Genetics of

hip dysplasia, elbow dysplasia and patellar luxation

in purebred dogs

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Genetica van

heupdysplasie, elleboogdysplasie en patella luxatie

bij rashonden

(met een samenvatting in het Nederlands)

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 woensdag 21 mei 2014, des ochtends om 10.30 uur

door

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geboren op 26 maart 1982 te Reusel

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Table of contents

Chapter 1

Aims and scope of the thesis

Aim of the study

Hereditary developmental orthopaedic diseases pose a serious threat to the quality of life of dogs. The pain as well as the detrimental effect on mobility that can accompany these disorders have a major impact on the dogs as well as their owners. The studies described in this thesis aim to give more insight into the incidence of the most relevant of these diseases, as well as a better understanding of how they develop. This knowledge is crucial in order to devise and maintain effective screening and breeding programs.

In **chapter 2** an overview is given of the specifics of hip dysplasia, elbow dysplasia and patellar luxation as far as relevant anatomical details, pathophysiological background, details about screening of the trait in The Netherlands and possible influences on the phenotype, is concerned.

The aim of the study reported in **chapter 3** is an inventory of dog breeds involved in hip dysplasia. In addition the results of screening of Dutch breed population for elbow dysplasia is included in this study. The results of screening are according to the screening procedure organized by the Dutch Kennel club as executed in The Netherlands, on request of breeders and according to regulations of the Federation Cynologique International (Brass, 1989) and the International Elbow Working Group (www.vet-iewg.org). The analysis of the phenotypes that underlie these developmental disorders, as well as any correlations between the two diseases are presented.

In **chapter 4** the results of population genetic research performed in four breeds most affected with fragmented coronoid process (medial coronoid disease) are presented: the phenotypic and genetic correlations between signs of osteoarthritis in certain locations in the elbow joint are calculated and the best radiographic indicator for elbow dysplasia is determined.

The epidemiology of the third major orthopaedic disease, i.e. patellar luxation, has been screened according to regulations as set by Prof. Meutstege for the Dutch Flat-coated Retriever during the last 20 years. The population genetic screening results are presented in **chapter 5**.

In **chaper 6**, the chromosomal locations associated with hip dysplasia in the Labrador Retriever are presented using molecular genetic techniques. **Chapter 7** describes the regions on the canine genome associated with patellar luxation in a cohort of Flat-Coated Retrievers.

The findings and overall conclusion of these studies are discussed in **chapter 8** and a Dutch summary is available in **chapter 9**.

Chapter 2

Hip dysplasia, elbow dysplasia and patellar luxation, pathophysiology, screening and genetic aspects

General introduction

Developmental orthopaedic diseases have been frequently identified in dogs (Lafond et al., 2002). Breed susceptibility for well-described developmental orthopaedic diseases, especially when revealed at a set age, may suggest a genetic component in the disease aetiology (Patterson et al., 1989). Genetically susceptible dogs may be more at risk for influences from the environment on the skeletal development. Optimization of the environment and thus decreasing the incidence of the trait, does not improve the genotype of the animals of the population at risk and therefore the trait can manifest easily in future generations. Knowledge of the aetiology of the disease, screening of animals at risk, and identification of the genes involved may be indispensable in eliminating developmental orthopaedic diseases with a threat for the quality of life of the animal involved.

Here the anatomy of the joints involved, the pathophysiology as far is known from literature of the traits, the screening methods and factors which possibly influence the screening outcome, as well as genetic aspects are discussed for the most relevant developmental diseases in companion animal orthopaedics, i.e. hip dysplasia, elbow dysplasia and patellar luxation.

Hip dysplasia

The normal hip joint is characterised by a round femoral head, fitting perfectly inside the hip socket and covered for approximately 50% by the dorsal acetabulum. The word dysplasia originates from the Greek language and refers to an abnormal formation or development. Canine hip dysplasia (HD) was first described by Schnelle (1935). He initially featured it as a "congenital and potentially hereditary condition", but Henricson et al. (1966) concluded that HD could not be diagnosed at birth and therefore was probably not congenital, but caused by joint laxity in early life. Riser and Shirer confirmed in a cohort of 95 dogs, that HD is not congenital (since the hip was indeed normal at birth) but developmental (Riser and Shirer, 1967). Joint laxity present early in life that leads to subluxation of the femoral head may result in failure of the acetabulum to develop as a deep cup that holds the femoral head in position (Mansson and Norberg, 1961). By definition of Henricson, HD is "a varying degree of laxity of the hip joint permitting subluxation during early life, giving rise to varying degrees of shallow acetabulum and flattening of the femoral head, finally inevitably leading to osteoarthritis" (Henricson, 1966).

Normal hip bone development

The fundamental plan of the skeleton is formed by cartilage cells and cartilaginous matrix. Cartilage cells (i.e. chondrocytes) have the ability to grow and divide and thus to increase the dimensions of the skeleton. At a certain embryonic age, the skeleton starts to ossify by a well-structured sequence of events, i.e. endochondral ossification. First the diaphyses of long bones are ossified, followed by the ossification, and thus radiological appearance, of secondary ossification centres (apophyses and epiphyses; Table 2.1).

Table 2.1 Appearance of ossification centres and growth plate fusion of pelvic bones in dogs.

Hip bones	Appearance of ossification centres on radiographs *	Growth plate fusion **
Os ilium, os ichium, os pubis	62 - 69 days	4 – 6 months
Epiphysis proximalis ossis femoris	13 – 17 days	6 – 9 months
Trochanter major	20 – 24 days	9 – 11 months
Trochanter minor	13 – 17 days	9 – 10 months

**Schebitz and Wilkins, 1986. ** Summerlee, 2002.*

This process continues after birth, with the highest growth rate till 4 months of age. Ossified parts will be connected by cartilaginous areas, i.e. growth plates, till the age of maturation where all growth plates eventually ossify and growth ceases (Figure 2.1). Cartilage has more flexibility than bone, but is less rigid to withstand deformation by overloading. Timely ossification of the more vulnerable cartilage is therefore important, in relation to growth in size and weight, and increased activity. Endochondral ossification of the femoral head starts at the age of 2 weeks (Schebitz and Wilkens, 1986) and is completed at 3-4 months of age whereas the bones of the acetabulum will fuse at the 12th postnatal week (Evans and Christensen, 1979). A delay in endochondral ossification has been noticed in Labradors, which developed HD (Todhunter et al., 1997).

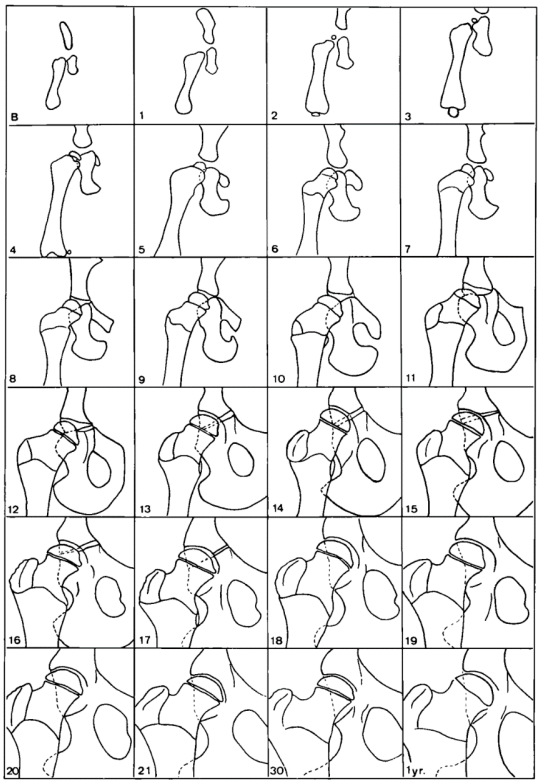


Figure 2.1 Skeletal maturity of pelvic bones and proximal femur from birth till 35 weeks of age. Drawings copied from radiographic overlay tracings of normal hip joint growth and development from birth (first frame) to one year of age (last frame). Other numbers indicate the age of the dog in weeks. (From Riser. 1975)

Both the configuration of the hip joint, but also the musculature surrounding the hip joint will keep the femoral head tight inside the acetabulum. Insufficient muscle force (in relation to increase in body weight and activities) or imbalanced muscle force (excessive contraction of one or more muscle groups) may result in insufficient stability of the hip joint, and may even force the femoral head outside the acetabulum. The latter can especially be the case if the not perfectly fitting femoral head causes increased pressure on the joint cartilage, and on the acetabular rim.

Pathophysiology of hip dysplasia

The first stage of hip joint dysplasia is typically asymptomatic (Lust and Summers, 1981) and puppies that are susceptible to develop HD have normal hips at birth (Riser and Shirer, 1967). By as early as two weeks of age the first signs of joint laxity can be observed in a stretching of the joint capsule and of the round ligament (Mansson and Norberg, 1961). By four weeks in the dog developing HD, the round ligament can reveal fibroplasia and a mild synovitis with joint effusion is present (Riser et al., 1985).

Due to the joint effusion and stretching of the connective tissue of the joint, and unbalanced action of periarticular musculature the femoral head is subluxated during weight-bearing. This results in abnormal pressure points on the dorsal acetabular rim and the medial part of the femoral head. The ventromedial part of the acetabulum and the lateral part of the femoral head, which now lack appropriate compressive forces to develop normally, show an increased ossification rate (Riser et al., 1985) while ossification in the dorsal rim and medial femoral head is delayed (Madsen et al., 1991). These changes in ossification pattern result in a shallower acetabulum and a flatter dorsal rim, further contributing to joint instability.

The abnormal load on the hip joint will eventually result in microfractures in the subchondral bone of the acetabular rim and the femoral head. Healing of these microfractures leads to an increased bone density in these areas, corrupting their shock absorbing characteristics. The cartilage on the articular surface now has to absorb the shocks, resulting in cartilage degeneration. By 60-90 days of age the articular surface cartilage is damaged: there are microfissures present and at places there are no chondrocytes left to cover the bone. The subchondral bone layer is exposed, which now becomes sclerotic (Morgan 1992; Riser et al., 1985).

Risk factors for hip dysplasia

A variety of factors are increasing the risk to develop HD or worsen the secondary signs of osteoarthritis (OA). The most important factors are here reviewed.

- Growth rate and muscle mass

Although HD has been identified in most breeds, it is more frequent in the larger breeds (Lust et al., 1973). Height, weight, pelvic muscle mass and growth rate are factors that were suggested to influence the development of HD. These factors relate to the balance between the strength of the material (muscle, tendon, bone) and the biomechanical forces, which are applied to it. Normal development of the hip relies on the coordinated growth of all tissues involved in hip stability. The selection for dogs with an accelerated growth rate or a specific body type may have disrupted this balance, making some breeds susceptible to HD. A significant correlation

between body mass index (i.e. body-weight divided by height at shoulder, kg/m^2) and the prevalence of HD has been demonstrated, but not between height and prevalence of HD (Comhaire and Snaps, 2008). A recent Norwegian study in 133 Labradors indicated however only minor differences in growth rate found between HD-affected and non-affected Labradors (Krontveit et al., 2012) Weight of these dogs at 3 months of age was correlated to hip development, with the heavier dogs - which at that age was supposedly to be an expression of more muscle mass – having better outcome for hip development (Krontveit et al., 2012).

- Housing at young age

The living environment (avoidance of stairs, and off-leash walking in moderately rough terrain) early in life correlated well with normal hip development in young Labradors (Krontveit et al., 2012). These conditions might be beneficial for strengthening muscle mass, without overloading the vulnerable cartilaginous acetabulum (Millard et al., 2010).

- Season of birth

A seasonal effect for the incidence of HD has been reported in different studies. In Norway HD-status revealed an odd ratio of 2.13 for dogs born in the winter (compared to dogs born in autumn) and a positive effect for born in spring (OR 0.54) or summer (OR 0.62) (Krontveit et al., 2012). These findings are in correspondence with results of others (Ohlert et al., 2002; Wood and Lakhani, 2003a), although there might be a difference between breeds (Hanssen, 1991). In New Zealand, studies revealed a protective effect of being born in autumn (Worth et al., 2012). The authors suggest the diminished exercise in winter time and the less muscle development in that period as the main cause of future joint laxity in these dogs (Worth et al., 2012; Krontveit et al., 2012). However, an increased food intake due to increased calorie requirements in cold periods can increase the excessive intake of other dietary factors (Durrer and Hannon, 1962, Hedhammar et al., 1974; Voorhout and Hazewinkel, 1987) and thus to skeletal development.

- Sex predisposition

From a study of Mansson and Norberg (1961) it became known that injections of oestrogens during skeletal growth coincided with HD development, possibly due to influence of the collagen stiffness of the joint capsule and tendinous attachments.

Experimental findings correspond with evaluation of populations affected (Henricson and Olsson, 1959; Hedhammar et al., 1979; Swenson et al., 1997) who found more females than males affected with HD, although Martin et al. (1980) found a male female ratio for HD of 1.2:1. Differences in the incidence between genders of dogs of the same breed could originate from differences in musculature balances, growth rate, sex hormones or behaviour (Swenson et al., 1997).

-Nutrition

Dogs of comparable size in the same category may present very different energy requirements, due to a variety of factors including thickness of the fur and skin, body composition (ie. the lean mass/fat ration), the housing (indoor vs. outdoor), and gender. The latter, since males are generally less fat than the females of the same breed, resulting in a greater (approximately 10%) expenditure (Kienzle and Rainbird, 1991). Neutering, although not playing a major role in HD development, will reduce expenditure as well for approximately 30% (Robertson, 2003). Not only the quantity of food, but also quality influences skeletal growth and development (Kealy et

al., 1992). From a variety of studies in clinical and laboratory circumstances, it has been demonstrated that excessive food and/or calcium intake disturbs the process of endochondral ossification in large breed dogs, including Labradors (Hedhammar et al., 1974; Kasström, 1975).

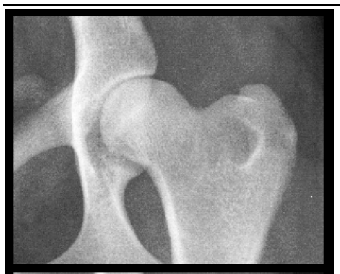
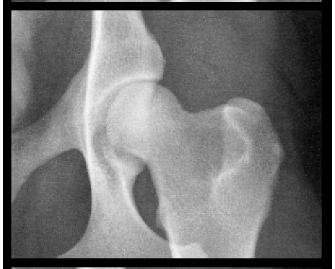
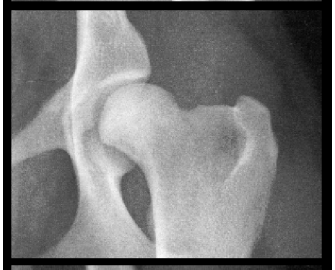
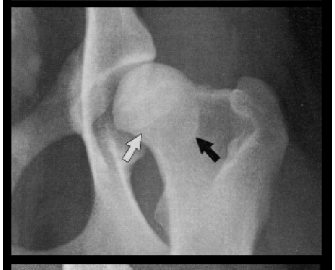
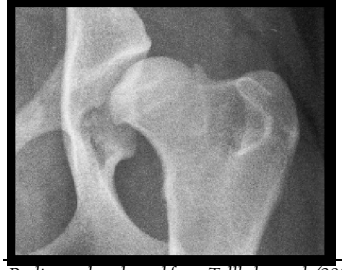
Screening for hip dysplasia in The Netherlands

In The Netherlands, screening for HD is organized according to the regulations of the FCI, the umbrella organization of national Kennel Clubs. Dogs will be radiographed at the age of 12 months (or 18 months only in giant breed dogs). Sedation has been recommended. The dogs are laid down in dorsal recumbence, with both hind legs extended. The pelvis is symmetrical projected, both femurs are parallel to each other and the patella is projected in the middle of the femur, to guarantee a standardized and symmetrical position.

In The Netherlands the hip status is screened by a panel of three specialized, independent scrutinizers who judge the scanned radiographs. Three criteria are separately examined: a) presence (and degree) of osteophytosis as sign of osteoarthritis, b) laxity of the femoral head and incongruity of the hip joint, and c) depth of the acetabulum. In addition, the Norberg angle (NA) is measured for each hip joint separately. In order to measure the NA, the angle between the craniolateral aspect of the acetabular rim, the center of the ipsilateral femoral head, and the center of the contralateral femoral head is determined. In The Netherlands both NAs are taken together and given as the Norberg Value of the dog. The most severe grading of each of the hips determines the final scoring of the dog. Together these criteria will determine the HD-status of the dog, according to the FCI- scheme (Table 2.2).

Since May 2002 the second radiographic view recommended by the FCI (i.e. both hip joints flexed with the greater trochantor not overlapping the femoral head), has been abandoned by the Dutch Kennel Club. The inability to observe small osteophytes around the femoral head and the overestimation of the congruency on the radiographic view with extended hind legs (the femoral head is pulled into the acetabulum due to twisting of the joint capsule and thereby shortening of the joint capsule) has resulted in a shift in HD scores in the period of transition.

Table 2.2 HD-status with the corresponding description according to FCI-standards

HD scoring scheme	
	**HD-A: no signs of HD** The femoral head and the acetabulum are congruent. The craniolateral acetabular rim appears sharp and slightly rounded. The joint space is narrow and even. The Norberg angle is about 105°. In excellent hip joints the craniolateral rim encircles the femoral head somewhat more in caudolateral direction.
	**HD-B: near normal** The femoral head and the acetabulum are slightly incongruent and the Norberg angle is about 105° or the femoral head and the acetabulum are congruent and the Norberg angle is less than 105°.
	**HD-C: mild HD** The femoral head and the acetabulum are incongruent, the Norberg angle is about 100° and/or there is slight flattening of the craniolateral acetabular rim. No more than slight signs of osteoarthrosis on the cranial, caudal, or dorsal acetabular edge or on the femoral head and neck may be present.
	**HD-D: moderate HD** There is obvious incongruity between the femoral head and the acetabulum with subluxation. The Norberg angle is more than 90° (only as a reference). Flattening of the craniolateral rim and/or osteoarthrotic signs are present.
	**HD-E: severe HD** Marked dysplastic changes of the hip joints, such as luxation or distinct subluxation are present. The Norberg angle is less than 90°. Obvious flattening of the cranial acetabular edge,

Radiographs adapted from Tellhelm et al. (2008).

Factors influencing HD scoring

The same hip joints can be screened differently to the consequence that the hip of the same dog score worse or better. Some of the known factors are:

Age of screening:

Smith (1997) performed a longitudinal study in a group of Labradors and demonstrated a significant age effect (Kealy et al., 1992; Kealy et al., 1997; Wood and Lakhani, 2003b), and therefore the age of screening can have an important influence on the judgment of the hip status. Not too many studies have been performed describing what the sensitivity and consistency of radiological screening is.

Radiological technique:

Not only the symmetrical positioning of the pelvis and the position of the patella in the middle of the femur, but also the rotational force during positioning of both hind legs, influences the projection of the femoral heads inside the acetabulum. This rotation was seen in almost 30% of more than 7000 evaluated radiographs and it therefore a matter of concern (Genevois et al., 2007). By a different technique the laxity can be decreased, and thus influence the final grading of the hip status (Flückiger et al., 1999).

Descriptive criteria of radiological findings:

There is a lack of precision of methods for assessing HD due to the use of descriptive criteria, such as those used by the FCI; this limits the sensitivity of predicting value for HD (Comhaire et al., 2009). Specific methods for estimating hip-joint laxity by means of stress or distraction and dorsolateral subluxation, quantitative scores may be better reflect HD-status (Comhaire et al., 2009; Smith, 2004; Flückiger et al., 1999).

However, since this distraction has to be (re-)produced by the force of the radiologist, inter-observer agreement between experienced assessors is reportedly only fair (Verhoeven et al., 2007). Because this technique is not in use in the FCI screening protocol, it will not be further discussed here.

Norberg angle:

The NA is another measure of hip joint laxity, and some studies have revealed it efficiently predicts the risk of HD in dogs. However, differences between breeds can occur: within the Retrievers, there were differences as well as between breeds (Comhaire et al., 2009). For example, the mean of the lowest of both NAs were 101.1°, 103.4°, and 108.9° for Golden Retrievers, Labrador Retrievers, and Flat-Coated Retrievers, respectively (Comhaire et al., 2009). It has already been mentioned that hip extended views stress the fibers of the joint capsule, lowering the chance to properly evaluate passive hip joint laxity in extended position (Smith et al., 1990).

Anesthesia during radiological investigation:

The muscle contraction in non-anaesthetized dogs may cause an underestimation of hip joint laxity as a consequence, since HD-grading was worse when the extended hips were radiographed with the dog under general anesthesia: this was not due to difference in acetabular or femoral morphology, but due to a significant higher percentage of dogs with hip joint laxity as expressed by NA (<105 degrees). (Genevois et al., 2006).

Table 2.3 Environment and genetic influences on the grading of canine hip dysplasia

Environment	Breed	Reference
Overweight	Labrador Retriever	Kealy et al., 1992, 1997
Overweight	German Shepherd Dog	Kasström, 1975
Over supplementation	Great Dane	Hedhammar et al., 1974
Sedation		Malm 2007; Genevois et al., 2006
Body weight at 3 months of age	Labrador Retriever	Krontveit et al., 2011
Off-leash exercise at 3 months of age	Labrador Retriever	Krontveit et al., 2011
Season of birth		Hanssen 1991; Krontveit et al., 2011; Worth 2011; Ohlert et al., 2002; Wood et al., 2002

In conclusion, it can be stated that HD is an inheritable disease, with an h^2 varying between breeds and different studies. Environment might have a large influence on the expression of genes involved in this disease and/or in the influence on the progress of the abnormalities and the clinical signs. This is especially the case in the severe forms of HD with a high score. The hip status of dogs with slight abnormalities can be influenced by a variety of factors including the amount of views, positioning and sedation of the dog, rotation and manual distraction of the femur, methodology of measuring the Norberg angle and determining the osteophytosis and incongruity of the joint. Several attempts have been made to objectivise the scoring but breeders are best served by consistent grading and screening methods which are not sensitive to manipulation, so that HD positive dogs can be correctly recognised as such.

Elbow dysplasia

Normal elbow joint development

The elbow joint is formed by the proximal end of the radius and ulna and the distal end of the humerus. The joint between radius-ulna and humerus allows for extension and flexion, whereas the radio-ulnar joint allows for pronation and supination. In the juvenile skeleton a diversity of secondary ossification centres can be recognised in the distal humerus and proximal radius and ulna, including the lateral and medial part of the humeral condyle which fuse at the age of 16 weeks, and the anconeal process which fuses before the age of 5 months (Voorhout and Hazewinkel, 1987). The anconeal process develops by apposition, by a separate ossification centre, or a combination of both (Breit et al., 2004). It is demonstrated that the medial coronoid process develops exclusively by appositional ossification and is completed earlier in smaller than in larger dogs, this unlike the anconeal process (Breit, 2004). The ossification of the coronoid process was completed at 16 weeks (in small breed dogs) till 20 weeks (in large breed dogs) (Breit et al., 2004) and in week 18 in Labrador Retrievers (Lau et al. 2013). The age of fusion of the secondary ossification centres varies in dogs of different breeds, within litters (Hare, 1961), and quantity of calcium intake (Voorhout and Hazewinkel, 1987), but is not influenced by gender (Hare, 1961).

Medial coronoid process bears 60% of the weight executed by the humerus on the elbow joint.

Pathophysiology of elbow dysplasia

Elbow dysplasia (ED) is a term used to describe a group of lesions, which are thought to arise from pathological incongruence in the elbow joint (Michelsen, 2012), which can be divided in:

- ulna is too long,
- radius is too long,
- the humeral notch has an elliptical configuration instead of a circular notch running parallel with the humeral condyle.

These malformations can all lead to altered pressure points within the joint during weight-bearing that can lead to cartilage trauma at different locations and thereby manifest in the following lesions: fragmented coronoid process (FCP), ununited anconeal process (UAP) and osteochondritis dissecans (OCD) of the medial part of the humeral condyle. All these entities may have a shared aetiology as hypothesized by Olsson (1981), with a disturbance of endochondral ossification as a common factor. During the process of endochondral ossification, chondrocytes divide, differentiate, mature and hypertrophy with eventually mineralisation of the cartilage matrix followed by blood vessel in-growth and introduction of osteoblasts on the mineralized cartilage spiculae thus forming primary cancellous bone. This process where cartilage is transformed into bone takes place in the secondary ossification centres in a randomly way, and in the growth plates in an orderly way. Disturbance of the process of endochondral ossification due to delay of maturation of the chondrocytes (so-called osteochondrosis) causes an increased amount of immature chondrocytes embedded in unmineralized cartilage matrix. With increased loading in growing animals due to physiological or abnormal joint incongruity, this increased area of unmineralized cartilage forms a vulnerable spot which may easily break. When this disturbance of endochondral ossification occurs in a focal area in the elbow joint, and when in the cartilage cracks will develop into a cartilage flap, this entity is named osteochondritis dissecans (OCD); when it occurs in the cartilage separation between anconeal process and olecranon it causes an ununited anconeal process (UAP) and when it occus in the radio-ulnar area of the medial coronoid process it causes fragmented coronoid process (FCP) (Lau et al., 2013). Olsson hypothesizes that both the OCD lesion and the FCP lesion are expressions of delayed endochondral ossifications, i.e. osteochondrose. According to the International Elbow Working Group (IEWG) incongruity is a fourth disorder of the elbow joint also captured under the term ED, without making assumptions as to cause and effect.

Fragmented Coronoid Process

Damage to the medial coronoid process can vary from fissures to complete fragmentation. Even chondromalacia is an expression form of pathology in this area, and therefore are all different forms of medial coronoid pathology included in the term Medial Coronoid Disease (MCD). When the medial coronoid process is still cartilaginous when fissuring of fragmentation occurs, the ossification may be delayed or aborted, but may also occur in time. The fragments can remain in place or become dislocated from the fracture bed. As long as the fragments are still connected to the joint capsule and thus the blood supply is maintained, the fragments can still ossify and even grow in size (Olsson, 1981). When such a dogs grows older, the endochondral ossification in the loose coronoid process can complete till the fragment is

ossified, and is than only covered by a layer of articular cartilage (Olsson, 1981). Fragmentation of the medial coronoid process can create abnormal pressure points on the opposing humeral condyle and result in a superficial erosion of the articular surface, a so-called "contact lesion" (or "kissing lesion"). Breeds that are often affected with MCD are Labrador Retrievers, Golden Retrievers, German Shepherd Dogs, Rottweilers and Bernese Mountain Dogs (Olsson 1981, Carpenter et al., 1993). The frequency of MCD has been reported higher in males than in females, though other reports claim there are some indications that this might be breed dependent. Clinical signs of ED usually start around 6 months following a rapid growth spurt around 4-5 months of age. These signs include lameness and pain, probably due to the fragmentation or fissure in bone or cartilage and due to developing degenerative joint disease. In older dogs the clinical signs are often more related to the secondary osteoarthritis (OA): the patient is stiff after rest (especially after heavy exercise) and improves after short exercise and/or analgesia.

Osteochondrosis of the humeral condyle

Osteochondrosis (OC) is caused by a delay in endochondral ossification which results in a thickening in the articular cartilage layer in the developing epiphysis (Olsson, 1981). OC in the elbow joint is thought to arise from increased contact pressure on the humeral condyle by the medial coronoid process as a result of an underdevelopment of the ulnar trochlear notch (Morgan et al., 2000) in animals predisposed to develop OCs. At this point OC may progress to osteochondritis dissecans (OCD), in which a partial or complete separation of the top layer of cartilage occurs. The cartilage fragment can either remain at the point of separation and may mineralise (but not ossify) at a later stage, and thus can be detectable on radiographs. An unmineralised OCD flap, which is only thickened joint cartilage, and a substantial contact lesion will all present similarly on radiographs, and scored as "OCD-like" lesion. Clinical signs of OCD are first seen at 3-4 months of age, but average age of referral is 5-8 months (Hazewinkel et al., 1998). OCD will result in degenerative joint disease and its clinical signs mimic those of MCD. Clinical signs of lameness often do not correlate to the radiographic evaluation (Grøndalen and Lingaas, 1982)

Different entities of ED can occur in the same breed, even in the same dog like UAP and FCP (Meyer-Lindenberg et al., 2006; Remy et al., 2004; Hazewinkel et al., 1998), and FCP and INC (Samoy et al., 2012). In Labradors, the most frequent form of ED is the fragmented coronoid process (FCP), whereas OCD and elbow incongruity are diagnosed less frequently. The ununited anconeal process is very seldom occurring in this breed (Meyer-Lindenberg et al., 2006) but especially in German shepherd dogs, and will thus not be further discussed here. Incongruity of the elbow joint (INC) due to radial shortening is seen in Bernese Mountain Dogs in 80% of the dogs with osteoarthrosis in the elbow joint (Ubbink, 1999; Samoy, 2012). In a survey of a large group of Bernese Mountain dogs this type of incongruity was seen in all cases with elbow lameness in conjunction with a fragmented coronoid process. Population analyses revealed that the disease was introduced right after WW II by a limited number of founding fathers and from there introduced in the breed (Ubbink, thesis).

Table 2.4 Breed specific prevalence (in %) of elbow dysplasia

	Belgium [a]		USA [b]	
	N° evaluated	% affected	N° evaluated	% affected
Bernese Mountain dog	266	20	11,685	28.3
Labrador	227	13	59,832	10.7
Golden Retriever	126	18	28,923	11,0
German Shepherd dog	130	12	32,937	19,1
Rottweiler	135	33	14,172	39.7

[a] *Coopmans et al., 2008: Incidence in Belgium in the period 2002-2006*

[b] *Orthopaedic Foundation for Animal (OFA) period January 1974- December 2011*

Degenerative joint disease

The amount of degenerative joint disease can be limited as was demonstrated recently by Van Bruggen et al. (2010) in a group of older dogs with MCD proven by bone scintigraphy and surgery but (often) not detectable by radiological investigation. In general can be stated that there is a poor correlation between the radiological finding in case of FCP and the pathology as present and visible during surgery (Fitzpatrick et al., 2009). In addition to plain radiology in different (preferably four projections) views (Cook and Cook, 2009), and the here mentioned bone scintigraphy, other techniques and modalities have been developed for imaging the complex articulations of the canine elbow, including ultrasonography (for visualisation of soft tissues and of an abnormal, FCP, even unmineralized), magnetic resonance imaging (in multiple planes and 3-dimensional reconstructions for visualisation of subtle changes and incongruities) (Cook and Cook, 2009). Nuclear scintigraphy is quit sensitive, although not much is known of negative findings in case of FCP with necrotic bone and without degenerative joint disease, but not specific (Cook and Cook, 2009, Van Bruggen et al., 2010). CT is increasingly in use in veterinary practices and offers great advantages with large sensitivity and specificity, only exceeded by invasive surgery or arthroscopy (Table 2.5)

Table 2.5 Imaging modalities in use for diagnosis of canine elbow dysplasia entities

Modality	Sensitivity(%)	Specificity(%)	reference
Radiography (for INC)	99.3	42.4	Wagner et al., 2007 Carpenter et al., 1993
CT (for INC)	85	45,8	Wagner etal.,, 2007
CT (for FCP)	86.7	88.2	Carpenter et al., 1993
Arthroscopy (for INC)	94	81,9	Wagner et al., 2007

Risk factors for elbow dysplasia

A variety of risk factors have been mentioned in literature, although the pathophysiological mechanism behind these factors on the manifestation of ED should be elucidated.

Food quality

In epidemiological and experimental studies it has been proven that overnutrition (Hedhammar et al., 1974) and excessive calcium (Goedegebuure et al., 1986), calcium and

phosphorous (Schoenmakers et al., 1999) and excessive vitamin D (Tryfonidou et al., 2003) intake can disturb the process of endochondral ossification to the extent that osteochondrosis and retained cartilage cones with consequently radius curvus syndrome developed in Great Dane dogs, when compared with Great Danes fed a control diet.

Food quantity

Ad libitum feeding caused at older age overweight Labradors in comparison with Labradors fed 70% of the ad libitum amount: in all dogs at the age of 11-13 years osteoarthritis of the elbow joint was diagnosed at necropsy, without any signs of MCD, OCD, UAP or INC (Huck et al., 2009)

Sex predisposition

In a variety of studies the prevalence of ED and FCP in male dogs has been reported (Padgett et al., 1995; Sjostrom, 1998; Ryssen and Van Bree, 1997), however in a study in Bernese Mountain dogs could not reveal a gender predisposition (Ubbink et al., 1999)

Family groups

In dendrograms, Ubbink et al. (1999) demonstrated that there was a close relationship within the Labrador breed of dogs diagnosed with MCD. These days the disease seems to be more disseminated throughout the breed. In Bernese Mountain dogs, FCP and INC were present in the whole cohort of dogs, irrespective of the family cluster, which coincided with a high percentage (45-64%) of occurrence of the disease. Common ancestors associated with FCP differed from those associated with INC (Ubbink et al., 1999), thus it seems that FCP and INC both originated from different ancestors more than eight generations ago.

The heritability estimates (h^2) are between 0.24-0.43 for Bernese Mountain Dogs, 0.77 for Labradors and 0.45 for Golden Retrievers (Guthrie and Pidduck, 1990; Stuttert et al., 1998). For Retrievers, these figures are for osteochondritis dissecans of the medial humeral condyle (OCD) plus FCP, and thus found to be polygenetic in addition to multifactorial. For FCP and OCD alone, these figures are not calculated yet.

Humeral condyle osteochondrosis is known to be a polygenic disorder in Labrador retrievers and other breeds (Padgett et al., 1995; Mäki et al., 2002; Mäki et al., 2004; Janutta and Distl, 2006), however, the genes responsible are unknown.

DNA analysis focused on collagen genes in Labradors with FCP, did not reveal any indication of the involvement of these candidate genes in this skeletal disease. A genome wide scan of affected siblings, using 300 microsatellite markers gave more promising results with loci on CFA01 and CFA13 that warranted further research (Salg et al., 2006). It is to be expected that DNA-analysis of the population will detect dogs with the implicated gene(s) who did not express the disease due to optimal environmental circumstances or are heterozygous for the disease. DNA analysis of the potential breeding stock will prevent frustration of breeders who now experience positive offspring from negative parent dogs and thus a slow decrease of the incidence of these hereditary diseases in next generations.

Screening for elbow dysplasia.

The disease is known to be heritable in many dog breeds (h^2 = 0·25 to 0·28) (Beuing et al., 2000), and a breeding program to control it has been successful in Rottweilers and Bernese

Mountain dogs (Swenson et al., 1997). Also in The Netherlands the incidence of ED (here FCP and INC) in Bernese Mountain dogs decreased from 64% cases in 1992 to 45% cases in 1995. In the Scandinavian countries, United Kingdom and in the USA, dogs are diagnosed with the disease if they are positive for the secondary lesions, whereas in Germany, The Netherlands, Switzerland, Belgium and France, dogs with only a primary defect may also be diagnosed with the disease.

Table 2.6 Breed specific prevalence (in %) of elbow dysplasia

	Belgium[a] (2002-2006)		USA[b] (1974-2011)	
	n° evaluated	% affected	n° evaluated	% affected
Bernese Mountain dogs	266	20	11,685	28.3
Labrador	227	13	59,832	10.7
Golden Retriever	126	18	28,923	11,0
German Shepherd dog	130	12	32,937	19,1
Rottweiler	135	33	14,172	39.7

[a]*Coopmans et al., 2008: Incidence in Belgium in the period 2002-2006*

[b]*Orthopaedic Foundation for Animal (OFA) period January 1974- December 2011*

More than 25 years ago, in Davis (CA, USA) an International Elbow Working Group has been founded by veterinarians and breeders with the intention to investigate and reduce the incidence of ED. Since 15 years a screening scheme has been introduced that is made available on internet and is in use in many countries. Only few conditions are prescribed for screening i.e. the dog should be mature (minimum 12 months of age), and identified, and at least one radiograph (mediolateral flexed view) of good quality has to be available for judging.

In The Netherlands, ED-screening is now organised by the Dutch Kennel Club which prescribes four views in selected breeds (including Labrador and Golden Retriever) and two views for other breeds where ED does not form a selection criteria for breeding. The four views include: the 90° flexed mediolateral, the extended mediolateral, the craniocaudal, and the craniolateral–caudomedial radiographic views of both elbows made by the technique as described before (Voorhout and Hazewinkel, 1987). The ED panel of the Dutch Kennel Club reviews all radiographs. Each radiograph is simultaneously assessed by three of five experienced European board-certified orthopaedic surgeons or radiologists. Scores are obtained by way of majority consensus. Both OA and primary ED are scored. For OA, the height of the osteophytes are estimated and graded 0 (no OA), 1 (<2 mm), 2 (2-5 mm), or 3 (>5 mm) at sites a, b, d, f and g as described by the International Elbow Working Group (IEWG) (Figure 2.2). Sclerosis is assessed at the trochlear notch at the base of the coronoid process (site e). The presence of indentation in the medial part of the humeral condyle is recorded (site h). Site c was not consistently scored due to its badly defined criteria (although it form recently an important criterion included in the final decision). The primary diseases are categorized and coded as: absent (0), suspect (1), or present (2) for UAP, OC(-like lesion), FCP, and INC. In the category 'suspect', the primary disease causing ED is not explicitly visible, but its presence is suspected based on secondary changes (Tellhelm, 2010).

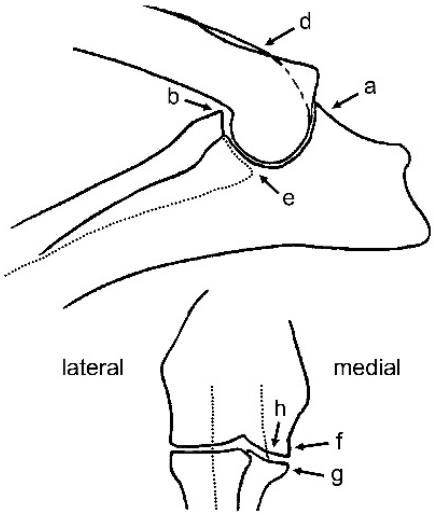


Figure 2.2 Locations that were graded for osteoarthrosis, a) proximal surface of the anconeal process, b) cranial aspect of the radial head, c) cranial edge of the medial coronoid process, d) caudal surface of the lateral condylar ridge, e) sclerosis of the ulnar notch at the base of the coronoid process, f) the surface of the medial epicondyle, g) medial edge of the medial coronoid process, h) indentation of the subchondral bone. Copied from IEWG.

Table 2.7 ED scorings scheme according to the International Elbow Working Group

ED scoring scheme	
ED-0 No signs of ED	No evidence of incongruency, sclerosis or arthritis
ED-1 Mild arthritis	Presence of osteophytes < 2 mm high or minor sclerosis at the base of the coronoid process.
ED-2 Moderate arthritis or suspected primary lesion	Presence of osteophytes 2-5 mm high, obvious sclerosis at the base of the coronoid process or indirect signs for the presence of a primary disease (INC, UAP, FCP or UAP)
ED-3 Severe arthritis or evident primary lesion	Presence of osteophytes > 5 mm high or obvious primary disease

Sclerosis of the ulnar trochlea.

The origin of the sclerosis, bordering the ulnar trochlea, is not well understood. It is associated with FCP (rather than with OCD) is not always present in case of MCD. Since sclerosis is hard to measure with objective criteria and is proportional to the degree of exposure of the object (Burton et al., 2007) with an observer sensitivity for trochlear sclerosis of 72 %, and with a specificity of only 22% (Burton et al., 2007)

Both the inter-observer agreement to detect sclerosis is only 'fair' and its identification has only a 'moderate' sensitivity, whereas the intra-observer agreement was moderate-substantial. (Burton et al., 2008). The sclerosis might originate from excessive loading as can be expected in INC elbow joints (Samoy et al., 2006) and is seen already early in the development of FCP, in young dogs even before obvious osteophytosis is developed (Schulz and Krotscheck, 2003). However, in certain cases it is not present in older dogs (Van Bruggen et al., 2010), where it is not known if it disappeared or was never formed. Investigation with the use of CT gave better insight about the location, the appearance of the sclerosis. Lau et al. (2013) demonstrated the intramedullar location of the bone formation in case of advanced (but not early) cases of FCP in

Labrador Retrievers. Histo-pathological investigation, with the use of fluorochromes is needed to understand the dynamics of the bone formation and remodelling in this area. Intramedullary osteosclerosis has been described in case of stress fractures, osteomyelitis, metabolic or endocrine disorders and malignancy (Ihde et al., 2011)

Factors influencing ED scoring at screening

There is a variety of circumstances which will influence the outcome of the screening on elbow joints of a certain population or of an individual dog. The following circumstances should be taken into account:

Age at screening

Based on the studies of Huck et al. (2009) it becomes clear that also dogs without primary cause may develop osteophytosis in the elbow joints, not due a primary disease, but as a form of primary osteoarthritis (i.e. due to old age). This becomes also obvious in population screening where the age of the dogs is taken into account, in cases where a primary disease is present (or suspected).

Number of radiological views

In different studies where the value of different radiological views were studied, it has been demonstrated that more views give more insight. For diagnosing OCD, the APMO view was excellent and the CrCd view good for detection of the lesion, whereas the ML, ML flexed and an additional distomedial-proximolateral oblique view give no diagnostic information (Chanoit et al., 2010) The experience and training of the radiological investigation of the elbow joint plays a crucial role in reaching a high sensitivity, reliability and repeatability for the radiographic diagnosis of MCD (Rau et al., 2011) Experience radiologists reach the sensitivity level which can be reached with CT scanning (Rau et al., 2011).

Comparison of radiographic an CT pictures of elbow joints of Belgium shepherd dogs revealed that the small radiopaque ridge dorsal of the anconeal process is not always pathological, but rather anatomical and thus should not be scored as a positive OA sign (Lappalainen et al., 2009)

Screening for HD and ED in The Netherlands

According to the Dutch Kennel Club (Dr Laura Roest is kindly acknowledged) are breeders of the following dog breeds obligated to screen the breeding stock for both HD and ED at present times:

Australian Shepherd, Bernese Mountain dog, Bouvier des Flandres, Bracco Italiano, German Shepherd dog, Labrador Retriever, Landseer ECT, Mastiff, Newfoundlander, Nova Scotia Duck Tolling Retriever, Rhodesian Ridgeback, Rottweiler, St Bernard, Swiss White Shepherd. Owners and breeders of other full bred dogs are allowed to send radiographs for screening to the Dutch Kennel Club who organises the panel screening and making available the certificate and central administration.

Patellar luxation

Normal stifle development

The patella forms a sesamoid bone within the patellar tendon, which has direct contact with the articular cartilage in the femoral trochlea. Due to its fit in the femoral trochlea it prevents tendon wear during flexion and extension of the stifle joint. Due to the fact that it increases the distance between tendon and rotation points of the femur, it increases the leverage arm and thus the forces exerted by the quadriceps muscle. This muscle, consisting of four muscle groups (i.e. rectus femoris, vastus lateralis and medialis, and vastus intermedius), is an extensor of the stifle joint and when contracted it allows weight bearing of the hind leg. The vastus lateralis and medialis have attachments with the medial and lateral para-patellar fibrocartilage, respectively, which articulates with the trochlear ridges bordering the femoral trochlea. During skeletal development, the contact between patella and trochlea is of importance for a proper development of both structures with a perfect fit between the concave femoral condyles with its convex trochlea and the convex patella. Stifle joint extension and flexion is accomplished by the extensor mechanism of the stifle joint, including the quadriceps muscles, the straight patellar tendon and patella, the trochlear groove and the tibial tuberosity, the latter being the insertion point of the patellar tendon. In case of extension of the stifle joint the patella is not forced into the trochlea by the quadriceps as is the case during flexion, but the patella is in addition kept within the trochlear groove by the joint capsule and the retinaculum (as thickened structure running from patella to fabellae), and the trochlea with its ridges. When the extensor mechanism of the stifle joint forms a straight line from the origin of the rectus femoris muscle to the tibial tuberosity, patella stability is maintained (Evans and Christensen, 1979).

Patellar luxation (PL) can be the cause of trauma due to rupture of the retinaculum, or to femoral fracture or malunion, or hip joint luxation with femoral torsion. Non-traumatic PL can develop due to a congenital or developmental malalignment of the extensor mechanism of the stifle joint (Harasen, 2006).

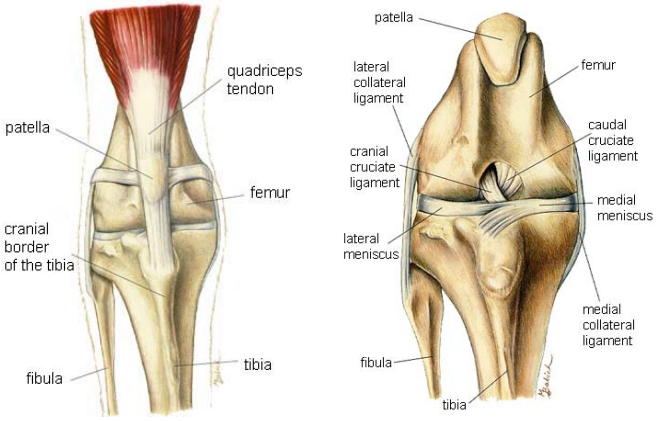


Figure 2.3 Anatomy of canine stifle joint indicating the horizontal stabilizing structures (femeropatellar ligament = retinaculum) and the vertical alignment (Quadriceps muscle, patella, patellar tendon, cranial tibia (i.e. tibial tuberosity). The pictures in this section are reprinted with permission by the copyright owner, Hill's Pet Nutrition, from the Atlas of Veterinary Clinical Anatomy.

Pathophysiology of patellar luxation

Fascia lata and the medial and lateral retinaculum (femoropatellar ligament) and fibrocartilage play a role in the stability of the normal patella position. In case of non-traumatic PL, fascia lata and retinaculum progressively get stretched due to malalignment of the extensor mechanism of the stifle joint. Petazzoni (2011) hypothesizes that the patella can luxate or subluxate because of two reasons: (1), the patella being out of position because of the medial or lateral traction of the distal insertion of the patellar tendon (tibial tuberosity) or, (2) because the distal femur being out of position on its frontal (distal varus or valgus) or transverse plane (i.e. internal or external femoral torsion).

Other causes of medial PL are described by Putman in his original thesis in 1968. Based on his investigations he hypothesized that coxa vara (ie a decreased angle of inclination of the femoral neck, or in other words a collo-diaphyseal angle which more straight than usual) together with a decreased femoral neck anteversion, ie relative retroversion (a femoral head pointing in a caudal rather than a slight cranial direction) can hold responsible for a medial PL (Putman, 1968).

Immature dogs have open physeal growth plates in the distal femur and proximal tibia till the age of 330 (range 136-392) days and 249 (range 143-435) days, respectively (Newton, 1985). The effect of compression on the physis is generally defined by the Hueter-Volkmann principle, in which decreased linear growth of the physis results from increased compression, and growth rate is undisturbed at the lateral side where static pressure is decreased (Bries et al., 2012). Hypoplasia of the distal femoral epiphysis was found to occur in dogs with PL, and was hold responsible for the bowing of the distal femur (Putman, 1968) however this can also be considered a cause of the eccentric quadriceps loading (Hulse, 2011). The degree of varus malformation of the femur depends on the duration of the luxation and the growth rate of the dog (Hulse and Shires, 1985). In addition the tuberositas tibiae will rotate in a medial direction together with varus deformation of the proximal tibia and external rotation of the distal tibia. (Hulse and Shires, 1985).

In addition to the inability to use the leg for weight bearing and jumping the following consequences of PL play a role:

- The severity of the grading of PL can progress (from grade 2 to grade 3 and from grade 3 to grade 4; for grading see later), especially in immature dogs (Hulse, 2011). The trochlear ridge will be lowered and the shape of the patella changed due to repetitively luxations with as a consequence that the patella will luxate even more easily (Hulse, 2011).
- The patella, which development depends on the contact with the trochlea, will be malformed in case of permanent PL at young age: the patella will stay flat or might even not be formed (Bonath and Prieur, 1998). In case of medial PL in mature dogs with closed growth plates, the whole tibia will endorotate, contrarily to the lateral PL with exorotation with lateral displacement of the tuberositas tibiae (Bonath and Prieur, 1998).

- The trochlea patellaris develops according to the Hueter-Volkmann's principle under the constant pressure by the patella. In case of PL at a very young age, the trochlea will not develop and the joint cartilage in the trochlea is convex rather than concave. With plain radiological investigation, with a special skyline view, this aspect can be missed since the thickening is only cartilage (Bonath and Prieur, 1998).
- In case of low grade PL (i.e. grade 2), the patella moves out of the trochlear groove; this can disturb articular cartilage at the articular side of the patella and of the trochlear ridge. When cartilage is surgically removed, the subchondral bone is exposed to inflammatory substances and osteoarthritis will develop. In this situation the dog is not only lame but also has a painful condition (Brinker, 1993).
- PL concurrent with cranial cruciate ligament rupture is seen in 15-20% of the stifles of middle-aged and older dogs with chronic PL (Brinker 1993) which is explained by the extreme stress put on the cranial cruciate ligament in case of medial PL due to endorotation of the tibia (Brinker, 1993). In a series of Pomeranians, a co-mobidity of PL a cranial cruciate ligament rupture was seen in a cohort of 124 Pomeranians (Wangdee, personal communication).

Risk factors for patellar luxation

For non-traumatic PL the following risk factors can be taken into account:

Age of the dog

Young to mature dogs with grade 2 or 3 PL will exhibited abnormal or intermittent lameness, and thus will seldom be offered for PL screening tests. In most cases the diagnosis can be made within the first 6 months of life (Newton, 1985; Nunamaker, 1985).

Older animals with grade 1 and 2 may reveal clinical signs due to further breakdown of soft tissues and developing osteoarthritis, but at an earlier stage these dogs can stay unnoticeable till offered for PL screening. Since dogs are skeletal mature at the age of 12 months, it has been decided not to screen dogs officially before that age.

Sex of the animal

A sex predisposition of females being 1.4-1.9 times more affected with PL than males (Priester, 1972; Hayes et al., 1994; Alam et al., 2007)

Breed

There is a breed predisposition for PL, with a registered incidence up to 43% in Pomeranians (OFA). In general, smaller breeds are more likely to develop PL than larger breeds (Priester, 1972; Hayes et al., 1994; Chase et al., 2009). Medial and lateral PL both occur in dogs, with medial PL more common than lateral PL in all breed sizes, and lateral PL more common in large and giant breeds (Hayes et al., 1994). In congenital PL involvement of both stifles is common (Hayes et al., 1994), although evaluation of large groups is lacking (see Table 2.8) There are no actual publications about the incidence of PL in different breeds or types dogs and the different manifestations. Medial PL is more common (70-80%) than lateral luxation in all breeds, with bilateral involvement in 20-25% (Priester, 1972)

The breed predisposition for PL (Table 2.8), together with an early age of onset, has led to the assumption that PL is a heritable trait with the pattern of segregation pointing towards a polygenic, multi-factorial disorder. Although there are several theories about the pathophysiology of PL, no underlying mechanism has yet been identified to explain the susceptibility of certain dogs to this disorder.

Table 2.8 Number of dogs of a variety of breeds screened for patellar luxation in the U.S.A.

Breed	Rank number	N° evaluated dogs between January 1974 and December 2011	Percent dogs affected with patellar luxation
Pomeranian	1	559	41.1
Yorkshire Terrier	2	333	24,3
Cocker spaniel	4	742	15,8
Chow chow	9	352	10,2
Shetland sheepdog	12	67	9.0
Labrador	15	555	6.8
Schipperke	32	336	4.5
Flatcoated retriever	71	1833	1.6
NewFoundland	95	1371	0.6
Belgian Malinois	98	93	0.0

Results from screening in the United States of America as presented by the Orthopaedic Foundation for Animals of more than 100 breeds (www.offa.org)

Screening for patellar luxation in The Netherlands

PL is screened in a limited amount of breeds in The Netherlands, often at a voluntary basis. Breeder clubs decide to screen for PL when an unacceptable amount of dogs of that breed suffers from hind limb lameness and seeks veterinary help. No umbrella organisation (FCI, Raad van Beheer) or legislative body has any rules on this issue in The Netherlands.

PL scoring can be divided in grade 0-4 (Table 2.9), whereas the grading "loose" has been introduced in certain breeds. Unlike the screening for HD or ED, this skeletal disease has to be screened by physical examination of the animal, and not by reading the radiographs, since PL can occur in a grading where the patella can be positioned inside the trochlea although it can also luxate (grade 1-3).

Since 1994 a form designed by Prof. F.J. Meutstege, is in use in The Netherlands for PL screening in order to screen and grade in a uniform way. A limited amount of board certified companion animal orthopaedic surgeons are invited to participate in the program, with the restriction that annual meetings for discussion, repeated evaluation of the process, adaptations of the form and feed-back are attended. This is to keep the variance in judging in the screening procedure as limited as possible. The form includes the following items:

Identification of the animal

Including breed, age, chip number or tattoo number, and the name of the owner.

History taken from the owner

Including any locomotive problems in the past or abnormalities noticed in gait. Dogs with grade 2 PL may demonstrate intermittent or more permanent limb carrying, respectively. In

grade 1 cases there is only slight rotation of the tibial tubercle and slight torsion of the tibia, but in grade 2 cases this can be with tibial tubercle medial deviation and with 30 degrees medial torsion of the tibia and abducted hock joint (Brinker, 1993). In case of PL grade 3, the patella is permanently luxated with torsion of the tibia and deviation of the crest between 30 and 60 degrees. Although the luxation is permanent, the dog may use the limb with the stifle kept in a semi-fixed position (Brinker, 1993). In case of medial PL grade 4 the tibia is medially twisted and the tibial tuberosity may deviate from 60-90 degrees, with a patella permanently luxated and the trochlea absent or even convex. The limb in carrier or, when bilateral, the dog will move in a crouched position (Brinker, 1993).

Investigation of the standing animal

The dog will be calmed to reach an optimum muscle relaxation. In standing position the patellae are localized, and forced in a medial and lateral direction by pressure with the thumbs and index fingers, respectively, and/or the depth of the trochlea is checked when the patella is or can be luxated.

Investigation of the animal in lateral recumbence

The physical examination is continued with the dog in lateral recumbence with the investigation of the upper positioned leg, by palpating for crepitation and for PL during extension and flexion of the stifle, by internal and external rotation of the tibia with control for PL, and in addition with pressure with the thumb in medial direction and pulling with the index finger on the patella in lateral direction to control for medial or lateral PL, respectively.

This investigation leads to the grading 0-4 per leg (Table 2.9). At the form, kept for achieve, final grading for each stifle joint is given by a code for normal, loose (to lateral and/or medial), luxable grade 1 without or with torsion to lateral and/or medial, luxation grade 2 to lateral or medial, have been operated and extra comments.

Table 2.9 Grading of patellar luxation with corresponding findings

PL scoring	Characteristics during investigation
grade 0	patella is moving inside trochlear groove and cannot be manually ab- or adducted.
loose patella*	patella can be manually positioned on the ridges of the trochlear groove, but not out of the groove completely
grade 1	manually luxable patella with spontaneous repositioning
grade 2	spontaneous luxation with repositioning upon active extension (with or without contemporarily rotation of the tibia)
grade 3	constant spontaneous PL which can be manually reduced
grade 4	constant spontaneous PL which cannot be manually reduced

The scheme is the version of Putnam's scoring system adapted by Meutstege and in use since 2002 by a group of certified orthopaedic surgeons in The Netherlands. Grades 1-2 can occur in a medial and/or lateral direction. In case of grade 1-4 the direction of the luxation (medial, lateral or both) is recorded for each stifle separately. Meutstege introduced an additional category, "loose patella", to identify slightly abnormal patellae within Putnam's grade 0. In The Netherlands, PL is performed on request by the owner of the dog.*

At the certificate handed over to the owner the following conclusion can be given for the dog: free of PL, loose patella (left and/or right), grade 1 (left and/or right), grade 2 (left and/or right),

additional (grade 3, 4, previous surgery) with name and signature of the specialist who performed the screening.

Influences on the degree of patellar luxation at screening

Excessive contraction of the quadriceps muscle could keep the patella inside the trochlea whereas in more relaxed stages and/or under sedation the patella can be luxated manually (i.e. PL grade 1 or 2). Therefore muscle tension is registered on the form, which is in use in The Netherlands, and in case of extreme muscle contraction re-investigation under sedation is advocated.

In case of torsion of the femur along its long axis in case of hip luxation or (mal-union of a) femoral fracture the patella can luxate out of the trochlea. This unusual co-morbidity will be mentioned by the owner or noticed by the specialist before and during the screening process.

In case of rupture of the lateral or medial retinaculum due to trauma, the patella can reveal a medial or lateral PL, respectively. Co-morbidity with cranial cruciate ligament rupture has been reported (Newton, 1985; Nunamaker, 1985), but is noticed seldom.

In case of weakness of collagen as in (1) Cushings disease and (2) Ehler Danlos syndrome can coincide with weakness of the retinaculum, which normally keeps the patella fixed in a mediolateral direction. Even without additional trauma, the laxity of the collagen structure may allow for PL in these two disease states. In case of Cushings disease the overweight of the dog can aggravate the symptoms, especially in case of bilateral stationary luxation (Hazewinkel and Nap, 1998). Since both diseases can be diagnosed also with other characteristic findings, a secondary PL is unlikely to be overlooked.

Screening for patellar luxation in the Flat-coated Retriever

The Flatcoated Retriever, whose breed standard has been accepted by the British Kennel Club in 1923, is officially bred in The Netherlands with offspring documentation since 1960. The annual birth rate of Flatcoated Retrievers (FR) in The Netherlands is approximately 700. In a pilot study performed in 1989 including 354 Flatcoated Retrievers, all standardized investigated by the same orthopaedic specialist (Prof Meutstege), it revealed that 40% of the Flatcoated Retrievers were not free of PL and that only 11% of these dogs were bilateral affected. Luxations included grade 1 till 4 and were in 19% medial, 13% lateral, and in 8% medial and lateral. It was remarkable that both sexes were equally represented and that the incidence of PL in this breed is three times of what is registered in the USA (Table 2.8).

Dog genetics

The dog is one of the most diverse species in the world. An obvious examples of this is size: there is a huge difference in height between Great Danes and Chihuahuas. Besides size, dogs also show a wide variety in the shape of the muzzle, legs, ears and tail, in the colour of the coat and its texture, in the amount of musculature and many other traits.

Dogs are also very versatile. They can be trained as companion animal, but also as guide dogs for the visually or hearing impaired, therapy dogs, rescue dogs, search dogs, herding dogs, sled dogs, performing dogs, hunting dogs, guard dogs, fighting dogs, tracking dogs, detection dogs of

mines, termites, illegal substances or corpses and even 'autism' assistance dogs. These examples, regarding both appearance and behaviour, illustrate the remarkably wide phenotypic range seen in dogs.

Ancestry of the domestic dog

Dogs belong to the genus Canis which includes dogs, wolves, coyotes and jackals. The relationship between the dog and its wild relatives had been debated for some time, but in the early nineties mitochondrial DNA (mtDNA) was used to show that the dog was most closely related to the gray wolf, with only 0.2% sequence divergence (Wayne, 1993). If we compare this to the mtDNA sequence divergence between the gray wolf and the coyote of 4% and the divergence among coyotes of 2.5% (Lehman and Wayne, 1991), it is clear that dogs and wolves are very closely related and might even be considered one species.

In a more elaborate study published in 1997, Vilà and colleagues sequenced mtDNA from 140 dogs, 162 wolves, 5 coyotes and 12 jackals (Vilà et al., 1997). The maximum variation in haplotypes found in dogs (12 substitutions) was equal to the maximum variation between any wolf and dog haplotype, while dog and coyote haplotypes differed by a minimum of 20 substitutions and 2 insertions. These findings confirmed the intimate kinship between dogs and wolves. It is important to realize that all members of the Canis genus can interbreed and mtDNA is maternally inherited and therefore only reveals matriarchal ancestry. If a female wolf would interbreed with a coyote, dog or jackal this would not be detected in the mtDNA of her offspring.

Dogs have been part of human society for a long time. Exactly when and where domestication occurred is under discussion. The oldest alleged dog fossils are radiocarbon dated to 13-17,000 years ago (Deguilloux, 2009). The oldest signs of dogs buried alongside humans (which is a clear indication of domestication), date from 12-14,000 years ago [Morey 2006]. It is interesting to consider a major historic landmark, which also occurred in this timeframe, namely the first agricultural revolution. Around 10-15,000 years ago there was a gradual shift from hunting/gathering to a farming lifestyle which created new habitats for many animals.

Vilà et al. (1997) suggested that the first domesticated wolves were initially morphologically identical to their wild counterparts and that the agricultural revolution was the initiator for the morphological divergence of dogs from wolves (Vilà et al., 1997). This theory is strengthened by a haplotype analysis of mtDNA which indicated that the haplotypes present in today's dog population have probably derived from multiple wolf haplotypes around 15,000 years ago (Savolainen et al., 2002). The same analysis also showed that East Asian dogs have the largest genetic variation, the largest number of haplotypes and the largest proportion of unique haplotypes compared to Southwest Asian or European dogs. These three qualities strongly imply that the East Asian population was the ancestral population and that other dog populations were derived from it by subsets of dogs migrating from East Asia to other locations.

Random and selective breeding

The domesticated dogs slowly evolved into distinct types, by adapting to their natural and cultural environment. These so-called landraces were subjective to evolutionary pressure and human guidance in breeding was minimal (random breeding), resulting in more diverse

phenotypes and genotypes compared to modern breeds. Genetic drift might also have contributed to differences between landraces, since it can cause certain characteristics to get fixed in a population, especially in small reproductive populations.

In Greek and Roman writing, different types of dogs are mentioned bred for hunting, carrying, guarding or as pets. During the Middle Ages the nobility exchanged rare and useful dogs (especially hunting dogs) as described in "Livre de la Chasse" by Gaston Phoebus in 1387. The physician of Queen Elizabeth I, John Keyes described 25 different types of dogs in his publication in 1570.

Selective breeding is a concept that has been used since late 18th century to improve a population of domesticated animals by breeding with those individuals that carry certain desirable traits. The British agriculturalist Robert Bakewell (1725-1795) was the first to abandon random breeding by separating the males from the females and only allow breeding between animals with certain qualities. By applying this concept he succeeded in producing superior livestock. Between 1700 and 1786 the average weight of a bull sold for slaughter increased from 170 to 380 kg. He mainly used sire selection, also offering his male animals for stud services. Charles Darwin (1809-1882) later referred to this idea as 'artificial selection' in his 1859 publication of 'On the Origin of Species'. Unlike natural selection, which is the main drive for speciation in Darwin's evolution theory and favours traits that are advantageous in certain environments, artificial selection does not necessarily benefit the species (and might even harm it), but is profitable to the breeder. Less than a decade later Gregor Mendel presented his observations on the experiments he had been conducting using garden peas. He was the first to understand that organisms carry more characteristics (genotype) than they actually show (phenotype) and introduced the terms dominant and recessive to describe the effect of genotype on phenotype. Unfortunately his work didn't get accepted by the scientific community until after his death, when a Dutch botanist named Hugo de Vries rediscovered it and published in 1900.

The formation of dog breeds

Selective breeding of dogs resulted in an explosion of new breeds, created by mixing different existing types or landraces. The genetic isolation in the kennels and inbreeding brought out a wide variety of characteristics, which were displayed in dog shows. To keep track of all these new breeds, the British founded the Kennel Club in 1873, soon followed by many other countries including the Royal Dutch Hunting Club Nimrod in 1874 (Koninklijke Nederlandsche Jacht Vereeniging Nimrod) and the American Kennel Club in 1884. After a while the kennel clubs adopted the “breed barrier rule”, which stated that (once a breed was established) a dog can only become a registered member of a breed if both its parents are registered members. This is also referred to as breeding with a closed studbook. In 1911 the Fédération Cynologique Internationale (FCI) was established, initially a corroboration of the German, Austrian, Belgian, French and Dutch kennel clubs to ‘promote and protect kynology and pure bred dogs’. Nowadays the FCI counts 86 member countries and recognises 343 different breeds, with several 'new' breeds in development.

Evidence of the mixing of ancestral types to create new breeds can still be found in the genetic structure of modern purebred dogs. A phylogenetic structure analysis using 96 microsatellites

in 85 breeds conducted by Parker et al. (2004) revealed that most modern dog breeds cluster together. This indicates that these breeds were constructed using a common founder population or that there was a strong gene flow before the breed barrier was raised. This cluster contained breeds with presumed modern European origin. The only breeds that didn't cluster with these 'modern' breeds are some Azian spitz-type breeds (Shar-Pei, Shiba Inu, Akita, Chow Chow), an ancient African breed (Basenji), two Arctic spitz-type breeds (Alaskan Malamute, Siberian Husky) and two Eastern sight hounds (Afghan, Saluki) which display a more ancient genetic heritage (Parker et al., 2004).

Mapping the canine genome

In 1990 the 'Human Genome Project' started to sequence the entire human genome. In the decade that followed there was a rapid increase in new DNA sequencing and data mining technologies. These new technologies made sequencing genomes of other organisms less expensive and time-consuming than sequencing of the human genome. The mouse genome quickly followed and in 2003 the first dog genome was sequenced (CanFam1.0, Kirkness et al., 2003). This build had 77% coverage of the genome of a male standard poodle (1.5 x sequence coverage). Later a second build of the dog genome was released, based on the genome sequence of a female boxer (7.5 x sequence coverage), covering ~99% of the genome (CanFam2.0, Lindblad-Toh et al., 2005). The latest assembly, CanFam3.1, was released in September 2011.

In addition a single nucleotide polymorphism (SNP) database was assembled containing >2.5 million SNPs in eleven dog breeds, publicly available via the Broad Institute webpage (www.broad.mit.edu/mammals/dog).

Platforms have been developed (by Illumina and Affymetrics), which allow genotyping of many SNPs simultaneously. These SNP arrays are used for both segregation analysis in family samples (linkage) and allele frequency analysis in unrelated population samples (association), to identify genomic regions that influence phenotype.

Association analysis

Any de novo mutation that occurs in the genome arises on a certain genomic background. The chromosome on which it occurs is unique by its combination of variations along its entirety. When this new mutation is passed onto offspring, not only the mutation will be passed on, but also part of the chromosome on which it originally occurred. As the mutation is passed on from generation to generation, recombination events will diminish the stretch of DNA that segregates with the mutation. Association studies use this knowledge and compare variation frequencies in groups of cases and controls, in search of genomic regions that are shared more often among cases than among controls.

A simple method to compare allele frequencies for each variation is a $\chi 2$-based statistic test. A correction for the number of comparisons performed is necessary and the Bonferroni correction, which sets the significance level by dividing the standard α error (usually 0.05) by the number of variations tested, is a very stringent correction. A less stringent correction is the use of permutations to generate an empirical probability that the observed frequency differences would occur irrespective of the phenotype. To achieve this, phenotype labels are randomly swapped within the data set many times. These simulations produce a normal distribution and

provide a probability estimate for the true frequency difference between cases and controls to occur by chance.

As an example, coat colour was analyzed in the Labrador Retriever, Golden Retriever and Flat-coated Retriever (van Steenbeek and Lavrijsen, 2010, unpublished results), all of which were genotyped for 22,000 SNPs.

Table 2.10 Distribution of coat colour in a multi breed sample set

	Black coat	Brown coat	Yellow coat
Genotypes per coat colour			
E locus	EE/Ee	EE/Ee	ee
- Mc1r gene			
- Chr5:66Mb			
B locus	BB/Bb	bb	BB/Bb/bb
- Tyrp1 gene			
- Chr11:36Mb			
Number of dogs in this study:			
Labrador Retriever	169	73	118
Flat-Coated Retriever	81	13	-
Golden Retriever	-	-	49

Comparison of 73 brown and 118 yellow Labrador Retrievers revealed two significant peaks, one on chromosome 5 and one on chromosome 11, with p-values of $1*10^{-32}$ and $1*10^{-25}$ after Bonferroni correction, respectively. An additional 13 brown Flat-coated Retrievers and 49 Golden Retrievers were added to the analysis resulting in a total of 86 brown retrievers and 167 yellow retrievers. This increased the significance for both peaks to $1*10^{-51}$ and $1*10^{-41}$ after Bonferroni corrections, respectively (Figure 2.4). The most strongly associated SNP on chromosome 5 is located 2.7 kb upstream of the Melanocortin 1 receptor (Mc1r) gene, and the most associated SNP on chromosome 11 is located 511 kb upstream of the Tyrosinase-related protein 1 (Tyrp1) gene. Both genes are known to be involved in coat colouration in dogs.

As demonstrated by this example, using multiple breeds can be advantageous when the mutation causing the phenotype is shared and has the same genetic heritage. In complex diseases, multiple genes are involved and risk-increasing mutations are usually not only present in the affected group, but also in the control group at a lower frequency. Although susceptibility loci might be shared between breeds, their allele frequencies might differ and the use of multiple breeds in association analysis is therefore largely dependent on study design and the phenotype studied.

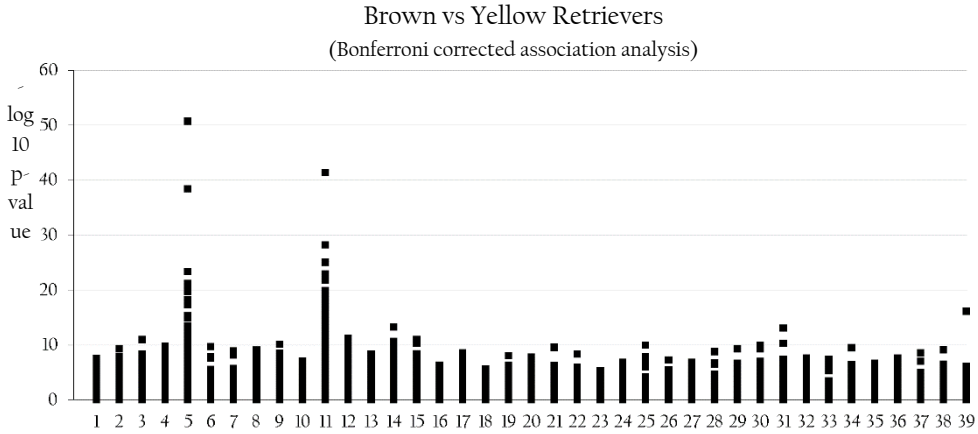


Figure 2.4 Multi-breed association analysis between 86 brown and 167 yellow retrievers (P-values are Bonferrroni corrected)

Population stratification

Association studies depend on the assumption that the only difference between the studied cases and controls is their disease status. Other differences between the groups, like age or sex, can interfere with the results. Another of those interfering factors is population stratification. An "identical-by-state" (IBS) plot can be used to check for stratification. This plot is generated by comparing each sample to every other sample in the data set, creating a multi-dimensional matrix. Using a principal component analysis the two most distinguishing components are determined and can be plotted.

To illustrate the principle, 682 dogs of the KNGF (the Royal Dutch Guide Dog Association) cohort, which was genotyped for 4500 SNPs, are used as an example (Figure 2.5). This cohort consisted of Labrador Retrievers, Golden Retrievers, German Shepherd Dogs and crosses between these breeds. The Labrador is considered the most suitable as a guide dog and of all the pure-bred samples the most abundantly present in this data set. The first generation crosses, are nicely located between the parent populations, and the same is true for the second-generation back-crosses to the Labrador (depicted with ◊). The mixed breed litter of a first generation Labrador/Golden cross and a German Shepherd Dog is clearly located in the middle of the plot.

Even within the pure-bred populations we see stratification. The Golden Retrievers seem to be divided into two sub-populations; pedigree examination reveals a population from American descent and one from European descent. [Two out of the seven Shepherd Dogs were actually White Shepherd Dogs; they cluster together below the five German Shepherd Dogs (two of which are overlapping).]

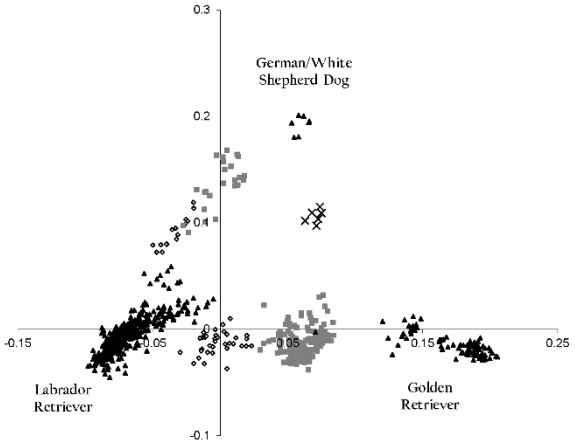


Figure 2.5 First 2 components of the identical-by-state principal component analysis. ▲ *= pure-bred dogs,* ■ *= 1st generation cross-bred animals,* ◇ *= 2nd generation animals cross-bred back to the Labrador,* × *= Labrador-Golden- Shepherd cross-bred animals.*

The limited information available for the Labrador/German Shepherd crosses (n=24) and Labrador back-crosses (n=16) is evident in the overlapping populations; increasing either sample size or number of SNPs genotyped will likely result in better distinction between populations.

Sometimes the collected affected dogs are derived from multiple populations, or even multiple breeds. In that case it is important to population-match the controls to the cases, before making comparisons. Including various populations/breeds in the analysis can be advantageous in a sense that when association is found across population or breed boundaries it is more likely to be a true association. However, genes with smaller effects will be harder to identify due to the increased variability from adding populations with different allele frequencies.

Selective sweeps

The dog is sometimes considered the largest experiment of mankind and it is a still on-going process. Selection has driven breeds towards extreme phenotypes and in the last few years, dog genetics has focused on breed-defining gene variants. Breed standards have been the cause of strong selection pressure in dogs since the formation of breeds. This has resulted in a reduction of variability in genomic regions that harbour mutations responsible for breed-defining traits. This process is referred to as "selective sweeps".

Evidence of selective sweeps in dogs was first reported for a genomic region spanning the IGF1 gene (Sutter, 2007). The region was associated with body size in Portuguese Water Dogs and showed strongly reduced heterozygosity in many small and toy breeds. Breeds that exhibit traits like chondrodysplasia (Parker, 2009) and brachycephaly (Bannasch, 2010) have also been shown to display evidence of selective sweeps for these traits and there are many morphological and behavioural traits to be mapped.

Risks of inbreeding

Selective breeding and the use of closed stud books resulted in uniformity of both exterior and behaviour within breeds, but unfortunately there is also the other side of the coin to consider. In the 1870's when the first kennel clubs were founded Darwin's theory on the effects of selection (natural or artificial) was generally accepted, but the implications of Mendel's observation that individuals can carry 'hidden' traits which they can pass on to their offspring was not widely understood. The use of closed stud books has created a genetic isolation of each breed population. The influx of new genetic material into the breed's gene pool is blocked and with each generation, artificial selection and the widespread use of only a few breeding dogs (especially sires) decreases the gene pool further. Only 5-6 % of the dogs are used for breeding (Dutch Kennel Club pedigree records). Dramatic changes in population size can also contribute to decrease in genetic diversity within a breed.

This continued inbreeding increases the homogeneity of alleles. Unfortunately also pathogenic alleles can get widely distributed in a dog population. Dominantly inherited diseases, which become apparent before sexual maturity are, once the disease is recognised, fairly easily dealt with by excluding the animals from breeding, but late-onset and recessive traits can reach high frequencies in certain breeds. Most breeds are known for a stronger predisposition to certain genetic diseases compared to most other breeds. Beagles are predisposed to develop pulmic stenosis, Yorkshire Terriers to portosystemic shunts, retinal dysplasia, tracheal collapse and PL, Boxers to aortic stenosis, cardiomyopathy and corneal dystrophy and Border Collies to Collie Eye Anomaly and deafness [Canine Inherited Disorders Database] to just name a few. Currently over 500 hereditary diseases are recognised in dogs (OMIA, 2012). For some of those genetic disorders the underlying cause is identified and genetic tests are available, including centronuclear myopathy in Labradors (PTPLA gene), Exercise-Induced Collapse in Labradors (DNM1), dwarfism in the German Shepherd Dog (LHX3) and severe combined immunodeficiency disease in the Wettterhoun (RAG1) (Pelé, 2005; Patterson, 2008: Voorbij, 2011). For diseases where the molecular mechanism is not yet understood but are prevalent in many breeds, like HD and ED, phenotypic screening programs have been set up to reduce incidence.

Locomotor disorders are the third most common cause of death in dogs, and include developmental orthopaedic diseases (DOD). Eight percent of all dogs referred to teaching hospital had DOD (LaFond, 2002), which included hip dysplasia, elbow dysplasia and patella luxation.

References

Alam MR, Lee JI, Kang HS, Kim IS, Park SY, Lee KC, Kim NS. 2007. Frequency and distribution of patellar luxation in dogs. 134 cases (2000 to 2005). Vet Comp Orthop Traumatol 20(1):59-64.

Bannasch D, Young A, Myers J, Truve K, Dickinson P, Gregg J, Davis R, Bongcam-Rudloff E, Webster MT, Lindblad-Toh K, et al. 2010. Localization of canine brachycephaly using an across breed mapping approach. PLoS One 5(3):e9632.

Beuing R, Mues C, Tellhelm B, Erhardt G. 2000. Prevalence and inheritance of canine elbow dysplasia in german rottweiler. J Anim Breed Genet 117:375-83.

Bonath KH, Prieur WD. 1998. Patellaluxation in: Kleintierkrankheiten. Band 3. Ulmer, Stuttgart, Germany, pp. 672-680.

Brass W, 1989. Hip dysplasia in dogs. J Small Anim Pract 30, 166-170. (reference in Aim and scope of this thesis)

Breit S, Künzel W, Seiler S. 2004. Variation in the ossification process of the anconeal and medial coronoid process of the canine ulna. Res Vet Sci 77:9-14.

Bries AD, Weiner DS, Jacquet R, Adamczyk MJ, Morscher MA, Lowder E, Askew MJ, Steiner RP, Horne WI, Landis WJ. 2012. A study in vivo of the effects of a static compressive load on the proximal tibial physis in rabbits. J Bone Joint Surg Am 94:1111-1110.

Brinker WO, Piermattei DL, Flo GL, 1993. Patellar Luxations. In: Handbook of small animal orthopeds & fracture treatment (2nd edition). Editors: Brinker WO, Piermattei DL, Flo GL. Publisher: Saunders, Philadelphia.

Bruggen LW van, Hazewinkel HAW, Wolschrijn CF, Voorhout G, Pollak YW, Barthez PY. 2010. Bone scintigraphy for the diagnosis of an abnormal medial coronoid process in dogs Vet Radiol Ultrasound 51:344-348.

Burton NJ, Comerford EJ, Bailey M, Pead MJ, Owen MR. 2007. Digital analysis of ulnar trochlear notch sclerosis in Labrador retrievers. J Small Anim Preact 48:220-224.

Burton NJ, Toscano MJ, Barr FJ, Owen MR. 2008. Reliability of radiological assessment of ulnar trochlear notch sclerosis in dysplastic canine elbows. J Small Anim Pract 49:572-576.

Carpenter LG, Schwarz PD, Lowry JE, Park RD, Steyn PF. 1993. Comparison of radiologic imaging techniques for diagnosis of fragmented medial coronoid process of the cubital joint in dogs. Journal of American Veterinary Medical Association 203(1):78-83.

Chanoit G, Singhani NN, Marcellin-Little DJ, Osborne JA. 2010. Comparison of five radiographic views for assessment of the medial aspect of the humeral condyle in dogs with OCD. Am J Vet Res 71:780-783.

Chase K, Jones P, Martin A, Ostrander EA, Lark KG. 2009. Genetic mapping of fixed phenotypes: Disease frequency as a breed characteristic. J Hered 100 Suppl 1:S37-41.

Cickomeit MJ, Bötcher P, Hecht S, Liebich H-G, Maierl J. 2011. Topographic and age-dependent distribution of subchondral bone density in the elbow joints of clinically normal dogs. AJVR 72:491-499.

Comhaire FH, Snaps F. 2008. Comparison of two canine registry databases on the prevalence of hip dysplasia by breed and the relationship of dysplasia with body weight and height. Am J Vet Res 69:330-333.

Comhaire FH, Criel AC, Dasst CA, Guévar PJ, Snaps FR. 2009. Precision, reproducibility and clinical usefulness of measuring the Norber angle by means of computerized image analysis. Am J Vet Res 70:228-235.

Cook CC, Cook JL. 2009. Diagnostic imaging of canine elbow dysplasia: a review. Vet Surg 38:144-153.

Coopmans F, Verhoeven G, Saunders J, Duchateau L, Van Bree H. 2008. Prevalence of hip dysplasias, elbow dysplasia and humeral head osteochondrosis in dog breeds in Belgium. Vet Rec 163:654-658.

Deguilloux MF, Moquel J, Pemonge MH, Colombeau G. 2009. Ancient DNA supports lineage replacement in European dog gene pool: insight into Neolithic southeast France. J Arch Sci 36:513-519.

Durrer JI, Hannon JP. 1962. Seasonal variations in caloric intake in dogs living in an arctic environment Am J Physiology 202:375-378.

Evans HE, Christensen GC. 1979. Anatomy of the dog. Publisher: Saunders, London.

Fitzpatrick N, Smith TJ, Evans RB, Yeadon R. 2009. Radiographic and artroscopic findings in the elbow joints of 263 dogs with medial coronoid disease. Vet Surg 38:213-223.

Fluckiger MA, Friedrich GA. Binder H. 1998. Correlation Between Hip Joint Laxity and Subsequent Coxarthrosis in Dogs J Vet Med A 45:199-207.

Flüchiger MA, Friedrich GA, Binder H. 1999. A radiographic stress technique for evaluation of coxofemoral joint laxity in dogs. Vet Surg 28:1-9.

Genevois JP, Chanoit G, Carozzo C, Remy D, Fau D, Viguier E. 2006. Influence of anaesthesia on canine hip dysplasia score. J Vet Med A Physiol Pathol Clin Med 53:415-417.

Genevois JP, Cachon T, Fau D, Carozzo C, Viguier E, Collard F, Remy D. 2007. Canine hip dysplasia radiographic screening. Prevalence of rotation of the pelvis along its length axis in 7,012 conventional hip extended radiographs. Vet Comp Orthop Traumat 20:296-298.

Goedegebuure SA, Hazewinkel HA. 1986. Morphological findings in young dogs chronically fed a diet containing excess calcium.Vet Pathol 5:594-605.

Grøndalen J, Lingaas F. 1982. Arthrosis of the elbow joint amoung Rottweiler dogs. Results from investigations into hereditary disposition. Tijdsch Diergeneeskunde 111, S1: 49-51.

Guthrie S, Pidduck HG. 1990. Heritability of elbow osteochondrosis within a closed population of dogs. J Small Anim Pract 31:93-96.

Harasen, G. 2006. Patellar Luxation. Can Vet J 47(8):817-8.

Hare WCD. 1961. The ages at which the centers of ossification appear rontgenographically in the limb bones of the dog. Am J Vet Res 22:825-835.

Hanssen I. 1991. Hip dysplasia in dogs in relation to their month of birth. Vet Rec 128:425-426.

Hazewinkel HA, Meij BP, Theyse LF. 1998. Surgical treatment of elbow dysplasia. Vet Q 20 Suppl 1:S29-31.

Hazewinkel HAW, Nap RC. 1998. Hyperadrenokortizismus (Cushing-Syndrom) in: Kleintierkrankheiten band 3. Ulmer, Stuttgart (Germany), pp. 437-439.

Hazewinkel HAW, Meij BP, Theyse LFH. 1998. Surgical treatment of elbow dysplasia Vet Quart 20 (Suppl 1):29-31.

Hayes AG, Boudrieau RJ, Hungerford LL. 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). J Am Vet Med Assoc 205(5):716-20.

Hedhammar A, Wu F, Krook L, Schryver HF, Delahaunta A, Whalen JP, Kallfelz FA, Nunez EA, Hintz HF, Sheffy BE, Ryan GD. 1974. Overnutrition and skeletal disease. An experimental study in growing Great Dane Dogs. Cornell Vet 64(suppl5):5-160.

Hedhammar A, Olsson SE, Andersson SA, Persson L, Pettersson L, Olausson A, Sundgren PE. 1979. Canine hip dysplasia: study of heritability in 401 litters of German Shepherd dogs. J Am Vet Med Assoc 174:1012-1016.

Henricson B, Olsson SE. 1959. Hereditary acetabular dysplasia in German shepherd dogs. J Am Vet Med Assoc 15:207-210.

Henricson B, Norberg I, Olsson SE. 1966. On the etiology and pathogenesis of hip dysplasia: a comparative review. J Small Anim Pract. 7:673-88.

Huck JL, Biery DN, Lawler DF, Gregor TP, Runge JJ, Evans RH, Kealy RD, Smith GK. 2009. A longitudinal study of the influence of lifetime food restriction on development of osteoarthritis in the canine elbow. Vet Surg 38:192-198.

Hulse DA. 1981. Medial patellar luxation in the dog Ch 71 in: Pathophysiology in small animal surgery, MJ Bojrab (ed) Lea&Febiger, Philadelphia, pp. 631-637.

Hulse DA, Shires PK, 1985. The stifle joint. In: Textbook of small animal surgery. Editor: Slatter DH. Publisher: Saunders, Philadelphia.

Hulse D. 2011. Treatment of patellar luxation in immature dogs Proceedings of the Seminar on Patellar Luxation. ESVOT, Lyon (France), pp. 55-56.

Ihde LL, Forrester DM, Gottsegen CJ, Masih S, Patel DB, Vachon LA, White EA, Matcuk GR. 2011. Sclerosing bone dysplasias: review and differentiation from other causes of osteosclerosis. RadioGraphs 31:1865-1882.

Janutta V and Distl O. 2006. Inheritance of canine hip dysplasia: Review of estimation methods and of heritability estimates and prospects on further developments. Dtsch Tierarztl Wochenschr 113(1):6-12.

Kasström H. 1975. Estrogens, nutrition and hip dysplasia in the dog. Diss Royal Veterinary College, Stockholm.

Kealy RD, Olsson SE, Monti KL, Lawler DF, Biery DN, Helms RW, Lust G, Smith GK. 1992. Effects of limited food consumption on the incidence of hip dysplasia in growing dogs. J Am Vet Med Assoc. 201(6):857-63.

Kealy RD, Lawler DF, Ballam JM, Lust G, Smith GK, Biery DN, Olsson SE. 1997. Five-year longitudinal study on limited food consumption and development of osteoarthritis in coxofemoral joints of dogs J Am Vet Med Assoc 210:222-225.

Kienzle E, Rainbird A. 1991. Maintenance energy requirement of dogs: what is the correct value for the calculation of metabolic body weight in dogs? J Nutr 121(Suppl):S39-40.

Kirkness EF, Bafna V, Halpern AL, Levy S, Remington K, Rusch DB, Delcher AL, Pop M, Wang W, Fraser CM, et al. 2003. The dog genome: Survey sequencing and comparative analysis. Science 301(5641):1898-903.

Krontveit RI, Trangerud C, Sævik BK, Skogmo HK, Nødtvedt A. 2012.

LaFond E, Breur GJ, Austin CC. 2002. Breed susceptibility for developmental orthopaedic diseases in dogs. AAHA 38: 467-477.

Lappalainen AK, Mölsä S, Liman A, Laitinen-Vapaavuori O, Snellman M. 2009. Radiographic and computed tomography findings in Belgian shepherd dogs with mild elbow dysplasia. Vet Radiol Ultrasound 54:364-369.

Lehman N, Wayne RK. 1991. Analysis of coyote mitochondrial DNA genotype frequencies: estimation of the effective number of alleles. Genetics 128:405-416.

Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, Chang JL, Kulbokas EJ,3rd, Zody MC, et al. 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 438(7069):803-19.

Lust G, Geary JC, Sheffy BE. 1973. Development of hip dysplasia in dogs. Am J Vet Res 34:87-91.

Lust G and Summers BA. 1981. Early, asymptomatic stage of degenerative joint disease in canine hip joints Am J Vet Res 42:1849-1855.

Madsen JS, Reimann I, Svalastoga E. 1991. Delayed ossification of the femoral head in dogs with hip dysplasia. J Small Anim Pract 32:351-354.

Mäki K, Groen AF, Liinamo AE, Ojala M. 2002. Genetic variances, trends and mode of inheritance for hip and elbow dysplasia in finnish dog populations. Anim Science 75:197-207.

Mäki K, Janss LL, Groen AF, Liinamo AE, Ojala M. 2004. An indication of major genes affecting hip and elbow dysplasia in four finnish dog populations. Heredity 92(5):402-8.

Malm S, Strandberg E, Danell B, Audell L, Swenson L, and Hedhammar A. 2007. Impact of sedation method on the diagnosis of hip and elbow dysplasia in Swedish dogs. Prev Vet Med 78:196-209.

Mansson J, and Norberg I. 1961. Höftledsdysplasi hos hund. Medlemsbl för Sv Vetförb. 1: 330-339.

Martin SW, Kirby K, Pennock PW. 1980 Canine hip dysplasia: breed effects. 21(11):293-6.

Meyer-Lindenberg A, Fehr M, Nolte I. 2006. Co-existence of ununited anconeal process and fragmented medial coronoid process of the ulna in the dog. J Small Anim Pract. 47:61-65.

Michelsen J. 2012. Canine elbow dysplasia: Aetiopathogenesis and current treatment recommendations. Vet J ?????

Millard RP, Headrick JF, Millis DL. 2010. Kinematic analysis of the pelvic limbs of healthy dogs during stair and decline slope walking. J Small Anim Pract 51:419-422.

Morey DF. 2006. Burying key evidence: The social bond between dogs and people. Journal of Archaeological Science 33:158-75.

Morgan JP, Wind A, Davidson AP, 2000. Elbow dysplasia In: Hereditary Bone and Joint Diseases in the Dog - Osteochondroses, hip dysplasia, elbow dysplasia -. Editors: Morgan JP, Wind A, Davidson AP. Publisher: Schlütersche, Hannover. pp. 41-68.

Morgan SJ. 1992. The pathology of canine hip dysplasia. Vet Clin North Am Small Anim Pract 22: 541-550.

Newton CD. 1985. Canine and feline epiphyseal plate cloruse and appearance of ossification centers; appendix C In: Textbook of small animal orthopaedics. Editors: Newton CD, Nunamaker DM. Publisher: Lippincott, Philadelphia, pp. 1107-1113.

Nunamaker DM. 1985. Patellar luxation. In: Textbook of small animal orthopaedics. Editors: Newton CD, Nunamaker DM. Publisher: Lippincott, Philadelphia, pp. 941- 947.

Ohlerth S, Lang J, Busato A, Gaillard C. 2002. Estimation of genetic population variables for six radiographic criteria of hip dysplasia in a colony of Labrador retrievers. Am J Vet Res 63:948-953.

Olsson SE. 1981. Pathophysiology, morphology, and clinical signs of osteochondrosis in the dog. In: Pathophysiology in small animal surgery. MJ Bojrab (ed) Lea&Febiger, pp.604-618.

Padgett GA, Mostosky UV, Probst CW, Thomas MW, Krecke CF. 1995. The inheritance of osteochondritis dissecans and fragmented coronoid process of the elbow joint in Labrador Retrievers. J Am Anim Hosp Assoc 31:327-330.

Parker HG, VonHoldt BM, Quignon P, Margulies EH, Shao S, Mosher DS, Spady TC, Elkahloun A, Cargill M, Jones PG, et al. 2009. An expressed fgf4 retrogene is associated with breed-defining chondrodysplasia in domestic dogs. Science 325(5943):995-8.

Parker HG, Kim LV, Sutter NB, Carlson S, Lorentzen TD, Malek TB, Johnson GS, DeFrance HB, Ostrander EA, Kruglyak L. 2004. Genetic structure of the purebred domestic dog. Science 304:1160-1164.

Patterson DF, Aguirre GA, Giger U, Green PL. 1989. Is this a genetic disease? J Small Anim Pract 30:127-139.

Patterson EE, Minor KM, Tchernatynskaia AV, Taylor SM, Shelton GD, Ekenstedt KJ, Mickelson JR. 2008. A canine DNM1 mutation is highly associated with the syndrome of exercise-induced collapse. Nat Genet 40(10):1235-9.

Pelé M, Tiret L, Kessler JL, Blot S, Panthier JJ. 2005. SINE exonic insertion in the PTPLA gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. Hum Mol Genet 14(11):1417-27.

Petazzoni M. 2011. Patellar luxation: when the tibia is guilty (Assessment of the tibia in dogs with MPL). Proceedings of the Seminar on Patellar Luxation ESVOT, Lyon (France), pp. 3-7.

Priester WA. 1972. Sex, size, and breed as risk factors in canine patellar dislocation. J Am Vet Med Assoc 160(5):740-2.

Putman RW. 1968. In: Handbook of small animal orthopeds & fracture treatment (2nd edition). Editors: Brinker WO, Piermattei DL, Flo GL. Publisher: Saunders, Philadelphia.

Rau FC, Wigger A, Tellhelm B, Zwick M, Klumpp S, Neumann A, Oltersdorf B, Amort K, Failing K, Kramer M. 2011. Observer variability and sensitivity of radiographic diagnosis of canine medial coronoid disease. Tierarztl Prax Ausg Kluintiere Heimtiere 39:313-322.

Remy D, Neuhart L, Fau D, Genevois JP. 2004.Canine elbow dysplasia and primary lesions in German shepherd dogs in France. J Small Anim Pract 45:244-248

Riser WH, Shirer JF. 1967. Correlation between canine hip dysplasia and pelvic muscle mass: a study of 95 dogs. Am J Vet Res. 28:769-77.

Riser WH. 1975. Growth and development of the normal canine pelvis, hip joints, and femur from birth to maturity. Veterinary Pathology 12:264-278.

Riser WH, Rhodes WH, Newton CD. 1985. Hip dysplasia. In: Textbook of small animal orthopaedics. Editors: Newton CD, Nunamaker DM. Publisher: Lippincott, Philadelphia, pp. 953- 980.

Robertson ID. 2003. The association of exercise, diet and other factors with owner-perceived obesity in privately owned dogs from metropolitan Perth. Prev Vet Med 58:75-83.

Ryssen B van, Van Bree H. 1997. Arthroscopic findings in 100 dogs with elbow lameness. Vet Rec 140:360-362.

Salg KG, Temwitchitr J, Imholz S, Hazewinkel HA, Leegwater PA. 2006. Assessment of collagen genes involved in fragmented medial coronoid process development in Labrador Retrievers as determined by affected sibling-pair analysis. Am J Vet Res 67:1713-1718.

Samoy Y, Van Ryssen B, Gielen I, Walschot N, van Bree H. 2006 Review of the literature: elbow incongruity in the dog. Vet Comp Orthop Traumatol. 19(1):1-8.

Samoy Y, Gielen I, Van Caelenberg A, van Bree H, Duchateau L, Van Ryssen B. 2012. Computed tomography findings in 32 joints affected with severe elbow incongruity and fragmented medial coronoid process. Vet Surg 41:486-494.

Savolainen P, Zhang YP, Luo J, Lundeberg J, Leitner T. 2002. Genetic evidence for an East Asian origin of domestic dogs. Science 298:1610-1613.

Schebitz H, Wilkins H, 1986. An Atlas of Radiographic Anatomy of the Dog and Cat. 4th edition. Publisher: Verlag Paul Parey, Berlin.

Schnelle GB. 1935. Some new diseases in dog. American Kennel Gazette 52:25-26.

Schoenmakers I, Hazewinkel HA, van den Brom WE. 1999. Excessive Ca and P intake during early maturation in dogs alters Ca and P balance without long-term effects after dietary normalization. J Nutr 129:1068-1074.

Schulz KS, Krotscheck U. 2003. Canine elbow dysplasia in: Textbook of small animal surgery Slatter DS (ed). WB Saunders Elsevier, Philadelphia (PA) pp. 1927-1952.

Smith GK, Biery DN, Gregor TP. 1990. New concepts of coxofemoral joint stability and the development of a clinical stress-radiographic method for quantitating hip joint laxity in the dog. J Am Vet Med Assoc 196:59-70.

Smith GK. 1997. Advances in diagnosing canine hip dysplasia. J Am Vet Med Assoc. 210(10):1451-7.

Smith GK. 2004. New paradigms for hip dysplasia prevention and control performance and ethics of CHD screening as an indication for preventive strategies. Proceedings ESVOT, München, pp. 125-131.

Sjostrom. 1998. Ununited anconeal process in the dog. Vet Clin Nth Am/SAC 28:75-86.

Stuttert VP, Lavelle RB, Beilharz RG, Mason TA. 1998. Clinical features and heritability of osteochondrosis of the elbow in Labrador retrievers. J Small Anim Pract 32:557-563.

Summerlee AJS, 2002. Bone formation and development. In: Bone in clinical orthopaedics. Editor: Sumner-Smith G. Publisher: Thieme Verlag, Stuttgart.

Sutter NB, Bustamante CD, Chase K, Gray MM, Zhao K, Zhu L, Padhukasahasram B, Karlins E, Davis S, Jones PG, et al. 2007. A single IGF1 allele is a major determinant of small size in dogs. Science 316(5821):112-5.

Swenson L, Audell L, Hedhammar A. 1997b. Prevalence and inheritance of and selection for hip dysplasia in seven breeds of dogs in sweden and benefit: Cost analysis of a screening and control program. J Am Vet Med Assoc 210(2):207-14.

Tellhelm B, Distl O, Wigger A. 2008. Hüftgelenksdysplasie (HD) – Entstehung, Erkennung, Bekämpfung. Kleintierpraxis 53(4):246-260.

Tellhelm B. 2010. Grading primary ED-lesions and elbow osteoarthrosis according to the IEWG protocol: www.vet_iewg.org/joomla/images/proceedings/proceedings2010iewg.pdf

Todhunter RJ, Zachos TA, Gilbert RO, Erb HN, Williams AJ, Burton-Wurster N, Lust G. 1997. Onset of epiphyseal mineralization and growth plate closure in radiographically normal and dysplastic Labrador retrievers. J Am Vet Med Assoc 210:1458-1462.

Tryfonidou MA, Holl MS, Stevenhagen JJ, Buurman CJ, Deluca HF, Oosterlaken-Dijksterhuis MA, van den Brom WE, van Leeuwen JP, Hazewinkel HA. 2003. Dietary 135-fold cholecalciferol supplementation severely disturbs the endochondral ossification in growing dogs. Domest Anim Endocrinol 4:265-85.

Ubbink GJ, Hazewinkel HAW, van de Broek J, Rothuizen J. 1999. Familial clustering and risk analysis for fragmented coronoid process and elbow incongruity in Bernese Mountain dogs in The Netherlands. Am J Vet Research 60:1082-1087.

Ubbink GJ. 1998. Some aspects of breed-associated canine disease in The Netherlands. Dissertation thesis, Utrecht University, Utrecht, The Netherlands.

Verhoeven G, Coopman F, Duchateau L, et al. 2007. Interobserver agreement in the diagnosis of canine hip dysplasia using the standard hip-extended radiographic method. J Small Anim Pract 48:387–393.

Vila C, Savolainen P, Maldonado JE, Amorim IR, Rice JE, Honeycutt RL, Crandall KA, Lundeberg J, Wayne RK. 1997. Multiple and ancient origins of the domestic dog. Science 276:1687-1689.

Voorbij AM, van Steenbeek FG, Vos-Loohuis M, Martens EE, Hanson-Nilsson JM, van Oost BA, Kooistra HS, Leegwater PA. 2011. A contracted DNA repeat in LHX3 intron 5 is associated with aberrant splicing and pituitary dwarfism in German shepherd dogs. PLoS One. 6(11):e27940.

Voorhout G, Hazewinkel HAW. 1987. A radiographic study on the development of the antebrachium in Great Dane pups on different calcium intakes. Vet Rad 28:152-157.

Wagner K, Griffon DJ, Thomas MW, Schaeffer DJ, Schulz K, Samii VF, Necas A. 2007. Radiographic, computed tomographic, and arthroscopic evaluation of experimental radio-ulnar incongruence in the dog. Vet Surg. 36(7):691-8.

Wayne RK. 1993. Molecular evolution of the dog family. Trends Genet 9:218-224.

Wind A. 1986. Elbow incongruity and developmental elbow diseases in the dog: Part I. J Am Anim Hosp Assoc 22:711-24.

Wind A and Packard ME. 1986. Elbow incongruity and developmental elbow diseases in the dog: Part II. J Am Anim Hosp Assoc 22:725-30.

Wood JL, Lakhani KH, Rogers K. 2002. Heritability and epidemiology of canine hip-dysplasia score and its components in Labrador retrievers in the United Kingdom. Prev Vet Med 55:95-108.

Wood JL, Lakhani KH. 2003a. Effect of month of birth on hip dysplasia in Labrador retrievers and Gordon setters Vet Rec 152:69-72.

Wood JL, Lakhani KH. 2003b. Hip dysplasia in Labrador retrievers: the effects of age at scoring. Vet Rec 152:37-40.

Worth AJ, Bridges JP, Cave NJ, Jones G. 2012. Seasonal variation in the hip score of dogs as assessed by the New Zealand Veterinary Association Hip Dysplasia scheme. New Zealand Vet J. 60:110-114.

Chapter 3

Prevalence and co-occurrence of hip dysplasia and elbow dysplasia in Dutch pure-bred dogs

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Abstract

Hip as well as elbow dysplasia (HD, ED) are developmental disorders leading to malformation of their respective joints. For a long time both disorders have been scored and targeted for improvement using selective breeding in several Dutch dog populations. In this paper all scores for both HD and ED, given to pure bred dogs in The Netherlands from 2002-2010, were analysed. Heritabilites and correlations between HD and ED were calculated for 4 most frequently scored breeds. Heritabilities ranged from 0.0 to 0.37 for HD related traits (HD-score according to the Fédération Cynologique Internationale, osteoarthritis, congruity, shape and laxity (Norberg angle) and from 0.0 to 0.39 for ED related traits (ED-score according to the International Elbow Working Group, osteoarthritis, sclerosis and indentation). HD related traits showed high genetic and residual correlations among each other, but were only to a minor extent correlated with ED related traits, which also showed high correlations among each other. Genetic correlations were higher than residual correlations. Phenotypic and genetic trends since 2001 for the four most scored breeds were slightly positive (decreasing over time) indicating that selection over the past decade has not been effective.

Keywords: hip dysplasia, elbow dysplasia, Dutch purebred dogs

Introduction

Hip and elbow dysplasia are two common developmental orthopaedic disorders in dogs which can cause lifelong disability. Both are considered complex diseases with multiple genes as well as environmental factors influencing susceptibility to these disorders (Distl et al., 1991; Swenson et al., 1997a,b; Mäki et al., 2000; Mäki et al., 2002; Malm et al., 2008; Stock et al., 2011; Lewis et al., 2011).

The prevalence of hip dysplasia (HD) ranges from 0 to 74% (OFA) within the different breeds and heritability estimates have been reported ranging from 0.1-0.6. Heritability indicates which part of the differences observed between dogs is due to genetics. Elbow dysplasia (ED) shows similar diversity in reported prevalence from 0-64% (Orthopaedic Foundation for Animals: OFA), and heritability estimates from 0.1 to 0.77 (Guthrie and Pidduck, 1990; Grøndalen and Lingaas, 1991; Dist et al., 1991; Swenson et al., 1997a,b; Mäki et al., 2000; Mäki et al., 2002; Malm et al., 2008; Hou et al., 2010; Stock et al., 2011; Lewis et al., 2011).To reduce prevalence, screening programs have been implemented for both HD and ED in The Netherlands. For several breeds HD scoring is mandatory for breeding, although the maximum score allowed differs between breeders' clubs, depending on the prevalence of HD and the size of the HD-free breeding population. Screening for ED is not so common yet and is restricted to a few breeds in which the breeders club is actively involved in reducing the prevalence of ED. HD and ED may become clinically evident during or just after the fast growth period. Breed differences in growth rate during this period might partly explain the differences in frequency between breeds and even between sexes of the same breed (Mäki et al., 2002). Although HD and ED are observed in all sizes of dogs, they are especially frequent in large breed dogs, which have a relatively high rate of longitudinal bone growth.

Little is known about the co-occurrence of HD and ED in dogs, though a slight phenotypic correlation has been reported in a Finnish cohort of Rottweilers (Mäki et al., 2000) and in a limited French multiple-breed data-set including Bernese Mountain Dogs, Rottweilers and other breeds (Cachon et al., 2010). Genetic correlations between HD and ED within one breed have also been reported, ranging widely between breeds from -0.09 in Golden Retrievers to 0.37 in Rottweilers (Mäki et al., 2000; Mäki et al., 2002; Malm et al., 2008; Hartmann et al., 2010; Stock et al., 2011; Lewis et al., 2011).

The objective of this study is to estimate the prevalence of HD and ED in The Netherlands, and to assess whether there are differences in prevalence between the sexes. In addition, the phenotypic and genetic relationship between HD and ED scores are reported in the four breeds most frequently screened for both HD and ED, i.e. the Labrador Retriever (LR), Golden Retriever (GR), Bernese Mountain Dog (BMD), and Newfoundland (NF).

Materials and methods

Animals

From 2002 to 2010 a total of 35,046 pedigree dogs of various breeds were screened for HD, ED, or both (Table 3.1). In total dogs of 214 breeds were screened for HD, and dogs of 117 breeds

were screened for ED. Heritability, and phenotypic and genetic correlations were determined in the four breeds most frequently screened for both HD and ED, i.e. LRs, GRs, BMDs and NFs. In these four breeds, screening for both disorders is mandatory in order to allow breeding with these dogs. The Dutch Kennel Club (www.raadvanbeheer.nl) provided pedigree files, which included the sex of dogs. Pedigrees from 1990 onwards were electronically available.

Phenotyping

Official HD grading as regulated by the Fédération Cynologique Internationale (FCI, 2010) requires a ventrodorsal radiographic view of the hip joints with extended hind limbs; the HD-score ranges from A (free of HD) to E (severely affected by HD) (Brass 1989; Morgan 2000). The Dutch screening panel takes four different characteristics into account, i.e. osteoarthritis (OA) (6 levels), joint congruity (8 levels), shape/contour of the acetabulum and femoral head (4 levels), and laxity of the hip joints (Norberg angle, continuous scale). Only laxity is registered for both hip joints separately; other characteristics have one overall assessment each. The HD scoring was performed on a weekly basis by a team of three experts (diplomats in radiology or othopedic surgery) simultaneously. Team members independently scored the anonymous radiographs. The final score was obtained by majority vote. When animals were scored with HD-C or higher, they were considered dysplastic.

Official ED screening as regulated by the International Elbow Working Group (IEWG) requires at least two, but preferably four radiographic views of each elbow. In The Netherlands, four radiographic views are required for scoring in LRs, GRs, BMDs, Rottweilers, German Shepherd Dogs and Bordeaux Dogs; a medio-lateral view with flexed elbow (MLflexed), a medio-lateral view with extended elbow (MLextended), a craniocaudal view (CrCd) and a craniolateral–caudomedial view (CrLCdM) (Voorhout and Hazewinkel, 1987). All other breeds minimally require a MLextended and a CrLCdM radiographic view. Elbow radiographs were scored according to IEWG guidelines; for each elbow the degree of OA was assessed (4 levels) at five standardized locations, the presence of osteosclerosis (2 levels), and the presence of an indentation of the humeral condyle (2 levels) was recorded. In case any of the four primary causes for ED (i.e. fragmented medial coronoid process (FCP), elbow incongruity (INC), osteochondritis dissecans (OCD) and/or ununited anconeal process (UAP)) could be observed or was suspected, this was also recorded (free, suspect or affected). These characteristics together determined the final ED grade (IEWG). Similarly to the HD-scoring a team of three experts scored the radiographs independently and the final grade was obtained by majority voting. Dogs which were scored grade 1 (or higher) were considered dysplastic. HD and ED screening occurred separately in time.

All characteristics that were scored for both HD and ED, except for laxity (Norberg angle), were scored using an ordinal scale. Distances between levels are not necessarily equal and to estimate these distances, a normal distribution underlying all categories was assumed. All available HD records (n = 34,620) and ED records (n = 9,788) were used to ascertain the prevalence and calculate the category mean for each level (Van Grevenhof et al., 2009).

Heritability and correlation calculations

Single trait and multi-trait (bivariate) analyses were conducted using the program ASReml (Gilmour et al., 2009). A single trait analysis using model 1 tested whether breed and age at screening were significantly associated with HD and ED, and the underlying characteristics that were scored for HD (including OA, congruity, shape and laxity) or ED (including OA, sclerosis and indentation) for all breeds (yijk).

$$yijk = \mu + breedi + agej + eijk \qquad \text{(model 1)}$$

where μ represents the mean, breed is a fixed effect, age at screening (in days) is a covariate (ageHD or ageED depending on the category), and e is the residual. Data on sex of the animal was available only for the four most scored breeds.

In the four breeds that were most frequently screened for both HD and ED, heritabilities and estimated breeding values (EBVs) were calculated using a single trait analysis (model 2) including the factor sex (2) while genetic correlations were calculated with a bivariate analysis for two traits at the time.

$$yijk = \mu + breedi + sexj + agek + animalk + eijk \qquad \text{(model 2)}$$

Model 2 included a mean (μ), sex as fixed effect, age (in days; according to HD or ED trait) as covariate, animal and residual (e) as random effects. Random animal effects were assumed to be normally distributed N(0, Aσa2), where A represents an additive genetic relationship matrix. It takes all relationships based on the pedigree into account. Random residual effects were also assumed to be normally distributed N(0, Iσe2). For the breed specific heritability the breed factor was omitted from the model. Heritability was calculated by dividing the variance component of the animal effect by the total variance. Estimated breeding values were obtained simultaneously. Residual correlations were calculated with the same model, without the animal effect.

Results

The total prevalence of HD (score HD-C or higher) in all screened dogs (n = 34,620) of various breeds was 15% when categorising the breeds according to the FCI classification (FCI, 2010). HD was most prevalent among the Mastiff-like breeds (Table 3.1). The three breeds most affected by HD all belong to this category. Prevalence per breed was highly variable among breeds (Supplement S 3.1). The prevalence in the Bullmastiff (51.9%), Italian Corso Dog (32.8%) and Boxer (26.8%) was much higher than the prevalence in the Rhodesian Ridgeback (6.4%) and Belgian Shepherd Dog varieties (4 – 6%).

Screening for ED was less common than for HD and the overall prevalence of ED in all screened dogs (n = 9,788) was 8.9% (Table 3.1). ED was also most prevalent in the Mastiff-like breeds (Supplement 3.2). The highest prevalence of ED in this screening population was observed in the Dogue de Bordeaux (32.9%), the lowest in the Rhodesian Ridgeback (3.9%). The prevalence of the different forms of ED for LRs, GRs, BMDs and NFs are shown in Table 3.2. FCP was by far the most frequent form of ED in this multiple-breed data set, with 94% of positive ED cases diagnosed with FCP, followed by INC (18%) and OCD (10%). UAP was rarely

reported (1.5%). Four percent of dogs were diagnosed with OA of the elbow joint without any signs of primary disease. In total, 26% of all cases were diagnosed with multiple forms of ED.

Table 3.1 The number of breeds (n_b), number of evaluations (n_e) and percentage positive of hip and elbow dysplasia (HD, ED) per breed type (FCI-classification) with › 50 evaluations from 2002 until 2010 in The Netherlands.

	HD				ED		
Breed type (FCI[1] classification number)	n_b	n_e	%HD	Breed type (FCI[1] classification number)	n_b	n_e	%ED
Mastiff type Molossoid breeds (2.2.1)	18	4171	29.6	Mastiff type Molossoid breeds (2.2.1)	15	751	19.7
Bull type Terriers (3.3)	3	259	25.5	Mountain type Molossoid breeds (2.2.2)	13	957	18.6
Water Dogs (8.3)	6	523	24.1	Swiss Mountain and Cattle Dogs (2.3)	4	1328	13.3
British and Irish Setters (7.2.2)	4	430	20.0	Cattle Dogs (except Swiss) (1.2))	2	170	6.5
Flushing Dogs (8.2)	9	1450	17.6	Sheepdogs (1.1)	29	987	5.8
Mountain type Molossoid breeds (2.2.2)	20	2627	17.5	Retrievers (8.1)	6	5033	5.2
Schnauzers (2.1.2)	3	165	17.0	Bull type Terriers (3.3)	2	120	4.2
Nordic Watchdogs and Herders (5.3)	7	250	16.8	Scenthound related breeds (6.3)	1	229	3.9
Swiss Mountain and Cattle Dogs (2.3)	4	1821	15.5	Breed types with ‹ 50 evaluations	45	213	9.9
Cattle Dogs (except Swiss) (1.2)	2	678	15.2				
Spaniel type Pointing Dog (7.1.2)	9	1882	13.3				
Poodles (9.2)	4	243	12.3				
Sheepdogs (1.1)	48	8526	11.8				
Retrievers (8.1)	6	7580	11.3				
'Griffon' type Pointing Dogs (7.1.3)	5	214	10.7				
Pinschers (2.1.1)	3	376	9.8				
Asian Spitz and related breeds (5.5)	10	599	9.3				
Continental type Pointing Dogs (7.1.1)	10	1009	8.2				
Large & medium-sized Terriers (3.1)	5	285	6.0				
Scenthound related breeds (6.3)	2	670	5.7				
Nordic Sledge Dogs (5.1)	3	405	5.4				
Tibetan Companion and Toy Dogs (9.5)	2	180	4.4				
Rough-haired Sighthounds (10.2)	1	50	4.0				
Breed types with ‹50 evaluations	30	227	30.0				
Total for all HD evaluations	214	34,620	15.0	Total for all ED evaluations	117	9,788	8.9

[1]*FCI: Fédération Cynologique Internationale*

Table 3.2 Distribution of primary diseases encompassing ED; overall and for the four most screened breeds shown as percentage of the total number of cases.

Primary diseases	Total population	Labrador Retriever	Golden Retriever	Bernese Mt. Dog	Newfound-land
OA without primary disease	4.1	2.9	6.0	1.8	5.0
only OCD	0.9	2.3	2.4		
only FCP	68.0	81.0	65.5	48.2	73.9
only UAP	0.6				0.8
only INC	0.6			2.4	
FCP & OCD	7.7	8.6	16.7	1.2	10.9
FCP & UAP	0.7				0.8
FCP & INC	15.8	4.0	4.8	45.3	8.4
FCP & INC & OCD	1.4	1.1	4.8	1.2	
FCP & INC & UAP	0.1				
FCP & INC & UAP & OCD	0.1				
OCD	10.1	12.1	23.8	2.4	10.9
FCP	93.8	94.8	91.7	95.9	94.1
UAP	1.5				1.7
INC	18.0	5.2	9.5	48.8	8.4
Total population size	9788	3333	1503	1221	622
Number of cases	868	174	84	170	119
Percentage of cases (%)	8.9	5.2	5.6	13.9	19.1

OA = Osteo Arthritis; OCD = osteochondritis dissecans; FCP= Fragmented Coronoid Process; UAP = Ununited Anconal Process; INC = elbow incongruity

The four breeds, most frequently screened for HD and ED, were tested for a sex predisposition (Table 3.3), which revealed that GRs had significantly higher prevalence of HD in females than in males, with a male to female ratio of 1:1.3. The other three breeds analysed, showed no significant differences in prevalence for HD between the sexes. In LRs a significant sex predisposition for ED was observed in males, but not in the GR, BMD or NF. Significantly more male LRs were affected with ED than females, with a male to female ratio of 1.5:1.

Table 3.3 Distribution of HD and ED between the sexes in frequently screened breeds. Sex fractions (male | female) are relative to the total amount of dogs screened, while the fraction affected animals is relative per sex.

			HD	
Breed	N	male | female	affected	p-value
Labrador Retriever	3746	0.28 | 0.72	0.097 | 0.106	0.148
Golden Retriever	2412	0.35 | 0.65	0.119 | 0.158	**0.009**
Bernese Mountain Dog	1479	0.21 | 0.79	0.141 | 0.145	0.886
Newfoundland	788	0.34 | 0.66	0.259 | 0.251	0.797
			ED	
Breed	n	male | female	affected	p-value
Labrador Retriever	3332	0.26 | 0.74	0.071 | 0.046	**0.004**
Golden Retriever	1503	0.37 | 0.63	0.067 | 0.049	0.148
Bernese Mountain Dog	1221	0.20 | 0.80	0.147 | 0.137	0.697
Newfoundland	622	0.31 | 0.69	0.221 | 0.178	0.211

A chi^2-test was calculated for affected (HD-C/D/E and ED-1/2/3) versus unaffected (HD-A/B and ED free) animals. Significant p-values (<0.05) are presented in bold.

Table 3.4 Association between HD and ED for dogs scored for both diseases, frequencies and the average normalized score of HD (ED) scores for each ED (HD) score.

Disease status	ED unaffected	ED affected	p-value χ^2 test
HD unaffected	7541	656	$8.6 * 10^{-11}$
HD affected	927	150	
HD score	n	frequency of ED[1] (in percentage)	severity of ED in HD cases (average normalized ED score)
HD-A	7499	8	1.82
HD-B	698	11	1.80
HD-C	775	11	1.86
HD-D1	138	22	1.82
HD-D2/E1	154	19	1.90
HD-E2	10	30	1.94
ED score	n	frequency of HD[2] (in percentage)	severity of HD in ED cases (average normalized HD score)
no ED	8468	11	1.48
ED grade I	20	10	1.52
ED grade II	159	13	1.52
ED grade III	627	20	1.61

[1]*ED score >= 1;* [2]*HD score >=C*

Table 3.5 Heritability estimates (with standard errors) for four breeds for HD, ED and underlying phenotypes based on their normalized score except for the Norberg score.

	All 4 breeds combined	Labrador Retriever	Golden Retriever	Bernese Mt. Dog	Newfoundland
Hip dysplasia (n)	8238	3687	2350	1422	759
FCI score	0.20 ± 0.02	0.10 ± 0.03	0.18 ± 0.04	0.31 ± 0.06	0.23 ± 0.08
OA	0.17 ± 0.02	0.07 ± 0.03	0.14 ± 0.04	0.25 ± 0.06	0.26 ± 0.08
Congruity	0.22 ± 0.02	0.22 ± 0.03	0.28 ± 0.05	0.18 ± 0.05	0.23 ± 0.07
Shape	0.05 ± 0.01	0.06 ± 0.02	0.06 ± 0.03	0.00 ± 0.00	0.01 ± 0.04
Laxity	0.30 ± 0.02	0.26 ± 0.04	0.33 ± 0.05	0.37 ± 0.06	0.22 ± 0.08
Elbow dysplasia (n)	6652	3317	1498	1215	622
IEWG score	0.18 ± 0.02	0.13 ± 0.03	0.12 ± 0.04	0.16 ± 0.05	0.33 ± 0.09
OA	0.20 ± 0.03	0.19 ± 0.04	0.12 ± 0.04	0.16 ± 0.05	0.39 ± 0.09
Sclerosis	0.15 ± 0.02	0.13 ± 0.03	0.09 ± 0.04	0.12 ± 0.05	0.29 ± 0.09
Indentation	0.06 ± 0.02	0.24 ± 0.04	0.05 ± 0.04	0.00 ± 0.00	0.00 ± 0.00

Table 3.6 Phenotypic and genetic correlations (with standard errors) between several HD and ED phenotypes based on an analysis of all 4 breeds combined. Genetic correlations are shown above the diagonal while phenotypic residual correlations are given below the diagonal.

	HD				ED		
	OA	congruity	shape	laxity	OA	sclerosis	indentation
HD -OA	-	0.77 ± 0.04	0.98 ± 0.04	0.61 ± 0.05	0.06 ± 0.10	0.06 ± 0.11	0.06 ± 0.15
HD - congruity	0.53 ± 0.01	-	0.83 ± 0.07	0.75 ± 0.03	0.14 ± 0.09	0.17 ± 0.09	0.19 ± 0.14
HD - shape	0.55 ± 0.01	0.44 ± 0.01	-	0.64 ± 0.08	0.19 ± 0.15	0.16 ± 0.16	0.03 ± 0.23
HD - laxity	0.48 ± 0.01	0.57 ± 0.01	0.47 ± 0.01	-	0.09 ± 0.08	0.08 ± 0.09	0.28 ± 0.13
ED - OA	0.06 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	-	*	0.84 ± 0.08
ED - sclerosis	0.05 ± 0.01	0.00 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.93 ± 0.00	-	0.78 ± 0.09
ED - indentation	0.00 ± 0.01	0.02 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.41 ± 0.01	0.37 ± 0.01	-

** did not converge*

In total 9,274 dogs of various breeds were examined for both hip and elbow dysplasia (Table 3.4). Overall there were significantly more dogs affected by both diseases than expected based on the overall frequencies of HD and ED ($\chi 2$ p-value < 0.001). The Pearson correlation coefficient between HD and ED, both measured as binary traits with HD-C to -E and ED grade 1 to 3 being affected, was 0.07 in the overall data set (n = 9,274). With increasing severity of HD there was an increase in the prevalence of ED, as well as a slight increase in severity of ED (Table 3.4). Also, with increasing severity for ED there was an increase in both prevalence and severity for HD.

Heritability estimates were calculated for the four breeds that were most frequently screened for both HD and ED, i.e. LRs, GRs, BMDs and NFs (Table 3.5). Heritabilities range from 0 to 0.39. The most heritable characteristic underlying the HD score was laxity in three out of four breeds, while arthritis (OA) was the most heritable characteristic underlying ED in three out of four breeds. Age of the dog at radiographic examination was significantly associated in both HD and ED, but was more significant in HD.

The four characteristics that determine the final HD score (OA, congruity, shape and laxity), show only a moderate residual correlation to each other (Table 3.6, below the diagonal) when corrected for age at radiographic examination and breed, indicating that scoring them separately gives additional information. Of the three characteristics that underlie the ED score (OA, sclerosis and indentation), the residual correlation between sclerosis and OA is very high (r_{phen} = 0.93), while they both correlate only moderately to an indentation of the humeral condyle. Residual correlations between HD and ED characteristics were universally low. Phenotypic correlations (uncorrected for breed and age; data not shown) were only slightly higher than the corrected ones, indicating that these correlations are not very breed dependent. Genetic correlations are a bit higher than the residual correlations, but follow the same trend as the residual correlations (Table 3.6, above the diagonal), with moderate to high genetic correlations between characteristics of the same disease, and only low genetic correlations

between HD- and ED-traits. The genetic and residual correlation between the overall HD and ED score were -0.03 (±0.10) and 0.04 (±0.01), respectively, determined in the analyses encompassing the four breeds.

Within the population of dogs screened for HD (n = 34,620), the incidence of HD had decreased for dogs born between 2001 and 2009 (**Error! Reference source not found.**A, dashed line). This was also true for the four individual breeds screened most often for both disorders, i.e. the LRs, GRs, BMDs and NFs. The average Estimated Breeding Value (EBV) for the corresponding years of birth (**Error! Reference source not found.**C), showed the same downward trend, most notably for the LRs. The population screened for ED (n = 9,788) showed little improvement in the incidence of ED (**Error! Reference source not found.**B). Of the four individually depicted breeds, only the BMDs showed a decline in incidence of ED. This decline was also seen in the average EBV for this breed (**Error! Reference source not found.**D). The other three breeds showed no clear decline, although the average EBV for LRs born in 2008 and 2009 indicate a downward trend.

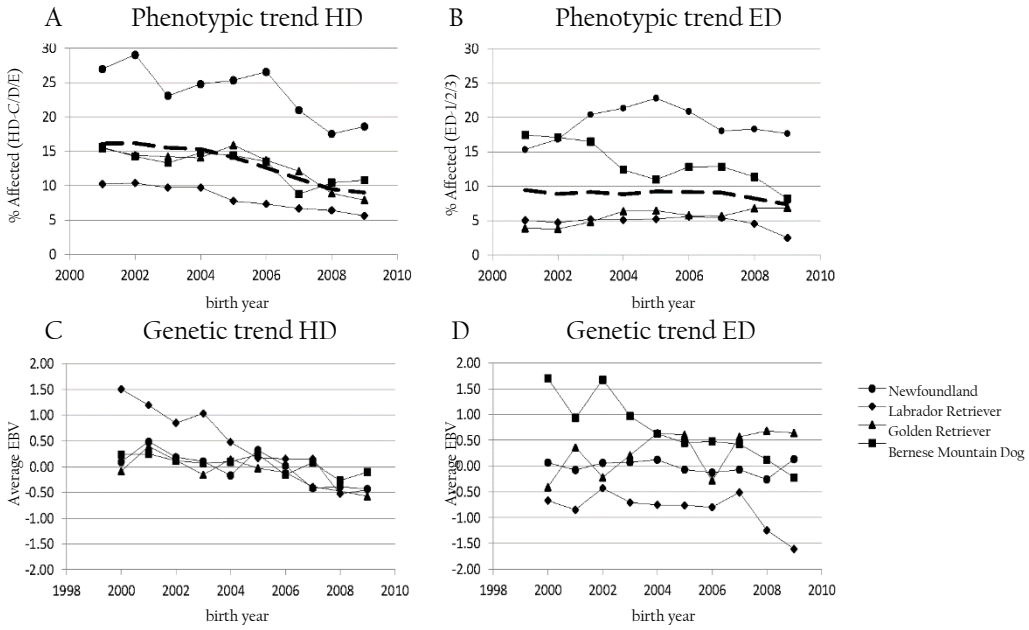

Figure 3.1 Phenotypic (A, B) and genetic (C, D) trend for hip dysplasia (HD) (A, C) and elbow dysplasia (B, D) for Labrador Retriever (LR), Golden Retriever (GR), Bernese Mountain Dog (BMD) and Newfoundland (NF) dogs.

Discussion

Both HD and ED were most prevalent among the Mastiff-like breeds. These breeds are relatively closely related (Parker, 2012) and share, besides common ancestors, also similar qualities/features. The breed characteristics: robust body type, weight, and skeletal maturation

rate, might be genetic risk factors that predispose to skeletal dysplasia and might be fixed in these breeds, making them more susceptible to develop these disorders.

In Golden Retrievers more females than males were affected with HD, in accordance with others (Henricson and Olsson, 1959; Hedhammar et al., 1979; Swenson et al., 1997b), but Wood and Lakhani (2003) found in Labradors, and Martin et al. (1980) found in a survey in twenty breeds a male predisposition of HD whereas Torres de la Riva et al. (2013) found a strong influence of early-neutering in male, but not in female Golden Retrievers in a hospital cohort. Hou et al. (2010) observed no differences among males and females in Labrador Retrievers. In our data set, a negligible percentage of dogs participating in the screening process are neutered. However, more females were evaluated in all breeds studied, most likely because they can generate less offspring than males and more are used for breeding. This implies that males are under stronger screening selection than females, which might result in slightly better scores for screened males than for screened females. Although we cannot exclude a systematic sex-based screening bias in the incidence of HD or ED, the breed with the most significant sex difference for HD (German Shepherd Dog, p-value = 0.0001), was also the breed with a similar amount of males and females screened although this sex prevalence was not demonstrated by Leppänen et al. (2000) in this same breed. In our data set a prevalence for ED was observed in male Labrador dogs, and others demonstrated the same prevalence in this breed (Guthrie and Pidduck 1990) and other breeds (Grøndalen and Lingaas, 1991; Beuing et al., 2000; Mäki et al., 2000; Malm et al., 2007), but not by all (Kronveit et al., 2011). These observations suggest that in these breeds, genetic risk factors might interact with sex-specific characteristics like sex chromosomes, hormone levels, juvenile skeletal development or differences in body weight gain, making one sex more susceptible to disease than the other.

Age at radiographic examinations has previously been reported to be associated with both HD and ED grading (Distl et al., 1991; Swenson et al., 1997a, 1997b; Mäki et al., 2000; Malm et al., 2007, 2008; Hou et al., 2010). Because the grading of both orthopaedic disorders is interdependent on the development of OA, the positive regression of HD and ED on age at examination, as demonstrated in both entities (Distl et al., 1991; Leppänen et al., 2000; Maki et al., 2000; Kealy et al., 2000; Huck et al., 2009; Wood and Lakhani, 2003) should be interpreted with care. On radiographs, there is no clear distinction between OA due to aging, overweight, increased sensitivity (genetically susceptible), or due to joint misalignment or fragments. Screening programs are aimed at the latter two causes for OA, because the genetic component might be more important. Age at examination (and weight or body condition score if available), could be used to distinguish between the causes for OA. The prevalence of OA in hip and elbow joints shows a linear correlation with age at examination in dysplastic joints (Malm et al., 2007) and therefore any distinction based on age would be very subjective. Currently, age at examination is noted, but not corrected for in the screening programs for HD or ED in The Netherlands. Longitudinal studies in more breeds are warranted to define an age slot for screening, for ED especially when scoring is solely based on the degree of OA, rather than on the primary cause.

Phenotypic and genetic correlations between HD and ED have been reported previously and phenotypic correlations ranged from 0.1 to 0.24 (Cachon et al., 2010; Mäki et al., 2000; Malm et al., 2008), while genetic correlations ranged from -0.09 to 0.42 (Mäki et al., 2002; Stock et al.,

2011; Lewis et al., 2011; Wooliams et al., 2011) in various breeds and populations. The low phenotypic (0.04±0.01) and genetic (-0.03±0.10) correlations observed in this study imply that, at least in these four populations, HD and ED do not share the same genetic risk factors. An intensified selection effect, which is expected in breeds with multiple screening programs for disorders that are genetically correlated, is lacking as well. While the prevalence of HD slowly but steadily declines, there is little indication for breeding progress against ED in the LR, GR or the NF, but a genetic improvement for both traits in BMDs, as also revealed in the study of Malm et al. (2008). Genetic correlations between the two orthopaedic disorders were reported for Finnish and Swedish BMDs (0.26 and 0.06, respectively), Finnish GRs (-0.09), and Finnish and UK LRs (0.31 and 0.41, respectively) (Mäki et al., 2002; Malm et al., 2008; Woolliams et al., 2011). The large differences within and between breeds, might in part be due to population differences (due to genetic drift), but other contributing factors are the large differences in screening protocols between countries, including sedation requirements during radiography, number and orientation of radiographic views required for scoring, percentage of the total population screened and the scoring system itself. A universal scoring system for both disorders with higher efficacy would be required in order to compare results across populations. Implementation of the use of estimated breeding value as well as genome-wide association mapping and quantitative trait loci mapping to elucidate the genetic basis of both entities (Malm et al., 2008) could bring the effect of screening on prevalence on a higher level.

Conclusions

In summary prevalence canine hip and elbow dysplasia varies considerably among Dutch breeding populations. Both traits were low to moderately heritable. Phenotypically there is a slight positive correlation but genetically these traits did not seem to be correlated based on the four most recorded breeds. Phenotypic and genetic trends were non-existent or tended to be decreasing over time indicating that use of the screening results in breeding programs has up to 2010 not been taken up to a large extent.

Acknowledgements

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Supplementary data

Supplement S 3.1 Hip dysplasia scores for breeds with >100 evaluations. The total number (n) and the percentage HD (HD-score > C) for breeds according to the FCI-system. HD grading is according to FCI-standard.

	HD scores							
Breed	A	B	C	D1	D2/E1	E2	n	% HD
Bullmastiff	182	63	147	50	64	3	509	51.9
Dogue de Bordeaux	141	45	92	48	48	2	376	50.5
Italian Corso Dog	293	43	80	36	46	2	500	32.8
Saint Bernhard Dog	151	15	40	22	18	0	246	32.5
- Saint Bernhard Dog (longhaired)	*84*	*8*	*29*	*15*	*14*	*0*	*150*	*38.7*
- Saint Bernhard Dog (shorthaired)	*67*	*7*	*11*	*7*	*4*	*0*	*96*	*22.9*
Mastiff	55	19	17	9	4	0	104	28.8
Wetterhoun (Frisian Water Dog)	141	13	43	4	14	1	216	28.7
Boxer	563	198	233	23	22	1	1040	26.8
American Staffordshire Terrier	129	17	43	5	4	0	198	26.3
Newfoundland	564	25	99	31	65	5	789	25.3
Entlebucher Mountain Dog	86	33	31	7	2	0	159	25.2
Polish Lowland Sheepdog	90	11	24	5	4	1	135	25.2
Great Dane	323	44	84	21	14	1	487	24.6
Spanish Waterdog	72	11	14	8	5	0	110	24.5
Gordon Setter	81	9	15	4	8	1	118	23.7
Welsh Springer Spaniel	216	29	30	7	24	1	307	20.2
English Springer Spaniel	130	33	19	1	19	1	203	19.7
Stabyhoun (Frisian Pointing Dog)	403	15	60	18	20	0	516	19.0
Irish Red Setter	178	21	27	3	15	1	245	18.8
German Shepherd Dog	1460	209	256	33	77	6	2041	18.2
Icelandic Sheepdog	124	3	16	4	4	1	152	16.4
Sharpei	229	16	35	4	7	1	292	16.1
English Cocker Spaniel	437	171	100	5	8	0	721	15.7
American Cocker Spaniel	96	14	18	2	0	0	130	15.4
Bouvier des Flandres*	488	40	71	11	10	0	620	14.8
German Longhaired Pointer	194	31	29	3	7	0	264	14.8
Golden Retriever	1874	191	214	41	90	4	2414	14.5
German Wirehaired Pointer	110	26	19	0	3	1	159	14.5
Bernese Mountain Dog	1059	208	135	36	39	3	1480	14.4
Briard	182	26	23	1	10	1	243	14.4
Vizsla (shorthaired)	163	16	24	1	3	0	207	13.5
Poodle (standard)	194	15	20	1	8	0	238	12.2
Nova Scotia Duck Tolling Retriever	315	7	37	4	3	0	366	12.0
Old English Sheepdog	139	10	10	2	8	0	169	11.8
Border Collie	1207	61	98	11	53	3	1433	11.5
Alaskan Malamute	108	3	12	1	1	0	125	11.2
Labrador Retriever	3226	163	259	41	68	8	3765	10.0
Small Munsterlander	196	30	20	1	3	1	251	10.0
Leonberger	642	50	43	12	20	0	767	9.8
Collie (rough)	580	10	31	3	30	0	654	9.8
Rottweiler	506	78	38	10	13	1	646	9.6
White Swiss Shepherd Dog	396	16	27	8	7	1	455	9.5
Dobermann	246	34	24	1	4	0	309	9.4
Hovawart	395	41	27	7	9	1	480	9.2
Drentsche Patrijshond	609	19	35	12	10	2	687	8.6
Flat-coated Retriever	721	160	64	1	13	1	960	8.2
Dutch Shepherd Dog	259	16	20	0	3	0	298	7.7
- Dutch Shepherd Dog (longhaired)	*88*	*7*	*9*	*0*	*1*	*0*	*105*	*9.5*

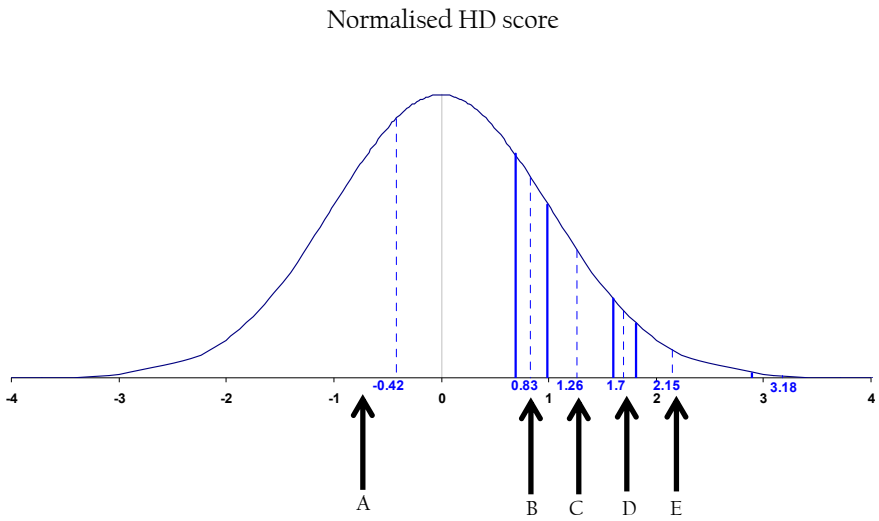
- Dutch Shepherd Dog (shorthaired)	*171*	*9*	*11*	*0*	*2*	*0*	*193*	*6.7*
Shetland Sheepdog	183	2	10	1	4	0	200	7.5
Shiba	147	9	10	1	1	0	168	7.1
Samoyed	89	4	7	0	0	0	100	7.0
Australian Kelpie	91	3	5	1	1	0	101	6.9
Rhodesian Ridgeback	449	35	22	3	8	0	517	6.4
Eurasier	104	2	4	0	3	0	113	6.2
Saarloos Wolfdog	122	3	4	2	1	0	132	5.3
Belgian Shepherd Dog	1100	48	38	8	8	0	1202	4.5
- Tervuren	*306*	*11*	*11*	*2*	*4*	*0*	*334*	*5.1*
- Malinois	*512*	*21*	*22*	*1*	*2*	*0*	*558*	*4.5*
- Groenendael	*225*	*14*	*4*	*4*	*2*	*0*	*249*	*4.0*
- Laekenois	*57*	*2*	*1*	*1*	*0*	*0*	*61*	*3.3*
Australian Shepherd Dog	282	14	10	1	3	0	310	4.5
Weimaraner	267	13	11	1	0	0	292	4.1
Tibetan Terrier	161	8	6	0	1	0	176	4
Dalmatian	140	8	4	0	1	0	153	3.3
Airedale Terrier	145	30	2	0	3	0	180	2.8
German Shorthaired Pointer	118	21	4	0	0	0	143	2.8
Bearded Collie	261	6	4	1	2	0	274	2.6
Siberian Husky	173	6	1	0	0	0	180	0.6
Breeds with <100 records	2806	322	459	69	131	8	3795	17.6
Total	26,591	2,842	3,404	646	1,074	63	34,620	15.0

Supplement S 3.2 Elbow dysplasia scores for breeds with › 25 evaluations. The total number (n) and the percentage ED (ED-score › = 1) for breeds according to the FCI-system. ED grading is according to IEWG standards.

	ED scores					ED affected
Breed	free	1	2	3	n	(in %)
Sharpei	25	0	3	10	38	34.2
Dogue de Bordeaux	98	2	5	41	146	32.9
Mastiff*	36	2	4	4	46	21.7
Saint Bernhard Dog	178	1	9	38	226	21.2
Saint Bernhard Dog (longhaired)*	*105*	*0*	*5*	*27*	*137*	*23.4*
Saint Bernhard Dog (shorthaired)	*73*	*1*	*4*	*11*	*89*	*18*
Bullmastiff	115	1	7	20	143	19.6
Newfoundland*	503	5	22	92	622	19.1
Rottweiler*	270	1	18	25	314	14
Bernese Mountain Dog*	1051	1	18	151	1221	13.9
Landseer*(European continental type)	34	1	2	2	39	12.8
Bracco Italiano* (Italian Pointing Dog)	23	0	1	1	25	8
Appenzell Cattle Dog	24	0	0	2	26	7.7
German Shepherd Dog*	447	3	5	25	480	6.9
Bouvier des Flandres*	153	0	4	7	164	6.7
Great Swiss Mountain Dog*	72	1	2	2	77	6.5
Belgian Shepherd Dog	114	0	1	6	121	5.8
Belgian Shepherd Dog (Laekenois)	40	0	1	4	45	11.1
Belgian Shepherd Dog (Groenendael)	9	0	0	1	10	10
Belgian Shepherd Dog (Tervuren)	14	0	0	1	15	6.7
Belgian Shepherd Dog (Malinois)	51	0	0	0	51	0
White Swiss Shepherd Dog	164	1	4	5	174	5.7
Golden Retriever	1419	4	21	59	1503	5.6
Labrador Retriever*	3159	1	33	140	3333	5.2
Rhodesian Ridgeback*	220	0	1	8	229	3.9
American Staffordshire Terrier	107	0	1	3	111	3.6

Chesapeake Bay Retriever	31	0	0	1	32	3.1
Leonberger	32	0	0	1	33	3
Flat-coated Retriever	92	0	0	2	94	2.1
Border Collie	48	0	1	0	49	2
Nova Scotia Duck Tolling Retriever*	66	0	0	1	67	1.5
Australian Shepherd Dog*	71	0	1	0	72	1.4
Bearded Collie	25	0	0	0	25	0.0
Breeds with <25 records	343	1	6	28	378	9.3
Total	8,920	25	169	674	9,788	8.9

**= Obligated to screen for elbow dysplasia in addition to hip dysplasia screening, by the respective national breeders club of that dog breed, facilitated by the Dutch Kennel Club.*



Supplement S 3.3 Normalization of scores based on percentage of animals in each score-class as described by van Grevenhof et al. (2009). Numbers on the x-axis indicate standard deviations. A-E is the percentage of animals in each class. The linearized score is the mean of each class given a standard normal distribution. The example shown was based on the overall HD-score. The method is also applied to other scores for linearization of these scores.

References

Brasss W, 1989. Hip dysplasia in dogs. J Small Anim Pract 30, 166-170.

Beuing R, Mues CH, Tellhelm B, Erhardt G, 2000. Prevalence and inheritance of canine elbow dysplasia in German Rottweiler. J Anim Breed Genet 117:375-383.

Cachon T, Genevois JP, Remy D, Carozzo C, Viguier E, Maitre P, Arnault F, Fau D, 2010. Risk of simultaneous phenotypic expression of hip and elbow dysplasia in dogs. A study of 1,411 radiographic examinations sent for official scoring. Vet Comp Orthop Traumatol 23:28-30.

Distl O, Grussler W, Schwarz J, Kräusslich H, 1991. Analysis of environmentally-conditioned and genetic influences on the frequency of hip joint dysplasia in German Shepherd dogs. Zentralbl Veterinarmed A. 38:460-471.

FCI Federation Cynologique Internationale, 2010. Standards and Nomenclature. Available at http://www.fci.be/nomenclature.aspx. Accessed January 2013.

Gilmour AR, Gogel BJ, Cullis BR, Thompson R, 2009. ASReml User Guide Release 3.0 VSN International Ltd, Hemel Hempstead, HP1 1ES, UK, www.vsni.co.uk.

Guthrie S, Pidduck HG, 1990. Heritability of elbow osteochondrosis within a closed population of dogs. Journal of Small Animal Practice 31:93-96.

Grøndalen J, Lingaas F, 1991. Arthrosis in the elbow joint of young rapidly growing dogs: a genetic investigation. Journal of Small Animal Practice 32:460-464.

Hartmann P, Stock KF, Distl O, 2010. Multivariate genetic analysis of canine hip and elbow dysplasia as well as humeral osteochondrosis in the Bernese mountain dog. Berl Munch Tierarztl Wochenschr. 123:488-495.

Hedhammar A, Olsson SE, Andersson SA, Petterson L, Olausson A, Sundgren PE, 1979. Canine hip dysplasia: study in 401 litters of German Shepherd dogs J Am Vet Med Assoc 174: 1012-1016.

Henricson B, Olsson SE, 1959 Hereditary acetabular dysplasia in German shepherd dogs. J Am Vet Med Assoc. 135:207-210.

Hou Y, Wang Y, Lust G, Zhu L, Zhang Z, Todhunter RJ, 2010. Retrospective analysis for genetic improvement of hip joints of cohort labrador retrievers in the United States: 1970-2007. PLoS One 5:e9410.

Huck JL, Biery DN, Lawler DF, Gregor TP, Runge JJ, Evans RH, Kealy RD, Smith GK, 2009. A longitudinal study of the influence of lifetime food restriction on development of osteoarthritis in the canine elbow. Vet Surg. 38:192-198.

IEWG, International Elbow Working Group, www.vet-iewg.org.

Kealy RD, Lawler DF, Ballam JM, Lust G, Biery DN, Smith GK, Mantz SL, 2000. Evaluation of the effect of limited food consumption on radiographic evidence of osteoarthritis in dogs. J Am Vet Med Assoc. 217:1678-1680

Krontveit RI, Trangerud C, Nødtvedt A, Dohoo I, Moe L, Sævik BK, 2012. The effect of radiological hip dysplasia and breed on survival in a prospective cohort study of four large dog breeds followed over a 10 year period. Vet J. 193:206-211.

Leppänen M, Mäki K, Juga J, Saloniemi H, 2000. Factors affecting hip dysplasia in Geman shepherd dogs in Finland J Anim Breed Genet 117:97-103.

Lewis TW, Ilska JJ, Blott SC, Wooliams JA, 2011. Genetic evaluation of elbow scores and the relationship with hip scores in UK Labradors Retrievers. Veterinary Journal 189:227-233.

Mäki K, Liinamo AE, Ojala M, 2000. Estimates of genetic parameters for hip and elbow dysplasia in Finnish Rottweilers. J Anim Sci. 78:1141-1148

Mäki K, Groen AF, Liinamo AE, Ojala M, 2002. Genetic variances, trends and mode of inheritance for hip and elbow dysplasia in Finnish dog populations. Animal Science 75:197-207.

Malm S, Strandberg E, Danell B, Audell L, Swenson L, Hedhammar A, 2007. Impact of sedation method on the diagnosis of hip and elbow dysplasia in Swedish dogs. Prev Vet Med 78:196-209.

Malm S, Fikse WF, Danell B, Strandberg E, 2008. Genetic variation and genetic trends in hip and elbow dysplasia in Swedish Rottweiler and Bernese Mountain Dog. J Anim Breed Genet. 125:403-412.

Martin SW, Kirby K, Pennock PW, 1980. Canine hip dysplasia: breed effects The Canadian Vet J. 21:293-296

Morgan JP, Wind A, Davidson AP, 2000. Hereditary bone and joint diseases in the dog. Schlütersche Verlag, Hannover, Germany.

Parker HG, 2012. Genomic analyses of modern dog breeds. Mamm Genome. 23:19-27

Stock KF, Klein S, Tellhelm B, Distl O, 2011. Genetic analyses of elbow and hip dysplasia in the German shepherd dog. J Anim Breed Genet. 128:219-229.

Swenson L, Audell L, Hedhammar A, 1997a. Prevalence and inheritance of and selection for elbow arthrosis in Bernese mountain dogs and Rottweilers in Sweden and benefit: cost analysis of a screening and control program. J Am Vet Med Assoc. 210:215-221.

Swenson L, Audell L, Hedhammar A, 1979b. Prevalence and inheritance of and selection for hip dysplasia in seven breeds of dogs in Sweden and benefit: cost analysis of a screening and control program. J Am Vet Med Assoc. 210:207-214.

Torres de la Riva G, Hart BL, Farver TB, Oberbauer AM, Messam LLM, Willits N, Hart L, 2013. Neutering Dogs: Effects on joint disorders and cancers in Golden Retrievers PLoS One 8:e55937.

Van Grevenhof EM, Ducro BJ, van Weeren PR, van Tartwijk JMFM, van den Belt AJ, Bijma P, 2009. Prevalence of various radiographic manifestations of osteochondrosis and their correlations between and within joints in Dutch Warmblood horses. Equine Vet. J. 41:11-16.

Voorhout G, Hazewinkel HAW, 1987. Radiographic evaluation of the canine elbow joint with special reference to the medial humeral condyle and the medial coronoid process. Veterinary Radiology 28:158-165.

Wood JLN, Lakhani KH, 2003. Hip dysplasia in Labrador retrievers: the effect of age at scoring Vet Rec 152:37-40.

Woolliams JA, Lewis TW, Blott SC, 2011. Canine hip and elbow dysplasia in UK Labrador retrievers. Vet J. 189:169-76.

Chapter 4

Phenotypic and genetic evaluation of elbow dysplasia in Dutch Labrador Retrievers, Golden Retrievers, and Bernese Mountain Dogs

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Abstract

Canine elbow dysplasia encompasses four developmental diseases: ununited anconeal process, osteochondrosis of the medial part of the humeral condyle, fragmented medial coronoid process, and incongruity of the elbow joint. Four radiographic views per joint were used to evaluate 2693 Labrador Retrievers, 1213 Golden Retrievers, and 974 Bernese Mountain Dogs for the presence of elbow dysplasia between 2002 and 2009 in The Netherlands. The views were also graded for signs of osteoarthritis and sclerosis. Heritabilities of the diseases were estimated using a sire model, using all available ancestors.

Fragmented medial coronoid process was diagnosed most frequently, with an incidence of 6%, 5%, and 15%, and a heritability of 0.17, 0.24, and 0.06 in Labrador Retrievers, Golden Retrievers, and Bernese Mountain Dogs, respectively. Sclerosis at the base of the medial coronoid process was the radiographic sign most strongly correlated with fragmented medial coronoid process ($r = 0.95$, 0.92, and 0.95 in Labrador Retrievers, Golden Retrievers, and Bernese Mountain Dogs, respectively). The sex of the dog was significantly correlated with the presence of osteoarthritis in Labrador Retrievers, but not in Golden Retrievers and Bernese Mountain Dogs. Male Labrador Retrievers were 1.7-fold more frequently, but not more severely, affected by osteoarthritis than female dogs. Age at radiographic examination was significantly associated with osteoarthritis in all three breeds. The heritability estimates in Retrievers were high enough to warrant excluding affected dogs from breeding, but until the biomechanical and genetic background of elbow dysplasia are better understood, correct phenotyping with multiple radiographic views is essential.

Keywords: Canine; Osteoarthritis; Osteosclerosis; Heritability; Fragmented medial coronoid process.

Introduction

Elbow dysplasia (ED) in dogs covers a number of developmental disorders leading to pain, lameness, and osteoarthritis (OA) of the elbow joint. Canine ED includes ununited anconeal process (UAP), osteochondrosis and/or osteochondritis dissecans (OC/OCD) of the medial part of the humeral condyle, fragmented medial coronoid process (FCP), and incongruity (INC) of the elbow joint, all leading to secondary osteoarthritis (Bedford, 1994).

The incidence of ED ranges from 0% to 55%, depending on breed, population, and screening technique (Swenson et al., 1997; Hazewinkel et al., 1998; Kirberger and Stander, 2007; Coopman et al., 2008; Temwichitr et al., 2010). The first clinical signs usually occur after the phase of rapid growth at four to six months of age, but can also become apparent later in life (Olsson, 1983; Voorhout and Hazewinkel, 1987; van Bruggen et al., 2010). ED is considered a hereditary disease with multiple genes and environmental factors influencing the phenotype (Guthrie and Pidduck, 1990; Mäki et al., 2002).

Several countries have implemented breeding programmes for breeds at risk of ED. A fundamental aspect of these breeding programmes is the screening of potential breeding animals for ED, employing 1, 2, or up to 4 radiographic views per elbow (Hazewinkel et al., 1988; Wosar et al., 1999). Differences between screening protocols are largely due to differences in national legislation, radiographic skills of veterinarians and technicians, and the need to limit the number of radiographs for financial reasons. The radiographic views always include a mediolateral view of the elbow in 15° or 90° flexion, to allow assessment of the outline of the anconeal process, one of the first sites of osteophyte formation (Bennett et al., 1981), and register osteosclerosis. Additional views may include the mediolateral view with the elbow in extension, to inspect the coronoid process and elbow congruity, and the craniocaudal and/or craniolateral–caudomedial views, to evaluate OC/OCD and OCD-like lesions (or kissing lesions), and osteophytes. However, even with four radiographic views, FCP can still be missed in some clinical cases, warranting other techniques to reach a reliable diagnosis, such as computed tomography, magnetic resonance imaging, scintigraphy, or arthroscopy (Carpenter et al., 1993; Snaps et al., 1997; van Ryssen and van Bree, 1997; Tromblee et al., 2007; van Bruggen et al., 2010).

The heritability of ED is reported to range from 0.1 to 0.77 (Guthrie and Pidduck, 1990; Grøndalen and Lingaas, 1991; Studdert et al., 1991; Mäki et al., 2002), depending on breed, sex, and dog population investigated. However, these studies have used different criteria to define ED and have usually not distinguished between the different primary diseases comprising ED (Guthrie and Pidduck, 1990; Beuing et al., 2000; Mäki et al., 2004). This phenotypic heterogeneity makes it difficult to determine the genetic background of the different diseases. Also the association between secondary changes in the elbow and the primary disease is not extensively investigated.

The aim of the present study was to determine the prevalence of the primary ED diseases and the variability in OA severity in Labrador Retrievers, Golden Retrievers, and Bernese Mountain Dogs (BMDs). Both the phenotypic and genetic correlations between the OA screening sites and the primary diseases were studied.

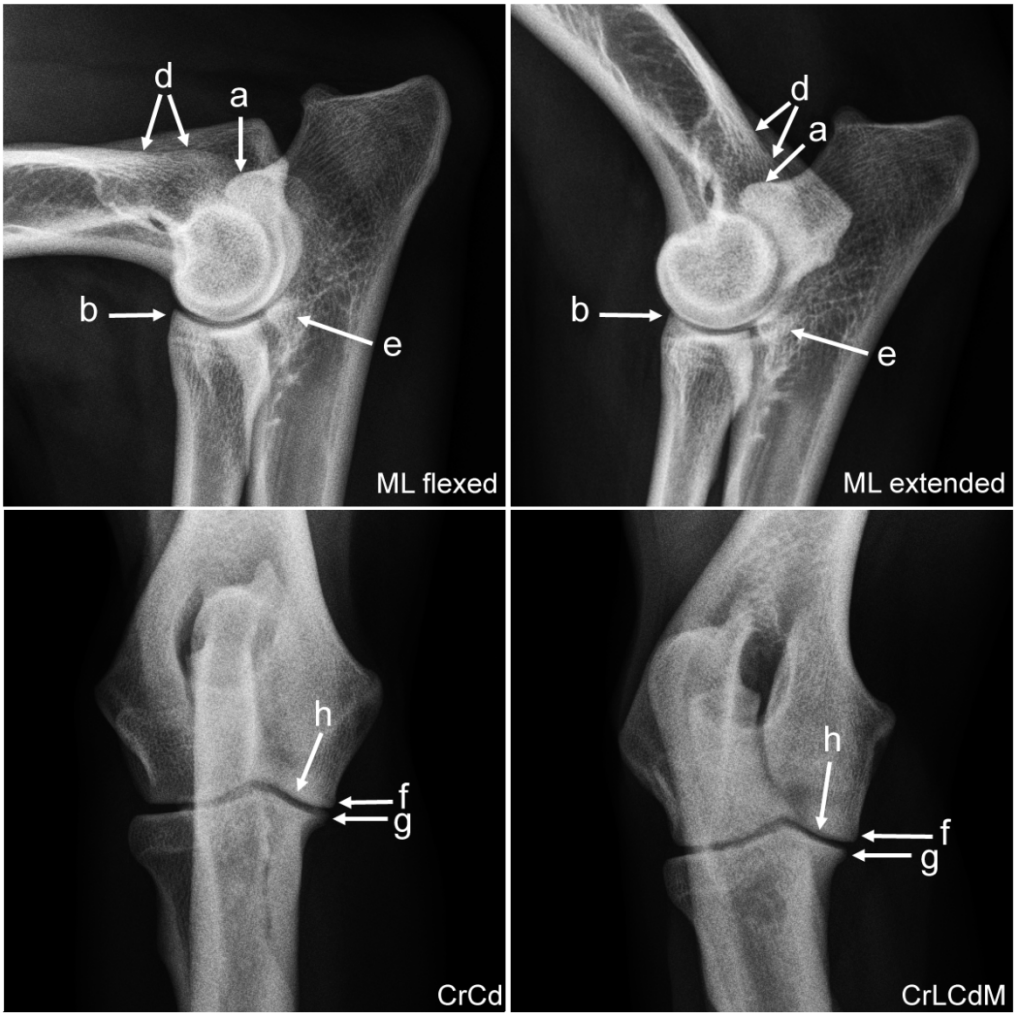

Figure 4.1 The radiographs were assessed for osteoarthritis at the proximal surface of the anconeal process (a), the cranial aspect of the radial head (b), the caudal surface of the lateral condylar ridge (d), the medial contour of the humeral condyle (f), and the medial contour of the medial coronoid process (g). The trochlear notch at the base of the coronoid process was assessed for sclerosis (e) and the subchondral bone of the medial part of the humeral condyle was assessed for an indentation (h). Adapted from the IEWG (Hazewinkel, 2007).

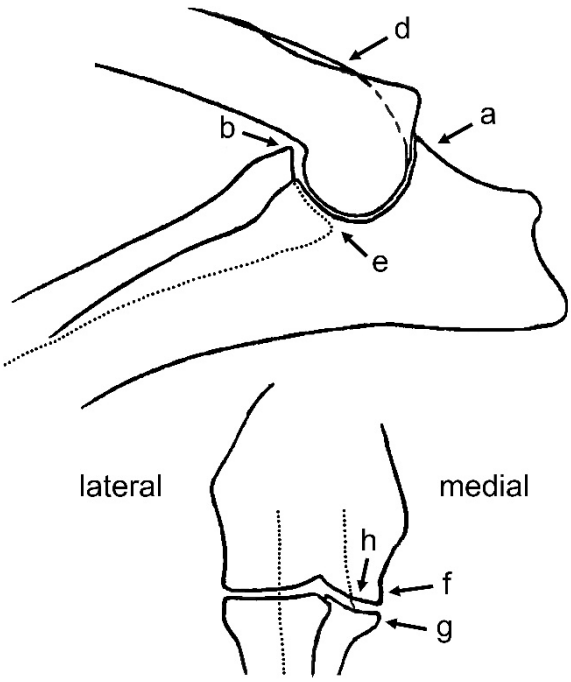

Figure 4.2. Locations that were graded for osteoarthrosis, a) proximal surface of the anconeal process, b) cranial aspect of the radial head, c) cranial edge of the medial coronoid process, d) caudal surface of the lateral condylar ridge, e) sclerosis of the ulnar notch at the base of the coronoid process, f) the surface of the medial epicondyle, g) medial edge of the medial coronoid process, h) indentation of the subchondral bone. Copied from IEWG..

Materials and methods

Population

Between 2002 and 2009, 2693 Labrador Retrievers, 1213 Golden Retrievers, and 974 BMDs were screened for ED in The Netherlands. The 90° flexed mediolateral, the extended mediolateral, the craniocaudal, and the craniolateral–caudomedial radiographic views of both elbows were made by the technique as described before (Voorhout and Hazewinkel, 1987), and were reviewed by the ED panel of the Dutch Kennel Club. Age at radiographic examination was recorded and pedigree data were obtained from the Dutch Kennel Club.

Scoring

Each radiograph was assessed by three of five experienced European board-certified orthopaedic surgeons or radiologists. Scores were obtained by way of majority consensus. Both OA and primary ED were scored. For OA, the height of the osteophytes was assessed and graded 0 (no OA), 1 (<2 mm), 2 (2-5 mm), or 3 (>5 mm) at sites a, b, d, f and g as described by the International Elbow Working Group (IEWG). Sclerosis was assessed at the trochlear notch at the base of the coronoid process (site e). The presence of indentation in the medial part of the humeral condyle was also recorded (site h) (Figure 4.1). Site c was not consistently scored and

therefore excluded from this study. The primary diseases were categorized and coded as: absent (0), suspect (1), or present (2) for UAP, OC(-like lesion), FCP, and INC. In the category 'suspect', the primary disease causing ED was not directly evident, but its presence was suspected based on secondary changes (Tellhelm, 2010).

Statistical analysis

Variance components (σ2) and heritability were calculated using ASReml software (Gilmour et al., 1995). Preliminary analysis showed a high genetic correlation between left and right OA scores and they were treated as repeated measurements and that UAP was absent in this study. A sire model was used to estimate the genetic and environmental variance, which were used to calculate heritability. The model calculated variance components for OA at the sites a, b, d, f, and g, sclerosis e, and indentation h (Figure 4.1) and the three traits OCD, FCP, and INC using the following model:

$y = \mu + sex + age + sire + kennel + e$

where μ is the overall mean and e is the residual. Fixed effects included sex (male or female), and age of the dog at radiographic examination (in number of quarter years). An F-statistic was used to test fixed effects; P values less than 0.05 were considered significant. The effect of age at examination was studied in more detail by making cross tabulations for age and OA score. The random effects in this model included the sire (s) of the animal and the kennel (k) where the animal was born. Random effects were assumed to be normally distributed: sire ~ N (0,Aσ2s), kennel ~ N (0,Iσ2k), e ~ N (0,Iσ2e), where A contains genetic relationships among sires and I is an identity matrix. The relation matrices were constructed using the pedigrees of 7283 Labrador Retrievers, 4913 Golden Retrievers and 2944 BMDs. Heritability was calculated using the model (Falconer, 1981):

$$h^2 = \frac{\sigma_s^{\,2} \times 4}{\sigma_s^{\,2} + \sigma_k^{\,2} + \sigma_e^{\,2}}$$

All records were included in the phenotypic correlation calculations, using Pearson's correlation coefficient. A semi-genetic correlation was calculated using Pearson's correlation coefficient of rank transformed estimated breeding values (EBVs). Probably due to the relatively low number of offspring per sire, correlation coefficients were located outside their parameter space (›1) when using the correction for reliability of EBVs proposed by Caro et al. (1973). Instead, the EBVs of sires with 5 or more offspring in our data set were used for calculating genetic correlations, thereby excluding so the least reliable EBVs. In total 177 Labrador Retrievers, 83 Golden Retrievers, and 65 BMD sires were included.

Results

In total 2693 Labrador Retrievers, 1213 Golden Retrievers and 974 Bernese Mountain Dogs were screened (Table 4.1).OA was more frequent in BMDs than in the other two breeds at all five sites screened for OA. OA at the caudal surface of the lateral condylar ridge (site d) was rarely observed in the Retriever breeds, but was more common in BMDs. FCP was the most

frequently observed primary disease in all three breeds (Table 4.2), INC was common in BMDs but was rare in Retrievers, whereas OCD was more common in Retrievers, but rare in BMDs.

Table 4.1 Breeding characteristics of three canine populations that were screened for elbow dysplasia.

Breed	Labrador Retriever	Golden Retriever	Bernese Mt. Dog
		Number	
Screened animals	2693	1213	974
- Screened males	720	448	190
- Screened females	1973	765	784
Sires	610	321	212
Dams	1465	663	557
Kennels	682	316	307
Total pedigree size	7283	4913	2944
		Mean (range)	
Screened offspring per sire	4.4 (1-66)	3.8 (1-39)	4.6 (1-32)
Screened offspring per dam	1.8 (1-10)	1.8 (1-18)	1.7 (1-11)
Screened dogs per kennel	3.9 (1-68)	3.8 (1-34)	3.2 (1-55)
Age at screening (months)	20.1 (12.2-101)	17.7 (12.2-77.3)	24.1 (14.4-70.1)

The data set included 27% male Labrador Retrievers, 37% male Golden Retrievers, and 20% male BMDs. The number of screened offspring per sire and number of screened dogs per kennel was highly variable. The age at radiographic examination ranged from 12 to 101 months (≈ 1 - 8.5 years), with a mean age between 1.5 and 2 years for the three breeds.

The heritability of FCP was 0.17 in Labrador Retrievers, 0.24 in Golden Retrievers, and 0.03 in BMDs (Table 4.3). Sclerosis of the trochlear notch (site e) was the radiographic sign most strongly correlated with FCP (r_{phen}=0.95 in Labrador Retrievers, r_{phen}=0.92 in Golden Retrievers, and r_{phen} =0.95 in BMDs), although screening sites a, b, f and g also showed high correlations with FCP in the three breeds (Table 4.4). Sclerosis also showed the strongest EBV correlation with FCP (r_{EBV}=0.96 in Labrador Retrievers, r_{EBV} =0.95 in Golden Retrievers, and r_{EBV} =0.97 in BMDs), indicating that sires with high breeding values for sclerosis, nearly always have high breeding values for FCP.

Table 4.2 Frequency and distribution of the primary diseases comprising elbow dysplasia in three dog populations.

	Labrador Retriever	Golden Retriever	Bernese Mt. Dog
ED cases:			
- Number	151	63	149
- % of total	5.6 %	5.2 %	15.3 %
ED cases with:			
- OCD[a]	13.2 %	25.4 %	2.7 %
- FCP[a]	98.0 %	96.8 %	96.6 %
- INC[a]	6.0 %	6.3 %	50.3 %

[a] *Cases were diagnosed with osteochondrosis or OCD(-like) lesions (OCD), fragmented medial coronoid process (FCP) and/or incongruity (INC), expressed as percentage of the total number of cases per breed.*

Table 4.3 Heritablities for seven screening sites and the primary diseases comprising elbow dysplasia in three dog populations.

Screening sites	Labrador Retriever	Golden Retriever	Bernese Mt. Dog
a	0.16 ± 0.06	0.14 ± 0.08	0.08 ± 0.07
b	0.17 ± 0.06	0.20 ± 0.08	0.01 ± 0.06
c	0.09 ± 0.05	0.15 ± 0.08	0.08 ± 0.07
e	0.12 ± 0.05	0.17 ± 0.07	0.05 ± 0.06
f	0.28 ± 0.07	0.22 ± 0.09	0.01 ± 0.06
g	0.25 ± 0.07	0.18 ± 0.09	0.04 ± 0.06
h	0.32 ± 0.07	0.05 ± 0.06	-
Primary diseases	**Labrador Retriever**	**Golden Retriever**	**Bernese Mt. Dog**
OCD	-	0.07 ± 0.05	-
FCP	0.17 ± 0.06	0.24 ± 0.08	0.03 ± 0.06
INC	-	-	0.10 ± 0.07

Heritabilities are followed by their standard deviations (± stdev). Heritability estimate is omitted where too few cases were available to reliably calculate it.

OC, OCD and OCD(-like) lesions were most prevalent in Golden Retrievers with a heritability of 0.07 (Table 4.3) and were most strongly correlated with indentation of the medial humeral condyle (site h) (r_{phen}=0.64). INC was most frequently observed in BMDs with a heritability of 0.10, but almost always co-occurred with FCP (Table 4.3) in this breed. This is consistent with the relatively high phenotypic (r_{phen} = 0.63) and EBV correlation (r_{EBV} = 0.79) between FCP and INC (Table 4.4).

Analysis of variance showed that sex was significantly associated with OA at multiple screening sites (data not shown) as well as with FCP in Labrador Retrievers, but not

significantly associated with FCP in Golden Retrievers or BMDs (Table 4.4). Compared with female Labrador Retrievers, male Labrador Retrievers were more often, but not more severely, affected by OA at screening sites a, b, e, f and g. The incidence of FCP (7.8% in males, 4.7% in females) was 1.7-fold higher in male than in female Labrador Retrievers.

The age of the dogs at radiographic examination was significantly associated with OA scoring at multiple screening sites in all three breeds (data not shown). When this effect was significant, for instance at screening site "g" in all three breeds, the number of elbows affected by OA tended to increase with age at radiographic examination (Figure 4.2). Age at screening was significantly associated with FCP in Labrador Retrievers (P = 0.002) and BMDs (P = 0.010), but not in Golden Retrievers (Table 4.4). Age at screening was also significantly associated with OC and OCD(-like) lesions in Golden Retrievers (p=0.011) and bordering association with INC in BMDs (P = 0.070).

Table 4.4 Phenotypic and estimated breeding value correlations between International Elbow Working Group screening sites and primary diseases in three dog populations.

Labrador Retriever

phenotypic corr. (2693 records) \ EBV correlation (177 sires, 1942 records)	a	b	d	e	f	g	h	OCD	FCP	INC
a	-	**0.93**	0.62	**0.86**	**0.87**	**0.88**	0.54	0.46	**0.89**	0.53
b	**0.87**	-	0.67	**0.89**	**0.92**	**0.91**	0.56	0.44	**0.92**	0.57
d	0.56	0.58	-	0.50	0.61	0.62	0.44	0.28	0.54	0.68
e	0.79	**0.82**	0.35	-	**0.89**	**0.85**	0.53	0.43	**0.96**	0.43
f	**0.81**	**0.85**	0.48	**0.85**	-	**0.96**	0.65	0.50	**0.92**	0.54
g	**0.80**	**0.83**	0.50	**0.83**	**0.95**	-	0.64	0.48	**0.89**	0.56
h	0.46	0.49	0.28	0.44	0.53	0.49	-	0.61	0.56	0.30
OCD	0.32	0.33	0.17	0.32	0.36	0.32	0.71	-	0.42	0.24
FCP	**0.81**	**0.85**	0.40	**0.95**	**0.88**	**0.87**	0.45	0.30	-	0.47
INC	0.24	0.31	0.34	0.23	0.28	0.29	0.15	0.10	0.25	-

Golden Retriever

phenotypic corr. (1213 records) \ EBV correlation (83 sires, 804 records)	a	b	d	e	f	g	h	OCD	FCP	INC
a	-	**0.93**	0.72	**0.86**	**0.87**	**0.83**	0.67	0.53	**0.87**	0.21
b	**0.89**	-	0.82	**0.84**	**0.90**	**0.88**	0.73	0.51	**0.85**	0.22
d	0.66	0.67	-	0.66	0.79	0.75	0.61	0.42	0.71	0.33
e	**0.82**	**0.80**	0.49	-	**0.87**	0.75	0.64	0.58	**0.95**	0.20
f	**0.86**	**0.87**	0.60	0.84	-	**0.95**	0.80	0.68	**0.87**	0.23
g	**0.80**	**0.85**	0.57	0.76	**0.93**	-	0.72	0.60	0.77	0.28
h	0.50	0.50	0.28	0.48	0.61	0.49	-	0.77	0.67	0.16
OCD	0.28	0.32	0.09	0.35	0.43	0.37	0.64	-	0.65	0.28
FCP	**0.84**	**0.83**	0.54	**0.92**	**0.88**	**0.83**	0.52	0.42	-	0.17
INC	0.23	0.21	0.15	0.27	0.23	0.21	0.32	0.33	0.28	-

Bernese Mountain Dog

phenotypic corr. (974 records) \ EBV correlation (65 sires, 711 records)	a	b	d	e	f	g	h	OCD	FCP	INC
a	-	**0.94**	**0.85**	**0.92**	**0.81**	**0.87**	0.16	-0.13	**0.91**	0.76
b	**0.93**	-	**0.82**	**0.91**	**0.84**	**0.88**	0.23	-0.04	**0.92**	0.74
d	**0.82**	0.80	-	0.76	**0.81**	**0.85**	0.28	0.03	0.77	0.74
e	**0.87**	**0.88**	0.74	-	0.77	**0.85**	0.13	-0.14	**0.97**	0.79
f	**0.85**	**0.87**	0.78	**0.82**	-	**0.95**	0.41	0.19	**0.81**	0.72
g	**0.82**	**0.84**	0.76	0.78	**0.91**	-	0.27	0.07	**0.87**	0.79
h	0.18	0.21	0.21	0.18	0.22	0.21	-	0.40	0.19	0.13
OCD	0.13	0.16	0.12	0.16	0.19	0.14	0.44	-	-0.09	-0.07
FCP	**0.87**	**0.88**	0.74	**0.95**	**0.85**	0.80	0.18	0.16	-	0.79
INC	0.66	0.64	0.56	0.63	0.61	0.61	0.10	0.08	0.63	-

Above the diagonal, EBV correlation coefficients (r_{EBV}) for seven screening sites and three primary elbow diseases are shown. Only sires with 5 or more offspring are included. Below the diagonal, phenotypic correlation coefficients (r_{phen}) based on scoring of radiographs at seven screening sites and three primary elbow diseases are given. All records are included in calculations. Correlation coefficients > 0.8 are presented in bold. OCD = osteochondrosos or OCD(-like) lesions, FCP = fragmented medial coronoid process, INC = incongruity of the elbow joint.

Discussion

In some screening programmes for canine ED, the flexed mediolateral radiographic view is the only view required for OA evaluation. However, multiple radiographic views facilitate the identification of the primary lesion, and consequently this approach identifies more cases of ED

than using only one radiographic view and is thus recommended (Lang et al., 1998). In a study of 108 BMDs, 17.6% of cases of FCP identified using four views were not identified when only the mediolateral flexed view was used (Hazewinkel et al., 1998).

In the combined Dutch ED database (n = 7309), FCP is the most common cause of ED. The radiographic sign most strongly correlated with FCP was sclerosis of the trochlear notch (site e) in the three breeds investigated. This correlation was slightly higher when radiographs with "suspect FCP" were excluded (data not shown), indicating this strong correlation is not due to a bias introduced by interpretation of radiographs of suspect FCP cases. The presence of sclerosis should therefore always be considered when screening for ED. To evaluate the contours of the humeral condyle (site f) and the medial coronoid process (site g), both of which are also highly correlated with FCP, a craniocaudal radiograph in addition to the standard mediolateral flexed view would be most appropriate for evaluating ED in these breeds. Moreover, OC and OCD(-like) lesions, diagnosed in 13.2% (Labrador Retrievers) and 25.4% (Golden Retrievers) of ED cases, can only be visualized on a craniocaudal or craniolateral–caudomedial view, which warrants inclusion of at least one of these views in the screening protocol for ED (Lang et al., 1998).

Increased subchondral bone stiffness, rather than cartilage damage, has been suggested to initiate primary OA (Dequeker et al., 1995), and therefore an increased subchondral bone density in the coronoid area may lead to fissures or fragmentation of the joint cartilage in this area (Temwichitr et al., 2010), ultimately leading to FCP. However, degeneration of articular cartilage may be accompanied by increased subchondral bone turnover, as suggested by bone scintigraphy (van Bruggen et al., 2010). This will also result in altered trabecular and osteoid thickness (Burr and Schaffler, 1997), highlighting the interdependence of articular cartilage and subchondral bone and the reciprocal influence of mechanical loading (Poole et al., 2007). Since the etiopathology of FCP is not yet known, one or both processes might explain the high correlation between FCP and sclerosis of the subchondral bone in the trochlear notch.

A sex effect for ED has previously been described in several breeds (Beuing et al., 2000; Mäki et al., 2000; Malm et al., 2008), including the Labrador Retriever (Guthrie and Pidduck, 1990; Everts, 2000). Differences in development, hormone levels, body weight gain or growth velocity between male and female dogs might in part account for sex differences in incidence of OA or ED in certain breeds. Also, some of the risk alleles that increase susceptibility to develop ED might be influenced by the sex of the animal, but frequencies of those alleles might differ substantially between breeds, and even between different populations of the same breed.

Age at radiographic examination has also been previously described to be significantly associated with OA and ED (Swenson et al., 1997; Mäki et al., 2000; Malm et al., 2008), although the precise implications of the effect are less apparent. A decade ago Mäki et al (2000) already suggested implementing an age correction or have all the dogs be screened at approximately the same age. However, also dogs without any form of ED show an increase in radiographic signs of OA with increasing age (Huck et al., 2009) and this could potentially lead to an increase in false positive ED evaluations in older dogs, especially when evaluations is based on OA alone, or when only one radiographic view is used to assess the elbow. In the current data set 79% of Labrador Retrievers and 90% of Golden Retrievers were graded before their second year, and

84% of BMDs (whose minimal age requirement is 18 months) were graded before they were 2.5 years of age.

Population screening for ED in The Netherlands seems to be primarily limited to potential breeding animals, probably due to the expenses involved. For these three breeds, only 7–9% of dogs born in 2002 and 2003 were submitted for evaluation, and approximately 60% of these dogs have since been used for breeding. This suggests not only that breeders tend to select dogs for breeding that have been screened, but also that the incidence of ED in this study probably underestimates the incidence in the total population. Several independent Dutch population studies, where dogs where randomly selected, support this assumption. In these studies, the incidence of FCP was 18% in Labrador Retrievers (Ubbink, 1998), 17% in Golden Retrievers (Hazewinkel et al., unpublished data), and 55% in BMDs (Ubbink et al., 1999), which is much higher than the 5.5%, 5.0%, and 14.8%, respectively, found in the present study. Although a time effect reflecting successful breeding measures cannot be ruled out, this difference in incidence is more likely the result of pre-selection by the owner or veterinarian (Ubbink, 1998).

The heritability factors reported for the three breeds in the present study are lower than those reported earlier (Guthrie and Pidduck, 1990; Studdert et al., 1991; Swenson et al., 1997; Malm et al., 2008), with the exception of 0.10 in Finnish Labrador Retrievers (Mäki et al., 2002). This may in part be explained by the selection bias for breeding animals in our study, which may result in an underestimation of a sire's EBV and therefore underestimation of the heritability of the trait. A simulation study by Stock and Distl (2010) showed that with increasing exclusion of disease information regarding offspring, a decreasing proportion of phenotypic variance in the offspring was explained by the predicted EBV of their sires. Other explanations are breeding population differences in the frequency of genetic risk factors for FCP, and the success of breeding programmes, resulting in a reduction in the frequency of genetic risk factors for FCP. Probably all three influences affect the heritability factor to some extent.

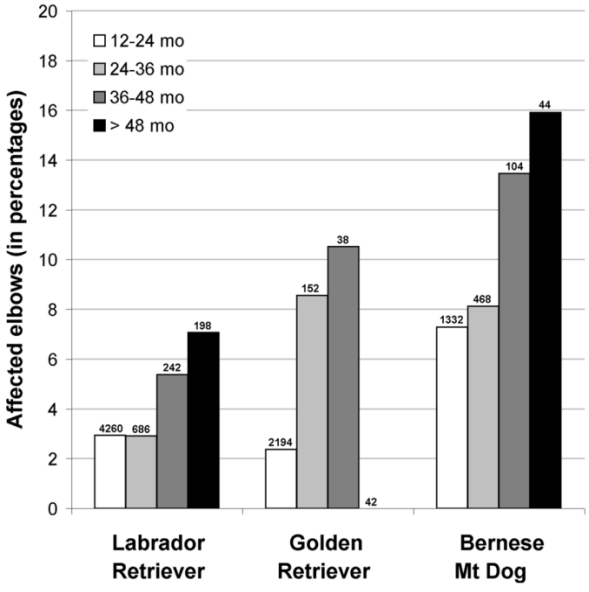

Figure 4.3. The percentage of affected elbows (OA grade >0) at screening site "g" in four age groups at radiographic examination (between 12-24 months, 24-36 months, 36-48 months and older than 48 months). Number of dogs per group are listed above the bars. No affected Golden Retrievers older than 48 months were screened (0 out of 42 dogs).

Conclusions

FCP was the most frequently diagnosed form of ED and sometimes accompanied by OCD in the Labrador and Golden Retriever, and regularly chaperoned by INC in the BMD. Sclerosis at the base of the coronoid process was the radiographic sign most strongly correlated with FCP in these three breeds, and concordantly the breeding values for sclerosis were also most strongly correlated with breeding values for FCP. The estimated heritability of FCP in the Retrievers was high enough to expect that excluding dogs with FCP from breeding will lead to some genetic progress, but because the mechanisms underlying the primary disease entities are poorly understood, correct phenotyping of patients seems fundamental for any type of genetic research.

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References

Bedford PGC, 1994. Control of hereditary elbow disease in pedigree dogs. Journal of Small Animal Practice 35, 119-122.

Bennett D, Duff SR, Kene RO, Lee R, 1981. Osteochondritis dissecans and fragmentation of the coronoid process in the elbow joint of the dog. Veterinary Record 109, 329-336.

Beuing R, Mues C, Tellhelm B, Erhardt G, 2000. Prevalence and inheritance of canine elbow dysplasia in German Rottweiler. Journal of Animal Breeding and Genetics 117, 375-383.

Burr DB, Schaffler MB, 1997. The involvement of subchondral mineralized tissues in osteoarthrosis: quantitative microscopic evidence. Microscopy Research and Technique 37, 343-357.

Calo LL, McDowell RE, Van Vleck LD, Miller PD, 1973. Genetic aspects of beef production among Holstein-Friesians pedigree selected for milk production. Journal of Animal Science 37, 676-682.

Carpenter LG, Schwarz PD, Lowry JE, Park RD, Steyn PF, 1993. Comparison of radiologic imaging techniques for diagnosis of fragmented medial coronoid process of the cubital joint in dogs. Journal of the American Veterinary Medical Association 203, 78-83.

Coopman F, Verhoeven G, Saunders J, Duchateau L, van Bree H, 2008. Prevalence of hip dysplasia, elbow dysplasia and humeral head osteochondrosis in dog breeds in Belgium. Veterinary Record 163, 654-658.

Dequeker J, Mokassa L, Aerssens J, 1995. Bone density and osteoarthritis. The Journal of Rheumatology Supplement 43, 98-100.

Everts RE, 2000. The inheritance of Fragmented Coronoid Process (FCP) in a Dutch population of Labrador retriever dogs. In: Molecular genetic studies in the dog: application to fragmented coronoid process (FCP) in the Labrador retriever. PhD thesis, Faculty of Veterinary Medicine, Utrecht University, the Netherlands, pp. 31-41.

Falconer DS, 1981. Heritability. In: Introduction to quantitative genetics, Second edition. Longman Group Limited, Essex, UK, pp. 148-169.

Gilmour AR, Thompson R, Cullis BR, 1995. Average Information REML, an efficient algorithm for variance parameter estimation in linear mixed models. Biometrics 51, 1440-1450.

Grøndalen J, Lingaas F, 1991. Arthrosis in the elbow joint of young rapidly growing dogs: a genetic investigation. Journal of Small Animal Practice 32, 460-464.

Guthrie S, Pidduck HG, 1990. Heritability of elbow osteochondrosis within a closed population of dogs. Journal of Small Animal Practice 31, 93-96.

Hazewinkel HA, Kantor A, Meij BP, Voorhout G, 1988. Fragmented coronoid process and osteochondritis dissecans of the medial humeral condyle. Tijdschrift voor Diergeneeskunde 113 Supplement 1, 41S-46S.

Hazewinkel HA, Meij BP, Theyse LF, 1998. Surgical treatment of elbow dysplasia. The Veterinary Quarterly 20 Supplement 1, S29-S31.

Hazewinkel HA, 2007. Elbow dysplasia, definition and known aetiologies. In: Proceedings of the 22nd annual meeting of the International Elbow Working Group, Munich, Germany.

Huck JL, Biery DN, Lawler DF, Gregor TP, Runge JJ, Evans RH, Kealy RD, Smith GK, 2009. A longitudinal study of the influence of lifetime food restriction on development of osteoarthritis in the canine elbow. Veterinary Surgery 38, 192-198.

Kirberger RM, Stander N, 2007. Incidence of canine elbow dysplasia in South Africa. Journal of the South African Veterinary Association 78, 59-62.

Lang J, Busato A, Baumgartner D, Flückiger M, Weber UT, 1998. Comparison of two classification protocols in the evaluation of elbow dysplasia in the dog. The Journal of Small Animal Practice 39, 169-174.

Mäki K, Groen AF, Liinamo AE, Ojala M, 2002. Genetic variances, trends and mode of inheritance for hip and elbow dysplasia in Finnish dog populations. Animal Science 75, 197-207.

Mäki K, Liinamo AE, Ojala M, 2000. Estimates of genetic parameters for hip and elbow dysplasia in Finnish Rottweilers. J Anim Sci 78, 1141-1148.

Malm S, Fikse WF, Danell B, Strandberg E, 2008. Genetic variation and genetic trends in hip and elbow dysplasia in Swedish Rottweiler and Bernese Mountain Dog. Journal of Animal Breeding and Genetics 125, 403-412.

Olsson S-E, 1983. The early diagnosis of fragmented coronoid process and osteochondritis dissecans of the canine elbow joint. Journal of the American Animal Hospital Association 19, 616-626.

Poole AR, Guilak F, Abramson SB, 2007. Etiopathogenesis of osteoarthritis. In: Osteoarthritis, Fourth edition. Wolters Kluwer, Philadelphia, USA, pp. 27-49.

Snaps FR, Balligand MH, Saunders JH, Park RD, Dondelinger RF, 1997. Comparison of radiography, magnetic resonance imaging, and surgical findings in dogs with elbow dysplasia. American Journal of Veterinary Research 58, 1367-1370.

Stock KF, Distl O, 2010. Simulation study on the effects of excluding offspring information for genetic evaluation versus using genomic markers for selection in dog breeding. Journal of Animal Breeding and Genetics 127, 42-52.

Studdert VP, Lavelle RB, Beilharz RG, Mason TA, 1991. Clinical features and heritability of osteochondrosis of the elbow in Labradors Retrievers. Journal of Small Animal Practice 32, 557-563.

Swenson L, Audell L, Hedhammar A, 1997. Prevalence and inheritance of and selection for elbow arthrosis in Bernese Mountain Dogs and Rottweilers in Sweden and benefit: cost analysis of a screening and control program. Journal of the American Veterinary Medical Association 210, 215-221.

Tellhelm B, 2010. Grading primary ED-lesions and elbow osteoarthrosis according to the IEWG protocol. In: Proceedings of the 25th annual meeting of the International Elbow Working Group, Bologna, Italy.

Temwichitr J, Leegwater PA, Hazewinkel HA, 2010. Fragmented coronoid process in the dog: A heritable disease. The Veterinary Journal 185, 123-129.

Tromblee TC, Jones JC, Bahr AM, Shires PK, Aref S, 2007. Effect of computed tomography display window and image plane on diagnostic certainty for characteristics of dysplastic elbow joints in dogs. American Journal of Veterinary Research 68, 858-871.

Ubbink GJ, 1998. Some aspects of breed-associated canine disease in the Netherlands. Thesis: Inherited disease in purebred dog populations: Predictions based on common ancestry. Utrecht University, the Netherlands, pp. 11-14.

Ubbink GJ, Hazewinkel HA, van de Broek J, Rothuizen J, 1999. Familial clustering and risk analysis for fragmented coronoid process and elbow joint incongruity in Bernese Mountain Dogs in the Netherlands. American Journal of Veterinary Research 60, 1082-1087.

van Bruggen LW, Hazewinkel HA, Wolschrijn CF, Voorhout G, Pollak YW, Barthez PY, 2010. Bone scintigraphy for the diagnosis of an abnormal medial coronoid process in dogs. Veterinary Radiology & Ultrasound 51, 344-348.

Van Ryssen B, van Bree H, 1997. Arthroscopic findings in 100 dogs with elbow lameness. Veterinary Record 140, 360-362.

Voorhout G, Hazewinkel HA, 1987. Radiographic evaluation of the canine elbow joint with special reference to the medial humeral condyle and the medial coronoid process. Veterinary Radiology 28, 158-165.

Wosar MA, Lewis DD, Neuwirth L, Parker RB, Spencer CP, Kubilis PS, Stubbs WP, Murphy ST, Shiroma JT, Stallings JT, Bertrand SG, 1999. Radiographic evaluation of elbow joints before and after surgery in dogs with possible fragmented medial coronoid process. Journal of the American Veterinary Medical Association 214, 52-58.

Chapter 5

Phenotypic and genetic trends of patellar luxation in Dutch Flat-Coated Retrievers

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Abstract

Canine patellar luxation has been described in various dog breeds, with high prevalence especially in smaller dogs. Most dogs suffer from medial displacement of the patella, although in larger dogs lateral displacement is also seen. A sex predisposition has been described for females. Patellar luxation is considered a polygenic, multi-factorial disorder.

From 1990 to 2007, in total 3834 Flat-Coated Retrievers were screened; 23.6% of those animals were affected with patellar luxation. Lateral displacement of the patella was most common in this breed (61% of cases), whereas medial (31% of cases) and lateral and medial (8% of cases) was less common. Unilateral involvement (51% of cases) was just as often observed as bilateral involvement (49% of cases). Females were more often affected with patellar luxation (30% of all tested females), than males (17% of all tested males). The heritability of patellar luxation was 0.17 ± 0.03 in this population, and breeding with one affected parent increased the prevalence of patellar luxation in offspring with 45% compared to two unaffected parents. Since the start of the screening program, there was an initial decrease from 28% to 18% in incidence, but this stagnated thereafter. The annual average estimated breeding values followed the same pattern.

With approximately a quarter of the Dutch Flat-Coated Retriever being affected with patellar luxation, this population shows unusually high prevalence compared to reports in other large-breed dogs. The heritability for patellar luxation in this population was moderate (0.17), indicating that environmental factors play a large role in the manifestation of the disorder. A screening program reduced the prevalence of patellar luxation in this breed, but improvement has recently stagnated. Inclusion of breeding values in the screening program could improve its effectiveness.

Keywords: Lateral patellar luxation, breeding values, heritability, prevalence, sex predisposition, phenotypic trend, genetic trend.

Introduction

The patella is a small sesamoid bone located at the junction of the patellar tendon and the quadriceps muscle group. Normally, the patella is located within the trochlear groove of the femur at a fixed distance to the tibial crest and has only limited sideways flexibility (Evans and Miller, 1993); however, in patellar luxation (PL) the patella dislocates out of the trochlear groove, a condition which can give rise to pain and lameness. Some dog breeds are predisposed to this disorder (LaFond et al., 2002), and in general small dogs are more likely than large dogs to develop PL (Priester, 1972; Hayes et al., 1994; Chase et al., 2009). For instance, in the USA, 43% of examined Pomeranians had PL, according to the Orthopaedic Foundation for Animals (website). Female dogs are 1.4-1.9 times more often affected than male dogs (Priester, 1972; Hayes et al., 1994; Alam et al., 2007). Both medial and lateral PL occur, and although lateral PL is observed relatively more often in large-breed dogs than in small-breed dogs, medial PL is more common than lateral PL in dogs of all sizes (Hayes et al., 1994). PL can be acquired by trauma or as a consequence of quadriceps mechanism misalignment secondary to the development of femoral and tibial deformities during skeletal growth (Hayes et al., 1994). Strong breed predisposition and frequent bilateral involvement have led to the assumption that PL can also be heritable. The lack of a clear segregation pattern points towards a polygenetic, multi-factorial disorder (Priester, 1972; Hayes et al., 1994).

The patella is stable if the extensor mechanism (including quadriceps muscle group, patella and patellar ligament) is aligned with the underlying skeletal elements (including femoral shaft, trochlear groove, and tibial tuberosity), the patella tendon is of normal length, and peri-articular soft tissues (including joint capsule and femoral-patellar ligaments) provide additional support to the articulation between the patella and the underlying femur. Bone deformities, such as coxa vara, genu varum, distal femoral varus or external torsion, proximal tibial varus or valgus, internal tibial torsion, and medial rotation of the tibial tubercle, can cause severe malalignment of the extensor mechanism and subsequent luxation of the patella out of the trochlea (Alam et al., 2007). Peri-articular soft tissues can rupture or weaken primary or secondary to the malalignment, thereby facilitating patellar luxation (Piras and Kowaleski, 2011). The mechanisms underlying the susceptibility of dogs of certain breeds to this disorder have not yet been identified.

In this study, we evaluated the prevalence of PL in Flat-Coated Retrievers in The Netherlands, estimated the heritability of the disorder in this breed, and we investigated the effect of selection over an 18-year time period.

Materials and methods

Animals and diagnosis

Between 1990 and 2007, the stifles of 3834 Flat-Coated Retrievers of at least 12 months of age with pedigree records were clinically examined in standing position and lateral recumbence, and graded for PL by a single certified orthopaedic surgeon using an adapted version of

Putnam's scoring system (Putnam, 1968). Each stifle was classified grade 0 (normal); "loose" (patella can be manually positioned on the ridges of the trochlear groove, but not out of the groove completely); grade 1 (manually luxable patella with spontaneous repositioning); grade 2 (spontaneous luxation with repositioning upon stifle extension); or grade 3 (constant spontaneous patellar luxation which can be manually reduced). Although "loose" was scored separately it was treated in the analysis as "normal". The direction of the luxation (medial, lateral or both) was also recorded for each stifle. The Dutch Kennel Club provided pedigree records. The pedigree included 4217 dogs over 10 generations sired by 449 sires and 821 dams from 1990 to 2007; approximately 7.5% of animals that were born were ultimately used for breeding.

Normalisation of scores

PL was scored using an ordinal scale. Distances between levels were not necessarily equal and to estimate these distances, the scores were normalised. For the transformation of categorical scores into quantitative values, a continuous normally distributed liability underlying the categorical scores was assumed. A liability model is a common way to analyse polygenic traits showing discrete phenotypic categories (Falconer 1981). All PL records were used to ascertain the prevalence and calculate a category mean for each PL grade. As described by van Grevenhof et al. (2009) liability values within a certain range correspond to a particular phenotypic category, the range being determined by the incidence of that category. Each categorical score was transformed into the mean liability value for that score.

Heritability and estimated breeding values

Variance components ($\sigma 2$) and the resulting heritability for PL in the Flat-coated Retriever was calculated with the program ASReml (Gilmour et al., 2006) using the following repeated measurements model:

$y = \mu + sex + animal + dam + pe + e$

where μ is the overall mean. Fixed effects included sex (male or female) and side (left or right stifle). Fixed effects were tested with an F-statistic, with p-values less than 0.05 considered significant, therefore side was removed from the final model. Random effects included animal, dam, permanent environment (pe), and residual (e). Dams are nested within kennels therefore this effect might reflect a common environmental effect as well as a common maternal effect. Permanent environment refers to environmental influences with a permanent effect on the animal, which are identical for both stifles of the same animal but different between animals. Normal distributions were assumed for the random effects: animal ~ N (0,Aσ2a), dam ~ N (0,Iσ2d), pe ~ N (0,Iσ2pe), e ~ N (0,Iσ2e), where A contains the additive genetic relationship between animals and I is an identity matrix of appropriate size. The relationship matrix was constructed using 4217 Flat-Coated Retriever pedigree records. Estimated Breeding Values (EBVs) were calculated according to the former model for all animals in the relationship matrix. The heritability and repeatability were calculated using the formulas (Falconer, 1981):

h^2 = additive genetic variation/phenotypic variation = $\sigma 2a / (\sigma 2a + \sigma 2d + \sigma 2pe + \sigma 2e)$

r = total genetic variation/phenotypic variation = (σ2a + σ2pe) / (σ2a + σ2d + σ2pe + σ2e)

Phenotypic and genetic trends

For each birth year for which data of more than 150 dogs was available, the incidence of PL grade 1, 2 and 3 combined was calculated. The estimated breeding values (EBVs) of animals born between 1992 and 2006 were grouped according to year of birth, and an average breeding value was calculated for each year, as well as a three-year average to investigate the genetic trend.

To determine the use of PL negative animals used in breeding over the years, all matings that resulted in registration of the pups by the Dutch Kennel Club from 1992 to 2006 were examined. The percentage of parental combinations where both sire and dam were screened and free of PL was calculated per year of birth of the offspring.

Results

A single examiner (FJM) tested approximately 30% of the Dutch Flat-Coated Retriever population for PL over the 18 years study period from 1990 to 2007 as part of a mandatory screening program installed by the breeders club. Of the screened population, 18.2% was ultimately used for breeding (compared to 7.5% of the total population). PL was observed in 23.6% of the 3834 screened dogs (Table 5.1). Of the dogs with PL, 93% had grade 1 PL, and only a small proportion of affected dogs was known to have undergone corrective surgery for PL, although reporting of operations was not mandatory and therefore data is likely incomplete. Most affected animals (61%) had lateral PL, 31% had medial PL, and 8% had both medial and lateral PL (including medial PL on one knee and lateral PL on the other) (Table 5.2). From 2.2% of affected knees, the patella could be luxated both medially and laterally. Unilateral and bilateral involvement was equally common. In total, 17% of male dogs and 30% of the female dogs were affected, giving a male: female ratio of 1:1.8 ($\chi 2$ P-value < 0.001).

Table 5.1 Prevalence of patellar luxation grades

	male[a] (n=1898)	female[a] (n=1936)	all (n=3834)
PL negative	83.0 %	70.0 %	76.4 %
normal	62.0 %	50.5 %	56.2 %
loose	21.0 %	19.5 %	20.2 %
PL positive	17.0 %	30.0 %	23.6 %
grade 1	15.7 %	28.2 %	22.0 %
grade 2	0.9 %	1.2 %	1.1 %
grade 3	0.1 %	0.1 %	0.1 %
operated onb	0.3 %	0.5 %	0.4 %

[a]*Male and female percentages are calculated using the total of the respective sex.*

Table 5.2 Direction of patellar luxation per dog.

	Unilateral	Bilateral	Total[a]
Unknown	11	9	-
Medial	165	113	31%
Lateral	281	257	61%
Medial & lateral	4	63[b]	8%
Total	51%	49%	

[a]*Percentage was calculated from known directions only.*

[b]*This number included dogs with medial luxation on one knee and lateral luxation on the other.*

Using a repeated measurement model the variance components for animal, dam, permanent environment and residual effects were 5.9, 1.24, 13.5 and 14.4, respectively. The heritability of PL was 0.17 (± 0.03) in this Flat-Coated Retriever population and the correlation between PL-status of the left and right stifle, i.e. the repeatability, was 0.55 (± 0.02).

Of the remaining 76.4% of dogs without PL, 20.2% had at least one stifle joint with a loose patella (i.e., the patella could be manually placed on the edge of the trochlear groove). As the hereditary implications of a loose patella were uncertain, we evaluated the effect of breeding with these animals. The total dataset of 3834 Flat-Coated Retrievers contained 2486 phenotyped parent-offspring trios in which a dog and both its parents had been screened and graded for PL. Breeding with animals with loose patellas did not significantly increase the prevalence of PL in the offspring ($\chi 2$ P-value= 0.91,Table 5.3). In contrast, breeding with one PL-positive parent significantly increased the incidence of PL in the offspring with 45% ($\chi 2$ P-value<0.001). Breeding with two PL-positive animals occurred in this cohort in 14 dogs originating from four different litters only, and therefore the effect of mating PL-positive animals could not be determined accurately.

Table 5.3 The effect of breeding with dogs with a loose patella and PL positive dogs

	Number of trios	PL positive offspring
Parental combinations		
normal x normal	963	18.8%
normal x loose	521	19.4%
loose x loose	144	20.1%
PL positive[a] x normal/loose	844	27.6%

[a]*All sires or dams with PL grade 1 or higher were considered PL positive.*

The incidence of PL decreased between 1992 and 1998 (Figure 5.1A) from 28% to 18%, and remained stable thereafter. Likewise, the average breeding value per birth year declined between 1992 and 1998, levelling off between 1998 and 2006 (Figure 5.1B). Of all the dogs born between 1992 and 1998; approximately 17.5% of dogs had one parent diagnosed with PL,

approximately 30% of dogs had PL free parents, 25% had only one parent screened and found free of PL, and approximately 27.5% had two unscreened parents (Figure 5.2). After 1998, fewer animals of unknown PL status were used for breeding, which coincided with an increase in the use of PL-positive animals for breeding.

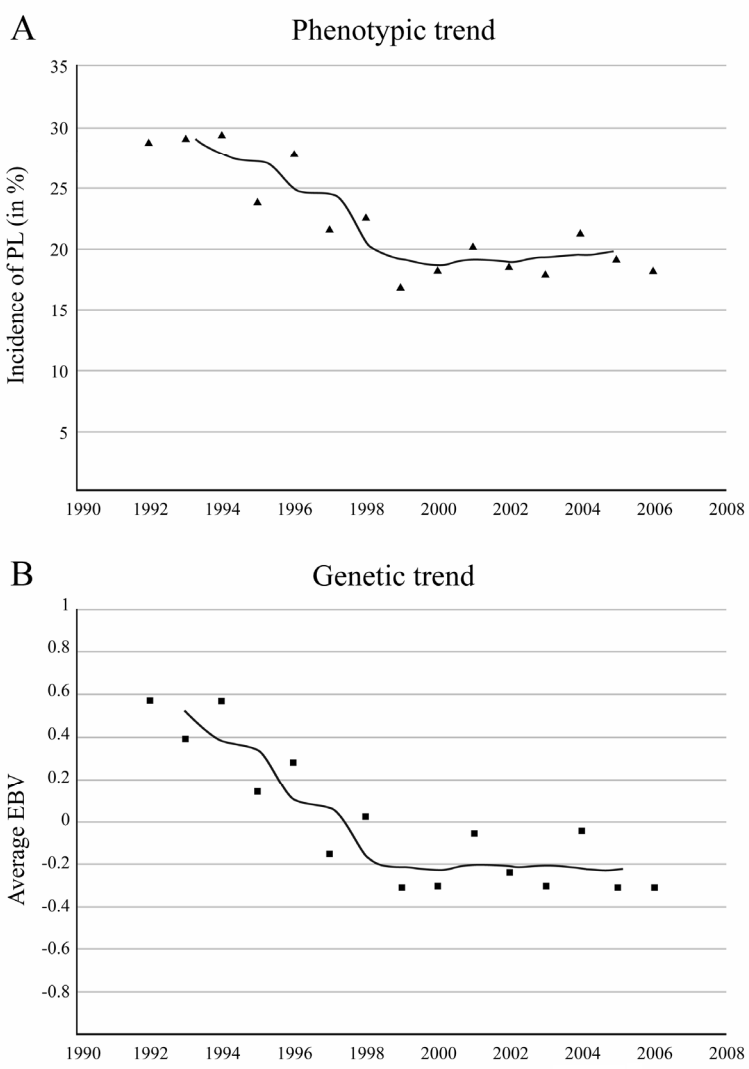

Figure 5.1 Incidence and average estimated breeding value for patellar luxation in Dutch Flat-Coated Retrievers. A) The incidence of PL and B) the breeding value average per year of birth from 1992 to 2006 in the screened Flat-Coated Retriever population. The line indicates the three-year average, calculated by including the preceding and following year.

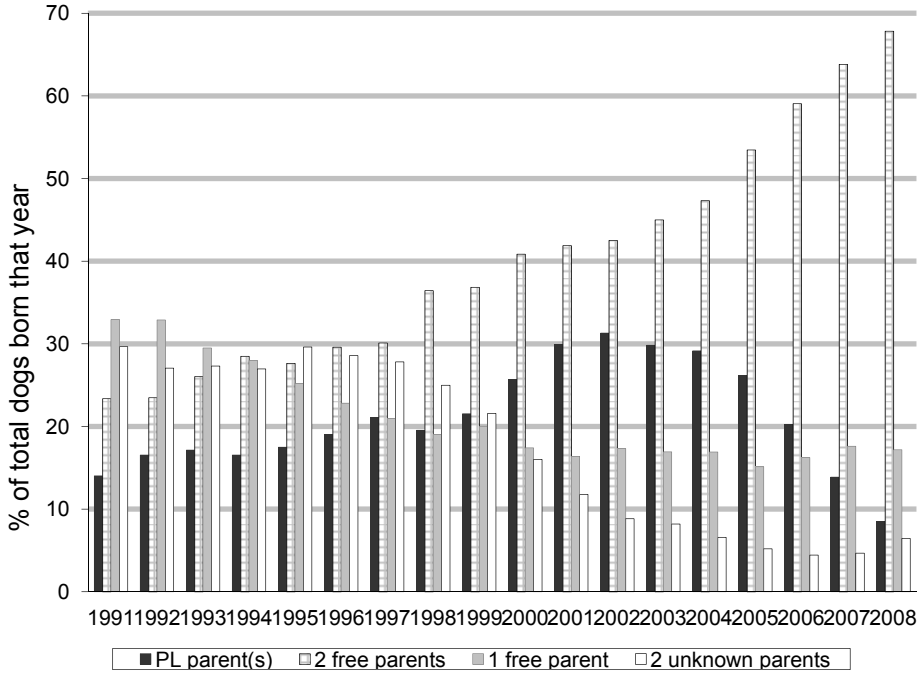

Figure 5.2 Trend in the use of patellar luxation negative animals for breeding from 1991 to 2008.
The percentage of matings where both sire and dam were screened and free of patellar luxation was calculated from all Flat-Coated Retriever matings registered at the Dutch Kennel Club for the corresponding year. The year of birth of the litter was used as the year the mating occurred.

Discussion

PL is less common in large-breed dogs than it is in small-breed dogs, and the relatively high incidence of PL in the Dutch Flat-Coated Retrievers prompted the breeder's club to implement a screening program. Thirty percent of dogs born each year were screened and 18.2% of these animals (compared to 7.5% of the total population) were ultimately used for breeding, indicating a 2-fold overrepresentation. Notwithstanding this, we consider the study sample to be representative of the overall Dutch Flat-Coated Retriever population.

The incidence of PL in this population was 23.6%, which is substantially higher than the 1.9% (n=1345) reported by the Orthopaedic Foundation for Animals for Flat-Coated Retrievers in the USA. LaFond et al. (2002) reported an increased risk of PL in American Flat-Coated Retrievers compared with mixed-breed dogs (odds ratio 3), but this ratio was based on only 14 dogs. The difference in incidence might be due to a reporting bias, but could also be due to genetic drift or as a result of differences in breeding goal between the USA and The Netherlands.

Approximately a quarter of the PL negative animals had a loose, but not luxable, patella. Comparison of breeding with animals with loose patellas and grade 1 PL suggested that loose patellas are of negligible clinical relevance, since loose patellas in the parents did not seem to predispose the offspring to PL. Most affected dogs had a relatively mild form of PL (grade 1),

which is different from findings from other studies involving large-breed dogs (Remedios et al., 1992; Gibbons et al., 2006). This might be because breeders, owners and veterinarians are more aware of the predisposition to PL in the Dutch Flat-Coated Retriever, so that severe cases are detected early and therefore seldom offered for screening.

Lateral PL was more common than medial PL in this population, which is contrary to other reports. Although lateral PL is more common in large-breed dogs than in small-breed dogs, the reported frequency of lateral PL is 3-17% of all PL cases in large-breed dogs (Hayes et al., 1994; Alam et al., 2007; Gibbons et al., 2006; Bound et al., 2009).

As described by others (Priester, 1972), PL tends to occur more often in female than male dogs. We found 1.8 fold more female dogs to be affected in our study, which is slightly higher than the 1.5 to 1.6-fold higher sex ratio for PL in female dogs previously reported in various breeds (Priester, 1972; Hayes et al., 1994), although this sex predilection is contradicted in other reports (Gibbons et al., 2006; Bound et al., 2009). Males tend to have better-developed muscles which could affect the tightness of the patella in the trochlear groove.

After the initial success in reducing the incidence of PL, over the years it appears to have plateaued at 18% in our population, as also indicated by the annual average breeding value, which suggests that measures other than screening and breeding selection are required to further reduce the incidence of PL. Dogs with PL were not necessarily excluded from breeding, probably because of other considerations, such as absence of hip dysplasia and hereditary eye diseases, and favourable behavioral and morphological characteristics. However, the percentage of matings that included a dog with PL was much lower than would be expected with random mating. Screening became more common from 1998 onwards, so that fewer dogs of unknown PL status were used for breeding. From 2002 onwards we also see a decline in the use of affected animals in breeding (while the prevalence remained constant), indicating that excluding PL positive animals from breeding had become more common. In 2008, only 8% of dogs were born from PL positive animals, indicating a clear preference for PL unaffected animals for breeding.

The stagnation of progress in the reduction of PL could in part be caused by the nature of the disorder. An estimated heritability of 0.17 for PL in the Dutch Flat-Coated Retriever indicates that environmental factors play a large role in the presentation of the disorder. These factors include screening sensitivity and reproducibility. Because all animals were examined by the same experienced, board certified veterinary orthopaedic specialist (FJM), the measurement error is expected to be relatively small. A heritability of 0.17 might limit the success of a breeding program, which is based on exclusion of affected animals from breeding. PL free animals carrying a high genetic susceptibility, but never being exposed to certain (as of yet largely unknown) environmental stimuli, could still transmit this high susceptibility to their offspring. The use of EBVs could reduce this problem as suggested in a review of selection for reduced hip and elbow dysplasia in UK Labrador retrievers (Woolliams et al., 2011). EBVs include phenotypic information of all screened relatives, and allows ranking on a continuous scale, which is especially useful for selection among PL free animals. EBVs calculated using a relatively low heritability puts more emphasis on family information and is therefore superior to phenotypic selection. Using family information could double the accuracy of selection and therefore double the genetic improvement per generation. We therefore suggest expanding the screening program to include EBVs as well as the PL status as considerations for breeding.

Conclusions

In summary, unlike in most breeds of large dogs, congenital PL is common (23.6%) in the Dutch Flat-Coated Retriever. Lateral displacement of the patella is observed most often, and females are 1.8 fold more likely to develop PL than males. The heritability of PL in the screened population was 0.17. A screening program managed to reduce the prevalence from 28% to 18%, but breed improvement has recently stagnated. Inclusion of EBVs in the screening program could improve its effectiveness.

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References

Alam MR, Lee JI, Kang HS, Kim IS, Park SY, Lee KC, Kim NS, 2007. Frequency and distribution of patellar luxation in dogs. 134 cases (2000 to 2005). Veterinary Compendium Orthopaedic Traumatology 20:59-64.

Bound N, Zakai D, Butterworth SJ, Pead M, 2009. The prevalence of canine patellar luxation in three centers. Clinical features and radiographic evidence of limb deviation. Veterinary Compendium Orthopaedic Traumatology 22:32-37.

Chase K, Jones P, Martin A, Ostrander EA, Lark KG, 2009. Genetic mapping of fixed phenotypes: disease frequency as a breed characteristic. Journal Heredity Suppl. 1:S37-41.

Evans HE, Miller ME, 1993). Miller's anatomy of the dog: WB Saunders.

Falconer DS, 1981. Heritability. In: Introduction to quantitative genetics. 2nd edition. Longman Group Limited, Essex, UK. pp 148-69.

Gibbons SE, Macias C, Tonzing MA, Pinchbeck GL, McKee WM, 2006. Patellar luxation in 70 large breed dogs. Journal Small Animal Practitioner 47:3-9.

Gilmour AR, Cullis BR, Harding SA, Thompson R, 2006. ASReml reference manual 2.00. Hemel Hempstead, UK: VSN International LTD.

Grevenhof EM van, Ducro BJ, Van Weeren PR, Van Tartwijk JMFM, Van den Belt AJ, 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.

Hayes AG, Boudrieau RJ, Hungerford LL, 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). Journal American Veterinary Medical Association 205:716-20.

LaFond E, Breur GJ, Austin CC, 2002. Breed susceptibility for developmental orthopaedic diseases in dogs. Journal American Animal Hospital Association 38:467-77.

Orthopaedic Foundation for Animals, www.offa.org.

Piras A, Kowaleski MP, 2011. Radiographs, what projections are needed. Proceedings of the Patellar luxation seminar, Lyon ESVOT:8-11.

Priester WA, 1972. Sex, size, and breed as risk factors in canine patellar dislocation. Journal American Veterinary Medical Association 160:740-2.

Putnam RW, 1968. Patellar Luxation in the Dog. University of Guelph, Ontario, Canada.

Remedios AM, Basher AW, Runyon CL, Fries CL, 1992. Medial patellar luxation in 16 large dogs. A retrospective study. Veterinary Surgeon 21:5-9.

Woolliams JA, Lewis TW, Blott SC, 2011. Canine hip and elbow dysplasia in UK Labrador retrievers. Veterinary Journal 189:169-176.

Chapter 6

Genome wide analysis indicates genes for basement membrane and cartilage matrix proteins as candidates for hip dysplasia in Labrador Retrievers

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Abstract

Hip dysplasia, an abnormal laxity of the hip joint, is seen in humans as well as dogs and is one of the most common skeletal disorders in dogs. Canine hip dysplasia is considered multifactorial and polygenic, and a variety of chromosomal regions have been associated with the disorder. We performed a genome-wide association study in Dutch Labrador Retrievers, comparing data of nearly 18,000 single nucleotide polymorphisms (SNPs) in 48 cases and 30 controls using two different statistical methods. An individual SNP analysis based on comparison of allele frequencies with a $\chi 2$ statistic was used, as well as a simultaneous SNP analysis based on Bayesian variable selection. Significant association with canine hip dysplasia was observed on chromosome 8, as well as suggestive association on chromosomes 1, 5, 15, 20, 25 and 32. Next-generation DNA sequencing of the exons of genes of seven regions identified multiple associated alleles on chromosome 1, 5, 8, 20, 25 and 32 ($p<0.001$). Candidate genes located in the associated regions on chromosomes 1, 8 and 25 included LAMA2, LRR1 and COL6A3, respectively. The associated region on CFA20 contained candidate genes GDF15, COMP and CILP2. In conclusion, our study identified candidate genes that might affect susceptibility to canine hip dysplasia. These genes are involved in hypertrophic differentiation of chondrocytes and extracellular matrix integrity of basement membrane and cartilage. The functions of the genes are in agreement with the notion that disruptions in endochondral bone formation in combination with soft tissue defects are involved in the etiology of hip dysplasia.

Introduction

Hip dysplasia is characterised by an abnormal formation of the hip joint, causing incongruity and/or laxity of the joint, which can lead to osteoarthritis. It has been observed in several mammals, including humans, where it is referred to as developmental dysplasia of the hip (DDH), and in dogs where the term hip dysplasia (HD) is used. In both species delayed femoral capital ossification, hip joint laxity and subluxation are observed in dysplastic hips (Todhunter, 2003; Boere-Boonekamp, 1998).

The reported incidence of DDH varies widely, depending on screening methods and DDH definition employed at medical centres. Between 0.5% and 4.2% of screened neonates and infants receive treatment (Boere-Boonekamp et al., 1998; Peled et al., 2008). Risk factors include a family history of DDH, female sex, other skeletal abnormalities and hormonal and environmental factors. CHD is one of the most common developmental skeletal disorders in dogs and affects predominantly breeds of medium and large sized dogs (Priester et al., 1972). In Bulldogs up to 72% of the screened animals are affected (www.offa.org/stats_hip.html). For some breeds a sex predisposition has been described (Malm et al., 2007). Hereditary and environmental factors are thought to play a role in the manifestation of this disorder. The involvement of one gene with a major effect in combination with genes with small contributions is considered likely in a number of breeds (Mäki et al., 2004; Janutta et al., 2006).

In recent years, several attempts have been made to gain understanding of the molecular genetic basis of hip dysplasia in man and dog. Occurrence of hip dysplasia in humans has been associated with variations in asporin (an extracellular matrix protein belonging to the small leucine-rich repeat protein family), interleukin-6 and transforming growth factor β1 (Kolundzic et al., 2011) (both involved in bone remodelling (Hinke et al., 2001; Kwan Tat et al., 2004)), growth differentiation factor 5 (Dai et al., 2008), T-box 4 (involved in hind limb development) (Wang et al., 2010), the vitamin D receptor and estrogen receptor 1 (Kapoor et al., 2007) and pregnancy-associated plasma protein A2 (Jia et al., 2012). In canines, genome-wide studies have been performed in Portuguese Water Dogs, German Shepherd Dogs, Labrador Retrievers and Greyhound/Labrador Retriever crossbreeds. Different definitions of CHD were used, including hip joint laxity measured as Norberg Angle or distraction index and hip scores according to the Fédération Cynologique Internationale (FCI) for CHD grading. These studies found quantitative trait loci (QTLs) on multiple chromosomal regions and a variant of the fibrillin 2 gene (FBN2) to be associated with CHD (Chase et al., 2004; Marschall et al., 2007; Zhu et al., 2008; Phavaphutanon et al., 2009; Zhou et al., 2010; Todhunter et al., 2005; Friedenberg et al., 2011). None of the loci could account for more than 18% of the genetic variance, confirming the polygenic nature of the disorder. Additional genes play an essential role in the correct formation of the hip joint, and identification of these genes would provide insight into the molecular processes that lead to CHD.

We describe a SNP-based genome-wide association study for CHD in Labrador Retrievers from The Netherlands, and subsequent fine mapping of the identified regions by case-control comparison of DNA sequences of gene exons included in the regions.

Materials and methods

Ethics statement

The dogs were privately owned and included with informed consent of the owners. They were handled by licensed veterinarians only. Thus we complied to the conditions set forth in the Dutch 'Wet op de Uitoefening van de Diergeneeskunde' (Law on the Practice of Veterinary Medicine) of March 21, 1990 and approval of an ethics committee for the use of samples of the animals was not necessary.

Animals

Hip radiographs and DNA from blood samples of 122 unrelated purebred Labrador retrievers born between 1980 and 2005 were assessed. All dogs were registered by the Dutch Kennel Club or descendants of registered dogs only. DNA was isolated from blood samples using a standard salt-extraction method (Miller et al., 1988). Blood samples were not available from 26 dogs and the DNA was isolated from buccal swabs from these dogs using the QIAamp DNA Blood Mini Kit (QIAgen, Hilden, Germany).

Phenotypes

A ventrodorsal hip radiograph of the dog in dorsal recumbency with extended hind limbs was made, examined by the CHD-panel of the Dutch Kennel Club and graded according to FCI guidelines, distinguishing CHD-A (normal), CHD-B (subnormal/borderline), CHD-C (mild), CHD-D (moderate) and CHD-E (severe). Most dogs were graded at age between 12 and 20 months. The six exceptions were one CHD-C graded dog of 50 months; three CHD-D dogs of 26, 27 and 40 months; and two CHD-D/E graded dogs of 33 and 63 months of age. We chose to analyze canine hip dysplasia as a binomial trait with affected (CHD-C/D/E) versus unaffected (CHD-A) dogs excluding CHD-B dogs from the study.

Generation of genotypes and quality control

The Illumina Infinium CanineSNP20 BeadChip was used to genotype more than 22,000 SNPs in our sample set. Of these, the 17,859 SNPs that had a minor allele frequency higher than 0.01 and that were successfully genotyped in more than 90% of the samples were included in the data analysis. PLINK software was used to generate an "identical by state" similarity matrix (Purcell et al., 2007). The first two principal components of this multidimensional similarity matrix were used to depict each individual in a 2-dimensional plot.

Association analysis

A $\chi 2$ statistic for the comparison of allele frequencies of 17,859 SNPs in 48 cases (21 males and 27 females) and 30 unaffecteds (12 males and 18 females) was generated using PLINK software (Purcell et al., 2007). Of the cases, 17 were graded CHD-C, 25 CHD-D and 6 CHD-D/E. The unaffecteds were all graded CHD-A.

A Bonferroni correction was used as multiple-testing correction, but because this is a rather stringent correction method, a series of 1,000 permutations were also run on our data set and

compared against the best result from all SNPs to estimate the significance of the peaks (EMP2 in PLINK).

Simultaneous analysis of multiple randomly selected SNPs was performed using a Bayesian procedure with the program iBay (George et al., 1993; Heuven et al., 2010), assuming a mixture model to identify associated SNPs. The model assumed that SNPs belong to one of two normal distributions; the first distribution contained SNPs that had very little effect on the phenotype and the second distribution comprised SNPs that had an effect on the phenotype. Bayesian analysis required values for the hyper parameters of the priors. It was assumed that less than 5% of our SNPs belonged to the second distribution explaining 99.5% of the genetic variance.

The genetic variance was obtained from the phenotypic variance assuming a heritability of 20%. This value was an average of the heritability derived for four dog breeds (unpublished results). The prior variances of the two distributions were calculated using these assumptions. The Bayesian procedure is based on Gibbs sampling and three million Bayesian iterations were run to calculate the posterior variances as well as the posterior probabilities that SNPs belong to either of the two distributions. Odds ratios were calculated for each SNP by dividing the posterior probability by the a priori probability, which was inherent to the assumptions made. A Bayes factor BF was calculated by dividing the posterior by the prior odds ratio for each SNP. BF larger than 3.2 indicate 'substantial' evidence for association while a BF larger than 10 indicates 'strong' evidence and a value larger than 100 as 'decisive' (Kass et al., 1995). We considered SNPs with a BF>3 as regions for follow up.

Next-generation sequencing and analysis

Five regions were selected for targeted exon enrichment and sequencing on a SOLiD4 at the University of Liverpool, UK. For each annotated gene in these regions, exons, intron/exon boundaries (30 bp) and the intergenic regions flanking both sides of each gene (2 x 500 bp), were targeted for enrichment. When the gene was smaller than 3 kb, the 5' (upstream) flanking region was increased until this criterion was met, to facilitate haplotype analysis. To minimize the chance of missing genes or exons due to a faulty annotation of the dog genome build, the synthenic regions in the human genome were compared and human cDNA of genes in these regions were compared with the reference dog genome using BLAST software. When the orientation of the alignments was corresponding to the homologous gene in the dog and located within 100 kb of the gene, this alignment was considered a putative non-annotated exon. These exons, as well as alignments of canine expressed sequence tags (ESTs) compared with the dog genome were targeted for enrichment. Microarray-based enrichment of all targeted loci was performed using a custom Comparative Genomic Hybridization Array by Roche NimbleGen.

A subset of "interesting" genes, based on their known function and position within the association peak regions, was selected and the exons were sequenced in 48 cases and 30 controls (priority genes), while the exons of remainder of the genes were sequenced in 15 cases and 15 controls (non priority genes).

Minor allele read frequencies for each sequenced location were calculated by dividing the number of observed reads for the less prevalent allele by the number of the reads that covered the position of the SNP. Genotypes were estimated using thresholds for minor allele read frequencies of more than 0.2 and less than 0.8 for heterozygotes. Individuals with a minor allele

read frequency of less than 0.2 at a certain location were considered homozygous for the major allele, and over 0.8 were considered homozygous for the minor allele. These inferred genotypes were used to calculate allelic association with a standard $\chi 2$ statistic as well as with iBay. All raw SNP data and DNA sequencing data are available upon request.

Results

Population stratification analysis revealed genetic divergence within the group of Labrador Retrievers. The Dutch Labrador Retrievers were identified as the main group, and samples that deviated from this group were dogs imported from the USA or their first generation descendants (n=44). There were no cases in this subpopulation and we excluded this group, leaving 48 cases (mild, moderate or severe CHD) and 30 controls (no signs of CHD) for further analysis.

Individual and multiple SNP association analysis

Individual SNP association to CHD was tested using a $\chi 2$-based statistic for the comparison of allele frequencies using PLINK software, and revealed association to multiple chromosomal regions, including chromosome 1, 3, 5, 8, 11, 12, 13, 15, 19, 20, 25, 28, 32, 34 and the X chromosome (P-value < 0.001, Figure 6.1). A Bonferroni correction of α=0.05 over 17,859 tests was performed to correct for multiple testing and only the region on chromosome 8 was significantly associated to CHD, with BICF2S23913508 located at position 33707642 bp of the reference genome CanFam2 as the most strongly associated SNP in this region (corrected P-value = 0.0007, Figure 6.1A). Permutations were performed as a less stringent correction for multiple testing, but it also resulted in significant association of the region on chromosome 8 only (corrected P-value = 0.002, Figure 6.1B).

A Bayesian variable selection method was used to study the combined effect of all SNPs simultaneously to distinguish between chromosomal regions likely to have an effect on the phenotype and false positives. The effect of a SNP is corrected for all other SNPs in the model. The underlying assumption in this simultaneous SNP association analysis is that SNPs belong to either of two distributions; one that contains SNPs that contribute to the phenotype, and one that contains SNPs that do not. The program iBay was used to generate posterior probabilities, expressed as Bayesian factors (BF), for each SNP to belong to the distribution of SNPs that had an effect on the phenotype. Bayesian factors larger than three were considered significant. Analysis of the SNP data of the 48 cases and 30 controls identified one genomic region located on chromosome 8 to have an effect on the phenotype with BF greater than 3. Additional regions were identified that have a minor contribution (BF>0.5) to the phenotype on chromosomes 1, 5, 15, 20, 25 and 32 (Figure 6.1C). In Table 6.1 the results of the 18 SNPs with a BF larger than 0.5 are summarized. At least one SNP in each of the seven regions passed this threshold. The strongest association with the highest number of SNPs and the highest BFs were observed on chromosome 8 and on chromosome 25 and only eight SNPs were identified in the other five regions together. Regions nominated for follow-up analysis were selected based on the individual and multiple SNP analyses, and included regions on CFA01 (70.7-71.9 Mb), CFA05 (58.6-63.5 Mb), CFA08 (28.0-34.5 Mb), CFA15 (32.2-33.6 Mb), CFA20 (46.3-51.2 Mb), CFA25 (47.1-51.9 Mb), CFA32 (11.2-13.0 Mb).

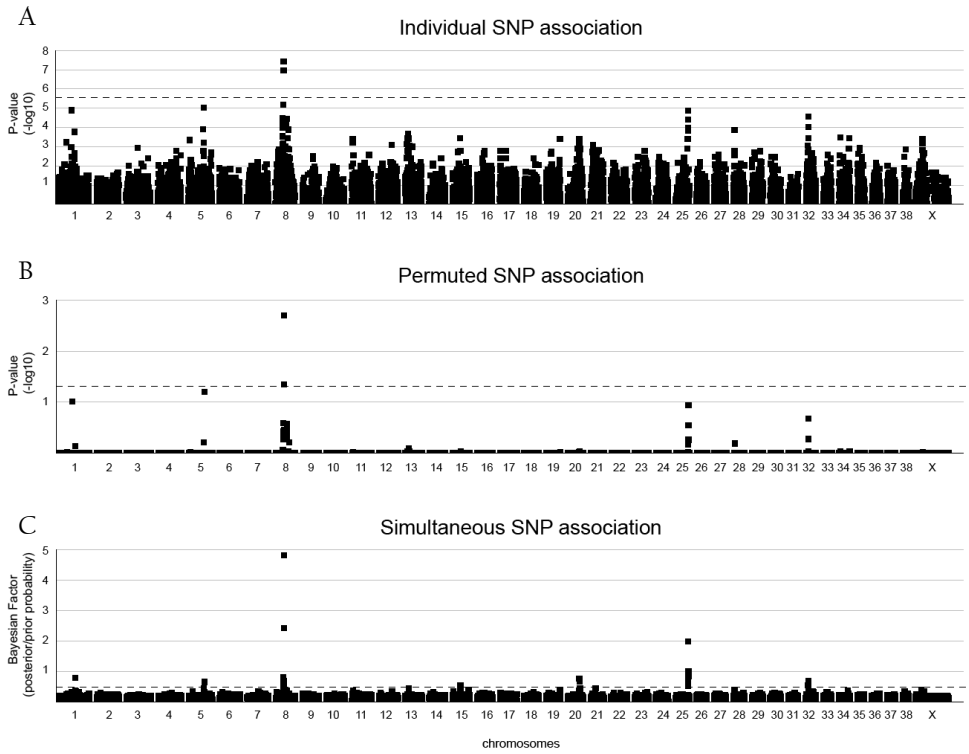

Figure 6.1 Genome wide association analysis of hip dysplasia in Labrador retrievers. Genotypes of 17,859 SNPs were compared between 48 cases and 30 controls. A. Allele frequency based $\chi 2$ statistics. The dotted line indicates the significance threshold after Bonferroni correction. B. Multiple testing correction of the $\chi 2$ statistics by 1,000 permutations of the phenotypes to determine empirical p-values. The dotted line indicates the significance threshold (α = 0.05). C. Genome wide association analysis using Bayesian variable selection to detect SNPs with a high probability to have an effect on the CHD phenotype. The dotted line indicates the significance level.

Targeted fragment enrichment and next-generation sequencing

The seven regions that displayed association were selected for microarray-based enrichment and DNA sequencing. The total size of these 7 regions was ~25 Mb. An exon sequencing approach was chosen to increase the coverage per analyzed position, resulting in 2.3 Mb of target DNA. Priority genes in the regions were selected based on the function of the encoded proteins. The processes involved were cartilage or bone development and synthesis or maintenance of extracellur matrix. The priority gene exons totaled 300 kb and these were analyzed in 69 DNA samples from the Dutch Labrador Retriever population. The exons of the remaining non-priority genes, 2 Mb in total size, were enriched and sequenced in 15 cases and 15 controls. For both sets, approximately 30% of the generated reads could be mapped to the targeted regions.

In the priority samples, approximately 81% of the targeted regions were sequenced at least once, but only 65% of the targeted regions were covered 10 times or more. For the non-priority samples, these percentages were slightly lower (80% and 60%, respectively) per sample.

Overall, 74.4% of the priority regions and 58.0% of the non-priority regions were sequenced at least 10 times in 10 cases and 10 controls. We identified 5388 variations that were covered at least 10 times in both cases and controls and that had a minor allele frequency higher than 0.01. Genotypes were inferred from the number of reads per allele and used to calculate $\chi 2$-based allelic association. The SNPs that were most strongly associated with CHD are listed in Table 6.2 together with the genes at the position or in the vicinity of these SNPs.

Table 6.1 Array SNPs associated with hip dysplasia in Labrador Retrievers a.

CFA	location	SNP[a]	MAF[b]		score	
			cases	controls	PLINK[c]	iBay[d]
1	70962581	BICF2P1446667	0.24	0.02	3.76	0.79
5	62363747	BICF2S23057444	0.22	0.57	5.02	0.66
8	29868871	BICF2P950415	0.28	0.62	4.47	0.81
8	31218201	BICF2S23118415	0.46	0.15	4.12	0.57
8	32367889	BICF2S2305197	0.56	0.23	4.26	0.68
8	33707642	BICF2S23913508	0.20	0.63	7.41	2.43
8	33990753	BICF2P601580	0.64	0.20	6.94	4.82
15	32865368	BICF2G630433123	0.28	0.57	3.42	0.54
20	46604376	BICF2P1445357	0.40	0.68	3.32	0.77
20	47759323	BICF2S22943564	0.40	0.67	3.00	0.68
20	47926376	BICF2P878084	0.34	0.63	3.39	0.75
25	50228063	BICF2G630160005	0.54	0.23	3.83	0.63
25	50458146	BICF2S23326254	0.28	0.03	3.97	0.52
25	50637313	BICF2G630159246	0.55	0.20	4.85	1.98
25	51127510	BICF2P909436	0.53	0.20	4.39	1.00
25	51155516	BICF2P770517	0.53	0.20	4.39	0.84
32	11802242	BICF2S23211582	0.07	0.33	4.54	0.54
32	12336449	rs8795055	0.20	0.47	3.43	0.68

a SNPs with a Bayes Factor > 0.5 were included

bMAF = minor allele frequency

c −10log p-values

d Bayes Factor

Table 6.2 Variant DNA sequences associated with hip dysplasia in Labrador Retrievers.

			number		MAF [a]				
CFA	location	alleles	cases	controls	cases	controls	score [b]	gene	position in gene [c]
1	70938018	[T/A]	36	30	0.28	0.02	4.35	LAMA2	intron
1	70997779	[A/T]	33	30	0.26	0.02	3.94	LAMA2	exon, synonymous
5	59194609	[G/-]	15	15	0.33	0.77	3.13	KLHL17	3'-UTR
5	62929475	[C/T]	14	8	0.04	0.63	4.85	NPHP4	5'-UTR
8	29247021	[C/T]	33	30	0.73	0.35	4.67	LRR1	3'-UTR
8	31496895	[C/T]	33	30	0.55	0.23	3.46	PTGDR	downstream
8	31496910	[C/T]	32	30	0.53	0.22	3.51	PTGDR	downstream
20	45260332	[A/G]	32	30	0.30	0.07	3.01	LTF	exon, non-synonym.
20	46671813	[C/G]	15	15	0.53	0.10	3.51	PBX4	inton
20	46706734	[A/G]	27	28	0.63	0.27	3.87	CILP2	3'-UTR
20	47636184	[G/A]	29	30	0.55	0.23	3.41	GDF15	3'-UTR
20	47714388	[G/A]	29	29	0.24	0.66	5.13	LSM4	5'-UTR
20	48071192	[G/A]	34	30	0.26	0.63	4.56	INSL3	upstream
20	48243399	[G/-]	12	13	0.17	0.65	3.31	UNC13A	3'-UTR
20	48804130	[G/A]	13	14	0.19	0.71	3.92	NWD1	exon, synonymous
20	49019261	[T/C]	29	30	0.57	0.25	3.38	CHERP	intron
20	49318725	[C/T]	7	12	0.64	0.04	4.31	CIB3	exon, synonymous
25	47629272	[C/T]	26	29	0.06	0.36	3.94	INPP5D	upstream
25	47666842	[G/A]	28	30	0.02	0.28	4.10	INPP5D	exon, synonymous
25	47685188	[C/T]	31	30	0.68	0.37	3.23	INPP5D	intron
25	48075297	[C/G]	15	15	0.37	0.00	3.62	LOC100688622	exon, non-synonymous
25	48076278	[G/A]	15	15	0.37	0.00	3.62	LOC100688622	downstream
25	48396330	[C/T]	25	30	0.54	0.23	3.03	SPP2	inron
25	50050076	[G/A]	9	6	0.78	0.08	3.71	ASB18	downstream
25	50228063	[C/G]	13	15	0.27	0.73	3.28	IQCA1	intron
25	51029326	[G/A]	28	30	0.57	0.27	3.06	COL6A3	intron
25	51031100	[A/G]	32	30	0.39	0.10	3.73	COL6A3	exon, synonymous
25	51040259	[A/G]	33	30	0.52	0.20	3.61	COL6A3	exon, synonymous
25	51046607	[G/A]	31	30	0.53	0.22	3.49	COL6A3	exon, synonymous
25	51736576	[T/C]	27	30	0.56	0.23	3.38	HES6	upstream
32	11265116	[-/T]	15	15	0.03	0.40	3.25	LOC487839	downstream

[a] minor allele frequency [b] –10log p-value [c] UTR lengths based on human cDNA data

Discussion

CHD is considered a complex trait and multiple chromosomal regions have been associated with the disease in various dog breeds (Chase et al., 2004; Marschall et al., 2007; Zhu et al., 2008; Phavaphutanon et al., 2009; Zhou et al., 2010; Todhunter et al., 2005; Friedenberg et al., 2011). Around the world different protocols are in use to classify the severity of the disease based on radiographs. In this study, the severity grade was not taken into account due to the limited number of samples and CHD was analysed as a binomial trait. Given that HD is a

complex disease two statistical analyses were applied. In contrast to the single SNP-analysis with PLINK software, the multiple-SNP analysis of iBay takes this complexity into account and therefore more weight was given to the results obtained with the latter program.

As could be expected for a complex disorder, our genome-wide analysis revealed multiple regions associated with CHD. Several of these regions had been implicated in CHD before. The region on CFA08 at position 28-34.5 Mb that was strongly associated in our study overlaps largely with the region of 29-34 Mb found by Marshall and Distl (2007) in the German Shepherd Dog. The less strongly associated region on CFA01 at 70.7-71.9 Mb overlaps with a region around position70 Mb identified by Phavaphutanon et al. (2009) in Labrador Retrievers and the region on chromosome 20 at 46.3-51.2 Mb overlaps with the region of 40-47 Mb described by Todhunter et al. (2005) in the offspring of affected Labrador Retrievers and unaffected Greyhounds. The minor associated region on CFA32 at 11.2-13.0 Mb is flanked by regions identified in Labrador Retrievers around 5 Mb (Phavaphutanon et al., 2009) and German Shepherd Dogs (16-20 Mb, Marschall et al., 2007).

We found no evidence for association of other regions on chromosomes 1, 2, 10, 20 (two regions around 30 Mb and 60 Mb) and CFA22 previously reported to be associated in Labrador Retrievers (Phavaphutanon et al., 2009). We also did not observe association to the region of CFA11 that contains FBN2 coding for fibrillin 2. A deletion in an intron of this gene was found to be associated with suppression of the gene and with hip dysplasia in Labrador Retrievers from the USA (Friedenberg et al., 2011). The discrepant results might be due to population differences. Indeed, the population stratification analysis indicated that the Dutch and American populations of Labrador Retrievers have diverged.

CHD is a common disorder occurring in many breeds, and alternate genetic factors may be involved in different breeds or even populations. The etiology of CHD is not understood, although two broad etiological categories have been proposed i.e. laxity of the peri-articular soft tissues (ligaments, muscles, joint capsule) (Paatsama, 2000; Ihemelandu et al., 1983; Lust et al., 1972; Cardinet et al., 1983), and an abnormal progression of the endochondral ossification in the hip joint, or a combination of both processes (Riser, 1975a; Riser, 1975b). A majority of 88% of the affected dogs in our sample was graded before the age of 20 months. We were not concerned with the possibility of including phenocopies by adding dogs that were diagnosed at a later age, because their number was small and these could not have led to falsely positive results.

One of the identified SNPs associated with CHD is located near the LRR1 gene on CFA08. The encoded protein has been described as down regulating the 4-1BB-mediated signal transduction pathways JNK1 and NfkappaB (Jang et al., 2001). JNK1 and NFkappaB play a role in proteoglycan synthesis by chondrocytes (Zhou et al., 2007; Chowdhury et al., 2008) making a role for LRR1 in cartilage development and physiology plausible. Functional studies are needed to corroborate the possible role of LRR1 in CHD.

Additional analysis of the data resulted in the selection of other SNPs of interest that were associated with CHD status together with LRR1. These SNPs were located in the top regions of the multiple SNP association analysis in or near the genes COL6A3 on CFA25, and LAMA2 on CFA01 (Table 6.2). The specific function of these genes in cartilage and muscle as described

below facilitates their implication in CHD. First, COL6A3 codes for the alpha 3 chain of collagen VI that forms a fine fibrillar network in many connective tissues (Groulx et al., 2011) and is a specific component of the peri-cellular matrix of chondrocytes with a protective role for these cells (Peters et al., 2011). Furthermore, one of the characterising features of myopathies related to mutations in the COL6A3 gene is joint hyper laxity (Demir et al., 2002). Second, laminins with a LAMA2 encoded chain are found in the basal lamina of basement membranes (Shibuya et al., 2003); the chain is also related to cartilage development (Durr et al., 1996) and has been implicated in congenital muscular dystrophy (Helbling-Leclerc et al., 1995). Third, the region of interest on CFA20 contains three genes involved in connective tissue integrity. This region displays strong linkage disequilibrium and it is not possible to discriminate between the genes based on the association data. Good candidate genes for involvement in CHD in this region are GDF15 encoding growth-differentiating factor 15, COMP for cartilage oligomeric matrix protein and the gene encoding cartilage intermediate layer protein 2 (CILP2). GDF15 is a gene with a role not only in bone remodelling (Hinoi et al., 2011), but has also been related to osteoarthritis in humans (Iliopoulos et al., 2008). Mutations in COMP are associated with pseudoachondroplasia (OMIM177170) and multiple epiphyseal dysplasia (OMIM132400). CILP-2 is expressed in cartilaginous tissues and muscles; specifically there are indications that it is associated with collagen VI microfibrils mediating interactions between matrix components in cartilage (Bernardo et al., 2011). It should be noted that our DNA sequencing strategy aimed at identification of mutations in exons and in intron/exon junctions. Intron mutations at positions not close to exons can affect RNA splicing and these were not captured by our strategy.

The associated regions support the view that cartilage, as a specified connective tissue, plays an important role in the pathophysiology of CHD. A delay in endochondral ossification of the femoral head has been demonstrated in dysplastic Labrador dogs (Todhunter et al., 1997; Foels et al., 2000; Madsen et al., 1991), in correspondence with earlier findings in German Shepherd dogs (Ihemelandu et al., 1983). Furthermore, CHD in American Labrador Retrievers is associated with a deletion in FBN2 that codes for fibrillin2, a component of the extracellular matrix in fibrous joint capsule and articular cartilage (Friedenberg et al., 2011). Another study observed a difference in muscle composition of two months old German Shepherd Dogs that developed CHD later in life compared to controls (Ihemelandu et al., 1983). In addition, structural alterations were found in collagen fibers of the joint capsule of the hip joint and of the round ligament of dysplastic Labrador Retrievers when compared to these structures in tight-hipped Labradors (Todhunter et al., 2003).

Conclusions

This study identified several SNPs associated with CHD in or near genes that are involved in extracellular matrix development. The combination of the candidate genes implicates bone and soft tissue development in CHD. First, disturbances during the process of endochondral bone formation attributing to the abnormal formation of the hip joint may well be related to disturbances in the hypertrophic differentiation of the chondrocytes. Second, there are indications for soft tissue involvement (cartilage, muscles, and ligaments). The association results described here need to be confirmed by analysis of an independent replication cohort.

Additional mechanistic studies are essential for the understanding of the molecular processes that lead to hip dysplasia.

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References

Bernardo BC, Belluoccio D, Rowley L, Little CB, Hansen U, et al, 2011. Cartilage intermediate layer protein 2 (CILP-2) is expressed in articular and meniscal cartilage and down-regulated in experimental osteoarthritis. J Biol Chem 286: 37758-37767.

Boere-Boonekamp MM, Verkerk PH, 1998. Screening for developmental dysplasia of the hip. Seminars in Neonatology 3: 49-59.

Cardinet GH,3rd, Guffy MM, Wallace LJ, Laben RC, 1983. Canine hip dysplasia in german shepherd dog-greyhound crossbreeds. J Am Vet Med Assoc 182: 393-395.

Chase K, Lawler DF, Adler FR, Ostrander EA, Lark KG, 2004. Bilaterally asymmetric effects of quantitative trait loci (QTLs): QTLs that affect laxity in the right versus left coxofemoral (hip) joints of the dog (canis familiaris). Am J Med Genet A 124: 239-247.

Chowdhury TT, Salter DM, Bader DL, Lee DA, 2008. Signal transduction pathways involving p38 MAPK, JNK, NFkappaB and AP-1 influences the response of chondrocytes cultured in agarose constructs to IL-1beta and dynamic compression. Inflamm Res 57: 306-313.

Dai J, Shi D, Zhu P, Qin J, Ni H, et al, 2008. Association of a single nucleotide polymorphism in growth differentiate factor 5 with congenital dysplasia of the hip: A case-control study. Arthritis Res Ther 10: R126.

Demir E, Sabatelli P, Allamand V, Ferreiro A, Moghadaszadeh B, et al, 2002. Mutations in COL6A3 cause severe and mild phenotypes of ullrich congenital muscular dystrophy. Am J Hum Genet 70: 1446-1458.

Durr J, Lammi P, Goodman SL, Aigner T, von der Mark K, 1996. Identification and immunolocalization of laminin in cartilage. Exp Cell Res 222: 225-233.

Foels WS, 2000. Secondary center of ossification differs in developmental dysplasia of the hip [abstract]. Proceedings of the 46th Annual Meeting, Orthopaedic Research Society.

Friedenberg SG, Zhu L, Zhang Z, Foels WS, Schweitzer PA, et al, 2011. Evaluation of a fibrillin 2 gene haplotype associated with hip dysplasia and incipient osteoarthritis in dogs. Am J Vet Res 72: 530-540.

George EI, McCulloch RE, 1993. Variable selection via gibbs sampling. Journal of the American Statistical Association 88: 881-889.

Groulx JF, Gagné D, Benoit YD, Martel D, Basora N, et al, 2011. Collagen VI is a basement membrane component that regulates epithelial cell-fibronectin interactions. Matrix Biol 30: 195-206.

Helbling-Leclerc A, Zhang X, Topaloglu H, Cruaud C, Tesson F, et al, 1995. Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. Nat Genet 11: 216-218.

Heuven HC, Janss LL, 2010. Bayesian multi-QTL mapping for growth curve parameters BMC Proc 4 Suppl 1:S12.

Hinke V, Seck T, Clanget C, Scheidt-Nave C, Ziegler R, et al, 2001. Association of transforming growth factor-beta1 (TGFbeta1) T29 --> C gene polymorphism with bone mineral density (BMD), changes in BMD, and serum concentrations of TGF-beta1 in a population-based sample of postmenopausal german women. Calcif Tissue Int 69: 315-320.

Hinoi E, Ochi H, Takarada T, Nakatani E, Iezaki T, et al, 2011. Positive regulation of osteoclastic differentiation by growth differentiation factor-15 up-regulated in osteocytic cells under hypoxia. J Bone Miner Res 27:938–949.

Ihemelandu EC, Cardinet GH,3rd, Guffy MM, Wallace LJ, 1983. Canine hip dysplasia: Differences in pectineal muscles of healthy and dysplastic german shepherd dogs when two months old. Am J Vet Res 44: 411-416.

Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A, 2008. Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. PLoS One 3: e3740.

Jang LK, Lee ZH, Kim HH, Hill JM, Kim JD, et al, 2001. A novel leucine-rich repeat protein (LRR-1): Potential involvement in 4-1BB-mediated signal transduction. Mol Cells 12: 304-312.

Janutta V, Hamann H, Distl O, 2006. Complex segregation analysis of canine hip dysplasia in german shepherd dogs. J Hered 97: 13-20.

Jia J, Li L, Zhao Q, Zhang L, Ru J, et al, 2012. Association of a single nucleotide polymorphism in pregnancy-associated plasma protein-A2 with developmental dysplasia of the hip: A case-control study. Osteoarthritis Cartilage 20: 60-63.

Kapoor B, Dunlop C, Wynn-Jones C, Fryer AA, Strange RC, et al, 2007. Vitamin D and oestrogen receptor polymorphisms in developmental dysplasia of the hip and primary protrusio acetabuli--a preliminary study. J Negat Results Biomed 6: 7. 10.1186/1477-5751-6-7.

Kass RE, Raftery AE, 1995. Bayes Factors. Journal of the American Statistical Association 90: 773-795.

Kolundzic R, Trkulja V, Mikolaucic M, Kolundzic MJ, Pavelic SK, et al, 2011. Association of interleukin-6 and transforming growth factor-beta1 gene polymorphisms with developmental hip dysplasia and severe adult hip osteoarthritis: A preliminary study. Cytokine 54: 125-128.

Kwan Tat S, Padrines M, Theoleyre S, Heymann D, Fortun Y, 2004. IL-6, RANKL, TNF-alpha/IL-1: Interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev 15: 49-60.

Lust G, Craig PH, Geary JC, Ross GE,Jr, 1972. Changes in pelvic muscle tissues associated with hip dysplasia in dogs. Am J Vet Res 33: 1097-1108.

Madsen JS, Reiman NI, Svalastoga E, 1991. Delayed ossification of the femoral head in dogs with hip dysplasia. Journal of Small Animal Practice 32: 351-354.

Mäki K, Janss LL, Groen AF, Liinamo AE, Ojala M, 2004. An indication of major genes affecting hip and elbow dysplasia in four finnish dog populations. Heredity 92: 402-408.

Malm S, Strandberg E, Danell B, Audell L, Swenson L, et al, 2007. Impact of sedation method on the diagnosis of hip and elbow dysplasia in swedish dogs. Prev Vet Med 78: 196-209.

Marschall Y, Distl O, 2007. Mapping quantitative trait loci for canine hip dysplasia in german shepherd dogs. Mamm Genome 18: 861-870.

Miller SA, Dykes DD, Polesky HF, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215.

Paatsama S, 2000. Fédération cynologique internationale (FCI). In: Morgan JP, Wind A, Davidson AP, editors. Hereditary Bone and Joint Diseases in the Dog - Osteochondroses, hip dysplasia, elbow dysplasia -. Hannover: Schlütersche. pp. 263-266.

Peled E, Eidelman M, Katzman A, Bialik V, 2008. Neonatal incidence of hip dysplasia: Ten years of experience. Clin Orthop Relat Res 466: 771-775.

Peters HC, Otto TJ, Enders JT, Jin W, Moed BR, et al, 2011. The protective role of the pericellular matrix in chondrocyte apoptosis. Tissue Eng Part A 17: 2017-2024.

Phavaphutanon J, Mateescu RG, Tsai KL, Schweitzer PA, Corey EE, et al, 2009. Evaluation of quantitative trait loci for hip dysplasia in labrador retrievers. Am J Vet Res 70: 1094-101.

Priester WA, Mulvihill JJ, 1972. Canine hip dysplasia: Relative risk by sex, size, and breed, and comparative aspects. J Am Vet Med Assoc 160: 735-739.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al, 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559-575.

Riser WH, 1975a. Growth and development of the normal canine pelvis, hip joints, and femur from birth to maturity. Veterinary Pathology 12: 264-278.

Riser WH, 1975b. The dysplastic hip joint: Radiologic and histologic development. Vet Pathol 12: 279-305.

Shibuya S, Wakayama Y, Inoue M, Kojima H, Oniki H, 2003. Merosin (laminin-2) localization in basal lamina of normal skeletal muscle fibers and changes in plasma membrane of merosin-deficient skeletal muscle fibers. Med Electron Microsc 36: 213-220.

Todhunter RJ, Lust G, 2003. Canine hip dysplasia pathogenesis. In: Slatter D, editor. Textbook of small animal surgery. Philadelphia: WB Saunders Co. pp. 2009-2019.

Todhunter RJ, Mateescu R, Lust G, Burton-Wurster NI, Dykes NL, et al, 2005. Quantitative trait loci for hip dysplasia in a cross-breed canine pedigree. Mamm Genome 16: 720-730.

Todhunter RJ, Zachos TA, Gilbert RO, Erb HN, Williams AJ, et al, 1997. Onset of epiphyseal mineralization and growth plate closure in radiographically normal and dysplastic labrador retrievers. J Am Vet Med Assoc 210: 1458-1462.

Wang K, Shi D, Zhu P, Dai J, Zhu L, et al, 2010. Association of a single nucleotide polymorphism in Tbx4 with developmental dysplasia of the hip: A case-control study. Osteoarthritis Cartilage 18:1592–1595.

Zhou Y, Millward-Sadler SJ, Lin H, Robinson H, Goldring M, et al, 2007. Evidence for JNK-dependent up-regulation of proteoglycan synthesis and for activation of JNK1 following cyclical mechanical stimulation in a human chondrocyte culture model. Osteoarthritis Cartilage 15: 884-893.

Zhou Z, Sheng X, Zhang Z, Zhao K, Zhu L, et al, 2010. Differential genetic regulation of canine hip dysplasia and osteoarthritis. PLoS One 5: e13219. 10.1371/journal.pone.0013219.

Zhu L, Zhang Z, Feng F, Schweitzer P, Phavaphutanon J, et al, 2008. Single nucleotide polymorphisms refine QTL intervals for hip joint laxity in dogs. Anim Genet 39: 141-146.

Chapter 7

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

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Abstract

Patellar luxation is an orthopaedic 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%. Here we report molecular genetic analyses of the condition in this breed population.

A genome-wide association analysis of 15,823 single nucleotide polymorphisms (SNPs) in 45 cases and 40 controls revealed a significant association of patellar luxation with a region on chromosome CFA07, as well as a suggestive associations with regions on CFA03, CFA31, and CFA36. The exons of the genes in these regions were selected for targeted massive parallel DNA sequencing. In total, 0.5 Mb of DNA from 15 cases and a pooled sample from 15 controls was targeted. Of the 7257 observed variations, 124 SNPs that showed the largest differences in the deduced allele frequency between the cases and the control pool were genotyped in 95 Flat-Coated Retrievers. Nine SNPs, in 8 genes on CFA07 and CFA31, were associated with patellar luxation with $P < 10^{-4}$, and these SNPs were genotyped in samples from a variety of breeds. The disease-associated allele of one SNP was not common in the other breeds. This was a synonymous SNP in a FMO6 pseudogene.

The genome-wide analysis followed by targeted DNA sequence analysis implies loci on chromosomes 7 and 31 in patellar luxation in the Flat-Coated Retriever breed.

Keywords: patellar luxation, knee, dog, canis, genome, association analysis, DNA sequence

Introduction

Patellar luxation (PL) is a common developmental orthopaedic disorder in dogs (Ness et al., 1996; Johnson et al., 1989; 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 et al., 1993). PL occurs when the patella moves out of the trochlear groove and it can lead to degenerative joint disease, lameness, and pain.

Developmental PL is most frequently seen in small dog breeds (Priester, 1972; Hayes et al., 1994), and the prevalence of PL in breeds seems to decrease with increasing body size (Hayes et al., 1994; Chase et al., 2009). The observed prevalence of PL in the Dutch population of Flat-Coated Retrievers (FCR) is 24%, much higher than reported for presumably mostly American FCR (1.6%) by the Orthopedic Foundation for Animals (www.offa.org). Both medial and lateral PL occur in dogs, and although medial PL is more common than lateral PL in all breeds regardless of breed size, lateral PL is more common in large breed dogs than in small breed dogs (Priester, 1972; Hayes et al., 1994; Remedios et al., 1992; Gibbons et al., 2006). There is no report on screening for patellar luxation in a purebred dog population where lateral PL is more common than medial PL except for the Dutch FCR (ICML, personal communications). Bilateral involvement occurred in half the cases and PL was more common in female than male FCR, as has been reported in other breeds (Priester, 1972; Hayes et al., 1994; Alam et al., 2007).

The predisposition of certain breeds to PL in combination with the high proportion of animals 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. The previously calculated heritability of 0.17 for the Dutch FCR population indicated that environmental factors play a role in the phenotypic appearance of the trait, in addition to genetic factors. Chromosomal regions or genes that predispose to PL have not yet been identified.

Here we report a genome-wide association analysis of PL in Dutch FCRs followed by massive parallel DNA sequence analysis. Our findings suggest that loci on CFA07 and CFA31 have a major influence on the phenotype. This is the first step towards identifying genes that are involved in the development of this disorder and may help us to gain insight into the aetiology of this crippling disability.

Materials and methods

Animals

The animals used in this study were part of a FCR cohort (n=3835) that had been screened for PL as adults between 1990 and 2007 by the same orthopaedic specialist (FJM). The grading system of the phenotype will be described elsewhere. An Estimated Breeding Value (EBV) was calculated for those animals for which pedigree information was available; the average EBV in 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). In the 93 animals 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 based on 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 FCR Breeders Club provided addresses of the owners of the selected dogs for the purpose of this project. The owners were contacted by letter, which was written on behalf of the breed club also, to inform them about the project and to ask them to contact their local veterinarian with the request to take a 4 ml blood sample from their dog for DNA isolation. The samples were sent to us by the veterinarians. In short, all dogs were privately owned and included with informed consent of the owners. The dogs were handled by licensed veterinarians only. Thus we complied to the conditions set forth in the Dutch 'Wet op de Uitoefening van de Diergeneeskunde' (Law on the Practice of Veterinary Medicine) of March 21, 1990 and approval of an ethics committee for the use of samples of the animals was not necessary.

Genotyping and data analysis

Blood was collected from the 45 PL-positive and 48 PL-negative animals for the isolation of DNA, 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 93 FCRs. Only SNPs that had a minor allele frequency of more than 1% and which 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 binomial trait and the EBVs of the animals as a quantitative trait. A $\chi 2$ based allelic association analysis with 45 cases and 40 controls was performed, as well as linear regression modelling 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 α=0.05 as the threshold for significance (P-value <1*10-5.5). Permutations were also performed (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 et al., 1993; Heuven et al., 2010). For detection of associated regions, we used a model that included a polygenic effect as well as all SNP 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 25 cases and 25 controls were purified using phenol/chloroform extraction and ethanol precipitation by standard techniques, and DNA concentrations were measured using Qubit® (Invitrogen). DNA from 15 cases was individually sequenced (1µg/sample), and equal amounts of DNA from the remaining ten cases were pooled and 1 µg of pooled DNA was used in subsequent steps. Equal amounts of DNA from control dogs were also pooled and 1 µg of pooled DNA was used. The 15 individual samples and the two pools 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 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 (Harakalova et al., 2011; Mokry et al., 2010).

Genotyping of selected SNPs

We genotyped selected variations detected by sequencing in 95 FCRs using the Komparative Allele Specific PCR (KASPar) assay (KBioscience, Hoddesdon, UK). Two differently labelled allele-specific primers and a common reverse primer were designed for each of the variations. 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 (KBioscience, 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.

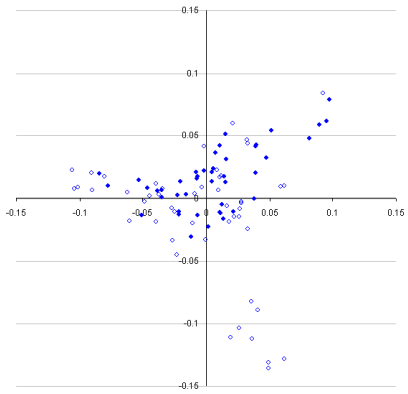

Figure 7.1 Identical-by-state plot of Flat-Coated Retrievers. The sample set of 93 dogs was genotyped with arrays for 22,000 SNPs. The first two principal components of a multidimensional identical-by-state matrix of 45 cases (filled symbols) and 48 controls (open symbols) were calculated with PLINK software. The cluster of 8 controls at the bottom right part of the plot was excluded from further analysis.

Results

Genome-wide association analysis

More than 22,000 SNPs were genotyped in 45 Flat-Coated Retrievers with signs of PL and 40 control dogs of the same breed. Microarray data are available in the ArrayExpress database under accession number E-MTAB-2040 (www.ebi.ac.uk/arrayexpress). 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 (Figure 7.1). Eight control samples that deviated from the main population were excluded from further analysis.

The SNP data were analyzed using two phenotypes and two statistical approaches. A discrete case–control phenotype was alternated with estimated breeding values (EBVs) for individual animals. A $\chi 2$ analysis of the allele distribution of individual SNPs over the groups of PL-positive and PL-negative FCRs, using PLINK software, indicated that PL is associated with a region on chromosome CFA07 (Figure 7.2A, Table 7.1). The uncorrected P-value reached $6.9*10^{-7}$ and after correction for multiple testing using permutations of the genotype data over the groups of dogs the empirical P-value was $1.0*10^{-3}$, the Bonferroni corrected P-value was $1.1*10^{-2}$.

A quantitative trait association analysis of individual SNPs with PLINK software using the EBVs of the 85 FCRs, instead of their PL status, indicated PL to be associated with CFA07 again (uncorrected P-value = 2.2×10^{-8}) and with CFA36 (uncorrected P-value = 1.9×10^{-6}). Both associations were significant after correction for multiple testing with permutations or the Bonferroni correction (Figure 7.2B, Table 7.1).

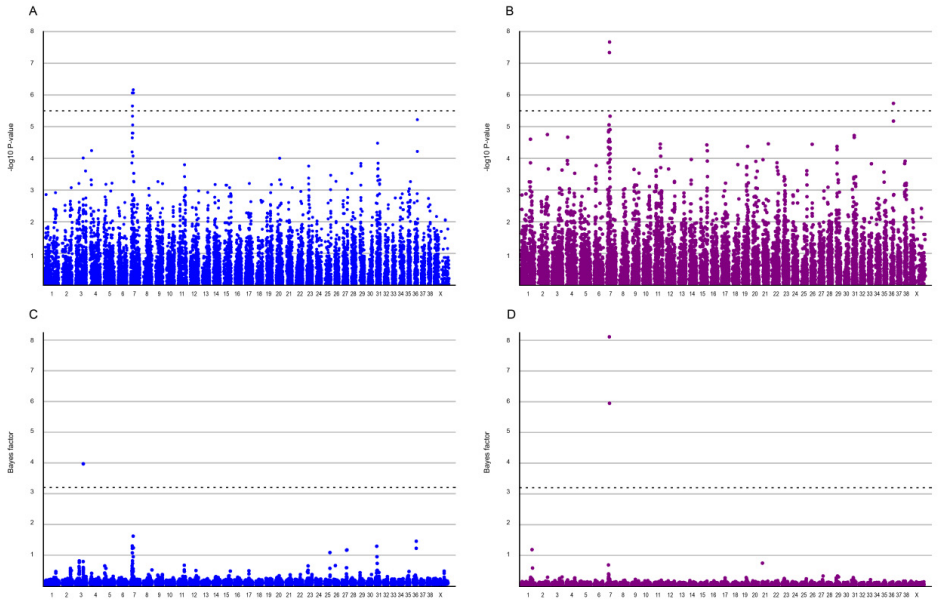

Figure 7.2 Genome-wide association analysis of patellar luxation in Flat-Coated Retrievers. (A) Association of individual SNPs was analyzed with PLINK software by comparing allele frequencies in the group of cases (n=45) and controls (n=40). (B) Association analysis of individual SNPs using the Estimated 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-values 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 [17].

Table 7.1 Comparison of top SNPs associated to patellar luxation defined as binary trait or by estimated breeding value.

			MAF		Single-SNP		Multiple-SNP	
CFA	Location	SNP	cases	controls	PL status	EBV	PL status	EBV
01	99888625	BICF2S2314252	0.11	0.45	6.16	7.34	1.62	8.11
03	67056782	BICF2P309055	0.12	0.46	6.06	7.67	1.23	5.95
04	16996349	BICF2S23034244	0.12	0.46	6.06	4.83	1.30	0.69
07	17648777	BICF2S2293048	0.13	0.46	5.65	4.52	1.07	0.31
07	18970233	BICF2G630553500	0.13	0.46	5.65	4.52	1.22	0.40
07	19071723	BICF2P1448362	0.13	0.45	5.33	4.87	0.81	0.31
07	19746349	BICF2G630553889	0.64	0.29	5.22	5.17	1.45	0.09
07	20109002	BICF2P1333659	0.19	0.51	5.05	4.11	0.73	0.20
07	20145907	BICF2P1335550	0.12	0.41	4.79	5.06	0.26	0.37
07	21065761	BICF2S23030368	0.12	0.41	4.79	5.06	0.33	0.24

07	22157845	BICF2P233561	0.12	0.41	4.79	4.54	0.57	0.21
07	23113211	BICF2P1060266	0.14	0.44	4.64	4.34	0.39	0.26
07	24186445	BICF2P424667	0.36	0.09	4.48	3.35	1.29	0.12
07	25490867	BICF2P205579	0.17	0.45	4.24	4.67	0.41	0.16
07	27099172	BICF2S2457585	0.51	0.21	4.22	5.73	1.22	0.07
07	28293222	BICF2P1386712	0.18	0.46	4.20	2.77	0.65	0.04
07	32710038	BICF2G630555333	0.23	0.53	4.07	3.88	0.46	0.15
25	49858895	BICF2P1461096	0.16	0.43	4.01	2.90	3.97	0.22
27	43484050	BICF2G630153501	0.58	0.33	3.02	1.98	1.16	0.17
27	46605159	BICF2G630154851	0.24	0.49	3.01	3.49	1.08	0.19
31	15166531	BICF2S23135348	0.58	0.34	2.77	2.74	1.17	0.33
36	29549762	BICF2S22944651	0.26	0.49	2.73	5.33	0.09	0.14
36	29608881	BICF2G630757990	0.42	0.23	2.20	2.94	0.42	1.18

The multiple SNP analysis using a Bayesian variable selection method (George et al., 1993), as implemented in iBay software, revealed regions associated with PL (Bayesian factor > 1) on chromosomes 3, 7, 25, 27, 31, and 36 when using PL as a binary trait (Figure 7.2C, Table 7.1). The region on CFA03 explained the largest part of the phenotypic variation (Bayesian factor = 3.97). When EBVs were used as phenotypic score, chromosomes 1 and 7 were associated with the PL phenotype (Bayesian factor >1, Figure 7.2D,Table 7.1).

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 64-69Mb), CFA07 (15-29.5Mb), CFA31 (13-21Mb), and CFA36 (27.5-32Mb).

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. We designed enrichment arrays that covered ~0.5 Mb of DNA. This selected DNA was sequenced in 15 individual dogs with PL and in pooled sample of 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 ~80x. 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 7.2.

Table 7.2 Top 40 variations associated with patellar luxation derived from DNA sequence data.

CFA	Position	Alleles	Ass.A.	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
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

Genotyping of candidate SNPs in a large cohort

A set of 124 SNPs was selected for further analysis on the basis of the deduced allele frequency differences between the cases and controls. Because the SNPs with the largest frequency differences were mainly located in the region on CFA07, a limited number of SNPs were added from the regions on CFA03, CFA31 and CFA36. The complete set of 144 selected SNPs is listed in Supplementary Table S1. These SNPs were genotyped in a cohort of 95 FCRs. This was done to expand the dataset and to ascertain the genotype deduced from the allele read frequencies. The sample set of dogs largely overlapped, but was not identical to, the sample set used in the

genome wide SNP analysis. This was due to the limited amount of DNA that was available of some dogs. In total, 127 SNPs were reliably genotyped, 30 of which were monomorphic. Single SNP $\chi 2$ based analysis of the remaining 97 SNPs revealed eight SNPs on CFA07 that were associated with the phenotype and one associated SNP on CFA31 (P-value $< 1.0*10^{-4}$, Table 7.3); the associated SNPs located on CFA03 and CFA36 were less significant (P-value $> 1.0*10-4$).

The eight SNPs on CFA07 that displayed association to PL were tested in 24 breeds (3-4 dogs per breed) to assess the variability in other dog populations. Most alleles associated with PL in the FCR breed were also detected in other breeds, with the exception of the synonymous SNP in the FMO6 pseudogene at position 30669327, which was seen infrequently in the other breeds.

Table 7.3 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_Synonym.	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

Discussion

In this study, we analysed the disposition to patellar luxation in two ways: we used the PL status of the animals as a binary trait and we used EBVs of all animals 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 fact that the analysis of the SNP data using EBVs resulted in more significant P-values than the association analysis of the binary trait indicates that a region on CFA07 is involved. The level of significance obtained for this complex disorder, with a relatively low number of cases and controls, suggests the presence of a major determinant of PL in this region.

The choice of the DNA sequencing strategy was influenced by two considerations. First, there was the large size of 9 Mb of the associated region on CFA07. 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 due to the small number of DNA barcode addresses that were available at the time. We chose to pool control samples because it was thought that the allele frequency of DNA sequence variants could be derived from their representation in the reads. However, analysis of the individually sequenced DNA of cases indicated that, at an average depth of 80 reads, the allele representation

was highly variable and therefore un unreliable indicator for the underlying genotype. Since a higher number of barcodes is now available, we advise the use of individually tagged DNA samples only.

Approximately 25% of the SNPs detected by the DNA sequence analysis that were genotyped using the KASPar assay turned out to be monomorphic. The high percentage of falsely detected DNA variations stresses the importance of confirmation of Next Generation Sequencing results by 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 causing malalignment of these elements have been suggested to be the basis of PL. Ventro-dorsal radiographs of the hip and knees in 8 Dutch FCRs with PL did not show signs of bony malalignment (Lavrijsen, unpublished data), and therefore involvement of muscles or ligaments in PL seems more likely as suggested by others (Mostafa et al., 2008).

We identified 9 DNA sequence variants in eight positional candidate genes for PL in affected FCRs. One of these, in 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 hyper extensibility, 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 hampering the splicing machinery or increased instability of the mRNA molecule. In combination with other genetic risk factors it might predispose FCRs to PL. However, the apparently exclusive expression of TNR in the brain seems to refute involvement in patellar luxation.

It should be noted that, although we reached an average coverage of between 80-90 DNA sequence reads per location, not all targeted regions were sufficiently covered. It is therefore possible that we missed relevant mutations. In addition we only analyzed exons and intron/exon boundaries and we cannot rule out that variants in promoter regions or introns contribute to the phenotype. To confirm the involvement of tenascin R in patellar luxation in FCR dogs, the TNR gene needs to be analyzed 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 associated regions on chromosomes 3, 31, and 36 may identify more genetic factors involved in PL.

Conclusions

We identified regions on chromosomes 3, 7, 31, and 36 that are associated with PL in the Dutch FCR. Fine-mapping of the most significantly associated region on CFA07 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.

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.

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References

Alam MR, Lee JI, Kang HS, Kim IS, Park SY, Lee KC, Kim NS: Frequency and distribution of patellar luxation in dogs. 134 cases (2000 to 2005). Vet Comp Orthop Traumatol 2007, 20:59-64.

Chase K, Jones P, Martin A, Ostrander EA, Lark KG: Genetic mapping of fixed phenotypes: Disease frequency as a breed characteristic. J Hered 2009, 100(Suppl 1):S37-41.

Evans HE, Miller ME: Miller's anatomy of the dog. Philadelphia: WB Saunders; 1993.

George EI, McCulloch RE: Variable selection via Gibbs sampling. J Am Stat Assoc 1993, 88:881-889.

Gibbons SE, Macias C, Tonzing MA, Pinchbeck GL, McKee WM: Patellar luxation in 70 large breed dogs. J Small Anim Pract 2006, 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 IJ, Kloosterman WP, Cuppen E: Multiplexed array-based and insolution genomic enrichment for flexible and cost-effective targeted next-generation sequencing. Nat Protoc 2011, 6:1870–1886.

Hayes AG, Boudrieau RJ, Hungerford LL: Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). J Am Vet Med Assoc 1994, 205:716-720.

Heuven HC, Janss LL: Bayesian multi-QTL mapping for growth curve parameters. BMC Proc 2010, 4(Suppl 1):S12.

Johnson JA, Austin CC, Breur GJ: Incidence of canine appendicular musculoskeletal disorders in 16 veterinary teaching hospitals from 1980 through 1989. Vet Comp Orthop Traumatol 1994, 7:56-69.

Kass RE, Raftery AE: Bayes factors. J Am Statl Assoc 1995, 90:773-795.

LaFond E, Breur GJ, Austin CC: Breed susceptibility for developmental orthopedic diseases in dogs. J Am Anim Hosp Assoc 2002, 38:467-477.

Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988, 16:1215.

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

Mostafa AA, Griffon DJ, Thomas MW, Constable PD: Proximodistal alignment of the canine patella: radiographic evaluation and association with medial and lateral Patellar luxation. Vet Surg 2008, 31:201-211.

Ness MG, Abercromby RH, May C, Turner BM, Carmichael S: A survey of orthopaedic conditions in small animal veterinary practice in Britain. Vet Comp Orthop Traumatol 1996, 9:43-52.

Priester WA: Sex, size, and breed as risk factors in canine patellar dislocation. J Am Vet Med Assoc 1972, 160:740-742.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007, 81:559-575.

Remedios AM, Basher AW, Runyon CL, Fries CL: Medial patellar luxation in 16 large dogs. A retrospective study. Vet Surg 1992, 21:5-9.

Chapter 8

General Discussion: Genetic improvement for orthopaedic diseases in dogs

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Genetic improvement for orthopaedic diseases in dogs.

The objective of this chapter is to discuss how breeding programs against orthopaedic diseases in dogs can be improved with emphasis on estimation of genetic ability using phenotypic and genotypic data.

Starting with the current practices and status of selection against orthopaedic diseases, also the additional value of DNA data shall be discussed and finally some recommendations be made.

Current practices

Orthopaedic diseases in dogs can be a major problem for the animals and owners, due to their effect on motility and thereby on the quality of life of dogs suffering from lameness. Orthopaedic diseases can be influenced by environmental factors such as rearing conditions, feeding and housing. Clinical cases can be treated by veterinarians, but often involve expensive and invasive surgery. The most effective way to reduce the prevalence of the skeletal developmental diseases is through the use of genetic selection (Woolliams, 2011). For that reason, dog breeders have a great interest in hereditary aspects of orthopaedic diseases, and implemented screening programs many years ago. The oldest screening program for canine diseases concerned hip dysplasia (HD) and started with a workshop in Utrecht in 1974. It was guided by the umbrella organization of kennel clubs, the Federation Cynologique International, but a worldwide, uniform protocol was never reached (Paatsama, 2000). After the first documented cases of elbow dysplasia (ED) (Tirgari, 1974; Olsson, 1983), screening for this group of diseases was initiated by the International Elbow Working Group (IEWG), founded in 1989 in Davis, California by a group of concerned veterinarians and breeders. A patella luxation (PL) screening program was initiated by Meutstege in The Netherlands in 1990 for a limited number of breeder clubs. However, such a program is not (yet) implemented by the FCI or national kennel clubs.

It is a drawback that still there is neither uniformity in radiographic techniques, nor in grading HD or ED at an international level. However, a high degree of national uniformity can be reached when the same panel evaluates the population for an extended period. In The Netherlands the HD- and ED-panel includes six specialists of which three are judging together the radiographs for hips and elbows by the same criteria for the last 12 and 20 years, respectively. For PL, one of a panel of certified orthopaedic specialists screens dogs according to a standard protocol. The currently used scoring methods are discussed in Chapter 2 of this thesis.

Current breeding programs in Labrador Retrievers (LR), Golden Retrievers (GR), Bernese Mountain dogs (BMD), Newfoundland (NF) and Flat-Coated Retrievers (FCR) take screening for HD into account when selecting breeding animals. It is not allowed to breed with dogs that have a HD-D or HD-E score. Breeding with HD-C is allowed with restrictions. For ED, selection rules apply for LR, BMD, and NF, but not for GR and FCR (according to the respective breeder club's breeding program). Breeding with dogs having an ED-score of 3 is not permitted, but rules for LR are stricter. Of these breeds PL screening is mandatory for FCR only. Breeding animals have to be 'free' or can have grade 1 if combined with a 'free' mate. All rules for all traits are based on phenotypic examination of the dogs. Selection is based on the phenotype of the

animal itself and neither the heritability nor the information from relatives is included to identify the best potential breeding animals.

The result of these breeding policies is shown in Figure 8.1 with phenotypic and genetic trends for HD, ED and PL for breeds studied in this thesis. Restrictions against breeding with affected animals have resulted in a drop in incidence for HD, but less so for ED. For PL initially progress was made until 10 years ago. The lack of progress is most likely due to the fact that selection for phenotypically sound breeding animals ignores the phenotypes of relatives and thereby the underlying susceptibility.

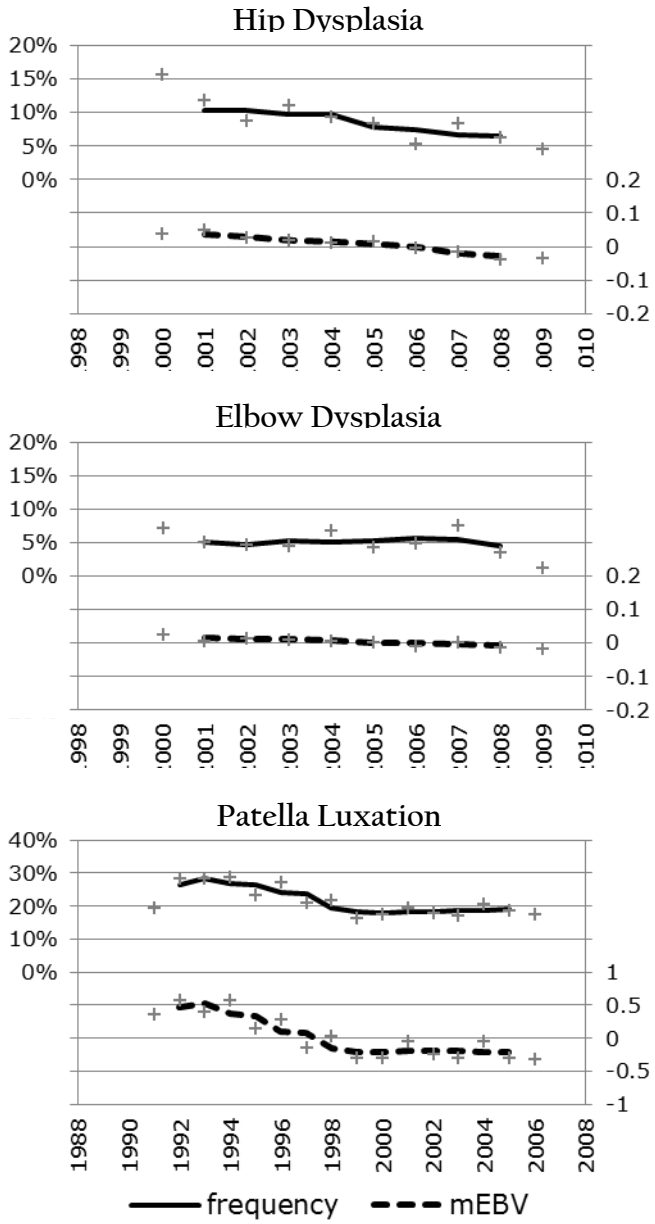

Figure 8.1 Phenotypic and genetic trend for HD (n=6627) and ED (n=6368) in LRs, GRs, BMDs and NFs (4-breed average), as well as for PL (n=3299) in FRs.

The frequency of each disease per year of birth is displayed on the left axis, with the 3-year average presented as a solid line (—).

The mean EBV (mEBV), on the right axis, is calculated by averaging the EBVs of all dogs born in a certain year. Again the dashed line (- - -) presents the 3-year average.

For more information see Chapter 3 (HD and ED) and Chapter 5 (PL). NB All three axes for PL differ from those for HD and ED.

The weakness of the current programs

Currently the selection of breeding animals is based almost exclusively on the phenotypic observations of the animals. On top of that, knowledge on family members is in many cases not collected and if collected, it is not always considered when selecting candidates. This is true for Dutch populations. In some countries, however, breeding values are estimated and being implemented into the breeding policies (like the German Shepherd Dogs in Germany and a few popular breeds in the UK). In many dog breeding programs potential breeding animals are informally pre-selected and on only a portion of the potential candidates, disease data is screened and recorded (Table 8.1). Although cost effective, it also reduces the genetic variation among the candidates and therefore the possible attainable progress. Furthermore using data on more relatives allows a more accurate estimation of the genetic ability of the candidates. Estimated breeding values are traditionally calculated using health/disease recordings combined with information of relatives. Offspring has the average breeding values of its parents until they are phenotyped or when they have offspring that have been screened for the disease.

Table 8.1 Proportion of dogs born between 2002 and 2006, which have since been screened and used for breeding.

		2002	2003	2004	2005	2006
Labrador Retriever	screened	10.3 %	9.9 %	9.9 %	10 %	10.1 %
(± 4500 pups / year)	bred with	6.3 %	6.2 %	5.9 %	6.7 %	5.7 %
Golden Retriever	screened	15.5 %	13.1 %	13.3 %	12.1 %	11.4 %
(± 2300 pups / year)	bred with	7.0 %	6.0 %	6.8 %	6.4 %	6.1 %
Bernese Mountain Dog	screened	10.0 %	9.9 %	9.3 %	10.1 %	9.3 %
(± 1700/year)	bred with	7.4 %	7.2 %	7.5 %	7.5 %	7.1 %
Flat-Coated Retriever	screened	28.4 %	32 %	32.5 %	26.1 %	26.9 %
(± 700/year)	bred with	7.1 %	9.1 %	6.9 %	5.6 %	5.6 %
Newfoundland	screened	22.7 %	22.6 %	16.9 %	17.2 %	18.9 %
(± 500/year)	bred with	9.6 %	9.4 %	8.4 %	7.7 %	9.1 %

Improvements for PL in the FCR-breed is a good example. Progress was made in the period from 1992-2000 but diminished afterwards because almost all candidates for selection were phenotypically acceptable, i.e. did not show PL., but the genetic ability was not estimated and therefore not used(Figure 8.1). Especially for a trait with a relatively low heritability of 0.17, the use of family information would have allowed a more reliable identification of the best candidates.

The second drawback mentioned above, of the current breeding policies, is the pre-selection of dogs to be screened for orthopaedic traits. Although it will be prohibitively expensive to

screen all candidates, more genetic progress can be made if at least some pups from each litter would be randomly screened. If all families have offspring scored, the differences among families are not biased by pre-selection. In addition, it would most likely result in more dogs being screened. This allows for a more accurate estimation of the genetic ability and also more genetic variation among the selection candidates. Ways to cover these costs, like demanding a 'breeding fee' for every pup sold to cover the screening costs, could be considered.

Swenson et al. (1997a, 1997b) investigated the costs and benefits of a screening program for HD and ED in Swedish dogs. It has been suggested that screening more dogs with lower accuracy is, from a genetic point of view, superior to screening very accurately but applied to a small part of the population. However, changes in protocols should not be done lightly because it might affect the utility of the data observed on dogs from the past as happened with HD in Labrador Retrievers. For ED a similar argument was used by Lappalaien (2013).

In summary the potential genetic improvement is not realized due to the limited numbers of dogs screened for orthopaedic diseases (~10% of all Labrador Retriever pups born in each generation, Table 8.1). Increasing the number of candidates allows for more intense selection, but also increases the costs. Secondly the use of the phenotype of the candidate for selection instead of his estimated genetic ability (EBV) and ignoring the information from relatives decreases the accuracy of selection, i.e. the genetically best candidates cannot be identified properly.

Possible ways to improve the effectiveness of breeding programs

Breeding programs can be used to diminish the occurrence and severity of orthopaedic diseases in dogs. The contribution of genetic improvement is usually small in most cases, but accumulates over generations and gives a permanent reduction. Therefore investment in these programs has a high return not only in terms of money but also in dogs that have a longer problem free life.

The start of this study focused on estimating the genetic variation of different orthopaedic diseases. The genetic variation among individual dogs for the orthopaedic diseases is the most important requirement for breeding programs to be effective. In chapters 3, 4 and 5 of this thesis, heritabilities have been calculated and these are summarized in Table 8.2. The heritability values indicate the proportions of the differences among dogs of the same breed due to differences in genetic ability, i.e. they carry different alleles at loci that affect the disease. As shown in Table 8.2 the orthopaedic diseases studied in this thesis have a significant heritability ranging from 0.10 to 0.33. The level of the heritability indicates that substantial progress is obtainable. The heritabilities are most likely underestimated due to pre-selection of candidates that are phenotyped, i.e. dogs which have visible problems at a young age, are not screened and therefore the 'extreme end' of the variation is not observed which causes the phenotypic and therefore the genetic variation to be underestimated.

Table 8.2 Heritabilities and genetic variation for HD, ED and PL by breed

Trait	Breed	n*	h^{2*}	Se*
Hip dysplasia	Bernese Mt. Dog	1422	0.31	0.06
(FCI score) [2]	Golden Retriever	2350	0.18	0.04
	Labrador Retriever	3687	0.10	0.03
	Newfoundland	759	0.23	0.08
Elbow dysplasia	Bernese Mt. Dog	1215	0.16	0.05
(IEWG score) [3]	Golden Retriever	1498	0.12	0.04
	Labrador Retriever	3317	0.13	0.03
	Newfoundland	622	0.33	0.09
Patellar Luxation	Flat-Coated Retriever	3834	0.17	0.03
(adjusted Putnam score)				

**Number of dogs (n) used for the calculation of the heritability (h^2) and it's standard error (se) for several breeds. [2]FCI = Federation Cynologique Internationale. [3]IEWG = International Elbow Working Group.*

Most dog breeder clubs have programs to select against orthopaedic abnormalities. Although the awareness amongst breeders is high, and the breed clubs often prohibit breeding with affected animals, still many puppies with these diseases are born every year indicating that programs must be improved.

The later chapters 6 and 7 of this thesis aimed, using DNA analysis, to identify chromosomal regions associated with the orthopaedic diseases (Table 8.3). Each identified region explains only a part of the genetic variation and more regions affect the observed occurrence of disease, which implies that these orthopaedic diseases have a polygenic inheritance. Due to the limited numbers of animals involved in these studies the power was lacking to identify more regions significantly associated with the diseases. This was especially the case for ED. Its low heritability might reflect heterogeneity of the phenotypical scoring.

Table 8.3 Chromosomal regions associated with orthopaedic diseases identified in this thesis

Trait	CFA	region	
Hip dysplasia (FCI score)	1	70.7 Mb - 71.9 Mb	suggestive
In Labrador Retriever	5	58.6 Mb - 63.5 Mb	suggestive
48 cases, 30 controls	8	28.0 Mb - 34.5 Mb	significant
	15	32.2 Mb - 33.6 Mb	suggestive
	20	46.3 Mb - 51.2 Mb	suggestive
	25	47.1 Mb - 51.9 Mb	suggestive
	32	11.2 Mb - 13.0 Mb	suggestive
Patellar Luxation (adjusted Putnam score)	3	64.0 Mb - 69.0 Mb	significant
In Flat-Coated Retriever	7	15.0 Mb - 29.5 Mb	significant
45 cases, 40 controls	36	27.5 Mb - 32.0 Mb	significant

The use of DNA-data in breeding programs

Rapidly new platforms for DNA analysis are coming available at reasonable costs for breeding programs of livestock and also of dogs. DNA can be obtained shortly after birth allowing selection of candidates at a young age. The breeder can preselect the best candidates for the next generation around the time the animals are put up for sale or castration. With DNA data, based on regions containing QTL (see Box 1), the genetic ability can be evaluated. DNA data is especially valuable for sex-dependent traits, e.g. litter size can be recorded for females, whereas semen quality is observable in males only. In addition, DNA-data can be valuable for traits that are measurable late in life, or are expensive to score. In case DNA-chips (containing 1000's of SNPs across the genome) are used, information is available for all traits of interest simultaneously.

For mono-genetically inherited diseases, gene or marker tests have been developed and are implemented in dog breeding programs, e.g. PRA-tests (progressive retina atrophy) are commercially available for a wide range of breeds. In principle these type of tests could easily be included in DNA chips.

The orthopaedic diseases studied in this thesis, however, have a polygenic inheritance. In Table 8.3 it is shown that more regions can be identified, each explaining a relatively small part of the total genetic variation. Therefore a simple gene-test cannot be developed for these diseases. Results from the human field (Visscher, 2010 on human height) and commercial production animals indicate that it might be even impossible to identify all chromosomal regions affecting a trait. However, it doesn't imply that DNA data cannot be used for genetic improvement of poly-genetically inherited traits such as orthopaedic diseases.

The landmark paper by Meuwissen et al. (2001) showed that knowledge of chromosomal regions affecting a trait, QTLs (see Box 1), is not required as long as there are sufficient number of markers, e.g. SNPs, genotyped in the candidates.

SNP arrays provide a large amount of information but some information is more useful. Markers in the regions that have been identified in GWAS-studies, e.g. chapters 6 and 7 could receive more weight when using DNA information for calculation of EBVs. Breeding values

Box 1: Quantitative Trait Loci and Linkage Disequilibrium.

Quantitative Trait Loci (QTL) are putative loci, which have an effect on the phenotype/trait. QTLs are identified using genome wide association studies (GWAS) using markers, e.g. SNPs. These markers have been chosen based on allele frequency genotyping efficiency and spacing along the chromosomes and are therefore most likely not causative mutations that affect the trait. However, these markers are significantly associated with the trait indicating that in the region surrounding the marker(s) there must be polymorphisms, e.g. SNPs, CopyNumberVariants (CNVs), InsertionDeletions (InDel), that have an effect on the trait. Since these polymorphisms are not known, they are called QTL.

Linkage Disequilibrium (LD) is the non-random association of alleles between markers, but also between markers and disease status. i.e. when markers show significant association with the trait because they are in LD with the causative polymorphisms. It means thatQTL-alleles show the same inheritance pattern as the marker allele. In case the LD is 100%, the marker allele is a perfect proxy for the QTL-allele. In those cases selection based on the marker has a similar effect than selection on the QTL-allele and for breeding the knowledge of the QTLs is not needed. However since the QTLs are not known, also the LD between QTL- and marker allele is not known. LD depends on the distance in base pairs between the marker and QTL-allele: the smaller the distance the higher the LD. The amount of LD is determined by the population history, predominantly the effective population size. The smaller the population has been over time the larger the LD-blocks. Thus denser SNP-panels are required for larger effective populations.

based on DNA data are called genomic EBVs (gEBVs). In Table 8.3 the regions that were identified in this thesis to have an effect on a selection of orthopaedic disorders are listed. These regions should receive more weight when DNA information is used to estimate EBVs.

Using DNA-chips with 1000's of SNPs, the prediction model can be calculated and used to rank the genotyped candidates with respect to the breeding goal, from bad to very good. The calculation of the prediction model requires a reference population (Figure 8.2).

Reference populations preferably consist of a random sample of the general population, including parents used in the past, that are phenotyped and genotyped so that a statistical model can be developed to estimate the effects of all DNA markers. The estimated effects are subsequently used to estimate the genetic ability of animals without phenotype as shown in Figure 8.2.

The populations genotyped in this thesis for the different traits are a good start for the reference population. However, due to their limited size the statistical power was too low to identify all regions associated with the traits. Therefore the reliability of the prediction equation based on these populations is still insufficient to be used in breeding programs.

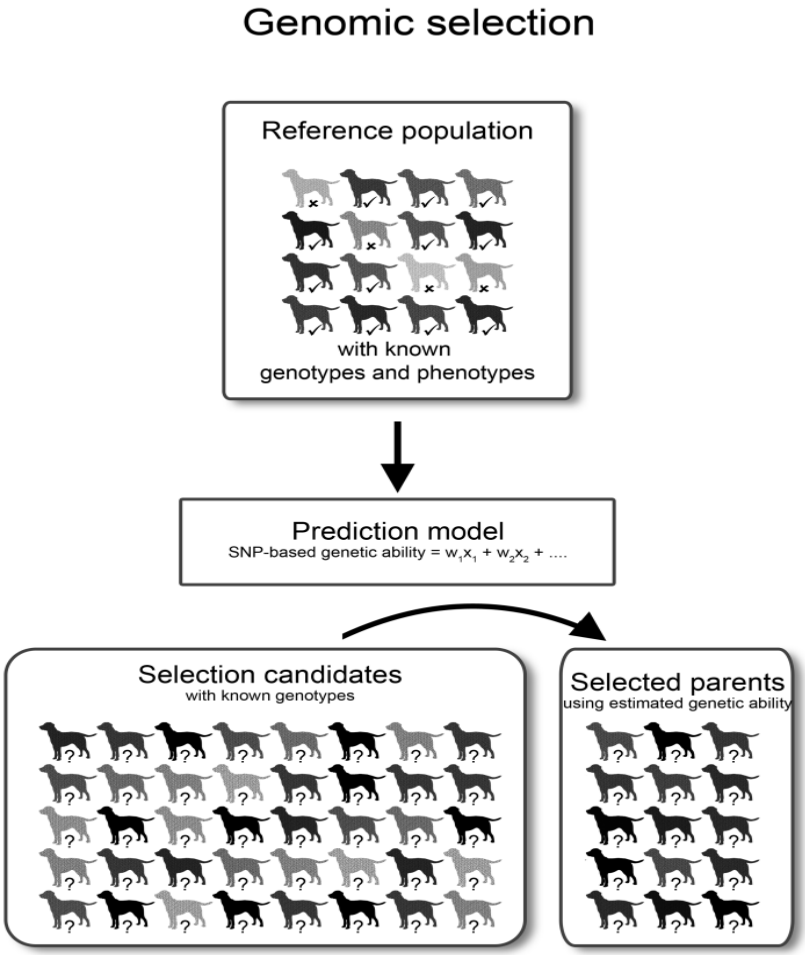

Figure 8.2 The use of reference populations to predict genetic ability. In the reference population on top, the disease status (✓ for healthy and ✗ for affected animals), for all dogs is known and used to calculate a SNP-based genetic ability (represented by different shades of gray). In a large pool of potential breeding animals without known phenotype (? in the figure), DNA is collected and the prediction model is used to estimate their genetic ability. The animals which are most likely to produce phenotypically sound offspring, are selected and used in breeding

Setting up an adequate reference population has been described by Daetwyler et al. (2013). It depends on the heritability, the effective population size, which determines the Linkage Disequilibrium between SNP and QTLs and the number of genes contributing to a trait, e.g. HD. For dog breeding populations it could be organized that a reference population is build up over generations. Enlarging the reference population allows for re-estimation of the prediction equation over time.

Setting up the initial reference population requires substantial funding, which might be covered by the general public funding. Testing all selection candidates with the SNP arrays might be prohibitively expensive. In case both parents have been tested with the array the candidates could be tested using a limited number of SNPs (3,000 to 4,000) and the omitted SNPs could be imputed using the parental information. This could substantially reduce the cost of genomic selection. Possibly prediction equations can be developed across breeds reducing the cost of setting up a reference population. Gene-tests for monogenic diseases could be included in this smaller SNP-dataset, which could lead to a further improvement of Genomic Selection. The additional value of DNA information is the possibility to manage genetic variability, i.e. prevent inbreeding. Using the SNP-chip, inbreeding and relatedness among potential mates can be calculated. However if genotyping costs are very high it is unlikely that many candidates will be genotyped which could have a negative effect on genetic variability. Although the use of DNA-data is useful, it could be counterproductive when it causes less animals to be screened.

When discussing the use of DNA data in breeding programs, five different aspects need to be considered:
1) Definition of the breeding goal
2) Data collection
3) Data analysis and estimation of the breeding values
4) Ranking of the parents for the next generation
5) Selection of mate pairs

Breeding programs are designed to improve a breed with respect to the traits of interest, e.g. reduction of the incidence of orthopaedic diseases. DNA-data has added value in both calculating the genetic ability more reliably (aspect 3) as well as in accurately ranking the potential breeding animals (aspect 4). However, DNA-data can also be used for the 5th aspect of a breeding program: combining the appropriate parents. Inbreeding and relatedness can be very well estimated and controlled using a limited number of SNPs (Windig, 2013 personal communication).

Recommended initiatives to improve effectiveness of breeding programs in dogs

Currently the preselection of potential breeding animals is only based on phenotype, and this introduced a major screening bias. A possible way to reduce this bias could be accomplished by randomly phenotyping one or several dogs from each litter.

Pedigree information is available for all kennel club registered dogs, but is currently not used in the selection of potential breeding animals. Calculating and, next to the phenotype of the animal, taking the estimated genetic ability into account when selecting animals for breeding would be the first step in integrating EBVs in Dutch breeding programs. The next improvement could be made by using DNA data (gEBVs).

Setting up a sufficiently large reference population is an expensive and timely endeavour. Therefore it is important to start collecting DNA of animals used for breeding, preferably both sires and dams, but at least of all sires. Then, when using DNA-assisted selection of breeding

animals is financially feasible, setting up a reference population would be much less time consuming.

Further research on the effectiveness of a reference population across breeds is needed. If the reliability of the prediction model is acceptable, setting up a joint, multi-breed reference population could reduce costs considerably.

Given the fast reduction in genotyping costs and increasing availability of statistical tools to analyse them, implementation of DNA data in breeding programs should be optimised, so that a combination of phenotypic data, pedigree data and DNA data can be used to give the best insight in responsible breeding, both for the individual animals, as well for the population as a whole.

References

Daetwyler HD, Calus MP, Pong-Wong R, de Los Campos G, Hickey JM., 2013. Genomic prediction in animals and plants: simulation of data, validation, reporting, and benchmarking. Genetics 193(2):347-65.

Lappalainen AK, Mölsä S, Liman A, Snellman M, Laitinen-Vapaavuori O. (2013). Evaluation of accuracy of the Finnish elbow dysplasia screening protocol in Labrador retrievers. J Small Anim Pract. 54(4):195-200.

Meuwissen TH, Goddard ME., 2001. Prediction of identity by descent probabilities from marker-haplotypes. Genet Sel Evol. 33(6):605-34.

Olsson SE. 1983. The early diagnosis of fragmented coronoid process and osteochondritis dissecans of the canine elbow joint. J Am Anim Hosp Assoc 19:616-26.

Paatsama S. 2000. Fédération cynologique internationale (FCI). In: Hereditary bone and joint diseases in the dog - osteochondroses, hip dysplasia, elbow dysplasia -. Morgan JP, Wind A,Davidson AP, Hannover: Schlütersche. 263-266 p.

Swenson L, Audell L, Hedhammar A. 1997a. Prevalence and inheritance of and selection for elbow arthrosis in bernese mountain dogs and rottweilers in sweden and benefit: Cost analysis of a screening and control program. J Am Vet Med Assoc 210(2):215-21.

Swenson L, Audell L, Hedhammar A. 1997b. Prevalence and inheritance of and selection for hip dysplasia in seven breeds of dogs in sweden and benefit: Cost analysis of a screening and control program. J Am Vet Med Assoc 210(2):207-14.

Tirgari M. 1974. Clinical radiographical and pathological aspects of arthritis of the elbow joint in dogs. J Small Anim Pract 15(11):671-9.

Visscher PM, McEvoy B, Yang J., 2010. From Galton to GWAS: quantitative genetics of human height.Genet Res (Camb) 92(5-6):371-9.

Woolliams JA, Lewis TW, Blott SC. 2011. Canine hip and elbow dysplasia in UK labrador retrievers. Vet J 189(2):169-76.

Chapter 9

Samenvatting
List of figures
List of tables
Dankwoord
Curriculum Vitae
List of publications

Samenvatting

Orthopedische ontwikkelingsstoornissen kunnen ernstige problemen veroorzaken bij honden. Betere inzichten in het ontstaan en verloop van deze aandoeningen zijn nodig om een goed screeningsprogramma en fokbeleid te kunnen handhaven In dit proefschrift wordt dieper ingegaan op heupdysplasie, elleboogdysplasie en patella luxatie, drie orthopedische aandoeningen die ernstige beperkingen kunnen creeren voor de mobiliteit van de hond.

Een uitgebreide beschrijving van de pathophysiologische achtergronden, als wel de manier waarop in Nederland op deze ziekten wordt gescreened wordt beschreven in **hoofdstuk 2**.

Tussen 2002 en 2010 zijn ruim 35,000 Nederlandse rashonden officieel gescreened op heupdysplasie, elleboogdysplasie of op beide. De rassen die het vaakst voor beide aandoeningen gescreened worden zijn de Labrador Retriever, de Golden Retriever, de Berner Sennenhond en de Newfoundlander. In deze vier rassen is het verplicht vast te stellen dat de honden vrij zijn van deze ziekten voordat er mee gefokt mag worden. In **hoofdstuk 3** werden de verschillende kenmerken die leiden tot de eindscore voor heupdysplasie (osteoarthritis, aansluiting, vorm en laxiteit) en elleboogdysplasie (osteoarthritis, sclerosis en "kuiltjes vorming") onderling vergeleken. Zoals verwacht was zowel de genetische als de residuele correlatie tussen kenmerken van de zelfde ziekte hoog. De correlatie tussen kenmerken van heupdysplasie en kenmerken van elleboogdysplasie was laag. Er is dus weinig bewijs dat dezelfde processen bijdragen aan het ontstaan van beide ziekten, hoewel dat niet gezegd kan worden van het verloop van de ziekten.

Erfelijkheidsgraden varieerden van 0.0 tot 0.37 voor HD-gerelateerde kenmerken en van 0.0 tot 0.39 voor ED-gerelateerde kenmerken. Voor beide ziekten is de phenotypische en genetische trend is maar licht gedaald sinds 2001, wat aangeeft dat de selectiepractijken zoals die in deze periode van 2001 tot 2009 toegepast zijn maar een licht positief effect hebben bereikt.

Elleboogdysplasie is een term die wordt gebruikt om een viertal ontwikkelingssstoornissen aan het ellebooggewricht te schrijven. In **hoofdstuk 4** wordt er dieper ingegaan op de manier van scoren en de verdeling van deze vier stoornissen in 2693 Labrador Retrievers, 1213 Golden Retrievers en 974 Berner Sennenhonden. Een "los processus coronoïdeus" (LPC) was de ontwikkelingsstoornis die het vaakst voor kwam, met een incidentie tussen de 5 en 15% bij deze drie rassen. De erfelijkheidsgraad varieerde van 0.06 tot 0.24. Sclerose aan de basis van de mediale processus coronoïdeus was radiographisch beeld dat het sterkst gecorreleerd was met LPC.

De term patella luxatie wordt gebruikt om een losse knieschijf te beschrijven. Deze aandoening wordt in verschillende hondenrassen gezien, maar het vaakst in de kleinere rassen. Een van de weinige grote rassen waar deze aandoening vaak gezien wordt is de Flat-coated Retriever (FCR). In **hoofdstuk 5** wordt de analyse van 3834 Nederlandse Flat-coated Retrievers beschreven, die gescreened werden tussen 1990 en 2007. In 23.6% van deze honden werd patella luxatie vastgesteld. Er was een duidelijk verschil tussen de sexes: 30% van de teven tegenover maar 17% van de reuen had patella luxatie. De erfelijkheidsgraad is vastgesteld op 0.17 in deze populatie, wat aangeeft dat omgevingsfactoren een grote rol spelen bij de ontwikkeling van deze aandoening. Het fokken met één aangedane ouder verhoogde de kans op patella luxatie

in de nakomelingen met 45% tegenover fokken met twee gezonde ouders. Sinds de start van het screeningsprogramma ging de incidentie van 28% naar 18%, maar de laatste jaren is er weinig vooruitgang geboekt.

De hoofdstukken 6 en 7 beschrijven de resultaten van twee genoomscans. In **hoofdstuk 6** wordt het genoom van 48 honden met heupdysplasie vergeleken met 30 gezonde honden. Zo´n 18.000 single nucleotide polymorphisms (SNPs) werden op met twee verschillende statistische methoden vergeleken. Significante associatie werd gevonden op chromosoom 8, als wel suggestieve associatie op de chromosomen 1, 5, 15, 20, 25 en 32. De exonen in deze regio's werden gesequenced, en resulteerden in verschillende kandidaatgenen die mogelijk de kans op het ontwikkelen van heupdysplasie verhogen. Deze kandidaatgenen, LAMA2, LRR1 en COL6A3, zijn betrokken bij de hypertrofe differentiatie van chondrocyten en de extracellulaire matrix integriteit van het basaal membraan en kraakbeen.

Hoofdstuk 7 beschrijft de genoom-brede studie van ruim 15.000 SNPs in 45 Flat-coated Retrievers met patella luxatie en 40 gezonde Flat-coated Retrievers. Een significante associatie werd gevonden op chromosoom 7, als ook suggestieve associatie op de chromosomen 3, 31 en 36. Net als in het vorige hoofdstuk werden ook hier de exonen in de geassocieerde gebieden gesequenced, deze keer in 15 patienten en een pool van controles. Ruim 7.000 variaties werden gevonden, waarvan 124 variaties een groot verschil in frequentie tussen de patienten en de pool van controles suggereerden. Deze werden gegenotypeert in een cohort van 95 Flat-coated Retrievers. Negen SNPs, in acht genen, waren gessocieerd met patella luxatie met een p-waarde van $<10^{-4}$.

Uit de studies bescheven in de hoofdstukken 3 en 5 van dit proefschrift blijkt dat de incidentie van heupdysplasie, elleboogdysplasie en patella luxatie de laatste paar jaar maar weinig is afgenomen. Dit lijkt erop te wijzen dat de huidige screeningsprogramma's en het fokbeleid dat eruit voortkwam, toe is aan een revisie. Immers, de ontwikkeling van alle drie de orthopedische aandoeningen in dit proefschrift zijn in sterke mate afhankelijk van omgevingsfactoren (zoals we af kunnen leiden uit de erfelijkeheidsgraden). Dit betekent dat de "gezonde" honden waarmee gefokt mag worden, nog steeds een hoge aanleg voor deze ziektes bij zich kunnen dragen, en dus ook door kunnen geven aan hun nakomelingen. In **hoofdstuk 8** wordt gediscussieerd over de mogelijke aanpassingen aan het huidige screeningsprotocol, waarmee de incidentie van deze aandoeningen mogelijk nog verder teruggedrongen kan worden.

List of figures

List of tables

Dankwoord

Het zit erop! Het enige wat nog rest is de vele mensen die me geholpen hebben bij het maken van dit boekwerk van harte te bedanken…

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Curriculum Vitae

Ineke Lavrijsen werd op 26 maart 1982 geboren in Reusel. Na het behalen van haar VWO diploma aan het Pius X College te Bladel, begon zij in 2000 aan de studie Biomedische Wetenschappen aan de Universiteit Utrecht.

In 2003 behaalde zij haar bachelor diploma in "Biomedical Sciences" na het schrijven van de bachelor thesis "The possible role of the PREP1 protein in Celiac Disease" onder leiding van dr. A. Monsuur and prof. C. Wijmenga.

Na goedkeuring van twee stageprojecten en een master thesis behaalde zij in 2006 ook haar master diploma in "Genomics & Bioinformatics". De master thesis werd geschreven onder leiding van dr. R. Hochstenbach en prof. D. Lindhout en had als onderwerp "X-inactivation in carriers of X-autosome translocations". De stages vonden plaats onder begeleiding van dr. R. Toonen en prof. M. Verhage ("The role of Munc 18-1 in secretory vesicle release") en dr. A. Monsuur en prof. C. Wijmenga ("Genetic background of Celiac Disease").

In juli 2006 begon zij haar promotie-onderzoek bij de faculteit Diergeneeskunde aan de Universiteit Utrecht, de resultaten daarvan zijn bescheven in dit proefschrift.

List of publications

Lavrijsen ICM, Leegwater PAJ, Wangdee C, van Steenbeek FG, Schwencke M, Breur GJ, Meutstege FJ, Nijman IJ, Cuppen E, Heuven HCM, and Hazewinkel HAW. Genome-wide survey indicates involvement of loci on canine chromosomes 7 and 31 in patellar luxation in Flat-Coated Retrievers. Submitted to BMC genetics,

Lavrijsen ICM, Leegwater PAJ, Martin AJ, Harris SJ, Tryfonidou MA, Heuven HCM, and Hazewinkel HAW. Genome wide analysis indicates genes for basement membrane and cartilage matrix proteins as candidates for hip dysplasia in Labrador Retrievers. PlosOne January 2014, DOI: 10.1371/journal.pone.0087735

Lavrijsen ICM, Heuven HCM, Breur GJ, Leegwater PAJ, Meutstege FJ, and Hazewinkel HAW. Phenotypic and genetic trends of patellar luxation in Dutch Flat-Coated Retrievers. Animal Genetics, December 2013, 44(6):736-41.

Lavrijsen ICM, Heuven HCM, Voorhout G, Meij BP, Theyse LFH, Leegwater PAJ, and Hazewinkel HAW. Phenotypic and genetic evaluation of elbow dysplasia in Dutch Labrador Retrievers, Golden Retrievers, and Bernese Mountain Dogs. The Veterinary Journal, August 2012, 193(2) pp 486-92.

Lavrijsen ICM, Heuven HCM, Meij BP, Theyse LFH, Nap RC, Leegwater PAJ, and Hazewinkel HAW. Prevalence and co-occurrence of hip dysplasia and elbow dysplasia in Dutch pure-bred dogs. Preventive Veterinary Medicine, January 2014, 114(2) pp 114-22.

Monsuur AJ, de Bakker PI, Alizadeh BZ, Zhernakova A, Bevova MR, Strengman E, Franke L, van't Slot R, van Belzen MJ, Lavrijsen ICM, Diosdado B, Daly MJ, Mulder CJ, Mearin ML, Meijer JW, Meijer GA, van Oort E, Wapenaar MC, Koeleman BP, Wijmenga C. Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect. Nature Genetics, September 2005, 37(12), pp. 1341-4.