxation and unilateral surgical repair. Veterinary Surgery 21, 475-479.

Singleton, W.B., 1957. The diagnosis and surgical treatment of some abnormal stifle conditions in the dog. Veterinary Record 69, 1387-1394.

Singleton, W.B., 1969. The surgical correction of stifle deformities in the dog. Journal of Small Animal Practice 10, 59-69.

Slocum, B., Slocum, D.B., Devine, T., Boone, E., 1982. Wedge recession for treatment of recurrent luxation of the patella: a preliminary report. Clinical Orthopaedics and Related Research 164, 48-53.

Slocum, B., Devine, T., 1985. Trochlear recession for correction of luxating patella in the dog. Journal of the American Veterinary Medical Association 186, 365-369.

Soparat, C., Wangdee, C., Chuthatep, S., Kalpravidh, M., 2012. Radiographic measurement for femoral varus in Pomeranian dogs with and without medial patellar luxation. Veterinary and Comparative Orthopaedics and Traumatology 25, 197-201.

Swiderski, J.K., Palmer, R.H., 2007. Long-term outcome of distal femoral osteotomy for treatment of combined distal femoral varus and medial patellar luxation: 12 cases (1999-2004). Journal of the American Veterinary Medical Association 231, 1070-1075.

Swiderski, J.K., Radecki, S.V., Park, R.D., Palmer, R.H., 2008. Comparison of radiographic and anatomic femoral varus angle measurements in normal dogs. Veterinary Surgery 37, 43-48.

Tomlinson, J., Fox, D., Cook, J.L., Keller, G.G., 2007. Measurement of femoral angles in four dog breeds. Veterinary Surgery 36, 593-598.

Temwichitr, J., Hazewinkel, H.A., van Hagen, M.A., Leegwater, P.A., 2007. Polymorphic microsatellite markers for genetic analysis of collagen genes in suspected collagenopathies in dogs. Journal of Veterinary Medicine A 54, 522-526.

Towle, H.A., Griffon, D.J., Thomas, M.W., Siegel, A.M., Dunning, D., Johnson, A., 2005. Pre- and postoperative radiographic and computed tomographic evaluation of dogs with medial patellar luxation. Veterinary Surgery 34, 265-272.

Vidoni, B., Sommerfeld-Stur, I., Eisenmenger, E., 2006. Diagnostic and genetic aspects of patellar luxation in small and miniature breed dogs in Austria. The European Journal of Companion Animal Practice 16, 149-158.

Wangdee, C., Leegwater, P.A., Heuven, H.C., van Steenbeek, F.G., Meutstege, F.J., Meij, B.P., Hazewinkel, H.A., 2014. Prevalence and genetics of patellar luxation in Kooiker dogs. The Veterinary Journal DOI 10.1016/j.tvjl.2014.05.036

Willauer, C.C., Vasseur, P.B., 1987. Clinical results of surgical correction of medial luxation of the patella in dogs. Veterinary Surgery 16, 31-36.

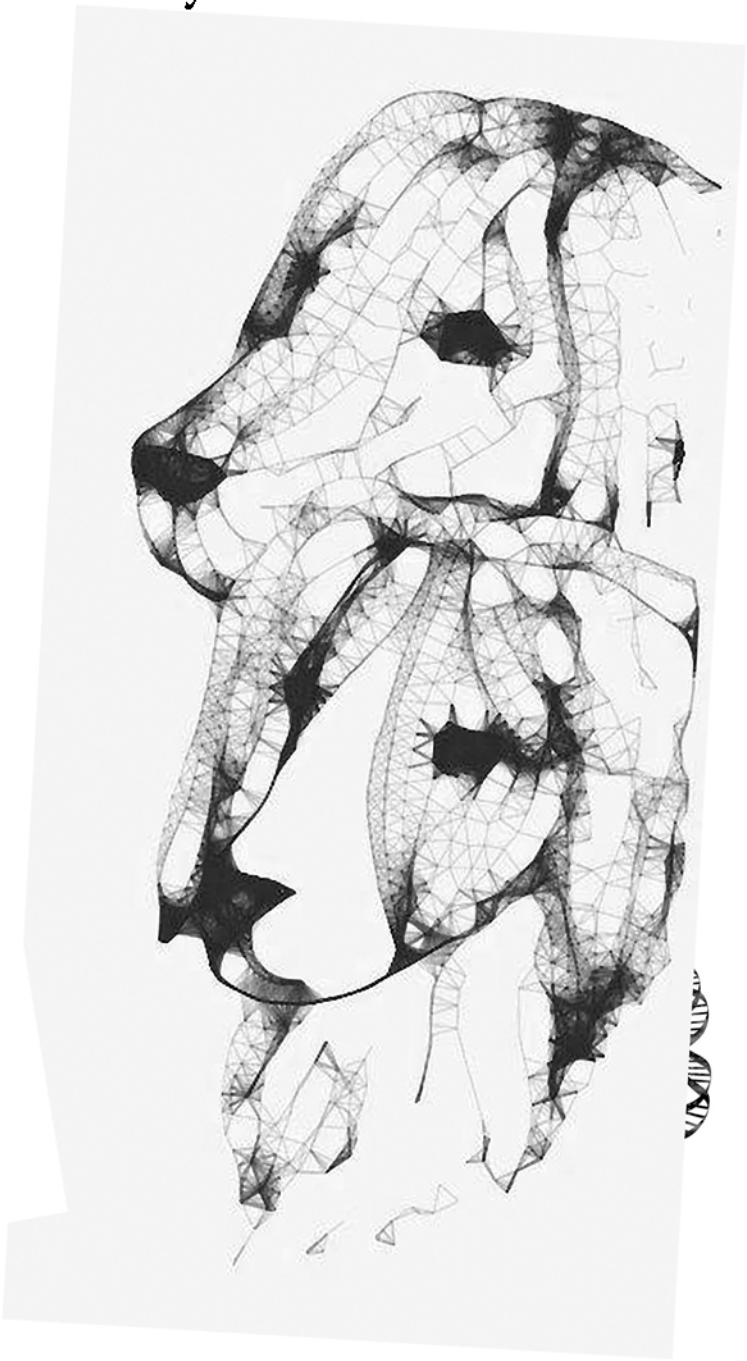
Zheng, Q., Wang, X., 2008. GOEAST: a web-based software toolkit for Gene Ontology enrichment analysis. Nucleic Acids Research 36, w358-w363.

Chapter 10

English summary

Nederlandse samenvatting

Thai summary



Summary

In dogs with a patellar luxation, the patella is or can be displaced – temporarily or permanently- to the inner side and/or the outer side of its original location. PL is a common cause of lameness in small-breed dogs. PL can result from a variety of anatomical abnormalities of the pelvic limbs. Many studies suggested that there is a genetic predisposition since there is high prevalence in particular dog breeds. Different explanations have been documented regarding the pathophysiology of PL, however the underlying cause of PL is not entirely understood.

In **Chapter 1,** the two aims of the study of this thesis are given: 1) to investigate the outcome of surgical treatment of medial patellar luxation (MPL) using standard techniques, and a novel surgical technique in Pomeranian dogs for treating bidirectional PL (BPL), and 2) to study the prevalence, heritability, and molecular genetics of PL using data sets from three dog breeds: Thai Pomeranians, Dutch Flat-Coated Retrievers, and Dutch Kooiker dogs in order to elucidate the pathophysiology of PL.

Several aspects of the pathophysiology of PL have been extensively reviewed in **Chapter 2**. The outcomes and complications of surgical treatments have been discussed. The study of prevalence of PL in particular breeds together with molecular genetic studies have been reviewed.

The study reported in **Chapter 3** evaluated prospectively the surgical treatment of MPL of 70 stifle joints in 55 Pomeranian dogs. The selected technique used depends on the depth of the trochlear sulcus and the alignment of the patella, patellar ligament, and tibial tuberosity. Trochlear block recession (TBR) alone was performed in 46 stifle joints, or in combination with tibial tuberosity transposition (TTT) in 24 stifle joints in dogs with MPL, including dogs with grade 2, 3, or 4 PL. Additional procedures were performed to restore lateral and medial retinacular function. The recurrence of PL and the degree of lameness were evaluated up to at least 16 weeks after surgery. The overall recurrence rate was 10%. The outcome of surgery was considered good for grade 2 MPL with a 100% success rate. Recurrence PL was

diagnosed in approximately 11% and in 36% of dogs with grade 3 and 4 MPL, respectively.

A novel surgical technique of extended proximal trochleoplasty for the treatment of BPL in 7 Pomeranians was described in **Chapter 4.** The study evaluated the clinical and radiological outcomes as well as the ratio between patellar ligament length (PLL) and patellar bone length (PBL). The ratio between PLL and PBL was not significantly different between Pomeranians with BPL and healthy stifle joints, therefore functional rather than anatomic patella alta might be associated with BPL in Pomeranians. The surgical outcome of extended proximal trochleoplasty was good-to-excellent in 87.5% of the stifle and all dogs achieved functional recovery. This technique is the first report on BPL in Pomeranians and its successful surgical treatment.

A high incidence of PL was reported in Pomeranian dogs in Thailand in **Chapter 5.** The genetic study of the possible co-segregation of the phenotype with polymorphic DNA markers situated close to the *COL6A1*, *COL6A3*, *COL9A1*, *COL9A2*, and *COL9A3* genes indicated that these collagen genes were not involved in the pathogenesis of PL in Pomeranians. In addition, an association study was performed with 1536 single nucleotide polymorphisms (SNPs) spread across the genome of Pomeranian dogs which indicated the involvement of a region on chromosome 7 in MPL. However, the low density of SNPs used in this analysis was not suitable to detect loci with a small effect on the phenotype.

In the studies reported in **Chapters 6 and 7**, the prevalence and genetics of PL in Thai Pomeranian dog and Dutch Kooiker dog populations were investigated and the heritability of PL analysed. Genome-wide association studies (GWAS) were performed to learn more about to identity of the chromosomal regions involved in the development and aetiology of PL in these dog breed populations. Subsequently, validated SNPs were tested in a large population of Pomeranians and Kooiker dogs. A cohort of 339 Pomeranian dogs, part of a four generation pedigree of 842 Pomeranians, was screened for PL from 2006 to 2011. Furthermore, a pedigree of 1737 Kooiker dogs from nine generations was screened for PL from 1994 to 2011.

The prevalence of PL was 77% and 24% of the screened Pomeranians and Kooiker dogs, respectively. Medial PL was more common than lateral or bidirectional PL

both in Pomeranians and Kooiker dogs. The relative risk of PL was similar in male and female dogs in Pomeranians and Kooiker dogs. The heritabilities of PL in the screened population were 0.44 and 0.27 in Pomeranians and Kooiker dogs, respectively. It indicated that the influence of genetic factors in Pomeranians is remarkably high in comparison to that in Kooiker dogs. A GWAS of PL in 48 cases and 48 controls of Pomeranian dogs using a high-density SNP array indicated the possible involvement of 15 chromosome regions, of which chromosome 5 and 32 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. A GWAS of PL in 48 cases and 42 controls of Kooiker dogs using the high-density SNP array suggested the possible involvement of a region on chromosome 3. However, the involvement of this region could not be confirmed in a validation group.

The study described in **Chapter 8** reported a genome-wide association analysis of 15,823 SNPs in 45 cases and 40 controls revealed that PL in Flat-Coated Retrievers was significantly associated with a region on chromosome 7 and possibly with regions on chromosome 3, 31, and 36. The exons of all genes in these candidate regions were selected for DNA sequence analysis in 15 cases and 15 controls. Nine of the detected DNA sequence variations in eight genes displayed association to PL in the complete cohort of Flat-Coated Retrievers. The most strongly associated SNP was located in *TNR* on chromosome 7 coding for tenascin R. In humans, mutations in *TNXB*, a paralog of *TNR*, have been described in Ehlers-Danlos syndrome type III, which is characterized by joint hypermobility and PL. *TNR* is an excellent candidate gene for PL in Flat-Coated Retrievers.

The findings of the studies involving the genetic background of PL in three different breeds by GWAS were discussed in **Chapter 9.** Combination of phenotypes and pedigree data for the calculation of estimated breeding values, could improve the effectiveness of breeding selection to reduce prevalence of PL. After correction for multiple testing, GWAS reached significance only in Flat-Coated Retrievers. However it must be considered that there may be many genes with a small effect in the complex aetiology of PL. Several regions on various chromosomes that displayed suggestive association with the phenotype should be investigated for their role in

PL. Therefore an integrative analysis was performed based on the line of thought that disease causing mutations in different genes in different breeds originating from one and the same pathway might result in the same phenotype. Integrating the associated chromosome regions of three different breeds confirmed the complex nature of PL pathophysiology. MicroRNAs (miRNAs) were identified in all three breeds and a pathway analysis on their predicted targets indicated that bone, cartilage, and/or fibroblast growth factors could be involved in the pathogenesis of PL in dogs. Future studies should focus on larger numbers of affected individuals and controls and the results will require confirmation with independent groups.

Nederlandse samenvatting

Bij honden met een patella luxatie (PL) is de knieschijf naar de binnenzijde en/of naar de buitenzijde van de knie - wisselend of permanent - verplaatst of te verplaatsen. PL is een veelvuldig voorkomende oorzaak van kreupelheid bij kleine rashonden. PL kan het gevolg zijn van verschillende anatomische afwijkingen van de achterpoten. In veel publicaties wordt gesuggereerd dat PL een genetische predispositie heeft omdat PL met hoge prevalentie voorkomt bij bepaalde hondenrassen. Verschillende verklaringen worden gegeven over de pathofysiologie van PL, alhoewel de onderliggende oorzaak van PL niet geheel wordt begrepen.

In **hoofdstuk 1** worden de twee doelstellingen van dit proefschrift beschreven: 1) onderzoek verrichten naar de resultaten van chirurgische behandeling van de mediale patella luxatie (MPL) met gebruikmaking van standard technieken, en van een nieuw-ontwikkelde techniek voor de behandeling van tweezijdige PL bij Dwergkeeshonden en 2) het bestuderen van de prevalentie, de erfelijkheidsgraden en de moleculair genetische aspecten van PL bij drie hondenrassen (Thaise Dwergkeeshondjes, Nederlandse Flatcoated Retrievers en Nederlandse Kooierhondjes) door middel van het onderzoeken van de chromosoomregio's of van genen die de aanleg van deze honden voor de ontwikkeling van PL verhogen om zo het pathofysiologische mechanisme van PL op te helderen.

In **hoofdstuk 2** wordt een uitgebreid literatuuroverzicht gegeven van de verschillende aspecten van de pathofysiologie van PL. De resultaten en complicaties van de verschillende chirurgische technieken worden besproken. De studie naar de prevalentie van PL bij bepaalde rashonden populaties, tezamen met moleculair genetische studies worden belicht.

In **hoofdstuk 3** worden de resultaten beschreven van de prospectieve studie naar de resultaten van de chirurgische behandeling van 70 kniegewrichten met MPL bij 55 Dwergkeeshondjes. De toegepaste techniek hing af van de diepte van de trochlea en de aansluiting van de knieschijf, van de kniepees en van de plaats van diens aanhechting aan het scheenbeen. Uitsluitend trochlea blok recessie (TBR) werd uitgevoerd in 46 kniegewrichten en in combinatie met tibia tuberositas transpositie (TTT) bij 24 kniegewrichten bij Dwergkeeshondjes met mediale PL graad 2, 3 of 4. Aanvullende maatregelen werden genomen om de functie van het laterale en

mediale retinaculum te herstellen. Recidive van PL en de mate van kreupelheid werden tenminste 16 weken na operatie geëvalueerd bij de patiënten. Het totale recidive percentage bleek 10 te zijn. Het behandelingsresultaat van de mediale PL graad 2 was goed met een succespercentage van 100. PL recidiveerde bij 11% en bij 36% van de Dwergkeeshondjes met respectievelijk graad 3 en graad 4 mediale PL.
In **hoofdstuk 4** wordt een nieuw ontwikkelde chirurgische techniek met een verlengingsplastiek van de proximale trochlea voor het behandelen van zeven Dwergkeeshondjes met tweezijdige PL beschreven. In deze studie werden de klinische en röntgenologische resultaten geëvalueerd, alsook de ratio tussen de patella-ligament-lengte (PLL) en de patella-bot-lengte (PBL) bij Dwergkeeshondjes. De ratio tussen PLL en PBL was niet significant verschillend tussen Dwergkeeshondjes met tweezijdige PL en Dwergkeeshondjes met gezonde kniegewrichten, daarom is een functionele patella alta waarschijnlijker dan een anatomische patella alta bij Dwergkeeshondjes met tweezijdige patella luxatie. Het operatieresultaat van de verlengde proximale trochleoplastiek was goed-tot-uitstekend met een functioneel herstel van 87,5% van de geopereerde hondjes. Dit is de eerste vermelding van tweezijdige PL bij Dwergkeeshondjes en de succesvolle chirurgische behandeling daarvan.

In **hoofdstuk 5** wordt een hoge incidentie van PL bij Dwergkeeshondjes in Thailand gemeld. De genetische studie van de mogelijke co-segregatie van het PL fenotype met polymorfe DNA-markers dichtbij de genen *COL6A1*, *COL6A3*, *COL9A1*, *COL9A2*, en *COL9A3*, geven een aanwijzing dat deze collageen genen niet betrokken waren bij de pathogenese van PL bij Dwergkeeshondjes. Bovendien werd een associatiestudie verricht met 1536 SNPs verspreid over het genoom van de Dwergkeeshondjes, die een aanwijzing gaven van de betrokkenheid van een gebied op chromosoom 7 in geval van mediale patella luxatie. De dichtheid van de SNPs die bij deze analyse gebruikt werden, was echter onvoldoende om plaatsen met een gering effect op het fenotype op te sporen.
In **hoofdstuk 6 en 7** worden studies beschreven naar de prevalentie en de genetica van PL bij populaties van Thaise Dwergkeeshondjes en Nederlandse Kooikerhondjes

en werd de erfelijkheidsgraad (h^2) van PL geanalyseerd. Genoom-brede associatie analyse (GWAS) werd uitgevoerd om de identiteit van de chromosomale regio's te achterhalen die betrokken zijn bij de ontwikkeling en de etiologie van PL bij deze rashondenpopulaties. Vervolgens werden geassocieerde SNPs getest in grotere populaties Dwergkeeshondjes en Kooikerhondjes. Een cohort van 339 Dwergkeeshondjes, onderdeel van een stamboom van vier generaties met 842 Dwergkeeshondjes, werd gescreend voor PL van 2006 tot en met 2011. Bovendien werden 1737 Kooikerhondjes afkomstig uit een populatie van 9 generaties Kooikerhondjes die door Prof. Meutstege werden gescreend van 1994 tot en met 2011.

De prevalentie van PL was respectievelijk 77% en 24% van de gescreende Dwergkeeshondjes en Kooikerhondjes. Zowel bij de Dwergkeeshondjes als bij de Kooikerhondjes kwam de mediale PL meer voor dan laterale of tweezijdige PL. Het relatieve risico van PL was onder mannelijke en vrouwelijke Dwergkeeshondjes en Kooikerhondjes gelijkelijk verdeeld. De erfelijkheidsgraad van PL bij de gescreende populaties Dwergkeeshondjes en Kooikerhondjes was respectievelijk 0.44 en 0.27. Dit geeft aan dat de invloed van genetische factoren bij Dwergkeeshondjes opmerkelijk hoog is in vergelijking tot die bij de Kooikerhondjes. Een GWAS van PL bij 48 lijders en 48 controle Dwergkeeshondjes werd met gebruikmaking van een SNP-array met hoge dichtheid uitgevoerd; deze toonde de mogelijke betrokkenheid van 15 chromosoomgebieden aan. De betrokkenheid van chromosoom 5 en 32 bleven over in een grotere studie met een extra 128 lijders en 7 controle Dwergkeeshondjes. Kandidaat genen in deze gebieden kunnen betrokken zijn bij de pathogenese van PL bij Dwergkeeshondjes. Een GWAS van PL bij 48 lijders en 42 controle Kooikerhondjes, waarbij gebruik werd gemaakt van dezelfde SNP-array, toonde de mogelijke betrokkenheid aan van een regio op chromosoom 3. Deze betrokkenheid kon echter niet worden bevestigd met een validatiegroep van Kooikerhondjes.

In **hoofdstuk 8** wordt een GWAS met 15.823 SNPs beschreven bij 45 lijders aan PL en 40 controle Flatcoated Retrievers waarbij werd aangetoond dat er een significante associatie bestaat tussen PL en een regio op chromosoom 7 en mogelijk regio's op chromosomen 3, 31 en 36 bij deze Flatcoated Retrievers. De exonen van alle genen van deze kandidaatregionen werden geselecteerd voor DNA sequentie analyse bij 15

aangedane en 15 controle Flatcoated Retrievers. Negen gedetecteerde variaties in de DNA sequentie bij acht genen vertoonden associatie met PL in het volledige cohort Flatcoated Retrievers. De meest geassocieerde SNP bleek gelokaliseerd te zijn in het *TNR* gen op chromosoom 7 dat codeert voor tenascine R. Bij de mens spelen mutaties van het *TNXB* gen, een gen dat overeenkomsten heeft met het *TNR* gen, een rol bij het Ehlers-Danlos syndroom type III bij de mens, een afwijking die gekarakteriseerd wordt door hypermobiliteit van gewrichten en patella luxatie. *TNR* is een bijzonder goed kandidaatgen voor PL bij de Flatcoated Retriever.

De bevindingen die werden verkregen met betrekking tot de genetische achtergrond van PL bij drie verschillende rashondenrassen met GWAS worden in onderlinge samenhang besproken in **hoofdstuk 9**. Combinatie van fenotypes met afstammingsgegevens voor de schatting van fokwaarden zouden effectief de fokkerijselectie kunnen verbeteren om de prevalentie van PL te reduceren. GWAS bereikte alleen significantie bij de Flatcoated Retrievers. Hierbij moet echter worden beseft dat er vele genen met kleine effecten een rol kunnen spelen in de complexe etiologie van PL. Verschillende regio's op verschillende chromosomen die een associatie deden vermoeden met het fenotype PL moeten nader onderzocht worden met betrekking tot hun mogelijke rol bij PL. Daarom werd een geïntegreerde analyse uitgevoerd, gebaseerd op de gedachtegang dat ziekmakende mutaties in verschillende genen bij verschillende hondenrassen een rol zouden kunnen spelen in één en hetzelfde netwerk dat bij verstoring resulteert in hetzelfde afwijkende fenotype. Door de genen van de betreffende chromosoom regio's van de drie verschillende rashonden te integreren in één analyse, wordt de ingewikkelde aard van de pathofysiologie van PL bevestigd. MicroRNAs (miRNAs) werden geïdentificeerd bij alle drie hondenrassen en de netwerkanalyse suggereerde dat het metabolisme van bot en/of kraakbeen of dat fibroblast groeifactoren betrokken kunnen zijn bij de pathogenese van PL bij honden.

In de toekomst zullen studies zich moeten richten op grotere aantallen lijders en controles en moeten de resultaten worden bevestigd in onafhankelijke groepen.

บทสรุปภาษาไทย

ในสุนัขที่เป็นโรคสะบ้าเคลื่อน พบการเคลื่อนของสะบ้าออกจากตำแหน่งปกติได้ทั้งแบบชั่วคราวหรือแบบถาวร การเกิดสะบ้าเคลื่อนเป็นสาเหตุของอาการขากระเผลกที่พบได้บ่อยในสุนัขพันธุ์เล็กและอาจเป็นผลมาจากการเกิดความผิดปกติของโครงสร้างทางกายวิภาคของขาหลัง จากการศึกษาพบว่าการเกิดโรคในสุนัขสายพันธุ์จำเพาะชี้ถึงปัจจัยโน้มนำทางพันธุกรรมของโรคสะบ้าเคลื่อน มีการอธิบายในหลายแง่มุมในด้านพยาธิสรีรวิทยาที่เกี่ยวข้องกับการเกิดโรคสะบ้าเคลื่อน อย่างไรก็ตามสาเหตุของการเกิดโรคยังไม่ชัดเจน

บทที่ 1 อธิบายเป้าหมายของการศึกษาในครั้งนี้ซึ่งประกอบด้วย 2 วัตถุประสงค์หลัก ได้แก่ 1) การประเมินผลของการรักษาทางศัลยกรรมของการเคลื่อนของสะบ้าเข้าทางด้านในด้วยวิธีการผ่าตัดที่ใช้เป็นมาตรฐานในปัจจุบัน และการรักษาทางศัลยกรรมด้วยวิธีการผ่าตัดวิธีใหม่ในสุนัขพันธุ์ปอมเมอเรเนียน เพื่อการรักษาแก้ไขสะบ้าที่มีการเคลื่อนไปในสองทิศทางในข้อเข่าเดียวกัน 2) การศึกษาความชุก อัตราการถ่ายทอดทางกรรมพันธุ์ (heritability) และการศึกษาพันธุกรรมระดับชีวโมเลกุลของโรคสะบ้าเคลื่อนในสุนัขโดยใช้ข้อมูลจากสุนัข 3 สายพันธุ์ ได้แก่ สุนัขพันธุ์ไทยปอมเมอเรเนียน สุนัขพันธุ์ดัสต์แฟลทโค้ท รีทรีฟเวอร์ และสุนัขพันธุ์ดัสต์คอยเคอร์ เพื่อที่จะอธิบายลักษณะทางพยาธิสรีรวิทยาของโรคสะบ้าเคลื่อน

ในบทที่ 2 ได้รวบรวมการศึกษาที่หลากหลายทางด้านพยาธิสรีรวิทยาของโรคสะบ้าเคลื่อน วิจารณ์ผลการรักษาและภาวะแทรกซ้อนของการทำศัลยกรรมรักษาโรคสะบ้าเคลื่อน รวมทั้งการศึกษาความชุกของโรคในสุนัขจำเพาะสายพันธุ์และการศึกษาพันธุกรรมระดับชีวโมเลกุลของโรคสะบ้าเคลื่อน

ในบทที่ 3 เป็นการประเมินผลของการทำศัลยกรรมรักษาโรคสะบ้าเคลื่อนเข้าด้านใน ใน 70 ข้อเข่าในสุนัขพันธุ์ปอมเมอเรเนียนทั้งสิ้น 55 ตัว วิธีการทางศัลยกรรมที่เลือกใช้ประเมินจากความลึกของร่อง trochlea และแนวของกระดูกสะบ้า เอ็นสะบ้าและตำแหน่งที่ยึดเกาะของเอ็นสะบ้าหัวเข่า (tibial tuberosity) การทำศัลยกรรมโดยวิธี trochlear block recession ทำใน 46 ข้อเข่า และวิธี trochlear block recession ร่วมกับ tibial tuberosity transposition ทำใน 24 ข้อเข่า ในสุนัขที่มีสะบ้าเคลื่อนเข้าด้านในระดับที่ 2, 3 และ 4 วิธีการทางศัลยกรรมอื่นที่ใช้ประกอบเพื่อซ่อมแซมหน้าที่แถบกระชับเอ็น (retinaculum) ทางด้านในและด้านข้าง การประเมินผลการผ่าตัดประกอบด้วยการกลับเคลื่อนของสะบ้าและระดับการกระเผลกของขาหลังที่ 16 สัปดาห์หลังการทำศัลยกรรม พบเปอร์เซ็นต์การกลับเคลื่อนของสะบ้าคิดเป็น 10% โดยผลการศัลยกรรมอยู่ในเกณฑ์ดีในข้อเข่าที่มีสะบ้าเคลื่อนเข้าด้านในระดับที่ 2 คิดเป็น 100% การกลับเคลื่อนของกระดูกสะบ้าคิดเป็น 11% และ 36% ในข้อเข่าที่สะบ้าเคลื่อนเข้าด้านในระดับที่ 3 และ 4 ตามลำดับ

บทที่ 4 รายงานผลการรักษาทางศัลยกรรมโดยวิธีใหม่คือ extended proximal trochleoplasty ในการแก้ไขสะบ้าเคลื่อนที่มีการเคลื่อนไปในสองทิศทางในข้อเข่าเดียวกัน ในสุนัขพันธุ์ปอมเมอเรเนียน จำนวน 7 ตัว โดยทำการประเมินผลจากการตรวจทางคลินิกและภาพถ่ายทางรังสีวิทยา รวมทั้งการประเมินอัตราส่วนของความยาวเอ็นสะบ้าและความยาวของกระดูกสะบ้า พบว่าอัตราส่วนของความยาวเอ็นสะบ้าและความยาวของกระดูกสะบ้าไม่มีความแตกต่างกันอย่างมีนัยสำคัญระหว่างสุนัขพันธุ์ปอมเมอเรเนียนที่มีการเคลื่อนของสะบ้าไปในสองทิศทางในข้อเข่าเดียวกันกับสุนัขที่มีข้อเข่าปกติ ดังนั้นการเคลื่อนไปทางด้านบนของกระดูกสะบ้าบนร่อง trochlea หรือ patella alta ในช่วงที่มีการเหยียดข้อเข่าอาจสัมพันธ์กับการเกิดสะบ้าเคลื่อนไปในสองทิศทางมากกว่าความผิดปกติของอัตราส่วนดังกล่าว ผลของการทำศัลยกรรมโดยวิธีใหม่นี้อยู่ในเกณฑ์ดีถึงดีมากคิดเป็น 87.5% ของข้อเข่าทั้งหมด โดยสุนัขทุกตัวกลับมาใช้ขาได้เป็นปกติ การศัลยกรรมด้วยวิธี extended proximal trochleoplasty นี้เป็นรายงานแรกและประสบความสำเร็จในสุนัขพันธุ์ปอมเมอเรเนียนที่มีภาวะการเคลื่อนของสะบ้าไปในสองทิศทางในข้อเข่าเดียวกัน

บทที่ 5 รายงานอุบัติการณ์การเกิดโรคสะบ้าเคลื่อนสูงในสุนัขพันธุ์ปอมเมอเรเนียนในประเทศไทย การศึกษาทางพันธุกรรมของการส่งผ่านลักษณะฟีโนไทป์สู่เซลล์ของรุ่นลูกโดยเป็นการศึกษาตัวบ่งชี้จากดีเอ็นเอที่มีความหลากหลายที่อยู่ในตำแหน่งใกล้กับยีนคอลลาเจนโปรตีนต่างๆ ได้แก่ COL6A1, COL6A3, COL9A1, COL9A2 และ COL9A3 การศึกษานี้พบว่าคอลลาเจนโปรตีนดังกล่าวไม่มีความเกี่ยวข้องกับพยาธิกำเนิดของโรคสะบ้าเคลื่อนในสุนัขพันธุ์ปอมเมอเรเนียน นอกจากนี้การศึกษาความสัมพันธ์ของสนิป (single nucleotide polymorphism or SNP) หรือความหลากหลายทางพันธุกรรมจากความแตกต่างของลำดับนิวคลีโอไทด์เบสเพียง 1 ตำแหน่ง จำนวน 1,536 SNPs ที่อยู่บนจีโนมของสุนัขพันธุ์ปอมเมอเรเนียน พบความเกี่ยวข้องของตำแหน่งบนโครโมโซมที่ 7 ในสุนัขที่มีสะบ้าเคลื่อนเข้าด้านใน อย่างไรก็ตามจำนวนสนิปที่ใช้ในการวิเคราะห์ครั้งนี้ไม่เหมาะสมในการหาตำแหน่งยีนที่มีผลกับฟีโนไทป์เพียงเล็กน้อย

ในบทที่ 6 และ 7 เป็นการศึกษาความชุก อัตราการถ่ายทอดทางกรรมพันธุ์และการศึกษาพันธุกรรมระดับชีวโมเลกุลของโรคสะบ้าเคลื่อนในกลุ่มประชากรสุนัขพันธุ์ไทยปอมเมอเรเนียนและสุนัขพันธุ์ดัสต์คอยเคอร์ โดยใช้ genome-wide association study (GWAS) เพื่อหาตำแหน่งบนโครโมโซมที่เกี่ยวข้องกับการเกิดและเป็นสาเหตุของโรคสะบ้าเคลื่อนในสุนัขกลุ่มนี้ แล้วทดสอบ validated SNPs ในกลุ่มประชากรที่ใหญ่ขึ้นของสุนัขพันธุ์ปอมเมอเรเนียนและสุนัขพันธุ์คอยเคอร์ สุนัขพันธุ์ปอมเมอเรเนียนจำนวน 339 ตัว ประกอบด้วยข้อมูลประวัติทางสายพันธุ์ทั้งหมด 4 รุ่น จากสุนัขพันธุ์ปอมเมอเรเนียนจำนวน 842 ตัว ได้รับการคัดกรองโรคสะบ้าเคลื่อนจากปี ค.ศ. 2006 ถึงปี ค.ศ. 2011 นอกจากนี้ข้อมูลประวัติทางสายพันธุ์ของสุนัขพันธุ์คอยเคอร์จำนวน 1,737 ตัว ประกอบด้วยสุนัขจากทั้งหมด 9 รุ่น

ที่ได้รับการคัดกรองโรคสะบ้าเคลื่อนจากปี ค.ศ. 1994 ถึงปี ค.ศ. 2011 พบความชุกของการเกิดโรคสะบ้าเคลื่อน 77% และ 24% ในสุนัขพันธุ์ปอมเมอเรเนียนและสุนัขพันธุ์คอยเคอร์ตามลำดับ พบการเคลื่อนของสะบ้าเข้าด้านในมากกว่าการเคลื่อนออกด้านข้างและการเคลื่อนไปในสองทิศทางในข้อเข่าเดียวกันทั้งในสุนัขพันธุ์ปอมเมอเรเนียนและสุนัขพันธุ์คอยเคอร์ สุนัขเพศผู้และเพศเมียมีโอกาสเสี่ยง (relative risk) ต่อการเกิดโรคสะบ้าเคลื่อนเท่ากันในทั้งสองสายพันธุ์ อัตราการถ่ายทอดทางกรรมพันธุ์ของโรคสะบ้าเคลื่อนคิดเป็น 0.44 และ 0.27 ในสุนัขพันธุ์ปอมเมอเรเนียนและสุนัขพันธุ์คอยเคอร์ตามลำดับ อธิบายได้ว่าปัจจัยทางพันธุกรรมที่มีอิทธิพลในสุนัขพันธุ์ปอมเมอเรเนียนมีมากกว่าเมื่อเปรียบเทียบกับสุนัขพันธุ์คอยเคอร์ จากผล GWAS ในสุนัขพันธุ์ปอมเมอเรเนียนที่เป็นโรคสะบ้าเคลื่อนจำนวน 48 ตัว และสุนัขปกติจำนวน 48 ตัว โดยใช้ high-density SNP array พบตำแหน่งบนโครโมโซมที่อาจเกี่ยวข้องกับการเกิดโรคสะบ้าเคลื่อนทั้งหมด 15 ตำแหน่ง โดยพบตำแหน่งบนโครโมโซมที่ 5 และ 32 แสดงความสัมพันธ์ในการศึกษาประชากรสุนัขพันธุ์ปอมเมอเรเนียนที่มากขึ้น ซึ่งประกอบด้วยสุนัขเป็นโรคสะบ้าเคลื่อนจำนวน 128 ตัว และสุนัขปกติจำนวน 7 ตัว ยีนในตำแหน่งบนโครโมโซมดังกล่าวอาจเกี่ยวข้องกับพยาธิกำเนิดของโรคสะบ้าเคลื่อนในสุนัขพันธุ์ปอมเมอเรเนียน ผล GWAS ในสุนัขพันธุ์คอยเคอร์ที่เป็นโรคสะบ้าเคลื่อนจำนวน 48 ตัว และสุนัขปกติจำนวน 42 ตัว โดยใช้ high-density SNP array สนับสนุนการเกี่ยวข้องที่เป็นไปได้ของตำแหน่งบนโครโมโซมที่ 3 อย่างไรก็ตามไม่พบความเกี่ยวข้องของตำแหน่งบนโครโมโซมดังกล่าวในการทดสอบในประชากรที่เพิ่มขึ้น

บทที่ 8 ได้รายงานผลการวิเคราะห์ GWAS จำนวน 15,823 SNPs ในสุนัขพันธุ์แฟลทโค้ท รีทรีฟเวอร์ที่เป็นสะบ้าเคลื่อนจำนวน 45 ตัว และสุนัขปกติจำนวน 40 ตัว พบความสัมพันธ์กันอย่างมีนัยสำคัญของตำแหน่งบนโครโมโซมที่ 7, 3, 31 และ 36 การวิเคราะห์ลำดับดีเอ็นเอของ exon ของยีนทั้งหมดบนตำแหน่งบนโครโมโซมดังกล่าวในสุนัขที่เป็นโรคสะบ้าเคลื่อนจำนวน 15 ตัว และสุนัขปกติจำนวน 15 ตัว พบว่าความแปรผันของลำดับดีเอ็นเอจำนวน 9 ตำแหน่งในยีนจำนวน 8 ยีน ซึ่งมีความสัมพันธ์กับโรคสะบ้าเคลื่อนในสุนัขพันธุ์แฟลทโค้ท รีทรีฟเวอร์ สนิปที่แสดงความสัมพันธ์มากที่สุดคือ TNR ซึ่งอยู่บนตำแหน่งบนโครโมโซม 7 ที่เป็นรหัสสำหรับ tenascin R ในคนพบว่าการกลายพันธุ์ของ TNXB หรือยีนที่สัมพันธ์กับ TNR ทำให้เกิดโรค Ehlers-Danlos syndrome type III ซึ่งจะมีข้อต่อเคลื่อนไหวได้มากกว่าปกติและการเกิดสะบ้าเคลื่อน TNR เป็นยีนตัวแทนที่ดีเยี่ยมของโรคกระดูกสะบ้าเคลื่อนในสุนัขพันธุ์แฟลทโค้ท รีทรีฟเวอร์

ในบทที่ 9 เป็นการวิจารณ์ผลการศึกษาความรู้พื้นฐานเกี่ยวกับพันธุกรรมของโรคสะบ้าเคลื่อนในสุนัข 3 สายพันธุ์ โดยวิธี GWAS การรวมข้อมูลของลักษณะทางฟีโนไทป์และประวัติสายพันธุ์เพื่อใช้ในการคำนวณค่าประเมินคุณค่าการผสมพันธุ์ (estimated breeding values) สามารถเพิ่มประสิทธิผลในการคัดเลือกพันธุ์เพื่อลดความชุกของการเกิดโรคสะบ้าเคลื่อน จากการวิเคราะห์ GWAS พบความมีนัยสำคัญ

แต่ในสุนัขพันธุ์แฟลทโค้ท รีทรีฟเวอร์เพียงพันธุ์เดียว อย่างไรก็ตามโรคสะบ้าเคลื่อนเป็นโรคที่มีสมุฏฐานของการเกิดโรคที่มีความซับซ้อน จึงอาจมียีนมากมายที่เกี่ยวข้องกับโรคแต่มีผลเพียงเล็กน้อยต่อการเกิดโรค พบความเกี่ยวข้องกับฟีโนไทป์หลายตำแหน่งบนหลายโครโมโซม จึงควรศึกษาบทบาทของตำแหน่งเหล่านี้ในการเกิดโรคสะบ้าเคลื่อน ดังนั้นการวิเคราะห์แบบผสมผสานโดยอาศัยแนวคิดที่ว่าโรคเกิดจากการการกลายพันธุ์ของยีนต่างๆ ในสุนัขต่างสายพันธุ์ซึ่งมีต้นกำเนิดมาจากเพียง 1 pathway หรือ pathway ที่เหมือนกันอาจทำให้เกิดลักษณะทางฟีโนไทป์ที่เหมือนกัน การผสมผสานของตำแหน่งบนโครโมโซมที่มีความเกี่ยวข้องกับโรคสะบ้าเคลื่อนในสุนัขทั้ง 3 สายพันธุ์ ยืนยันความซับซ้อนของพยาธิสรีรวิทยาของโรคสะบ้าเคลื่อน พบไมโครอาร์เอ็นเอ (miRNAs) แสดงความเกี่ยวข้องในสุนัขทั้ง 3 สายพันธุ์ และจากการวิเคราะห์ pathway ของ predicted target gene ของ miRNA บ่งชี้ว่า กระดูก กระดูกอ่อนและ/หรือ fibroblast growth factors อาจเกี่ยวข้องกับพยาธิกำเนิดของโรคสะบ้าเคลื่อนในสุนัข ดังนั้นการศึกษาในอนาคตควรมุ่งการศึกษาในประชากรที่มากขึ้นทั้งที่เป็นโรคสะบ้าเคลื่อนและสุนัขปกติและควรมีการยืนยันผลในกลุ่มประชากรที่เป็นอิสระ

Acknowledgements

Curriculum vitae

Acknowledgements

It is my great pleasure to write this part of my thesis as I have many people support me and made my study possible and complete. However, there are quite numbers to recall and express my gratitude to all of them. Therefore, I would like to thank everyone who involved and supported me during my study here. You have made the Netherlands as my second home, thank you for your friendship, for every word and for your smile.

I would like to express the deepest appreciation to Prof.dr. Herman Hazewinkel for accepting me as your PhD student, you have been a tremendous mentor for me. Thank you for your encouraging my research and for allowing me to grow as a research scientist. Your advices on both researches as well as on my career are priceless. I highly appreciate everything that you did for me.

I would like to thank my co-promoters; Dr. Peter Leegwater for introducing me to a research field on molecular genetics and Dr. Lars Theyse for guiding me a lot of aspects on surgery. I totally appreciate your encouragement, guidance, patience and dedication.

My deep gratitude goes to Prof.dr. Mongkol Techakumphu, ex-dean of Faculty of Veterinary Science, Chulalongkorn University, Thailand. You made lots of effort for helping me to study at Utrecht University and to get a scholarship. In addition, I sincerely thank to Dr. Robert Paling for giving me an opportunity to study here and for everything you did.

I am grateful to Chulalongkorn University, Thailand for financial support my study in the Netherlands. Thanks so much to the officers from Chulalongkorn University and from the Royal Thai Embassy, in Den Haag.
I would like to express my appreciation to Prof.dr. Marissak Kalpravidh, who advised me on the research field of patellar luxation and taught me how to perform surgery.

Dr. Jedee Temwichitr, Dr. Kumpanart Soontornvipart, Dr. Niyada Suwankong and Dr. Sudson Sirivaidyapong: thanks for all your support.

Many thanks to the staff of Bureau of International Contacten (BIC); Adja van Oers, Hellen van der Maarsen, Jean de Gooijer, Mariella Spitzers-Kirner and Rosita Kolader for making my study life in Utrecht University pleasant.

My sincere thanks also go to Prof.dr. Björn Meij, Dr. Frank van Steenbeek, Dr. Henri Heuven, and Dr. Marianna Tryfonidou for your assistance, your precious time and all useful comments and suggestions.

To my paranimphs, Manon Vos-Loohuis: thanks for your analysis and your coaching on genetic work. Thanks to Panithi, I was so happy to cook, eat, travel, and spend time with you.

Ineke Lavrijsen: thanks for being a good friend, for helping me on data files and computer stuff and for spending time with me. Seng Fong Lau: thanks for your help and for spending time with me. Thanks to Hille Fieten for helping and sharing on genetic stuff.

Special thanks to my friends and all colleagues in the Genomic lab; Adri Slob, Ana Gracanin, Anje Wiersma, Bart Spee, Bas Brinkhof, Baukje Schotanus, Bianca Kuster, Ellen Martens, Elpetra Timmermans-Sprang, Floryne Buishand, Frank Riemers, Gaby Hoffman, Hedwig Kruitwagen, Ingrid van Gils, Jan Mol, Jeannette Wolfswinkel, Kim Boerkamp, Louis Penning, Miriam Kool, Monique van Wolferen, Nicole Willems, Nagesha Rao, Sathidpak (Ja), Suttiwee (New). Thanks also to my colleagues in the Reproductive cell biology.

To my roommates; Alberto Miranda Bedate, Anna Tellegen, Annemarie Voorbij, Annemieke Vlijm, Chenli Lai, Frances Bach, Gayathi Thevi, Hannah van Velzen, Hendrik-Jan, Luc Smolders, Lucy Verdonschot and Michelle Teunissen. Thanks to all for sharing room no. 2.021 and your stories.

Thanks to Ies Nijman, Ewart de Bruijn, Pim Toonen (Hubrecht) for KASPar assay and Ruben van't Slot (UMCU) for Illumina genotyping.

I would like to thank Jane Sykes for proofreading in my English manuscripts. I would like to thank Pichai Jirawattanapong for the statistical consultancies and for enjoyable time.

To my Thai friends in the Netherlands, thanks for our great memorial and friendships, that I will never forget all of you. In addition, to my friends and my students in Thailand, thanks for your encouragement, I appreciate all of your concern.

Thanks to Anjolieke Dertien for cover design and Pajaree Sirotamarat for drawing picture on the cover. Thanks to my brother-in-law "Supachai Pongsri" for a great job of your drawing, as well as your creative advice for cover design.

To my landlord, Nico Dijkshoorn: thanks for the wonderful room at Utrechtseweg 50. My sincere thanks also go to Niels, Tom and your staff.

My appreciate My sincere gratitude goes to my colleagues and staff at Faculty of Veterinary Science, Chulalongkorn University, Thailand for their support and willingness to help during my study.

I am thankful to the owners of the patellar luxation patients who were involved in my study.

Last but not least, my deepest gratitude goes to my family; my parents, my brother and sisters, as well as their families. I truly appreciate your encouragement, support and love.

"A person who never made a mistake never tried anything new"

Albert Einstein

Curriculum vitae

Chalika Wangdee was born on 9 December 1977 in Bangkok, Thailand. She started her undergraduate education in Veterinary Sciences in 1995 at Chulalongkorn University, Bangkok, Thailand, and received her DVM degree in 2000. She was admitted to the Master's programme of Chulalongkorn University in 2001, where she investigated the surgical treatment of patellar luxation in dogs, using proximal tube realignment, in the Department of Veterinary Surgery under the supervision of Prof. dr. Marissak Kalpravidh. She became a staff member at the same department in 2003. In September 2006, funded by the Thailand Research Fund of Dr. Kumpanart Soontornvipart (MRG5080124), Chalika came to Utrecht to study the genetic background of patellar luxation at the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, the Netherlands, under the supervision of Prof. dr. H.A.W. Hazewinkel and Dr. P.A.J. Leegwater. She returned to Chulalongkorn University in August 2007 and was promoted to assistant professor in July 2010. In August 2010, she came back to Utrecht to follow a training programme in surgery and neurology given by the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine. During the MOU meeting in 2010, Prof. dr. H.A.W Hazewinkel, Prof. dr. Mongkol Techakumphu, Dr. Robert Paling, and Dr. Sudson Sirivaidyapong reached agreement about a PhD studentship in Utrecht, funded by a scholarship from Chulalongkorn University. This enabled Chalika to start her PhD study at the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University in May 2011, under the supervision of Prof. dr. H.A.W. Hazewinkel, Dr. P.A.J. Leegwater, and Dr. L.F.H. Theyse. Her research focused on patellar luxation in dogs from both a genetic and surgical perspective, the results of which are described in this thesis, which will be publically defended at the Academiegebouw, Utrecht, on 9 October 2014. Thereafter, Chalika will return to Thailand to continue her work as lecturer in the Department of Veterinary Surgery, Faculty of Veterinary Science (head Assist. Prof. dr. Sumit Durongpongtorn), Chulalongkorn University, Bangkok, Thailand.