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Identification of Quantitative Trait Loci (QTL) for radiological alterations in the navicular bone of Hanoverian warmblood horses

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To my family
and K. von Oertzen

Das größte Geheimnis ist der Mensch sich selbst

Novalis

Parts of this work have been submitted for puplication in the following journals:

1. Mammalian Genome
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Contents

List of abbreviations

ALPL	alkaline phosphatase
APS	ammoniumperoxidsulfat
ATM	animal threshold model
BLAST	basic local alignment search tool
bp	base pairs
CDH11	cadherin 11
cDNA	complementary deoxyribonucleic acid
CFB	core binding factor
CFBF	core binding factor, beta subunit
cM	centiMorgan
CMC	first carpometacarpal
COL16A1	type XVI collagen alpha chain
dATP	deoxy adenine triphosphate
DCS	deformed canales sesamoidales
dCTP	deoxy cytosine triphosphate
dGTP	deoxy guanine triphosphate
DIP	distal interphalangeal
DMSO	dimethylsulfoxid
DNA	deoxyribonuclein acid
dNTP	deoxy nucleoside triphosphate
dTTP	deoxy thymine triphosphate
ECA	chromosome of equus caballus
EDTA	ehtylenediamine-tetraaceticacid
EST	expressed sequence tag
GS	Gibbs sampling
HET	mean observed heterozygosity
HSA	chromosome of homo sapiens
HW	Hanoverian Warmblood
h^2	heritability
IBD	identical-by-descent
INRA	Institut National de la Recherche Agronomique

KWPN	Koninklijke Vereniging Warmbloed Paardenstamboek Nederland
LAM	linear animal model
LSM	linear sire model
MATN1, 3	matrilin 1, 3
Mb	megabases
MCP	metacarpophalangeal
MMP-2, -9, -15	matrix metalloproteinase 2, 9, 15
OA	osteoathritis
OMIN	Online Mendelian Inheritance in Man database
PCR	polymerase chain reaction
PIC	polymorphism information content
PIP	proximal interphalangeal
QTL	quantitative trait loci
RAC	radiological alterations in the contour of the navicular bone
RAS	radiological alterations in the structure of the navicular bone
RH	radiation hybrid
REML	residual maximum likelihood
SNP	single nucleotide poly morphism
STM	sire threshold model
Ta	annealing temperature
TBE	TRIS - Boric acid – EDTA
TE	tournment entries
TEMED	tetramethylendiamine
TIP	thump interphalangeal
TP	tournment placing

Introduction

Navicular bone disease is a hereditary defect which is a problem in many horse populations. The disease is characterised by a chronic and shifting forehand lameness which is often therapy-resistant and in the long term limits the durability and use of riding horses.

A correct lameness examination with diagnostic analgesia and radiography is the current method for the diagnosis of podotrochlosis. The radiographs of affected animals show either a high number of or deformed canales sesamoidales (DCS) or an irregular contour or loss of the normal fine-woven structure. Because of the complex anatomy there are also cases without pathological radiographic findings, where only bony components are visible in detail. Application of computed tomography or magnetic resonance imaging medicine in horses can depict the distal part of the limb in more detail but these apparatuses are expensive and are not part of the equipment of a normal veterinary practice.

The onset of navicular disease can sometimes be late in life, and first signs of the defect are often not noticed before the age of three to four years. Middle-aged riding horses in particular show the largest incidence of clinically manifest navicular bone disease at about seven to nine years of age. The first signs of navicular bone disease can be seen both in younger lameness patients as well as in clinically healthy horses of all ages, including foals. Thus, prevention of navicular disease cannot be achieved solely by exclusion of affected animals from breeding.

Consequently, there is an urgent need for a molecular genetic diagnosis of carriers. The objective of the present study was to identify the genomic regions harbouring the quantitative trait loci (QTL) responsible for radiographic alterations in the navicular bone. In order to achieve this goal, a whole genome scan was performed and refined in selected chromosomal regions to identify the genomic regions harbouring the gene loci responsible for radiographically visible navicular bone alterations.

Overview of chapter contents

Chapter 1 reviews the literature particularly for navicular disease and for radiological alterations in the distal sesamoid bone in horses, including the anatomy, clinical signs, aetiology, pathogenesis, prevalences and genetic factors.

Chapter 2 gives an overview of the prevalence of the different traits analysed here: DCS and radiological navicular bone alterations in the contour (RAC) and structure (RAS). Genetic parameters and influences on the prevalences of these traits were also analysed.

Chapter 3 describes the whole genome scan and the refined scan of chromosome regions performed on seventeen families of the Hanoverian Warmblood horse. The traits analysed were the occurrence of DCS, RAC and RAS.

Chapter 1

Genetic aspects of radiological alterations

in the navicular bone of the horse

Genetic aspects of radiological alterations in the navicular bone of the horse

Abstract

Lameness problems are the main reasons for premature retirement and culling of horses. Navicular disease or podotrochlosis has long been known to cause forelimb lameness. In addition to the detailed clarification of the structure and anatomy of the podotrochlea, hypotheses have been proposed to explain the development of podotrochlosis chronica aseptica which has similarities to the human osteoarthritis (OA) complex. The function of the podotrochlea is to distribute traction and pressure forces in the equine foot. Early diagnosis of navicular disease is possible only on the basis of radiographic findings. However, there are still difficulties in the interpretation of these radiological changes. Reports on the prevalences of navicular disease indicate that radiological alterations in the navicular bone are present in different warmblood populations at frequencies of between 14.9% and 87.6%. Genetic factors play an important role in the development of the radiological signs. Estimates of heritability using animal threshold models range from $h^2 = 0.09$ to $h^2 = 0.40$. Estimated additive genetic correlations between radiological changes in the navicular bone and other orthopaedic health traits indicated that they mostly develop genetically independently of each other. There was a negative genetic correlation between radiological changes in the navicular bone and the number of tournament entries and placings. It has also been shown that reduction of radiological changes of navicular bones and improvement of breeding values for performance of riding horses can be achieved if selection is based on breeding values for these traits simultaneously. An optimised markerset was developed to detect quantitative trait loci for the various traits (canales sesamoidales, contour and structure) describing the radiographic status of the navicular bone.

Introduction

Disorders of the musculoskeletal system are the main reason for premature retirement and culling of horses (PHILIPSSON et al. 1998, WALLIN et al. 2000). In many cases the distal part of the equine limb is affected by pain and an underlying pathological condition. Navicular disease is one of the main causes of chronic, often therapy-resistant forelimb lameness in middle-aged horses (LOWE 1974, ACKERMANN et al. 1977, BODENMÜLLER 1983, BRUNKEN 1986, WRIGHT 1993a). In the long term, this

condition may limit durability and use of riding horses (PHILIPSSON et al. 1998, WALLIN et al. 2000, STOCK and DISTL 2006a).

Navicular disease, which is also called navicular syndrome or podotrochlosis, is defined as a chronic and usually progressive, degenerative alteration of the equine podotrochlea (RIJKENHUIZEN 1989). Pathological alterations can primarily affect the navicular bone (os sesamoideum distale), the navicular bursa (bursa podotrochlearis) or the distal end of the deep digital flexor tendon. The disease may be part of the osteoarthritis complex (SVALASTOGA 1983, SVALASTOGA and SMITH 1983).

However, the specific aetiopathogenesis of navicular disease is not yet known. This paper will discuss the different aspects of alterations in the navicular bone and of navicular disease, and the factors that may play a role in its aetiopathogenesis.

Clinics

Function, anatomy and histology of the podotrochlea

The podotrochlea comprises the navicular bone (distal sesamoid bone), the insertion part of the deep digital flexor tendon and the bursa podotrochlearis. Together with coffin bone the navicular bone forms the socket for the joint roll of the pastern bone, enlarging the weigth bearing area (HERTSCH et al. 1982, HICKMAN 1989, WRIGHT 1993a, WISSDORF et al. 1998, LITZKE 1999). According to LITZKE (1999), the navicular bone together with its bursa builds a deflection plate for the deep digital flexor tendon. The dorsal joint faces for the coffin bone and pastern bone are coated with hyaline cartilage, while the palmar flexor surface is coated with fibrous cartilage (WINTZER and DÄMMRICH 1971, WRIGHT 1993a). The fibrous cartilage contains no nerves or blood vessels or lymph vessels (HICKMAN 1989). The shape of the proximal margin can be convex, undulating, linear or concave, depending on the age of the horse (DIK et al. 1999). According to WISSDORF et al. (1998), the proximal and distal margins contain numerous small nutritive foramina, which can be seen radiographically if extended. The canales sesamoidales distales are lined with normally structured synovial cells (HERTSCH et al. 1982, WINTZER 1964, WISSDORF et al. 1998) and are consequently regarded as part of the coffin joint. The vessels (arteriae nutritiae) always pass extra-articularly at the margins of the bony immersions (DROMMER et al. 1992).

The distal sesamoid impar ligament extends between the distal margin of the navicular bone and the coffin bone, near the insertion of the deep digital flexor tendon (CHRISTIANSON and REINERTSON 1984). The collateral ligaments originate from the distal part of the

pastern bone and insert at the proximal margin of the navicular bone with branches to the hoof cartilage (LEACH 1993).

The blood supply of the navicular bone emanates from the medial and lateral digital arteriae. The distal navicular ramus, the palmar phalangis mediae ramus and the anastomosing medial and lateral navicular rami provide the blood supply for the navicular bone (RIJKENHUIZEN 1989).

The podotrochlea and the surrounding structures are innervated from the lateral and medial digital palmar nervs, but anatomical variations exist (CHRISTIANSON and REINERTSON 1984).

Radiographic findings in the navicular region and their pathological evaluation

Radiographic examination is an important diagnostic tool in clinical practice. Radiographic examination of the navicular bone can be performed using three different projections. Lateromedial projection provides an overlap-free representation of the navicular bone. In this projection only alterations of the contour are of significance (KLESSINGER 1973). According to SEYREK-INTAS et al. (1999), this projection makes it possible to assess thickness, structure and surface of the bony endplate, structure of the spongiosa, and number and shape of the canales sesamoidales and the exophytes at the proximal and distal margins.

OXSPRING (1935) described two different methods for dorsopalmar projection: the high coronary route and the upright pedal route. The distal margin of the navicular bone superposes the coffin joint space. The dorsopalmar projection allows evaluating the canales sesamoidales, their number, depth and shape, and the contour, structure and bony thickness of the navicular bone.

The tangential projection was first described by MORGAN (1972) and is conducted to illustrate the flexor surface. This technique makes it possible to obtain an extensive, overlap-free picture of exophytes at the flexor surface, and to show the idensity and structure of the subchondral compacta of the sliding surface, the transition zone from the compact part to the spongyous part of the bone, and the structure of the spongiosa including the canales sesamoidales of the navicular bone. Furthermore, this projection permits distinction between break-ins of the tendon sliding surface and cystoid defects in the navicular bone (HERTSCH and HÖPPNER 1999). According to SEYREK-INTAS et al. (1999), changes in the contour of the lateral ends of the navicular bone can be also assessed with this projection.

The radiologically sound navicular bone shows undeformed and short canales sesamoidales, a finely woven, regular structure and a contour without exostoses. Radiographic findings have

been associated with navicular bone pathology such as branched or lollipop-shaped canales sesamoidales, an irregular, sclerosed or radiolucent structure, and a contour with exostoses (OXSPRING 1935, BRUNKEN 1986, HERTSCH and STEFFEN 1986, KASER-HOTZ and UELTSCHI 1992, WRIGHT 1993b).

Several evaluation schemes have been developed for radiographs of the navicular bone. DIK and VAN DEN BROEK (1995) classified navicular bones on the basis of the shape of the proximal border, whilst LUKAS (1987) considered both shape of the proximal border and of the medial and lateral extremities of the navicular bones. The evaluation scheme of BRUNKEN (1986) permits a highly differentiated recording of the different appearances of the navicular bone by classifying radiographic findings in the navicular bone according to size, shape and distribution of canales sesamoidales and the structure and contour of the navicular bone.

CAMPBELL and MACGREGOR (1984) found that horses with diagnosed navicular bone lameness had at least 8.1 ± 1.7 canales sesamoidales compared with normal horses, which had 5.5 ± 1.6 canales sesamoidales. Furthermore, these investigators showed that more canales sesamoidales were enlarged and of abnormal shape in horses with navicular disease than in clinically sound horses. According to WINTZER (1964) moderate changes in the navicular bone included the presence of more than nine canales sesamoidales with partly lollipop-shaped ends maximally reaching to the middle of the navicular bone; severe alterations of this bone were characterised by nutritive foramina with large inner diameters. According to HERTSCH (1984), an increased intra-articular pressure in the distal interphalangeal joint during the loading phase was responsible for the enlargement of the canales sesamoidales. DELIUS (1982) demonstrated that deep canales sesamoidales with bulb-shaped ends were associated with a high degree of degradation of the tendon sliding surface. HERTSCH and STEFFEN (1986) found out that moderate (up to one-fourth of the navicular bone width) and extensive (more than one-fourth of the navicular bone width) elongation of radiologically visible canales sesamoidales were associated with pathologic changes in 50% of the flexor surface. The higher the numbers of canales sesamoidales of a certain length, the more frequent were pathological changes in the tendon sliding surface. These pathological changes can include a rough, yellow to brownish tendon sliding surface with unchanged cartilage thickness; extensive rarefaction or translucency of the bone; complete rarefaction of the bone and adhesion between the flexor surface and the deep digital flexor tendon; and/or the breakdown of the bony border lamella. Only at this stage navicular disease is directly educible from radiography (HERTSCH 1984). Changes in the fibrous cartilage of the flexor surface

and subsequently in the deep digital flexor tendon caused pain due to microdisruptions during exercise.

Sprains and distensions of the insertion of the sesamoid ligament cause the formation of exophytes at the proximal margin, at the extremities and at the distal margin; the latter are known as insertion desmopathies (HERTSCH et al. 1982, HERTSCH and STEFFEN 1986). At this stage, the regular shape of the navicular bone is lost.

Abnormal colouration of the cartilage was seen twice as often in combination with coarse meshed structure of the navicular bone than with fine-meshed structure of the navicular bone. Navicular bones with a blank bone, adhesions with the tendon sliding surface and the deep digital flexor tendon and breakdown of the bony border lamella were six times more frequent in coarsely meshed navicular bones. With progressing disease, these alterations continued to rise in severity with radiologically visible sclerosis (HERTSCH and STEFFEN 1986).

However, radiological examination and evaluation of the navicular bone is considered to be one of the most demanding and controversial tasks in veterinary radiology. Radiographic alterations in the navicular bones that are considered to be pathological do not necessarily involve lameness problems or constrained gaits, but have also been observed in clinically healthy horses of all ages (BRANSCHEID 1977, TURNER et al. 1986, KASER-HOTZ and UELTSCHI 1992, HORNIG 1993). In light of the variation in the progress of the disease, any statements as to the prognosis will always be subjected to dispute if based on the result of only a single radiological examination (BRUNKEN 1986, SEYREK-INTAS 1993). On the other hand radiographic findings should always be taken into account in the connection with clinical findings.

Clinical signs and diagnosis

Often no specific conclusions can be drawn from the anamnesis of the patient with podotrochlosis syndrome. Most horses have a history of slight intermittent lameness (ACKERMANN et al. 1977). Diagnosis of navicular disease is to be considered if, during adspection of the standing animal, the horse places its front limbs alternately and inflected forward to relieve the painful rear part of the hoof (SILBERSIEPE et al. 1986). The axis of the toe is broken in one or both of the digital joints resulting in irregular loading of the navicular bone eventually promoting development of navicular disease. Decrease of stride length is accompanied by stepping on the tips of the toes, sometimes with a tendency to stumble (GIBSON and STASHAK 1990). These signs vary from minor gait irregularity to slight or moderate lameness (HERTSCH and HÖPPNER 1999). HERTSCH (1991) found no

hoof form typical of navicular disease. However, deficient or improper hoof care can result in a tapered hoof with twisted or rolled-up heels.

Marginal exostoses at the coffin and pastern joints are often responsible for positive results of hyperextension and rotation pain tests of the tower limb (HERTSCH and BEERHUES, 1988). Possible provocation tests may assist diagnosis of navicular disease, but they unspecifically indicate pain in the distal limb (CHRISTIANSON and REINERTSON 1984, , TURNER 1989, GIBSON and STASHAK 1990, HERTSCH and HÖPPNER 1999).

Anaesthesia of the digital nerves (rami tori digitales, deep palmar nerves), leads to analgesia of the coffin joint and the bursa podotrochlearis and is used for diagnosis of lameness originating from the navicular bone. Typical outcome is shifting of the lameness from left to right frontlimb or vice verse.

The measurement of pressure within the coffin joint is of particular interest (SVALASTOGA and SMITH 1983, HÖPPNER 1993). The changes in pressure during movement and standing affect the synovia, the viscoelasticity of which is reduced during an inflammatory process.

According to RIJKENHIUZEN (2006), treatment has focused on alleviating clinical signs rather than curing the underlying disease process.

Aetiology and pathogenesis of navicular disease

It is generally accepted that an abnormal limb position, excessive loading and genetic factors (ACKERMANN et al. 1977, MEYER 1979, DÄMMRICH et al. 1983, BOS et. al. 1986, KWPN 1994, PAGAN et al. 1996, WINTER et al. 1996, PHILIPSSON et al. 1998, WILLMS et al. 1999, STOCK et al. 2004) may promote the development of podotrochlosis. RIJKENHUIZEN (2006) described in detail the biomechanical stress supported by biochemical changes as causative factor for navicular disease. However, it is not only the centrally positioned navicular bone, on which the most biomechanical hypotheses were focused; all structures situated within the hoof were affected by similar biochemical influences and may therefore play some role inpathogenesis. Better understanding of the factors that cause pathological changes in the navicular bone may help to define hereditary factors.

Prevalence and age of onset

Prevalence

Radiographic examinations have been carried out in different horse populations to establish the prevalence of alterations in the navicular bone (Tables 1 and 2). Most studies did not

distinguish between deformed canales sesamoidales, and changes in the contour and structure of the navicular bone. HEINZ (1993), THOMSEN (1995) and KIRCHNER (1996) examined Holsteiner warmblood horses at the age of five to nine months (n=220), as yearlings (n=190), and at two years of age (n=151). These investigators showed that the occurrence of six or more canales sesamoidales increased with age (0.3%- 1.7%). The number of narrow and small canales with bulb-shaped ends and of expanded canales with large bulb-shaped ends also increased from foal age to age of two years (0.7%- 7.6%). WILLMS et al. (1999) used the data of the authors mentioned above and analysed them anew.

One reason for these findings may be the still incomplete growth of young horses, so that normal processes may be mistaken as signs of pathological remodelling activity. BOS et al. (1986) reported that 87.6% of Dutch warmblood horses between three and four years of age had alterations in the navicular bone. The study of the KWPN (1994) reported radiological navicular bone changes in 15.5% of 590 mares. WINTER et al. (1996) found that 78.7% of 3566 German riding horses of between three and eight years of age showed slight or moderate changes in the navicular bone. MÜLLER (1982) found changes in 83.8% of 754 Hanoverian warmblood horses examined. MEINERS (2004) classified changes in the number and length of the canales sesamoidales as slight changes; deformed canales sesamoidales and lucency of the navicular bone as moderate changes; and structural changes such as cystoid defects and branched or bulb-ended canales sesamoidales as severe changes and found mild to severe changes in 27.5% of 6184 Hanoverian warmblood horses of between three and eight years of age. Similar prevalences were reported by STOCK et al. (2004) for 3748 Hanoverian warmblood horses, 14.9% of which had slight, 5.3% moderate and 1.8% severe radiological changes in the navicular bone. STOCK and DISTL (2006b) reported in more detail about the prevalences of the different kinds of radiological alterations in the navicular bone. Of 5157 three-to seven-year-old horses, 59.43% showed no abnormal findings in the navicular bone, 20.48% had few (1–4) short and conical canales sesamoidales and 16.97% had few deformed canales sesamoidales. Deformed canales sesamoidales (DCS) were seen in 2.23% of the horses, radiological alterations in the contour of the navicular bone (RAC) in 0.5% and in its structure (RAS) 0.39% of the horses.

Age of onset

There are very few reports on prevalences of particular radiographic findings in the navicular bones of foals. The work of BRANSCHEID (1977) indicated that postnatal development of the navicular bones proceeds up to about 18 months of age. According to LUKAS (1987)

problems in the evaluation of the navicular bone structure in horses of less than about 12 months of age result from the superimposition of the coarse structure of the coffin bone (phalanx media) over the navicular bone. He also found few affected horses up to the age of about 16 months and low prevalences of mostly minor radiographic findings in horses of between 17 and 28 months of age. However, STOCK et al. (2004) found no significant influence of age on the prevalence of pathologic changes in the navicular bone in three- to seven-year-old German warmblood horses. KWPN (1994) also reported no significant effect of age in 590 Dutch warmblood mares. Navicular disease associated with lameness appears to be mainly a problem of middle-aged horses, with a maximum incidence at the age of about seven to nine years (ACKERMANN et al. 1977, BODENMÜLLER 1983, BRUNKEN 1986, WRIGTH 1993a). However, radiographically detectable changes in navicular bones also occur both in younger lameness patients as well as in clinically healthy horses of all ages including foals (BRANSCHEID 1977, KASER-HOTZ and UELTSCHI 1992, HORNIG 1993).

Genetics

Influence of exterior parameters, sex and breed

Exterior parameters

WILLMS et al. (1999) found no significant correlation of the height at withers with radiographic findings indicative of podotrochlosis. STOCK et al. (2004) found a significantly positive genetic correlation only for moderate alterations in the navicular bone. Larger horses were more likely to be classified with moderate alterations in the navicular bone than smaller horses. In Dutch warmblood mares, there was no significant effect of height at withers on alterations of the navicular bone (KWPN 1994). WINTER et al. (1996) showed a positive genetic correlation between alterations in the navicular bone and a better evaluation of front limb conformation.

Sex

WINTER et al. (1996) reported a higher proportion of affected female horses in different German warmblood horses. Other investigators reported that males were more often affected (LOWE 1974, ACKERMANN et al. 1977, MÜLLER 1982) or found no differences regarding sex (BODENMÜLLER 1983). MEINERS (2004) found a significant sex effect, with males being more likely to be affected by slight or moderate alterations of the navicular bone.

STOCK et al. (2004) found that male horses carried a significantly higher risk only for showing moderate changes in the navicular bone.

Breed and gene proportion

JORDAN (1996) reported significant differences between the radiographic appearances of the navicular bones in Freiberger horses and Swiss halfbred horses. Swiss halfbred horses affected by podotrochlosis had more often deformed canales sesamoidales and a distally elongated flexor surface. Reports for thoroughbreds were inconsistent. LOWE (1974) reported that thoroughbreds had a significantly greater risk of navicular disease than all examined breeds combined or any other examined breed. The studies of KWPN (1994) and STOCK et al. (2004) showed no correlation between the prevalence of radiologically visible navicular bone alterations and the proportion of English thoroughbred genes in Dutch and German warmblood horses, respectively. However, MEINERS (2004) and STOCK et al. (2004) came to the conclusion that a higher percentage of Hanoverian and Holsteiner warmblood genes significantly increased the risk of alterations in the navicular bone. Several studies have shown Quarter horses to be at a higher risk of developing podotrochlosis (LOWE 1974, ACKERMANN et al. 1977, TURNER et al. 1986) than Arabs and ponies (LOWE 1974).

Genetic factors

Population genetics

Many authors have speculated on genetic influences on navicular disease (ROONEY 1979, ADAMS 1980, LAUNER et al. 1990). In a study of the KWPN (1994) based on 590 mares sired by 30 stallions, the heritability of radiologically visible navicular bone changes was estimated at between $h^2 = 0.26$ and $h^2 = 0.32$. WILLMS et al. (1999) estimated heritabilities for radiographically visible navicular bone changes at between $h^2 = 0.20$ and $h^2 = 0.31$ in 472 three-year old mares descending from 97 different stallions and at $h^2 = 0.20$ to $h^2 = 0.25$ in 220 foals of Holsteiner warmblood horses. Some of the foals were re-examined as yearlings ($n = 190$) and as two-year-olds ($n = 151$). WINTER et al. (1996) analysed 3566 German warmblood horses descending from 862 stallions selected for auction sale without clinically manifest navicular disease. The heritability estimate of $h^2 = 0.06$ in a linear animal model for alterations of the navicular bone was underestimated because no threshold model or transformation onto the liability scale was used. STOCK et al. (2004) estimated heritabilities of $h^2 = 0.09$ to $h^2 = 0.21$ for radiological changes of different severity in the navicular bone. In a further study, using different definitions of categorical traits, STOCK and DISTL (2006b)

estimated heritabilities of between $h^2 = 0.10$ and 0.34 for radiographic findings in the navicular bone. Low heritability estimates were reported for radiographic findings generally considered to represent physiological variation. These analyses indicated that radiological changes of the navicular bone are genetically influenced.

Estimated genetic correlations between navicular bone alterations and other orthopedic health traits were not consistent. STOCK and DISTL (2006c) estimated additive genetic and residual correlations between osseus fragments in fetlock and hock joints, deforming arthropathy in hock joints and pathological changes in the navicular bones. In 5231 Hanoverian warmblood horses of three to seven years of age those authors found a negative additive genetic correlation between pathologic changes in the navicular bone and osseus fragments in hock joints. The genetic correlations were close to zero between pathologic changes in the navicular bone and other considered radiographic findings in the limbs. In further study on 3725 three to seven-year old horses the additive genetic correlations between radiologically visible alterations in the navicular bone and osseus fragments in fetlock and hock joints were close to zero STOCK and DISTL (2006a). However, there was a moderately negative additive genetic correlation between radiologically visible alterations in the navicular bone and deforming arthropathy in hock joints. Heritability estimates of radiographic findings implied that it is possible to include radiographic health traits in genetic evaluation and selection schemes.

STOCK and DISTL (2005a) came to the conclusion that it is feasible to develop a multitrait selection scheme to simultaneously account for performance parameters and for the most important radiological changes in the limb including the radiological status of the navicular bone. The expected relative decrease of radiological navicular bone alterations per generation was 7.3% and the breeding values of dressage as well as show jumping increased by 6.4% and 6.1% using equal weights for performance and radiographic traits. In contrast the prevalence of navicular bone alterations decreased by only 3.2% and the breeding values for dressage and show jumping increased by 7.3 and 7.1% if only performance traits were used for selection. These results were confirmed in a subsequent study on expected response to selection over two-generations (STOCK and DISTL 2005b). The study of KWPN (1994) used categorisation of horses in five grades (according to DIK et al. 1978; grade 0 = excellent, grade 4 = bad) and showed that the risk of being affected by third-grade and by third- to fourth-grade changes in the navicular bone was twice as high in the offspring of affected stallions as in the offspring of unaffected stallions. Mating a grade-1 stallion to a random mare caused a 9% risk of grade-3 navicular bone defects and a 1% risk of grade-4 defects in the offspring. When a grade-3

stallion was used instead, the risk of grade-3 and grade-4 offspring increased to as much as 20%. The same effect was shown if a grade-3 mare was mated to a grade-1 or grade-3 stallion. In 1997 the Dutch warmblood studbook started a selection programme for stallions on the basis of radiographs of the navicular bone together with related criteria such as conformation and athletic activity. This resulted in a reduction of the prevalence of third- to fourth-grade navicular bones from 11% in 1997 to 3% in 2002 (VAN DEN BELT et al. 2003).

STOCK and DISTL (2006a) analysed the relationships between radiologically visible changes in the navicular bone and the number of annual entries and placings as measures of performance of riding horses. They found negative additive genetic correlations between pathological changes in the navicular bone and the number of annual tournament entries (TE) and placings (TP) in both basic build-up competitions ($r_g = -0.48 \pm 0.09$ for TE and $r_g = -0.18 \pm 0.16$ for TP) and in show jumping competitions ($r_g = -0.24 \pm 0.05$ for TE and $r_g = -0.32 \pm 0.09$ for TP). The additive genetic correlations between radiologically visible navicular bone alterations and TE and TP for dressage competitions were close to zero, with $r_g = 0.05 \pm 0.16$ (TE) and $r_g = 0.02 \pm 0.25$ (TP).

Molecular genetics

We prepared a microsatellite marker set to be applied in Hanoverian warmblood horses for a whole genome scan and tested this set in 144 descendants of 17 Hanoverian warmblood stallions. The genotyped horses were randomly sampled from the whole Hanoverian warmblood breeding district. Table 4 shows the characteristics of the whole marker set for each chromosome including 161 microsatellite markers. Furthermore, the distances between the markers are given according to the linkage map of SWINBURNE et al. (2006). If the microsatellites were not located on this map, we used the linkage map of PENEDO et al. (2005) and the equine radiation hybrid map of CHOWDHARY et al. (2003).

Since the marker set was developed before the maps of PENEDO et al. (2005) and SWINBURNE et al. (2006) were available, the current marker set showed large gaps on equine chromosomes 14 and 28 (ECA), which were closed by adding new informative markers on these chromosomes. The estimated length of ECA14 increased to 152.8 cM (SWINBURNE et al. 2006) and 168.4 cM (PENEDO et al. 2005), whereas only 46 cM were covered by markers on the previous linkage maps reported by SWINBURNE et al. (2000a). A higher marker density also led to higher estimates for the length of chromosome ECA28. The estimated length of ECA28 was 74.9 cM in the map of PENEDO et al. (2005) and 63.1 cM in

the map of SWINBURNE et al. (2006), while previous maps included either only very few markers on ECA28 (SWINBURNE et al. 2000) or only one linkage group of approximately 180 cR (CHOWDHARY et al. 2003).

According to SVALASTOGA and SMITH (1983) increased bone marrow pressure and lengthened contrast passage indicate similarities between osteoarthritis (OA) in humans and navicular disease in horses. About 50 different positional candidate genes have been reported for OA in humans. These candidate genes encode different types of collagens, hormone receptors and interleukin receptors, growth factors and metalloproteinases. About 13,966 equine cartilage expressed sequence tags (ESTs) and further 23,171 ESTs from other cDNA libraries as well as BAC end sequences or whole genome sequences can be used for identification of single nucleotide polymorphisms in functional and positional candidate genes. However, since navicular disease does not occur in humans, it is not clear whether genes for OA are suitable candidates for navicular bone disease in horses.

Conclusions

The aetiology and pathogenesis of navicular disease in horses remain unclear. However, several genetic studies have shown the impact of genetic factors on radiological alterations in the navicular bone. In addition, there are some indications of negative genetic correlations between radiologically visible changes in the navicular bones and sport performance. A marker set was developed and tested for its suitability for a whole genome scan to identify genomic regions which contain quantitative trait loci for the various traits describing the radiographic status of the navicular bone.

Table 1 Prevalences of radiographic alterations in the navicular bone in different horse populations

Population	No. of horses examined	Age (years)	Prevalence (%)	Reference
Hanoverian warmblood horses	754	3–7	83.8	MÜLLER (1982)
Dutch warmblood horses	169	3–4	87.6	BOS et al. (1986)
Dutch warmblood horses	Mares: 590 Stallions: 30	3–4	15.1 23.3	KWPN (1994)
Dutch warmblood horses	586	3	14.9	DIK and VAN DEN BROEK (1995)
German riding horses	3566	3–8	20.1[a] 58.6[aa]	WINTER et al. (1996)
Holsteiner warmblood horses	Mares: 472	3	51.0[a] 11.0[aa] 15.0[aaa]	WILLMS et al. (1999)
	Foals: 220	0.4–0.8	18.5[a] 1.5[aaa]	
	Yearlings: 190	1	24.7[a] 0.5[aa] 1.1[aaa]	
	Two-year-olds: 151	2	38.4[a] 0.7[aa]	
Hanoverian warmblood horses	6184	3	18.35[a] 3.97[aa] 1.56[aaa]	MEINERS (2004)
		4	20.74[a] 4.75[aa] 1.54[aaa]	
		≥ 5	24.88[a] 5.26[aa] 2.27[aaa]	
		3–8	21.09[a] 4.67[aa] 1.71[aaa]	
			27.49[b]	
Hanoverian warmblood horses	3748	3–7	14.9[a] 5.3[aa] 1.8[aaa]	STOCK et al. (2004)

a: slight alterations; aa: moderate alterations; aaa: severe alterations;
b: central lucency in the navicular bone

Table 2 Prevalences of specific radiographic alterations in the navicular bone in different horse populations

Population	No. of horses examined	Age (years)	Prevalence of			Reference
			DCS (%)	RAS (%)	RAC (%)	
Hanoverian warmblood horses	754	3-7	48.67[a] 44.69[aa] 9.28[aaa]	3.98[d]	9.15	MÜLLER (1982)
Holsteiner warmblood horses	Mares: 363	3	11.02[b] 26.72[bb] 15.70[bbb]	1.65[d]	11.85	MÜLLER (1994)
Hanoverian warmblood horses	5157	3-7	1.26[c] 0.33[cc] 0.64[ccc]	0.39	0.50	STOCK and DISTL (2006a)

DCS: deformed canales sesamoidalessesamoid channels; RAS: radiologically visible alterations in the structure; RAC: radiologically visible alterations in the contour
a: 1 to 2 visible canales sesamoidales; aa: 2 to 4 clear expanded canales sesamoidales; aaa: 5 to 6 clearly expanded canales sesamoidales
b: 6 and more canales sesamoidales; bb: canales sesamoidales with a length > ¼ of the navicular bone width; bbb: canales sesamoidales with off-branching
c: few (1-4) deformed canales sesamoidales; cc: several (≥5) deformed canales sesamoidales; ccc: diverse markedly deformed canales sesamoidales
d: central lucency

Table 3 Heritability estimates with their standard errors for radiographic alterations in the navicular bone

Author Population and number (and sex) of horses investigated	Age (years)	Heritability estimates with their standard errors	Method of analysis
KWPN (1994) Dutch warmblood horses: Mares: 590 Stallions: 30	3–4	0.30 ± 0.14 0.31 ± 0.12	LAM (REML, DL-transformation[1]) STM (REML-type algorithm[2])
WINTER et al. (1996) German Riding horses: 3566 (862 sires) 2407 (236 sires)	3–8	0.06 ± 0.03	LAM (REML)
WILLMS et al. (1999) Holstein warmblood horses: Mares: 456 Two-year-olds: 144	3 2	0.31 ± 0.05 0.25 ± 0.04	ATM (GS) ATM (GS)
STOCK et al. (2004) Hanoverian warmblood horses: 3748	3–7	0.206 ± 0.044[a] 0.094 ± 0.051[aa] 0.126 ± 0.063[aaa]	LAM (DL transformation[1]) LAM (DL transformation[1]) LAM (DL transformation[1])
STOCK and DISTL (2006a) Hanoverian warmblood horses: 5175	3–7	0.10 ±0.02[b] 0.17 ± 0.09[bb] 0.25 ± 0.04[bbb] 0.14 ± 0.02 0.19 ± 0.02	LAM (DL transformation[1]) LAM (DL transformation[1]) LAM (DL transformation[1]) LAM (quasi-linear analyses) LAM (quasi-linear analyses)
STOCK and DISTL (2006b) Hanoverian warmblood horses: 5175	3–7	0.40 ± 0.04	LAM (DL transformation[1])

a: slight alterations
aa: moderate alterations
aaa: severe alterations
b: few (1-4) short and conical canales sesamoidales in the central part of the distal border
bb: deformed canales sesamoidales in the central part of the distal border, alterations of navicular bone contour and structure
bbb: several (≥ 5) short and conical canales sesamoidales and deformed canales sesamoidales in the central part of the distal border, alterations of the navicular bone contour and structure

ATM: animal threshold model; STM: sire threshold model
LAM: linear animal model
REML: residual maximum likelihood; GS: Gibbs sampling

[1]Dempster and Lerner (1950); [2]Misztal (1989)

Table 4 Marker information per chromosome of the equine marker set (161 microsatellite markers) developed for whole genome scans in horses

Equine chromosome	Average allele number	Average HET	Average PIC	Average distance (cM)	Chromosome Size (cM)	Number of markers
ECA1	5.67	63.33	57.10	16.1	193.5	12
ECA2	6.12	61.95	55.21	16.1	128.8	8
ECA3	8.00	66.32	61.30	17.2	120.2	7
ECA4	7.86	70.00	63.91	17.5	122.5	7
ECA5	4.33	67.75	60.50	33.4	100.1	3
ECA6	6.83	71.86	67.86	21.1	126.8	6
ECA7	5.00	56.01	51.82	20.4	102.1	5
ECA8	6.20	68.02	61.73	21.8	108.8	5
ECA9	6.00	67.72	57.80	17.5	104.9	6
ECA10	6.13	64.74	58.13	13.2	105.8	8
ECA11	8.00	73.01	69.45	16.2	64.9	4
ECA12	8.33	73.85	69.47	19.3	58.0	3
ECA13	5.50	70.47	62.65	14.5	58.0	4
ECA14	5.00	54.99	48.00	38.2	152.8	4
ECA15	7.83	76.38	69.38	16.1	96.7	6
ECA16	4.80	56.38	52.16	22.2	111.2	5
ECA17	4.50	60.88	54.24	17.8	71.3	4
ECA18	6.71	71.74	65.34	12.5	87.6	7
ECA19	6.17	59.18	54.13	9.3	55.7	6
ECA20	6.40	61.88	58.55	16.2	80.9	5
ECA21	5.43	61.92	57.21	10.8	75.8	7
ECA22	5.60	49.15	44.94	16.2	79.7	5
ECA23	6.60	59.79	55.05	11.2	56.2	5
ECA24	5.83	58.34	53.49	7.9	47.2	6
ECA25	6.33	63.28	58.95	16.2	48.7	3
ECA26	8.00	73.56	69.49	12.1	24.4	2
ECA27	6.50	68.54	61.87	23.3	93.0	4
ECA28	5.50	61.01	50.71	31.6	63.1	2
ECA29	7.67	75.44	64.45	25.0	75.0	3
ECA30	6.67	71.44	61.85	16.6	49.7	3
ECA31	7.50	74.76	68.65	20.6	41.1	2
ECA32(X)	9.00	80.40	77.18	16.3	65.2	4
Average	6.44	66.07	60.08	18.3	86.6	5.0

ECA: Equus caballus autosome; HET: observed mean heterozygosity (%)
PIC: mean polymorphism information content (%)
cM: centiMorgan, chromosome size according to SWINBURNE et al. (2006)

Supplemental table Optimised microsatellite marker set for horses containing 161 microsatellite markers

ECA	cM	Marker	Ta (°C)	Forward (F) /Reversed (R) Primer Sequence	Allele size (bp)
01	0.0	COR054	58	F: CAAGCAAAACAAGAAATCCC R: CTTTGTACACGTTGCAGTGG	229 - 243
01	23.0	ASB041	60	F: AAAGTTCACTTAGTCCTTGG R: CCACCTGTTTGCACTTGC	129 - 159
01	31.0	LEX020	55	F: GGAATAGGTGGGGGTCTGTT R: AGGGTACTAGCCAAGTGACTGC	192 - 213
01	35.6	1CA12	60	F: GGGAGTGGTGATTACTTCTTGC R: TAGCCGTGAGAAGGTGTGTG	101 - 109
01	62.3	COR079	58	F: TGCTGCCAGATCTTCTGAAT R: TGGAGAGCGTGAAATTAACC	204 - 212
01	71.5	ASB012	60	F: TCAGCAATAGAAGCCAGCTCC R: TCCTATGGAGGTGACCTTCCC	168 - 182
01	96.3	AHT021	60	F: TCCAAGTTGCTGAATGGATC R: ACGGCCTGATTCTCTCTTTG	199 - 215
01	110.0	1CA20	60	F: CTAAGCAGGTTCCCTATCATGG R: TCCACTACACAGGAAAACGAA	112 - 122
01	137.0	HTG012	60	F: CACTAGAGTCAGGGGGGGTGGGCT R: TTGGAGTACTCTTTCTCCCTTCCC	111 - 119
01	138.0	HMS015	60	F: ATATCTCTTGCTGTCCTACTTTCC R: AATGTGACACGTAAGATAGGCCTC	214 - 234
01	156.5	HMS007	60	F: CAGGAAACTCATGTTGATACCATC R: TGTTGTTGAAACATACCTTGACTGT	170 - 182
01	193.5	COR053	58	F: AATTGACTGTGGAAGCCTTG R: GGCTGAGGAGTAAGCTGAAAG	171 - 197
02	0.0	COR065	58	F: CAAAAGCACACACAAAGTGC R: TCCGGAAAGTGCAAAGTTAG	266 - 284
02	6.0	ASB018	60	F: TGCAGACAAAGCTGGACACTC R: CTGCTGAGAAAGCTTCTGC	190 - 206
02	26.9	COR090	62	F: GGTTTGTCTCTTTGAGGTGTG R: TGCTCATATCTTCACCCTGC	91 - 101
02	43.4	UM007	60	F: GGGAATAGAGAAAGGTGAAG R: TTAGAGTTCCTGCTCCTCC	119 - 157
02	64.0	AHT012	60	F: ACCCAAAGTCATGGGAATCA R: TTGTTGCCGACAACATGC	102 - 114
02	81.0	A-14	60	F: CAGCTGGGTGACACAGAGAG R: GTCATCACTACTCCCTACAC	208 - 236
02	100.0	100G3_ T7_MS	65	F: GGGTGAACAGTAGGGGAAAC R: CTGTTGTAGAGAGGGGGCTC	185 - 197
02	128.7	COR026	55	F: GGCGTCCAACGTAAAGTAGA R: CCTCTTCGGAAACTCTGACA	228 - 230
03	0.0	AHT036	60	F: TGCTGCTCCAGTGTCCT R: TAGATTTCACAGGCGGGTG	134 - 148
03	20.7	COR028	60	F: TAAAGAGGAAGGCAATGGAC R: ACCTTTTGTGCTAGGCACTG	229 - 243
03	35.0	AHT022	58	F : AAGCACAATGTGGGGGTTAG R : TCCACGTTCACACATACCTCA	189 – 201
03	71.6	LEX057	55	F : TGGTCCCCTAATCAAATCAGA R: ACGGCATCCCACATAAAATAG	157 – 171
03	85.5	UCD437	60	F : CTGTTCTGGGCAGGCTTCTCTA R : TTGCTGGCTTGGCTGGTC	165 – 187
03	107.9	LEX007	55	F : GGTAGGGCTCTGGGATGA R : AACACTGGGGAAAAGTCAG	192 – 200
03	120.5	AHT092	55	F : TGAGCATCTTGAAGATGAGCA R : CAACAGTTGTTAGCTCAGGTGC	254 – 296
04	0.0	AHT043	60	F : ACACAAGTGACAGGAGCGTG R : TGGAAGCATGCAAGAGGTC	156 – 190
04	10.1	HMS006	60	F : GAAGCTGCCAGTATTCAACCATTG R : CTCCATCTTGTGAAGTGTAACTCA	157 – 167
04	39.5	ASB003	60	F : AATTCATCTCAGTGCTCTACCAGC R : TTCATTTTCTACATGCACTACAGC	196 – 208

Supplemental table continued

ECA	cM	Marker	Ta (°C)	Forward (F) /Reversed (R) Primer Sequence	Allele size (bp)
04	57.8	LEX050	55	F: ATAGTCTGGGGTTAGGTAAGG R: TCTAGCCCAATGTAAATGC	112 – 124
04	71.0	ASB022	62	F: AGGAATGTGAAATACAGGAGG R: TTTGTGGTCTTCCGTGCACC	155 – 167
04	73.3	HTG009	55	F: TGTGGGAAGAGTGTCAATAGCTGT R: AGGCATCTGGTTTGCTGCAATTTC	118 – 138
04	117.7	SG23	58	F: GGCTTAAGATATGGGTGAGTAAGG R: GCCCACCCTCTTACTTTTCTCAA	198 – 236
05	44.3	UCD304	55	F: CGCTTTCCTGCTGTCACC R: GAGGGACTGTGGGGGAGGT	95 – 113
05	62.4	LEX034	55	F: GCGGAGGTAAGAAGTGGTAG R: GGCCTAAGATGAGGGTGAA	243 – 253
05	79.3	LEX014	55	F: CCTTACTCACTGGGGAATAAA R: AGACTGAACACCTAACTATGA	390 – 400
06	0.0	HTG031	55	F: CTCTGTAACCCTTATATCCTTA R: TGTTGATTGCTCCTCCCCT	135 – 145
06	37.0	NV082	60	F: TGTGGCAGCATCCCACAAAC R: CCTCCATTTTTGTCGGTTAGCG	123 – 137
06	56.0	UM015	55	F: AGTCTGGCTGAGGATACTG R: GGTGAGAAAGGAGATAAATG	298 – 312
06	96.7	COR070	62	F: CATCTGTTCCGTGGCATTA R: TTCAGGTGTGGGTTTTGAATC	273 – 299
06	98.0	TKY0028	55	F: TTCAGCAGGGTCTCATGCCAC R: TTCGGCTCTGGTTCAAGAGG	271 – 359
06	113.9	TKY0284	62	F: CTGGACTAGAGTCAGATTGC R: AACAGGATTCCCCCAATGCC	157 – 171
07	18.1	HTG020	55	F: CTGGTTTACCTTCCCTACAG R: CCAATGGTTCCTCTGAGAAG	144 – 156
07	43.0	LEX038	58	F: CTGCATTCCCATCATCACAT R: TGCCTTGCCTCTTTCTGTTTA	133 – 143
07	66.0	COR095	58	F: TACCTCTGGTGGTGATGCTT R: CCCACACTTACTCCCATCAC	206 – 320
07	74.0	SG28	62	F: CTGTGGCAGCTGTCATCTTGG R: CCCAATTCCAGCCCAGCTTGC	149 – 165
07	102.1	AHT019	62	F: CATTTCTCTGGTGTATCTCCCA R: GGAATAGTCATAGTCCACGACC	137 – 145
08	0.0	COR097	58	F: GGGATTTCTGAGATGCTGAA R: ATGGCTGGCTAGAGTTTGTG	236 – 244
08	39.0	UCD046	60	F: GCCAAACGCTGGAGGGTT R: CCACATTCACACACATGCACAC	228 – 234
08	56.0	COR012	58	F: TCTAGGAAAGACCCATCACG R: AGTAAGTGGAGGCCAAGGAT	166 – 180
08	79.3	COR003	58	F: TAGGGAAACTCCTCAAAGCC R: GAAACCAAAACCTTCATCCA	192 – 208
08	108.8	COR056	58	F: AGATTCCAGGCATTAGGACC R: TCAGGGACAATCTTCCTCAAG	190 – 212
09	0.0	HTG004	55	F: CTATCTCAGTCTTCATTGCAGGAC R: CTCCCTCCCTCCCTCTGTTCTC	127 – 137
09	29.5	HMS003	58	F: CCAACTCTTTGTCACATAACAAGA R: CCATCCTCACTTTTTCACTTTGTT	149 – 167
09	48.0	HTG008	56	F: CAGGCCGTAGATGACTACCAATGA R: TTTTCAGAGTTAATTGGTATCACA	178 – 190
09	49.0	COR098	55	F: GCAACAGATGTTGGCTCAG R: GGAGATGTCCTTGACCACAG	233 – 251
09	81.7	ASB004	56	F: TAAATTGTAAAAGCTGGAGCCG R: GCAAATAGTAGTTAAGTCCTC	120 – 144
09	101.1	ASB005	62	F: TCGAGGAGCTCATGACCTGG R: TTGTACAACTCTCCACCATAGC	105 – 117
10	4.8	HMS023	60	F: GATCCAATATTGTAAACCCCGCC R: CCTTCATAACCCTTATTGCAGCC	82 – 94
10	23.0	COR020	60	F: TCTCTACCGCAAGTGAAACC R: CTGAATTGTAGGACATCCCG	213 – 227

Supplemental table continued

ECA	cM	Marker	Ta (°C)	Forward (F) /Reversed (R) Primer Sequence	Allele size (bp)
10	31.5	ASB006	60	F: GGCACAGATGTTAGCTCAGC R: ATGGAACCAGCCTGGATTGC	190 – 204
10	49.0	COR015	58	F: GGTGTGGAAACATTCCGTAT R: ACTGCATGTGTGGGAGAGAT	215 – 231
10	64.0	ASB009	62	F: GTGCGCATGTATGTGCGTGCC R: ATTTCCACAAGGGACATGAGG	86 – 102
10	80.0	LEX009	55	F: AAAGCCGTAAGATTGGGACA R: TCCATTGTGAGGGTGTAACA	366 – 378
10	94.2	NV067	58	F: GCTCACTCAACTCCCAGAG R: GGATTAGATTACCCAGACAACT	212 – 220
10	105.8	AHT086	60	F: CCCAATGAAGTCCAAGATGG R: GAAATCTCTAGCAAGACCCAGG	187 – 217
11	17.7	LEX068	50	F: AAATCCCGAGCTAAAATGTA R: TAGGAAGATAGGATCACAAGG	154 – 168
11	24.5	SG24	58	F: CTACCATTGAAGAGGGGTGGC R: GAAACGAGCAGGAAGTGAATCTCC	107 – 127
11	46.0	SG13	60	F: GGACTAAAGCCCAACCATCCAGC R: CTCACCAGTAAGGGGTTATGGGGC	163 – 189
11	64.9	UCD457	60	F: GGGGCGTGAGCATAAAGG R: CGCTGGATGAGTGAGGGA	73 – 95
12	0.0	SG10	60	F: CATCCATCCTTTCCAGCTCGATATTC R: CAAGACCGTAACTCAGGAGCCC	173 – 183
12	16.8	SG08	58	F: GAGTTCATTCTTTTTCGTGGCTG R: GGAAACACCCTAAGTGTCCCTTG	121 – 135
12	25.6	COR058	58	F: GGGAAGGACGATGAGTGAC R: CACCAGGCTAAGTAGCCAAAG	208 – 230
13	0.0	COR069	58	F: AGCCACCAGTCTGTTCTCTG R: AATGTCCTTTGGTGGATGAAC	265 – 279
13	24.0	VHL047	55	F: GTTTGCTGTGGTTACCAGGCAGA R: GCAAATTGAATATTTGAAGTTGAGAC	126 – 142
13	30.0	LEX041	55	F: TATTTTCTGAATGCTTCTGTGC R: CTCTACACCCAATGCCTGAT	143 – 161
13	51.0	ASB001	60	F: AGCAGAAACCCACTCAAGCC R: GCATAATACCCTCAAGGTC	153 – 167
14	3.6	AHT029	60	F: ACTCATTCATTCACAAATCCCC R: AGAAAATTCCCTCCTGTCCC	262 – 284
14	27.8	LEX043	58	F: CATTAAGCAACAAAAAGCATC R: GGAAAAGCATGACAAGACACT	224 – 244
14	36.0	UM010	56	F: TACAGCCATTGGAAATCTAC R: CACCATTACATTTTCCCAG	106 – 120
14	131.0	LEX078	58	F: AATGTGCGCATTTAACCACTGTG R: CAAGCCATGCTGTGGAAACG	160 – 164
15	26.8	B-8	60	F: TCCTCAGTCCTTTCTCATGC R: AGCTGAAGGCAATCTGTACC	79 – 101
15	37.0	LEX046	58	F: ATAAGCCAATCCACTTTTCC R: ATTACCACCCCATTTCCTT	115 – 129
15	51.0	ASB002	60	F: CCTTCCGTAGTTTAAGCTTCTG R: CACAACTGAGTTCTCTGATAGG	234 – 254
15	63.2	HTG006	55	F: CCTGCTTGGAGGCTGTGATAAGAT R: GTTCACTGAATGTCAAATTCTGCT	82 – 104
15	90.3	HMS001	60	F: CATCACTCTTCATGTCTGCTTGG R: TTGACATAAATGCTTATCCTATGGC	170 – 180
15	96.7	COR014	58	F: CTATCATGTCAGGGACCAGG R: CTGCCCTAGTTAGCAACCAA	143 – 161
16	5.7	HTG003	55	F: TAACCTGGGTGCAAAGCCACCCAT R: TCAGGGCCAATCTTCCTCAC	114 – 124
16	49.0	LEX059	55	F: TGAAATGTCACCTTCTCAGAG R: GTGGACACTTGCCNTCAT	227 – 231
16	85.6	LEX056	55	F: GACCTACAGGCCACTCATCAA R: GGCAGTTTCCTCCATCCTTA	211 – 227
16	90.0	COR064	58	F: TCCATACATGTGTGAGGGC R: AAGATGGCTTACAAGGATTATG	192 – 202

Supplemental table continued

ECA	cM	Marker	Ta (°C)	Forward (F) /Reversed (R) Primer Sequence	Allele size (bp)
16	111.2	AHT091	62	F: TAGCTGTCTGCAAAGGCTCA R: CCAGTGTTCCACATGCCTC	108 – 126
17	4.4	COR105	60	F: TTTCCTCATTGCTTCCTGAG R: CCCAAGGTCTGTCTTGCTCTC	174 – 192
17	31.3	UCD014	60	F: GCATTTGCTCACTGGCTAC R: ACTCCTCCACTCCCACCTA	128 – 134
17	46.6	COR032	58	F: GCCCTCTTAGAGCATTTTCC R: CAGAGATGGCTGGAGTAAGG	249 – 255
17	65.0	HMS025	60	F: CAAACATAAAATATGCATGTCCATGT R: CTTTTGGATATGTAAGGCTTGAGG	124 – 128
18	0.0	UCD136	60	F: CTTTGGGCCTTTCCTCCAT R: CGAGCCTGGGAGTGATAC	111 – 119
18	10.3	TKY0019	56	F: CTTCTGCTGATTCCTGAATG R: GGATCTCCTTAAATGGAACA	144 – 164
18	20.6	LEX054	55	F: TGCATGAGCCAATTCCTTAT R: TGGACAGATGACAGCAGTTC	164 – 180
18	30.5	HMS046	60	F: GTCTCAGCCAAAAGGTATTCAAGC R: TGGCACCAATATAGGTCACCTGG	122 – 134
18	45.9	COR096	58	F: CCCCTCTTTTGCTTGAGAAT R: GCGTGTATGTGAGGATTGAAG	307 – 321
18	66.0	TKY0101	55	F: TCTGAAATACCGTGTGCCT R: TTCTGCCTCCCTCCAACTTT	197 – 217
18	87.6	UCD387	60	F: ACCCCCGCCCCAGCAC R: TGCCCCGTCATTCTGC	76 – 88
19	0.0	COR062	58	F: GTCATCCAGTGACGAACACA R: AGGAAGTGCGCAGTAGAGAA	208 – 236
19	2.0	AHT094	60	F: CACCTCCATCACATTGGTCA R: GGCTGGAGTCAGCTGACATT	232 – 240
19	21.1	LEX036	55	F: ATCAGCCCAGCCTCTTCA R: AACAACCGGCNAAATAGTGC	137 – 161
19	41.5	HMS008	58	F: GGTGAGGAATTATCTCTTTGAAGG R: GCAGGTAGGATTGGATAGGTACAT	207 – 215
19	55.0	NV011	60	F: GGCCCCACCCACTAAATATCACTG R: CGGGGTCTTGGAAATTTATGAAGG	120 – 130
19	55.7	AHT055	60	F: TGAAAATACACCCAGCTACGC R: GGGAGATATTTCTTGGCTTGC	147 – 159
20	2.5	HTG005	55	F: TGCTAAGCCTCAGCACATACA R: TGGAAATAAGGTTAGCAGGGATGC	79 – 89
20	11.0	LEX064	50	F: ACCCTTTCCGCAGACAA R: CACATCAGAGCCCATCTTCTC	192 – 207
20	27.6	UM011	56	F: TGAAAGTAGAAAGGGATGTGG R: TCTCAGAGCAGAAGTCCCTG	160 – 180
20	59.4	COR050	58	F: TCTGTTGCCTTTATCCACAA R: ATGAAAACCCTGGGAATAGC	287 – 297
20	68.5	HMS042	55	F: TAGATTTCTTAAGTGCCAATAGTGG R: GAACTGCTATAGATATACCTAACTC	111 - 133
21	0.0	SG16	55	F: AATTCTCAAATGGTTCAGTGA R: CTCCCTCCCTTCCTTCTA	146 - 192
21	16.0	UMNe229	60	F: CTTCTCTGGACAAAGGGGTG R: CATGAATTTGCCAGTTTGATG	122 - 124
21	24.5	HTG010	55	F: CAATTCCCGCCCCACCCCCGGCA R: TTTTTATTCTGATCTGTCACATTT	93 - 113
21	27.7	COR073	58	F: GCCAAGACATGGAAACAATC R: GTTCTCAAGGTGCATCCCTA	180 – 198
21	30.0	COR068	60	F: AACCAATTGTGAGATTTTTGCT R: GGCTAGTCCTGGATCATGTG	146 – 156
21	40.0	LEX031	58	F: CCCATTAAGAACTTTTCATCCTG R: GGCAAGCCCCACAAAATTAT	252 – 258
21	67.0	LEX037	55	F: GGATTCCTCAACCTCCTAAA R: AGGGATAAGTGACCACCAC	193 – 199
22	0.0	HTG014	55	F: CCAGTCTAAGTTTGTTGGCTAGAA R: CAAAGGTGAGTGATGGATGGAAGC	131 – 147

Supplemental table continued

ECA	cM	Marker	Ta (°C)	Forward (F) /Reversed (R) Primer Sequence	Allele size (bp)
22	20.6	HTG021	58	F: ATTACTTCCTCCAGGTATCTCAG R: AGGCAGGGCTGGGAGACGT	124 – 134
22	57.1	COR016	58	F: CAGCTCAGTAGATGATTGTCCA R: GCAAAGACAAGGAGGTTAAGTT	172 – 202
22	65.3	HMS047	60	F: CCTGCTGAGGACCTTGGAAGCT R: ATGTATTTTCAAGTCTAATATCTGCC	196 – 202
22	79.7	SG19	58	F: GCCCCCACCTGCTCCACC R: GGGGCAAAGTGGAAATCC	139 - 149
23	1.2	COR055	58	F: TAGTGACGCCTACGGATTTC R: CCCAAGAGGGCTTAGAAAGAG	228 – 256
23	3.0	UM019	55	F: TACTGCCAGCACTTGTACC R: TCTCTCAGTTTCTCTCTCTGTC	154 – 168
23	25.7	ASB039	60	F: ACAGCTGCCTGGATATGTGG R: GCAGAGAGAAATAGAGATGC	154 - 172
23	44.4	LEX053	55	F: TTATTCCTGCTTCGTANATGA R: ACACACTTGGGTTCAAATC	123 - 133
23	57.0	SG04	60	F: CGACGCCTCCTCCTAAAC R: CAGCTGTGTGCCTTTGATTAT	201 - 213
24	1.6	LEX042	55	F: ACATACAAACCTGCTCAACAT R: CCTACACATCGCTCATCAA	212 - 222
24	6.0	AHT004	60	F: AACCGCCTGAGCAAGGAAGT R: CCCAGAGAGTTTACCCT	148 - 164
24	8.4	EA2C4	50	F: ATGTATCTTCGAGGGATGAT R: GGCAGTTAATGGTGAGTAAG	142 - 162
24	31.0	LEX032	55	F: CGTAGTAGGGTTTTGGGTCC R: TTGCGTTTCAATTTTTAATGAC	249 - 261
24	35.5	COR024	58	F: CAAAAGTGATTGCCTTCGAT R: TTGGAAGCTGGGTGATTG	205 - 217
24	41.0	COR025	58	F: ACAGAGCTGACTGCCTATGG R: TCCTCTTCTCAGGGAGACCT	172 - 178
25	0.0	NV043	60	F: TGACACAAGATAAAAGCCCCAGG R: GATTGGGAAAAGAGCACAGCC	142 - 158
25	23.5	UCD405	60	F: ACCTCGTCTGGCTGTTGTAAG R: ACTTGCTGTGCGACTCTG	252 - 270
25	30.1	COR018	58	F: AGTCTGGCAATATTGAGGATGT R: AGCAGCTACCCTTTGAATACTG	253 - 275
26	6.9	COR071	58	F: CTTGGGCTACAACAGGGAATA R: CTGCTATTTCAAACACTTGGA	180 - 208
26	19.4	UM005	56	F: CCCTACCTGAAATGAGAATTG R: GGCAAAAGATCAGGCCAT	212 - 224
27	0.0	COR031	58	F: CAATTGCCATTTGTTCCAGTG R: GCTTAAGAAACACCAGGCAG	202 - 214
27	24.0	UCD005	56	F: AGCGGAAGTGCTGCGAAAG R: CCAGCATCTCTGGGCAGG	226 - 240
27	52.2	LEX005	55	F: AAGGCAATGCTTATCAAATGC R: TTACCCGCAGTGACTTCTATT	243 - 263
27	72.9	COR017	58	F: GAAGGCCTGAAGCATTTACA R: CGTAATGTTGACCAAACTTCA	239 - 253
28	7.0	UM003	56	F: GGAGGGACGATAGAGAGTAAG R: GCAGAGATAACGGACATGG	149 - 155
28	63.1	UCD425	55	F: AGCTGCCTCGTTAATTCA R: CTCATGTCCGCTTGTCTC	233 - 247
29	0.0	LEX018	60	F: TTTCATCACTTTCTGCTTCC R: TTCTCTTCCTTTGCTCATCCT	228 - 246
29	43.0	COR027	58	F: CAGCTCTGCAATTTCTCCTC R: AATGACCAAGGCATTGAAAG	229 - 245
29	61.0	ASB043	60	F: TCACTTAGTAGGGGCATGC R: GTGTTTGTCCTTGACTCTCC	85 - 99
30	0.0	LEX025	55	F: CAATCGTGGCCCGGTAAC R: TTCACTCCAATCCTCAGTCA	141 - 157
30	31.4	VHL020	60	F: CAAGTCCTCTTACTTGAAGACTAG R: AACTCAGGGAGAATCTTCCTCAG	88 - 106

Supplemental table continued

ECA	cM	Marker	Ta (°C)	Forward (F) /Reversed (R) Primer Sequence	Allele size (bp)
30	48.6	LEX075	55	F: TGAAAAGTTGCAGTTTGAGA R: CAACCTCTTGCTACCAGAATA	150 - 160
31	13.5	AHT033	58	F: CTGAGGGCGTAAGTCGAGTC R: GTTAATAGGAGCGGTTGTTTGG	145 - 165
31	41.1	AHT034	60	F: CTCAGGGCGAATGTTCCTC R: CCCCACCATGAGTCAAAAAC	121 - 141
X	9.0	LEX027	56	F: ACCACTGGGAAACTGTGTAA R: GCCCAGAATCCGAACC	187 - 201
X	36.7	AHT028	60	F: CCTGGCTTATAGATGGCTGC R: ATTTGGAGATGGGGGTCTTT	178 - 216
X	47.8	LEX024	55	F: GGGGGTAGAGGGAAAAAGAG R: TTGTTGGCAGATCCCAGG	132 - 150
X	67.0	LEX003	55	F: ACATCTAACCAGTGCTGAGACT R: GAAGGAAAAAAAGGAGGAAGAC	143 - 163

ECA: Equus caballus autosome; cM: centiMorgan according to SWINBURNE et al. (2000a), CHOWDHARY et al. (2003), PENEDO et al. (2005), SWINBURNE et al. (2006);
Ta: annealing temperature; bp: base pair

Chapter 2

Prevalences and estimation of genetic parameters for radiological alterations in the navicular bone of Hanoverian warmblood horses

Prevalences and estimation of genetic parameters for radiological alterations in the navicular bone of Hanoverian warmblood horses

Abstract

The results of a standardized radiological examination of 155 Hanoverian Warmblood horses with an age of between 22 months and 19 years (mean age 5.4 years) were used to quantify the factors associated with the prevalence of different radiological alterations in the navicular bones of the front feet. The radiographic findings were classified into three traits according to the evaluation scheme of BRUNKEN (1986) and were analysed as binary traits. The lowest prevalence was found for radiological alterations in the contour of the navicular bone (RAC; 23.2 %). More horses showed radiological alterations in the structure (RAS; 25.8 %) of the navicular bone. Most radiological alteration in the navicular bone were classified as deformed canales sesamoidales (DCS; 40.4 %). The prevalence of DCS and RAC was significantly influenced by age at the radiological examination, and RAS and RAC were significantly influenced by sex. The prevalence of RAC was significantly influenced by the interaction of sex and age at the radiological examination.

Heritabilities were estimated for DCS, RAS and RAC using Residual Maximum Likelihood (REML) under a linear animal model. After transformation to the underlying liability scale the heritability estimates were $h^2 = 0.63 \pm 0.19$ for DCS, $h^2 = 0.53 \pm 0.28$ for RAS and $h^2 = 0.24 \pm 0.13$ for RAC. Moderately positive additive genetic correlations were estimated between DCS and RAS ($r_g = 0.27 \pm 0.14$) and between DCS and RAC ($r_g = 0.38 \pm 0.08$). The additive genetic correlation between RAS and RAC was negative ($r_g = -0.79 \pm 0.10$).

We concluded from our results that there is a genetic influence on the development of the different radiological alterations in the navicular bone.

Introduction

Lameness problems play an important role in horse medicine and they are the main reason for premature retirement and culling (PHILIPSSON et al. 1998, WALLIN et al. 2000).

The navicular bone, os sesamoidale distale, is located in the rear part of the equine distal interphalangeal joint. Together with the bursa podotrochlearis, the distal part of the deep digital flexor tendon and ligamentous structures it forms the equine podotrochlea. Only the bony part of the podotrochlea is radiographically visible. Deviations from presumably normal radiographic appearance of the navicular bone occur in horses of all ages, including foals

(BRANSCHEID 1977, KASER-HOTZ and UELTSCHI 1992, HORNIG 1993). It has to be taken into account that the radiographic findings do not reliably reflect the pathohistological changes (DELIUS 1982, DROMMER et al. 1992). Clinical findings have to be taken into consideration when stating on the disease value of radiological alterations.

Studies, which were based only on radiographic examinations and not on clinical findings found slight radiologically visible alterations of the navicular bones in 14.9 % in three- to seven-year-old Hanoverian warmblood horses (STOCK et al. 2004) and in 51.0 % of three-year-old Holstein warmblood mares (WILLMS et al. 1999). Reported prevalences of severe radiologically visible alterations in the navicular bones ranged from 1.1 % to 15.0 % in Holstein warmblood mares (WILLMS et al. 1999).

Reported heritability estimates for radiologically visible alteration in the navicular bones of Warmblood horses varied between $h^2 = 0.06$ and $h^2 = 0.31$ (KWPN 1994, WINTER et al. 1996, WILLMS et al. 1999, STOCK et al. 2004). These large differences between heritability estimates might be caused by the different types of the applied estimation methods, the different schemes of data collecting and coding, and the different numbers and breeds of examined animals.

The aim of the present study was to investigate the distribution of different radiographic findings in the front navicular bones of two-year-old and adult horses. Analysis of variance was performed to test the influences of age at examination and sex influences on the prevalence of navicular bone alterations.

The estimation of genetic parameters for the prevalence of the different radiographic findings in the navicular bone should substantiate their role in a hereditary context.

Material and methods

Data collection and pedigree structure

Data from radiological examinations of 155 Hanoverian warmblood horses descending from 46 stallions were used for this study. The size of the examined half-sib groups ranged from one to 19. Of the examined horses 100 (64.52 %) were female and 55 (35.48 %) were male. At examination the horses had an age of between 22 months and 19 years (mean age 5.4 years). The mean age at examination was 6.4 years in the female horses and 3.6 years in the male horses. The animals were separated into three age classes. The first age class included 34 horses of up to 2.5 years of age, the second age class included 71 horses of more than 2.5 years of age up to 5.0 years of age and the third age class comprised 50 horses of more than 5.0 years of age.

The radiographic examinations were performed between 2001 and 2004 and included dorsoproximal-palmarodistal projections of the front toes (upright pedal route according to OXSPRING 1935). The radiographs were scrutinised by two radiologically experienced veterinarians.

The radiological appearance of the navicular bones of the front feet was classified according to the evaluation scheme of BRUNKEN (1986). The diagnostic criteria were size, shape and distribution of canales sesamoidales, and structure and contour of the navicular bone. The radiographic findings were analysed as three single binary traits for each animal: deformed canales sesamoidales (DCS), radiological alterations in the contour of the navicular bone (RAC) and radiological alterations in the structure of the navicular bone (RAS) (Table 1).

Statistical analysis

The prevalences of DCS, RAC or RAS were analysed as binary traits. For DCS, horses with grades C1, C2a or C2b for canales sesamoidales were classified as unaffected, and horses with grades C3a to C6 and with grade CP were classified as affected. For RAC, horses with grade K1 were classified as unaffected, horses with grades K2 to K5 were classified as affected. For RAS, horses with grades S1a and S1b were classified as unaffected and horses with grades S2a to S5c were classified as affected. Affection of animals with more than one kind of radiological alterations in the navicular bone was taken into account in the coding of the traits.

Analyses of variance were performed for the prevalences of DCS, RAS and RAC. The sex of the horse, the age class at radiological examination and the interaction between the sex of the horse and the age class at radiological examination were tested as fixed effects.

A generalised linear model was employed for the analysis of variance with a binomial function of distribution and the probit function as link function. The procedure GENMOD of the Statistical Analysis System (SAS), version 9.1.3 (SAS Inc., Cary, NC, 2005) was used for these analyses.

The estimation of genetic parameters was performed using Residual Maximum Likelihood (REML) with VCE5 Version 5.1.2. (Variance Component Estimation, KOVAČ et al. 2003). Multivariate analyses for DCS, RAC and RAS were performed using a linear animal model. Pedigree information on four ancestral generations was included. The multivariate model used for the genetic analyses included those effects, which were statistically significant in the multiple analyses of variance. Because sex (SEX), age at the radiological examination (AGE)

and the interaction between sex and age class at the radiological examination were significant, the following model was used for DCS, RAC and RAS:

$$y_{ijkl} = \mu + SEX_i + AGE_j + SEX_i*AGE_j + e_{ijkl}$$

with y_{ijkl} = binary dependent variate for DCS, RAC or RAS;

μ = model constant;

SEX_i = fixed effect of the sex of the horse (i = 2; male, female);

AGE_j = fixed effect of age class at the radiological examination included three levels (j: ≤ 2.5, >2.5 < 5, ≥ 5 years of age, with 34, 71 and 50 animals);

a_k = random additive genetic animal effect (k = 1536);

e_{ijkl} = random residual effect.

The estimated heritabilities (h^2_{obs}) were transformed according to DEMPSTER and LERNER (1950), and the residual correlations ($r_{e\ obs}$) were transformed according to VINSON (1976) from the observed scale to the underlying liability scale. Let p_i be the frequency of outcome 1 for trait i, z_i be the ordinate of a standard normal distribution at the threshold point corresponding to a fraction p_i, of the population having the character, h^2_{obs} be the heritability of trait i on the observed (binary) scale, $r_{e\ obs}$ be the residual correlation between traits i and j on the observed binary scale, h^2_{liab} be the heritability of the trait i on the underlying continuous scale, and $r_{e\ liab}$ be the residual correlation between traits i and j on the underlying continous scale, then

$$h^2_{liab} = h^2_{obs}\,[p_i(1 - p_i)]z_i^2 \text{ and } r_{e\ liab} = r_{e\ obs}\,\{[p_i\,(1 - p_i)]\,/\,z_i^{\,2}\}^{1/2}\,\{[p_j\,(1 - p_j)]\}.$$

Results

Prevalence of different radiographic navicular bone alterations

The distribution of DCS, RAS and RAC by sex and in total is shown in Table 2. From all 155 investigated horses, there were 72 horses (46.5 %) with no signs of any navicular bone alteration, 62 horses (40.0 %) were affected by DCS, 36 horses (23.2 %) were affected by RAC and 40 horses (25.8 %) were affected by RAS.

There were 12 horses (7.7 %) affected by DCS and RAC, 15 horses (9.7 %) affected by DCS and RAS, and 4 horses (2.6 %) affected by RAC and RAS. From all 155 investigated horses, there were 12 horses (7.7 %) affected by DCS, RAC and RAS.

The results of the multiple analyses of variance are given in Table 3. Sex had a significant influence on the prevalence of RAS and RAC. The age at the radiological examination had a significant influence on the prevalence of DCS and RAC. The interaction between sex and age at the radiological examination had a significant influence on the prevalence of RAC.

Estimation of genetic parameters

Genetic parameters estimated for the prevalences of DCS, RAC and RAS are given in Table 5. The linear heritability estimates for DCS, RAC and RAS were in the range of $h^2_{obs} = 0.12$ to $h^2_{obs} = 0.39$. After transformation onto the liability scale, the heritabilities ranged between $h^2_{liab} = 0.24$ to $h^2_{liab} = 0.63$. Corresponding standard errors were in the range of $SE_{h^2} = 0.07$ to $SE_{h^2} = 0.15$ before transformation and in the range of $SE_{h^2} = 0.13$ to $SE_{h^2} = 0.28$ after transformation. Moderately positive additive genetic correlations were estimated between DCS and RAC ($r_g = 0.38 \pm 0.27$) and between DCS and RAS ($r_g = 0.27 \pm 0.14$). The estimate for the additive genetic correlation between RAC and RAS was negative ($r_g = -0.79 \pm 0.21$). The residual correlations were $r_{e\,obs} = 0.13 - 0.39$ ($SE_{h^2} = 0.08 - 0.14$) before transformation and $r_e = 0.24 - 0.72$ ($SE_{h^2} = 0.14 - 0.24$) after transformation.

Discussion

The objective of this study was to investigate the distribution of different radiological alterations in the navicular bones of a sample of Hanoverian warmblood horses and to quantify the importance of the additive genetic variation for the prevalence of these alterations.

Depending on the investigated horse population and on the study designs, prevalences around 25 % were reported for the different kinds of radiologically visible changes of navicular bones. Reports on the distribution of specific radiographic findings in the navicular bones are rare. STOCK and DISTL (2006b) found a prevalence of 2.23 % for DCS in a study on 5157 Hanoverian warmblood horses preliminarily appointed for sale at auction with an age between three and seven years of age. The prevalences of RAC was 0.50 % and the prevalences of RAS was 0.39 % in the same study. In this study the prevalences of DCS, RAC and RAS were much higher. The reason for these higher prevalences of radiographically visible alterations in the navicular bone might be seen in the range of age of horses radiographically examined for this study. Broodmares with an age of seven to 19 years comprised about 21.3 % of all investigated horses. As opposed to previous studies which were based on samples of young riding horses, the population sample used in this study was mainly drawn from the breeding population.

In Dutch warmblood mares no significant effect of age on the radiographic findings in the navicular bones was determined (KWPN 1994). STOCK et al. (2004) found no significant influence of age on the prevalence of pathologic changes in the navicular bone of three- to

seven-year-old Warmblood riding horses. Although our material was sampled from the same horse breed an age effect was evident for DCS and RAC. The larger range of age of horses included in the present study appears to be responsible for the different results. An increase of navicular bone alterations with age was also seen by WINTER et al. (1996), which investigated German Riding horses with three to eight years of age.

In the study of WINTER et al. (1996) the proportion of mares affected by radiologically visible navicular bone alterations was higher than the proportion of affected stallions and geldings. Other investigators found males more often affected (ACKERMANN et al. 1977) or did not see any sex differences (BODENMÜLLER 1983). In the present study significant sex effects were identified for RAS and RAC, but not for DCS. However, these results should be verified in a larger sample of horses because only young males were included in this study.

Reported heritability estimates for radiological navicular bone alterations were low on the observed scale ($h^2 = 0.06$; WINTER et al. 1996) and ranged from $h^2 = 0.20$ to $h^2 = 0.46$ on the liability scale (KWPN 1994, WILLMS et al. 1999, STOCK et al. 2004, STOCK and DISTL 2006b). Possible reasons for the wide range of estimates included the different definitions of investigated traits, the different data structures in respect of age and sample populations, the different methods used for the genetic analyses. The heritability estimate for DCS, RAS and RAC ranged between $h^2 = 0.24$ and $h^2 = 0.63$ in our study, but the corresponding standard errors were high.

The genetic correlation estimates indicated the presence of some common genetic background of DCS and RAC and of DCS and RAS, whilst RAC and RAS seemed to be influenced by different genes. Detailed radiographic information on a larger number of horses should be used to verify these results.

Table 1 Evaluation of the radiographic appearance of the navicular bones of the front limbs on the basis of size, shape and distribution of canales sesamoidales and of structure and contour of the navicular bones, according to BRUNKEN (1986)

Type of radiographic finding	Code	Description of radiographic finding	Classification
Canales sesamoidales	C1	no canales sesamoidales distales	not-affected
	C2a	few (1-4) short indentations or conical canales sesamoidales distales	
	C2b	few (1-4) elongated, narrow and straight canales sesamoidales distales	
	C3a	several (≥ 5) short indentations or conical canales sesamoidales distales	affected
	C3b	several (≥ 5) elongated, narrow and straight canales sesamoidales distales	
	C4	few (1-4) lollypop-shaped and/or branched canales sesamoidales distales	
	C5	several (≥ 5) lollypop-shaped and/or branched canales sesamoidales distales	
	C6	extensive osteolysis at margo distalis, no discernible canales sesamoidales distales	
	CP	canales sesamoidales proximales	
Navicular bone structure	S1a	fine-structured and regular spongiosa	not-affected
	S1b	fine-structured, but irregular spongiosa	affected
	S2a	coarse, but regular spongiosa	
	S2b	coarse and irregular spongiosa	
	S3a	general sclerosis	
	S3b	sclerosis around canales sesamoidales	
	S4a	central osteolytic area with blurred margins	
	S4b	central osteolytic area with sharp margins	
	S5a	fracture at margo distalis (chip fracture)	
	S5b	sagittal fracture	
	S5c	pathological fracture	
Navicular bone contour	K1	no contour changes	not-affected
	K2	new bone formation in the central part of margo distalis	affected
	K3	new bone formation at margo proximales	
	K4	new bone formation at medial or lateral part of margo distalis	
	K5	angular or peaked new bone formation at medial or lateral extremity	

Table 2 Prevalence (%) and number of animals affected (n_{aff}) by deformed canales sesamoidales (DCS), by radiological alterations in the contour of the navicular bone (RAC) and by structure of the navicular bone (RAS) in the 155 Hanoverian warmblood horses included in this study

Radiographic finding	Female (n = 100)		Male (n = 55)		Total	
	n_{aff}	%	n_{aff}	%	n_{aff}	%
DCS	44	28.4	18	11.6	62	40.0
RAC	28	18.1	8	5.2	36	23.2
RAS	39	25.2	1	0.6	40	25.8

Table 3 Results of the multiple analyses of variance for the prevalence of deformed canales sesamoidales (DCS), radiological alterations in the contour of the navicular bones (RAC) and radiological alterations in the structure of the navicular bones (RAS)

Source of variation	DF	DCS		RAS		RAC	
		χ^2	*P*	χ^2	*P*	χ^2	*P*
Sex	1	0.34	0.561	12.67	0<0.001	8.19	0.004
Age	2	12.51	0.002	1.68	0.431	25.08	<0.001
Sex*Age	2	3.35	0.187	3.81	0.149	7.42	0.025

DF: degrees of freedom

Table 4 Genetic parameters with their standard errors estimated for the prevalences of deformed canales sesamoidales (DCS), radiological alterations in the contour of the navicular bones (RAC) and radiological alterations in the structure of the navicular bones (RAS) in 155 Hanoverian warmblood horses with heritabilities (transformed) in bold on the diagonal, additive genetic correlations in the upper off-diagonal and residual correlations (transformed) on the lower off-diagonal, standard errors were not estimable

Trait	DCS	RAS	RAC
DCS	**0.629 ± 0.185**	0.271 ± 0.142	0.382 ± 0.081
RAS	0.257 ± 0.244	**0.529 ± 0.282**	-0.786 ± 0.096
RAC	0.235 ± 0.141	0.724 ± 0.180	**0.241 ± 0.132**

Chapter 3

Genome-wide search for microsatellite markers associated with radiological alterations in the navicular bone of Hanoverian warmblood horses

Genome-wide search for microsatellite markers associated with radiological alterations in the navicular bone of Hanoverian warmblood horses

Abstract

The aim of this study was to identify quantitative trait loci (QTL) with significant linkage to pathologic changes in the navicular bone in Hanoverian warmblood horses. Seventeen paternal half-sib groups with at least three descendants were analysed in a whole genome scan. These families comprised 144 individuals of progeny and grandchildren, which were chosen randomly from the Hanoverian warmblood. Three different traits were considered: deformed canales sesamoidales, radiographic changes in the contour and in the structure of the navicular bone. The genome scan included 161 highly polymorphic microsatellite markers in the first step. In a second step the putative QTL regions on equine chromosomes (ECA) 2, 3, 10 and 15 were confirmed using 52 additional microsatellite markers. In total, QTL were found on 17 different equine chromosomes. These QTL were located on ECA2, 3, 4, 7, 10, 13, 14, 15, 17, 18, 20, 22, 24, 26, 29, 30 and 31. This study was a first step to get more insight into the molecular genetic determination of radiological changes in the equine navicular bone.

Introduction

Defects of the musculoskeletal system are the main reasons for premature retirement and culling in horses (PHILIPSSON et al. 1998, WALLIN et al. 2000). In many cases, pain with an underlying pathology is located at the distal part of the equine limb. Podotrochlosis is one of the main causes of chronic and often therapy-resistant forelimb lameness (PHILIPSSON et al. 1998, WALLIN et al. 2000). Navicular disease, also known as navicular syndrome or podotrochlosis, describes a chronic, generally progressive, degenerative alteration of the equine podotrochlea. Pathologic alterations might primarily affect the navicular bone (os sesamoideum distale), the navicular bursa (bursa podotrochlearis) or the distal end of the deep flexor tendon. Of these structures, only the bony components can be examined by radiography, which is the primary method to evaluate number, location, size and form of the canales sesamoidales as well as the contour and structure of the navicular bone (BRUNKEN 1986, MACGREGOR 1986, DIK and VAN DEN BROEK 1995). Increased numbers of canales sesamoidales, the appearance of branched canales sesamoidales, very deep or lollypop-shaped (bulbed ends) canales sesamoidales, a reduced radiographic density of the

navicular bone (cyst-like lesion) and spurs at the margins of navicular bone (insertion desmopathy) are regarded as pathologic (NUMANS and VAN DER WATERING 1973, O'BRIEN et al. 1975, HERTSCH et al. 1982, CAMPBELL and MACGREGOR 1984, BRUNKEN 1986, ROSE et al. 1978, KASER-HOTZ and UELTSCHI 1992, WRIGHT 1993). The navicular bones of younger lameness patients as well as of clinically healthy horses of all ages including foals may show radiographic findings, so that they have to be regarded as at least critical (ROSE et al. 1978, KASER-HOTZ and UELTSCHI 1992). WILLMS et al. (1999) could show in a study of Holsteiner warmblood foals up to two-year-old horses that the form and the numbers of canales sesamoidales increased. Of these 144 foals, 18.5 % had slight and 1.5 % severe radiological alterations in the navicular bone.

Some authors presumed hereditary factors to be involved in the pathogenesis of navicular disease (LOWE 1974, ACKERMANN et al. 1977). This could be confirmed by significant differences of prevalences between paternal progeny groups (BOS et al. 1986, DIK and VAN DEN BROEK 1995, PHILIPSSON et al. 1998). Furthermore, heritability estimates were found in the range of h^2= 0.06-0.32 (KWPN 1994, WINTER et al. 1996, WILLMS et al. 1999). A study in Hanoverian warmblood horses, which were appointed for auctions, indicated a heritability up to $h^2 = 0.23$ for deformed canales sesamoidales and $h^2 = 0.17$ for profoundly radiological findings in the navicular bone (STOCK and DISTL 2006b).

Selection of stallions on the basis of navicular radiographs has been successfully practised in the Dutch warmblood studbook. The incidence of horses with more severe grades of navicular bone changes was reduced from 11 % in 1997 to 3 % in 2002 (VAN DEN BELT et al. 2003). In addition, STOCK and DISTL (2005a) could show that selection response in pathological changes of navicular bones can be achieved via a multitrait selection index for performance in dressage and show jumping as well for the most important radiological changes of joints.

The aim of this study was to identify quantitative trait loci (QTL) that significantly influence radiological findings associated with the development of navicular disease. For this purpose, we carried out a whole genome scan on a random sample of 182 Hanoverian warmblood horses using the evaluation scheme of BRUNKEN (1986).

Material and Methods

Family material

The families analysed included 132 progeny and twelve grandchildren of 17 stallions of the Hanoverian warmblood horse population. Blood or hair samples were available for twelve stallions, all 144 progeny or grandchildren, and for 36 dams of the progeny. The number of

progeny and grandchildren per stallion varied from three to 23 with an average size of 8.47 progeny or grandchildren per stallion. Progeny, grandchildren and dams were radiographically examined. Most of the horses (68.02 %) had an age between two and six years (mean age: 3.77 years) at the time of radiological examination, 17 animals were between four and nine months old. Radiographic data of these 17 foals were not used due to the young age and the uncertainty on the manifestation of radiological changes in the navicular bone.

In addition, three dams with radiographic findings but without blood samples were included in our analyses. The 39 dams of the progeny were between four and 19 years old and on average 9.5 years when radiological examination was performed.

Phenotypic traits

Digital radiographs were obtained of both front limbs. The radiological examination comprised the dorsoproximo-palmarodistal projection (upright pedal route according to OXSPRING 1935). Radiological appearance of the navicular bones was classified according to the evaluation scheme of BRUNKEN (1986). Diagnostic criteria were size, shape and distribution of canales sesamoidales as well as structure and contour of the navicular bone (Table 1). The radiographs were analysed several times according to all criteria to get a standard evaluation. Subsequently, individual radiographic findings were combined in order to derive binary traits for canales sesamoidales, navicular bone structure and navicular bone contour. Horses with grades C1, C2a or C2b for canales sesamoidales were classified as unaffected, whereas horses with grades C3a up to grade C6 and CP were classified as affected by many short or elongated or deformed canales sesamoidales (DCS). Horses with grades S1a and S1b for navicular bone structure were regarded as unaffected, while horses with grades S2a up to S4a were classified as affected by radiologically visible alterations of the structure of the navicular bone (RAS). With regard to the contour of the navicular bone, only grade K1 was classified as unaffected, whereas horses with K2 till K5 were considered as affected by radiologically visible alterations of the contour of the navicular bone (RAC).

Of all progeny and grandchildren with radiological findings included in the present study (n = 127), 37.80 % of the horses were affected by DCS, 21.25 % were affected by RAC, and 18.10 % were affected by RAS. Some of the horses showed more than one single radiological change in the navicular bones (Table 2). The percentage of horses affected with regard to one single diagnostic entity was between 4.72 % and 17.32 %. About 4.72 % and 11.81 % of the horses were recorded as affected by at least two radiologically defined traits.

Marker selection and two-step analysis.

For the whole genome scan we selected 161 highly polymorphic microsatellite markers from published equine maps (SWINBURNE et al. 2000a, 2006; CHOWDHARY et al. 2003, BRINKMEYER-LANGFORD et al. 2005, PENEDO et al. 2005) and from the HORSEMAP database at the INRA Biotechnology Laboratories Home Page (http://locus.jouy.inra.fr) to achieve a uniform coverage of all equine autosomes and the X-chromosome. Markers were first selected according to their location on the equine linkage map to provide an even and equidistant coverage of the entire equine genome, and were then selected according to their possible information content for linkage analysis. Threshold was set at five alleles and an observed heterozygosity and polymorphism information content greater than 50 %. The majority of the selected markers were dinucleotide repeats.

For the second step of the genome scan the density of microsatellite markers was increased on selected equine chromosomes, which had the highest linkage test statistics. In total, 23 microsatellite markers on ECA2, eight microsatellite markers on ECA3, 13 microsatellite markers on ECA10 and eight microsatellite markers on ECA15 were added in regions which showed putative QTL with multipoint chromosome-wide error probabilities $p < 0.05$ for the linkage test statistics.

Genomic DNA was isolated from 75 µl EDTA blood or about 30 hair roots using the QIAamp 96 DNA Blood Kit (QIAGEN, Hilden, Germany). Hair roots were digested with proteinase K prior to DNA isolation.

PCR was performed in a total volume of 10 µl using 9 ng DNA, 10x incubation buffer containing 15 mM MgCl2, 5 % DMSO, 150 µM dNTPs and 0.5 U Taq Polymerase (Qbiogene, Heidelberg, Germany).

The amount of primers in the multiplex groups ranged from 1.0 to 18.0 pmol. All forward primers were fluorescence labelled at the 5' end with IRD700 or IRD800. To increase efficiency, 153 primer pairs were pooled into PCR multiplex groups of two to four markers. The eight remaining primer pairs were amplified separately. The primers for the second scan were also pooled into 20 multiplex groups of two to four markers. For amplification, PTC 100™ or PTC 200™ thermal cyclers (MJ Research, Watertown, MA, USA) and a general PCR program with variable annealing temperature (Ta) were used. The reaction started with denaturation at 94 °C for 4 min followed by 37 cycles comprising denaturation for 30 s at 94 °C, annealing for 60 s at Ta (50 – 65 °C) and extension for 30 s at 72 °C. The PCR was completed with a final cooling step at 4 °C for 10 min.

The multiplex groups and the separately amplified PCR products were pooled according to their size and labelling and diluted with formamide loading buffer in ratios from 1:10 to 1:30, that were determined empirically and carried out prior to electrophoresis.
For the analyses of the marker genotypes, the PCR products were size-fractionated by gel electrophoresis on an automated sequencer (LI-COR 4200/S-2, LI-COR 4300, Lincoln, NE, USA) using 4 % or 6 % polyacrylamide denaturing gels (Rotiphorese Gel 40, Roth, Karlsruhe, Germany). Allele sizes were scored against IRD700- and IRD800-labeled DNA ladders used as standards on every gel. Alleles were assigned by visual examination.

Linkage analysis

Multipoint non-parametric linkage (NPL) analyses were performed using Merlin software (multipoint engine for rapid likelihood inference, version 1.0.1) (ABECASIS et al. 2002). Linkage between the different radiological findings and microsatellite markers was estimated through the proportion of alleles shared identical by descent (IBD) for affected animals (WHITTEMORE and HALPERN 1994; KRUGLYAK et al 1996; KONG and COX 1997). The Whittemore and Halpern NPL test statistic, Zmean and the LOD score according to KONG and COX (1997) were used to calculate the error probabilities (p) for linkage. A multipoint chromosome-wide cosegregation of a marker allele among affected family members with the phenotypic expression of the particular radiological findings in the navicular bones was assumed for p-values < 0.05. Genome-wide error probabilities were obtained by applying a Bonferroni correction

$$P_{genome\text{-}wide} = 1 - (1 - P_{chromosome\text{-}wide})^{1/r}$$

with r being the length of the respective equine chromosome in cM divided by the total equine genome length of 2772 cM (SWINBURNE et al. 2006).

Quality of the used marker set and genotyping

The average polymorphism information content (PIC) of the whole markerset for the first genome scan with 161 microsatellite markers was 60.08 %. The PIC values per microsatellite marker showed a minimum of 11.9 % (HTG002, ECA3) and a maximum of 84.9 % (AHT092, ECA3). The mean PIC value per chromosome ranged from 44.94 % (ECA22) to 77.18 % (ECA32). The PIC was at least 50 % in 127 out of the markers (78.9 %). Only four markers showed a PIC ≤ 25 % (2.5 %). The mean observed heterozygosity (HET) in this study was 66.07 %. The HET of individual markers ranged from 11.4 % (HTG002) on ECA3 to 89.5 % (COR070) on ECA6. The mean HET per chromosome ranged from 49.15 %

(ECA22) to 80.4 % (ECAX). The highest average number of alleles of the markers per chromosome was 9.00 for ECA32 and the lowest was 4.33 for ECA5. The average marker distance was 17.2 cM. The highest average marker distance was on ECA14 (38.2 cM) and the smallest one on ECA24 (7.9 cM). Thus, the applied markerset was highly informative and suitable for linkage studies.

Results

We detected chromosome-wide significant QTL for DCS on five equine chromosomes (ECA3, 4, 10, 13 and 14). For the trait RAC, QTL were observed on ten chromosomes (ECA2, 3, 4, 7, 15, 17, 18, 22, 24 and 26). QTL for the trait RAS were found on six different equine chromosomes (ECA4, 7, 26, 29, 30 and 31; Tables 3 to 5). The chromosome-wide error probabilities for these QTL reached the significance level of $p < 0.05$ for Zmeans or LOD scores.

Genomic regions significant in both test statistics, were found for DCS on equine chromosome 3 (ECA3) at 30.20 cM (SG18), on equine chromosome 10 (ECA10) between 4.80 cM (HMS023) to 9.00 cM (COR048) and from 45.50 cM (SG30) to 55.00 cM (LEX017). For the trait RAC, QTL significant in both test statistics were located on equine chromosome 2 (ECA2) from 48.00 cM (UCD380) to 62.20 cM (TKY340) and on equine chromosome 3 (ECA3) at 0.00 cM (AHT036).

Genome-wide error probabilities were obtained by applying the Bonferroni correction for the QTL linked with DCS on ECA10. The chromosomal region from 45.50 cM to 49.80 cM reached the significance threshold ($p<0.05$) of the genome-wide error probability for the Zmean statistic test. For the trait RAC, the microsatellite marker UCD380 at 48.00 cM reached the significant threshold of the genome-wide error probability.

Discussion

The aim of this work was to detect QTL associated with radiographically visible alterations in the navicular bones of Hanoverian warmblood horses.

The high number of putative QTL on different chromosomes found for deformed canales sesamoidales (DCS), pathologic contour (RAC) and pathologic structure (RAS) in this study suggest that several genes are involved in the development of radiological alterations in the navicular bone of horses.

QTL on ECA2, 3, 10 and 15 identified in a whole genome scan using a mean marker distance of 18.3 cM showed the lowest error probabilities for the linkage test statistics and thus, these

QTL positions were refined by increasing the marker density for these regions to an average distance of 5.2 cM (ECA2), 9.3 cM (ECA3), 4.8 cM (ECA10) and 6.9 cM (ECA15). With exception of the QTL on ECA15, the positions of QTL identified in the first step of the whole genome scan could be refined by narrowing the marker distances.

According to the evaluation scheme of Brunken (1986) we considered three different diagnostic criteria (DCS, RAC and RAS). We found QTL on 14 equine chromosomes, which were unevenly distributed between the different traits.

QTL of DCS, RAC and RAS mapped to different chromosomal regions on ECA4. The same situation is given for QTL of RAC and RAS on ECA7 and for QTL on ECA26. The reason for these different positions of the QTL may be due to the number of affected horses per family for the traits analysed and the information content for the different trait analyses may vary. An increased marker density would possibly help to refine these QTL positions. Furthermore, DCS and RAC show a common QTL 30.2 cM on ECA3. These genomic regions may harbour genes, which are involved in both alterations or there may be different genes located closely together. All other QTL mapped to different equine chromosomes and so far these positions were not yet refined by additional markers, this has to be done in further studies. At some QTL regions microsatellite markers are available. For ECA4, there are seven microsatellite markers between LEX050 at 57.80 cM and HTG009 at 73.30 cM published on the equine linkage map (SWINBURNE et al. 2006). For those QTL positions, where no informative microsatellite markers are available yet, new markers have to be developed by using equine expressed sequence tags (ESTs), whole genome shotgun sequences (WGS) or BAC end sequences (BESs) for identification of single nucleotide polymorphisms (SNPs).

NCBI data bank search (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=nucleotide) retrieved 13,966 equine cartilage ESTs and further 23,171 ESTs from other cDNA libraries and steadily increasing numbers of WGS and BESs that can be used for SNP development.

According to SVALASTOGA (1983), SVALASTOGA and SMITH (1983) and SVALASTOGA et al. (1983) navicular disease resembles human osteoarthritis (OA) in general aspects. This finding is based on comparative analyses of the subchondral bone pressure and the changes of the fibrocartilage.

Human molecular genetic studies revealed different candidate genes for osteoarthritis. In this study, we found QTL associated with radiographically visible navicular bone alterations in genomic regions homologous to human genomic regions linked to human OA.

The genomic region on ECA2 at 48.00 cM is syntenic to a region at 31.8 Mb on HSA1p34-p35 and includes some candidate genes like *COL16A1* (coding for alpha chain of type XVI collagen). High levels of type XVI collagen have been found in fibroblasts, chondrocytes, keratinocytes and cornea (PAN et al. 1992, JUN et al. 2001, KASSNER 2004). A further candidate gene in this region is *MATN1* (matrilin 1, cartilage matrix protein), which was mapped on ECA2p15 (CHOWDHARY et al. 2003) and located on HSA1p35 at 30.9 Mb. Mutations of this gene have been associated with hip radiographically evident OA in man (MEULENBELT et al. 1997). These two genes are located proximally of the QTL region for RAC. More distal, near to the microsatellite UCD380 the gene *ALPL* (alkaline phophatase) is positioned. This gene, located on HSA1p34-p36.1 at 21.5 Mb, encodes the tissue non-specific form of this enzyme. Although the exact physiological function of this particular alkaline phosphatase remains to be determined, there is evidence for its function in the mineralisation of the cartilagenous matrix. The enzyme has been linked directly to a disorder known as hypophosphatasia, which is characterized by hypercalcemia and includes skeletal defects (FEDDE and WHYTE 1990, MORNET et al. 1998).

The QTL on ECA3 at 0 cM for RAC and the QTL on ECA3 at about 30.20 cM (SG18) for DCS and RAC is syntenic to HSA16q13-q21, where genes for the matrix metalloproteinase 2 and 15 (*MMP-2, -15*) are located. VIITANEN et al. (2003) showed that in damaged navicular bones of lame horses the content of matrix metalloproteinase 2 (MMP-2) in the hyaline cartilage is significantly raised. The concentration of matrix metalloproteinase 9 (MMP-9) is also significantly higher in tendons of lame horses with lesions in the fibre cartilage of the navicular bone than in tendons of sound horses without lesions in the fibre cartilage of the navicular bone. The gene coding for *MMP-2* is located on HSA16q13-q21 at 54.1 Mb and another gene coding for matrix metalloproteinase 15 (*MMP-15*) at 56.6 Mb of HSA16. This region is syntenic to ECA3p11-p16 (CHOWDHARY et al. 2003) where the marker AHT036 (0.00 cM) is located. Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodelling, as well as in disease processes, such as arthritis and metastasis. Most MMP's are secreted as inactive proproteins, which are activated when cleaved by extracellular proteinases (HAMACHER et al. 2004).

Cadherin 11 (*CDH11*) located in the region at 24.4 cM on ECA3 is another possible candidate gene linked to RAC. This gene encodes a type II classical cadherin from the cadherin superfamily and integral membrane proteins mediating calcium-dependent cell-cell adhesion. Expression of this particular cadherin in osteoblastic cell lines, and its upregulation during

differentiation, suggests a specific function in bone development and maintenance (OKAZAKI et al. 2004, TANIHARA et al. 1994). The gene is located at 63.5 Mb on HSA16q22.1 syntenic to ECARH03a. Near the microsatellite markers COR033 and SG18 the gene encoding the core-binding factor, beta subunit (*CBFB*) is already mapped on ECARH03a on the RH map of CHOWDHARY et al. (2003) and sytenic to HSA16q22.1 (65.6 Mb). *CBFB* belongs to the PEBP2/CBF transcription family, which mainly regulates the host of genes specific to hematopoesis and osteogenesis (Yoshida et al. 2002).

On ECA4 three positional candidate genes for RAC might be *COL1A2* (alpha 2 type I collagen), *PTHB1* (parathyroid hormone-responsive B1), and *BMPER* (bone morphogenetic protein-binding endothelial receptor). *COL1A2* is located at HSA7p22.1 (93.7 Mb) and was already mapped on the RH-map of CHOWDHARY et al. (2003) between the microsatellite markers ASB022 and HTG009. Mutations in this gene are associated with osteogenesis imperfecta, Ehlers-Danlos syndrome, idiopathic osteoporosis, and atypical Marfan syndrome (MYERS et al. 1985, PHILLIPS et al. 1990, NICHOLLS et al. 1992, WATSON et al. 1992). *PTHB1* expression is downregulated by parathyroid hormone in osteoblastic cells, and for that reason, *PTHB-1* is thought to be involved in parathyroid hormone action in bones (ADAMS et al. 1999). The gene encoding for *BMPER* has influence on the osteoblast and chondrogenic cell differentiation (BINNERTS et al. 2004). All three genes, *COL1A2, PTHB-1, BMPER*, are located on HSA7p14, a syntenic genomic region to ECA4. LEPPÄVOURI et al. (1999) found linkage between distal interphalangeal joint OA and HSA7p15-p21, while DEMISSIE et al. (2002) reported significant linkage 10-30 cM proximal of this region for hand OA. Another region linked to hand OA was found on HSA19q13.1 which includes the positional candidate gene for *TGFβ1* (transforming growth factor, beta 1). The dysregulation of *TGFβ* activation and signalling may result in apoptosis. This genomic region is syntenic with ECA10p where we found the chromosomal region from 45.5 cM to 55.0 cM linked to DCS.

The resemblances to human OA may help to find the genes which are causally involved in the development of navicular disease.

Table 1 Evaluation of radiographic appearance of the navicular bones of the front limbs on the basis of size, shape and distribution of canales sesamoidales and of structure and contour of the navicular bones, according to BRUNKEN (1986)

Type of radiographic finding	Code	Description of radiographic finding	Classification
Canales sesamoidales	C1	no canales sesamoidales distales	not-affected
	C2a	few (1-4) short indentations or conical canales sesamoidales distales	
	C2b	few (1-4) elongated, narrow and straight canales sesamoidales distales	
	C3a	several (≥ 5) short indentations or conical canales sesamoidales distales	affected
	C3b	several (≥ 5) elongated, narrow and straight canales sesamoidales distales	
	C4	few (1-4) lollypop-shaped and/or branched canales sesamoidales distales	
	C5	several (≥ 5) lollypop-shaped and/or branched canales sesamoidales distales	
	C6	extensive osteolysis at margo distalis, no discernible canales sesamoidales distales	
	CP	canales sesamoidales proximales	
Navicular bone structure	S1a	fine-structured and regular spongiosa	not-affected
	S1b	fine-structured, but irregular spongiosa	
	S2a	coarse, but regular spongiosa	affected
	S2b	coarse and irregular spongiosa	
	S3a	general sclerosis	
	S3b	sclerosis around canales sesamoidales	
	S4a	central osteolytic area with blurred margins	
	S4b	central osteolytic area with sharp margins	
	S5a	fracture at margo distalis (chip fracture)	
	S5b	sagittal fracture	
	S5c	pathological fracture	
Navicular bone contour	K1	no contour changes	not-affected
	K2	new bone formation in the central part of margo distalis	affected
	K3	new bone formation at margo proximales	
	K4	new bone formation at medial or lateral part of margo distalis	
	K5	angular or peaked new bone formation at medial or lateral extremity	

Table 2 Joint distribution (%) of the traits deformed canales sesamoidales (DCS), radiological alterations in the contour of the navicular bones (RAC) and radiological alterations in the structure of the navicular bones (RAS) of the progeny or grandchildren (n=127) of the 17 stallions used in this study

Trait	DCS	RAC	RAS
DCS	17.32	11.81	8.66
RAC	-	4.72	4.72
RAS	-	-	4.72

Table 3 Test statistics Zmean and LOD score of the non-parametric multipoint linkage analysis for deformed canales sesamoidales in the navicular bone (DCS) and their error probabilities (p_Z, p_L) for affected horses (DCS ≥ C3a)

ECA	POS in cM	Marker	Zmean	p_Z	LOD score	p_L
3	27.30	TKY937	1.56	0.06	0.67	0.04
	30.20	SG18	2.26	0.012	1.00	0.02
	34.00	UMNe158	1.78	0.04	0.83	0.03
3	71.60	LEX057	1.53	0.06	0.75	0.03
	72.00	HMS061	1.55	0.06	0.75	0.03
4	0.00	AHT043	1.23	0.11	0.74	0.03
10	4.80	HMS23	1.70	0.04	0.73	0.03
10	45.50	SG30	3.00	0.0014	1.29	0.007
	49.00	COR015	3.43	0.0003	1.46	0.005
	49.80	LEX008	3.46	0.0003	1.48	0.004
	50.10	TKY537	2.45	0.007	1.04	0.014
	50.50	TKY592	2.50	0.006	1.09	0.013
	52.30	LEX066	2.43	0.007	1.12	0.012
	55.00	LEX017	2.35	0.010	0.99	0.02
13	51.00	ASB001	1.07	0.14	0.93	0.02
14	3.60	AHT029	0.98	0.2	0.84	0.02
	27.80	LEX043	1.16	0.12	0.92	0.02

ECA: Equus caballus autosome; POS: position on the linkage maps (Swinburne et al. 2000a, 2006, Chowdhary et al. 2003, Brinkmeyer-Langford et al. 2005, Penedo et al. 2005);

p_Z: error probability for Zmean; p_L: error probability for LOD score

Table 4 Test statistics Zmean and LOD score of the non-parametric multipoint linkage analysis for the trait radiological alterations in the contour of the navicular bone (RAC) and their error probabilities (p_Z, p_L) for affected horses (RAC ≥ K2)

ECA	POS in cM	Marker	Zmean	p_Z	LOD score	p_L
2	48.00	UCD380	3.11	0.0010	1.20	0.009
	51.00	TKY784	1.71	0.04	0.69	0.04
	52.00	TKY352	1.71	0.04	0.69	0.04
	53.00	TKY474	1.71	0.04	0.69	0.04
	57.00	TKY605	1.69	0.05	0.69	0.04
	62.20	TKY340	1.70	0.04	0.69	0.04
3	0.00	AHT036	2.05	0.02	1.41	0.005
3	24.40	COR033	1.82	0.03	0.43	0.08
	27.30	TKY937	1.92	0.03	0.48	0.07
	30.20	SG18	2.29	0.011	0.54	0.06
3	71.60	LEX057	1.33	0.09	0.73	0.03
	72.00	HMS061	1.34	0.09	0.73	0.03
4	57.83	LEX050	1.41	0.08	1.07	0.013
	71.01	ASB22	1.63	0.05	0.71	0.04
	73.30	HTG009	1.64	0.05	0.66	0.04
7	74.00	SG28	1.05	0.15	0.68	0.04
	102.1	AHT019	1.28	0.10	0.88	0.02
15	51.011	SG06	1.30	0.10	0.78	0.03
	51.015	ASB019	1.31	0.10	0.79	0.03
	51.02	COR077	1.31	0.09	0.79	0.03
17	4.40	COR105	0.94	0.2	0.68	0.04
18	87.60	UCD387	0.74	0.2	0.65	0.04
22	79.70	SG19	0.73	0.2	0.68	0.04
24	1.60	LEX042	1.15	0.12	0.64	0.04
24	8.40	EA2C4	1.16	0.12	0.66	0.04
26	6.90	COR071	1.51	0.07	1.00	0.02

ECA: Equus caballus autosome; POS: position on the linkage maps (Swinburne et al. 2000a, 2006, Chowdhary et al. 2003, Brinkmeyer-Langford et al. 2005, Penedo et al. 2005);
p_Z: error probability for Zmean; p_L: error probability for LOD score

Table 5 Test statistics Zmean and LOD score of the non-parametric multipoint linkage analysis for the trait radiological alterations in the structure of the navicular bone (RAS) and their error probabilities (p_Z, p_L) for affected horses (RAS ≥ S2a)

ECA	POS in cM	Marker	Zmean	p_Z	LOD score	p_L
4	117.70	SG23	1.31	0.09	0.99	0.02
7	18.10	HTG020	1.18	0.12	1.00	0.02
	43.02	LEX038	1.05	0.15	0.85	0.02
	66.00	COR095	1.01	0.2	0.70	0.04
26	19.40	UM005	0.87	0.2	0.66	0.04
29	43.00	COR027	1.39	0.08	1.08	0.013
	61.00	ASB043	1.17	0.12	0.83	0.03
30	31.40	VHL020	0.85	0.2	0.60	0.05
31	41.10	AHT034	0.78	0.2	0.62	0.04

ECA: Equus caballus autosome; POS: position on the linkage maps (Swinburne et al. 2000a, 2006, Chowdhary et al. 2003, Brinkmeyer-Langford et al. 2005, Penedo et al. 2005);
p_Z: error probability for Zmean; p_L: error probability for LOD score

Summary

Ulrike Diesterbeck (2006)

Identification of Quantitative Trait Loci (QTL) for radiological alterations in the navicular bone of Hanoverian warmblood horses

The objectives of the present thesis were to collect a family material showing both a high incidence of radiologically visible alterations in the navicular bone as well as a genetic predisposition to these radiological changes for the identification of quantitative trait loci (QTL) responsible for radiologically visible alterations in the navicular bone in Hanoverian warmblood horses.

The family material comprised 155 horses descending from 46 stallions. The size of the examined half-sib groups ranged from three to 19. Radiographs were taken of both front limbs of the navicular region in the dorsoproximo-palmarodistal projection according to OXSPRING (1935). The radiographs of the navicular bone were analysed according to the scheme of BRUNKEN (1986) and appraised by experienced veterinariens. The following prevalences were determined: deformed canales sesamoidales (DCS), 40.0 %; for radiological changes in the contour (RAC), 23.2 %; and for radiological alterations in the structure (RAS), 25.8 %. The estimated heritabilities for the single characteristics were $h^2 = 0.63 \pm 0.19$ (DCS), $h^2 = 0.53 \pm 0.28$ (RAS), $h^2 = 0.24 \pm 0.13$ (RAC). Moderatly positive additive genetic correlations were estimated between DCS and RAS ($r_g = 0.27 \pm 0.14$) as and between DCS and RAC ($r_g = 0.38 \pm 0.08$), while RAC and RAS were negatively additively genetically correlated ($r_g = -0.79 \pm 0.10$).

The whole genome scan comprised 161 microsatellite markers equally distributed over all autosomes and the X-chromosome. The 17 half-sib families were comprised of on average 8.47 progeny or grandchildren and 181 animals were genotyped. The average information content (PIC) of the microsatellits used was 60.5 %.

Increasing the marker density with 52 additional markers, QTL with high Zmeans and LODscores on ECA2, 3, 10 and 15 were refined.

Non-parametric linkage analyses based on identical-by-descent (IBD) mapping were separately performed for the traits DCS, RAC and RAS. Chromosome-wide significant QTL

for the different traits were found on 17 equine chromosomes and the region from 45.50 cM to 49.80 cM on equine chromosome 10 linked with DCS and the microsatellitemarker UCD380 at 48.00 cM on ECA2 linked with RAC were also genome-wide significant. Chromosome-wide significant QTL were detected for DCS on five chromosomes. Chromosome-wide significant QTL for RAC were found on ten equine chromosomes and for RAS on six equine chromosomes. The uneven distribution of the QTL between the traits analysed indicates that different genes may be responsible for the different traits.

Positional candidate genes for osteoarthritis (OA) were identified in syntenic human chromosome regions for QTL on equine chromosomes 2, 3, 4 and 10. As navicular bone disease resembles human OA but is not identical to human osteoarthritis, it is not clear if osteoarthritis genes may be really useful candidates for equine navicular disease.

The genome scan carried out in this study was a first step towards the identification of candidate genome regions harbouring genes responsible for the development of radiologically visible alterations in the navicular bones of horses.

Erweiterte Zusammenfassung

Ulrike Sigrid Diesterbeck (2006)

Quantitative Merkmalsgenorte (QTL) für radiologische Veränderungen am Strahlbein beim Hannoverschen Warmblut

Einleitung

Lahmheitsprobleme spielen in der Pferdemedizin eine große Rolle und sind der Hauptgrund für den vorzeitigen Abgang des Pferdes (PHILIPSSON et al. 1998, WALLIN et al. 2000). Hierfür sind häufig krankhafte Veränderungen der Hufrolle, die sich aus dem Strahlbein (Os sesamoidale distale), dem distalen Anteil der tiefen Beugesehne und der Bursa podotrochlearis zusammensetzt, verantwortlich. Eine häufige degenerative Erkrankung der Hufrolle ist die Podotrochlose oder Hufrollenentzündung. Bis heute ist die Pathogenese dieser weit verbreiteten Erkrankung nicht geklärt. Als verantwortlich werden unphysiologische Belastungen im Hufrollenbereich, insbesondere übermäßige Stoß- und Druckkräfte angesehen (BRANSCHEID 1977, HERTSCH et al. 1982, DÄMMRICH et al. 1983). Diesen wiederum liegen Gliedmaßenfehlstellungen, pathologische Hufformen oder eine mangelnde Hufpflege zu Grunde. Die entstehenden Druck- und Zugkräfte führen dann zu kompensatorischen Reaktionen der knöchernden Strukturen und der Bänder. Eventuell können auch Durchblutungsstörungen auf Grund von Thromben, Proliferationen der Intima der Arterien und Arteriosklerose Ursache für Strahlbeinveränderungen sein (SVALASTOGA 1983).

Klinische Symptome für das Vorliegen einer Hufrollenentzündung sind häufig unspezifisch. In den meisten Fällen liegt eine geringradige intermittierende Lahmheit vor (ACKERMANN et al. 1977). Nach der Anästhesie des Rami tori digitalis kommt es in klassischen Fällen zu einem Umschwenken der Lahmheit auf das andere Vorderbein.

Radiologisch erfassbare Veränderungen am Strahlbein treten bei Pferden jeglichen Alters auf und auch bereits bei Fohlen (BRANSCHEID 1977, KASER-HOTZ und UELTSCHI 1992, HORNIG 1993). Allerdings spiegeln die radiologischen Befunde die pathohistologischen

Vorgänge nicht zuverlässig wider (DELIUS 1982, DROMMER et al. 1992) und müssen daher mit den Befunden der klinischen Untersuchung für eine zuverlässige Diagnose im Zusammenhang interpretiert werden.

Das gesunde Strahlbein zeigt nicht deformierte und kurze Canales sesamoidales, eine feinmaschige und gleichmäßige Struktur und eine alterstypische Form ohne Exostosen. Radiologische Befunde wie verzweigte oder erweiterte Canales sesamoidales oder eine große Anzahl dieser, eine unebene und sklerosierte oder aufgehellte Struktur sowie Zubildungen an der Kontur werden pathologischen Prozessen am Strahlbein zugeordnet (OXSPRING 1935, CAMPBELL und MACGREGOR 1984, BRUNKEN 1986, HERTSCH und STEFFEN 1986, KASER-HOTZ und UELTSCHI 1992, WRIGTH 1993b).

Auf Grund eines gehäuften Auftretens in verschiedenen Familien wurde auch eine erbliche Komponente für das Auftreten von Podotrochlose postuliert (DÄMMRICH et al. 1983, BOS et al. 1986, DIK und VAN DEN BROEK 1995). Die geschätzten Heritabilitäten lagen modellabhängig zwischen $h^2 = 0,06$ bis $h^2 = 0,31$ (WINTER et al. 1996, WILLMS et al. 1999). Die hier vorliegende molekulargenetische Untersuchung stellt einen ersten Schritt zur Identifizierung beteiligter Genorte dar.

Prävalenzen und Schätzung genetischer Parameter für radiologische Strahlbeinveränderungen beim Hannoverschen Warmblut

Familienmaterial und radiologische Untersuchung

Für diese Studie wurden 155 Tiere radiologisch untersucht. Diese sind Nachkommen von 46 Hannoveraner Hengsten. Die Halbgeschwistergruppen umfaßten eins bis 19 Tiere. Der Anteil weiblicher Pferde betrug 64,52 % (n = 100), 35,38 % (n = 55) waren männliche Tiere. Das Alter der Pferde lag zum Zeitpunkt der Untersuchung zwischen 22 Monaten und 19 Jahren (Mittel: 5,4 Jahre). Das mittlere Alter der weiblichen Pferde betrug 6,4 Jahre bzw. 3,6 Jahre bei den männlichen Pferden. Die Tiere wurden in drei Altersklassen eingeteilt. Die erste Altersklasse umfaßte 34 Tiere bis zu einem Alter von 2,5 Jahren, die zweite Altersklasse beinhaltete 71 Pferde älter als 2,5 Jahre bis 5 Jahre und die dritte Altersklasse setzte sich aus 50 Pferden älter als 5 Jahre zusammen.

Die Zehen der Vordergliedmaßen wurden in der dorsoproximal-palmarodistalen Projektion (OXSPRING 1935) geröngt. Die Röntgenbilder wurden von zwei erfahrenen Tierärzten nach dem Beurteilungsschema von BRUNKEN (1986) ausgewertet. Diagnostischen Kriterien

waren Größe, Form und Verteilung der Canales sesamoidales, die Struktur und Kontur des Strahlbeins. Für jedes Individuum erfolgte eine Analyse der folgenden drei Merkmale: deformierte Canales sesamoidales (DCS), Veränderungen in der Strahlbeinkontur (RAC) und Veränderungen der Strahlbeinstruktur (RAS) (Abbildung 1-3).

Statistische Auswertung

Die Prävalenzen von DCS, RAC und RAS wurden als binäre Merkmale ausgewertet. Für die Canales sesamoidales wurden Befunde mit ≥ C3a, für die Kontur ≥ K2 und für die Struktur ≥ S2a als pathologisch verändert bewertet. Alle anderen Tiere wurden als nicht betroffen von Strahlbeinveränderungen betrachtet. Jedes Tier konnte mehr als einen Befund aufweisen, dabei wurde jeder Befund jeweils separat analysiert.

Die Varianzanalyse wurde für die Prävalenz von DCS, RAC und RAS durchgeführt. Das Geschlecht des Pferdes, die Altersklasse und die Interaktion zwischen Geschlecht und Altersklasse wurden als fixe Effekte getestet.

Die Schätzung der genetischen Parameter erfolgte mittels REML (Residual Maximum Likelihood) im linearen Tiermodell. Die Pedigreeinformation beinhaltete vier Ahnengenerationen.

$$y_{ijkl} = \mu + SEX_i + AGE_j + SEX_i*AGE_j + e_{ijkl}$$

wobei y_{ijkl} die binär abhängige Variable für DCS, RAC und RAS ist; μ beschreibt die Modellkonstante; SEX_i ist der fixe Effekt des Geschlechts des Pferdes (i = 2; männlich, weiblich); AGEj ist der fixe Effekt der Röntgenaltersklasse ((j: ≤ 2,5 Jahre, > 2,5 bis ≤ 5 Jahre, > 5 Jahre); a_k ist der zufällige additiv-genetische Tiereffekt (k = 1536) und e_{ijkl} ist der zufällige Resteffekt.

Die geschätzten Heritabilitäten (h^2_{obs}) wurden nach DEMPSTER und LERNER (1950) und die geschätzten residual Korrelationen ($r_{e\ obs}$) nach VINSON et al. (1976) transformiert.

Ergebnisse

Prävalenzen radiologischer Strahlbeinveränderungen

Von allen 155 untersuchten Pferden wiesen 72 Pferde keine Veränderungen am Strahlbein auf, 62 Pferde (40,0 %) waren von DCS, 36 Pferde (23,2 %) von RAC und 40 Pferde (25,8 %) von RAS betroffen.

12 Pferde (7,7 %) wiesen sowohl DCS als auch RAC auf, 15 Pferde (9,7 %) waren betroffen von DCS und RAS und 4 (2,6 %) Pferde von RAC und RAS. Von allen 155 untersuchten Pferden waren 12 Pferde (7,7 %) von allen drei Veränderungen betroffen.

Die multiple Varianzanalyse zeigte einen signifikanten Einfluss des Geschlechts auf die Prävalenz von RAS und RAC. Die Röntgenaltersklasse hatte einen signifikanten Einfluss auf die Prävalenz von DCS und RAC. Die Interaktion von Geschlecht und Altersklassebeeinflusste signifikant die Prävalenz von RAC.

Schätzung der genetischen Parameter

Die linearen Heritabilitäten lagen für das Merkmal DCS bei $h^2_{obs} = 0,39 \pm 0.12$, für RAC bei $h^2_{obs} = 0,13 \pm 0.07$ und für das Merkmal RAS bei $h^2_{obs} = 0,29 \pm 0,15$. Nach der Transformation in das Schwellenmodell wurden Heritabilitäten von $h^2_{liab} = 0,63 \pm 0,19$ für DCS, $h^2_{liab} = 0,24 \pm 0,13$ für RAC und $h^2_{liab} = 0,53 \pm 0,28$ für RAS geschätzt.

Die geschätzten additiv-genetischen Korrelationen zwischen DCS und RAC ($r_g = 0,38 \pm 0,27$) und DCS und RAS ($r_g = 0,27 \pm 0,14$) waren positiv, während RAC und RAS ($r_g = -0,79 \pm 0,21$) dagegen negativ additiv-genetisch korreliert waren.

Die residualen Korrelationen lagen vor der Transformation zwischen $r_{e\ obs} = 0,13$ und 0,39 ($SE_{h^2} = 0,08 – 0,14$) und zwischen $r_e = 0,24$ und 0,72 ($SE_{h^2} = 0,14$ -0,244) nach der Transformation in das Schwellenmodell.

Diskussion

Das Ziel dieser Studie war die Verteilung verschiedener radiologischer Strahlbeinveränderungen an einer Stichprobe Hannoveraner Warmblutpferde zu ermitteln. Dabei sollte die Bedeutung des genetischen Einflusses für das Auftreten von diesen pathologischen Strahlbeinveränderungen quantitativ bestimmt werden.

Abhängig von der radiologisch untersuchten Pferdepopulation und dem Untersuchungsaufbau wurden Prävalenzen für röntgenologische Veränderungen im Strahlbein von 25 % beschrieben.

Studien über die Verteilung der speziellen radiologischen Strahlbeinveränderungen wurden bisher nur selten durchgeführt. STOCK und DISTL (2006) ermittelten Prävalenzen von 2,23 % für DCS in einer Studie mit 5157 drei- bis siebenjährigen Pferden, die für Auktionen vorausgewählt waren. Die Prävalenzen für RAC und RAS betrugen dabei 0,50 % und 0,39 %. In der hier vorgelegten Studie lagen die Prävalenzen deutlich höher, weil die Untersuchungsdaten von Zuchtstuten und deren Nachkommen genutzt wurden. Das Alter dieser Pferde lag zwischen 22 Monaten und 19 Jahren, und es gab einen Überhang an älteren weiblichen Pferden. Im Unterschied zu der Studie von STOCK und DISTL (2006), welche auf jungen Reitpferden basierte, spiegelt die Stichprobe dieser Studie die Zuchtpopulation wider.

Bisher konnte ein signifikanter Alterseinfluss für pathologische Strahlbeinveränderungen bei Holländische Warmblutstuten nicht nachgewiesen werden (KWPN 1994). Dies stimmt überein mit den Ergebnissen von STOCK et al. (2004), die ebenfalls keinen signifikanten Alterseinfluss auf die Prävalenz von röntgenologischen Strahlbeinveränderungen bei drei- bis siebenjährigen für Auktionen vorgesehene Pferde fanden. In dieser Studie konnte ein Alterseinfluss für DCS und RAC gezeigt werden. Unterschiede in Alters- und Geschlechtsverteilung könnten Ursache für die Diskrepanz bezüglich des Alterseinflusses sein. In Übereinstimmung mit den hier beschriebenen Ergebnissen, ergab sich auch bei WINTER et al. (1996) eine altersabhängige Zunahme von Strahlbeinveränderungen bei drei- bis achtjährigen Warmblutpferden.

In der Studie von WINTER et al. (1996) war der Anteil betroffener Stuten größer als der Anteil betroffener Hengste und Wallache. Bei anderen Untersuchern waren männlicher Tiere häufiger betroffen (ACKERMANN et al. 1977), oder ein Geschlechtseinfluss wurde nicht festgestellt (BODENMÜLLER 1983). In der vorliegenden Studie wurde ein signifikanter Geschlechtseinfluss nur für RAS und RAC, aber nicht für DCS aufgezeigt. Allerdings sollten diese Ergebnisse mit Vorsicht bewertet werden, da der Materialumfang sehr gering war.

Die berichteten Heritabilitäten für rradiologische Strahlbeinveränderungen waren im beobachteten Modell klein ($h^2 = 0,06$; WINTER et al. 1996) und erstrecken sich von $h^2 = 0,20$ zu $h^2 = 0,46$ im Schwellenmodell. Mögliche Gründe für die Unterschiede der Schätzwerte sind auf feine unterschiedliche Merkmalsdefinitionen, unterschiedliche Datenstrukturen in Bezug auf das Röntgenalter und die Stichprobe der Population und die verschiedenen Methoden für die genetischen Analysen zurückzuführen. In dieser Studie wurden zum ersten Mal Heritabilitäten für die unterschiedlichen radiologischen Strahlbeinveränderungen geschätzt. Diese lagen für RAC im unteren bis mittleren Bereich. Für DCS und RAS konnten mittlere bis hohe Heritabilitäten geschätzt werden. Die geschätzten additiv-genetischen Korrelationen weisen auf einen gemeinsamen genetischen Hintergrund für DCS und RAC und für DCS und RAS hin, während RAC und RAS von verschiedenen Genen beeinflusst zu sein scheinen. Zur Bestätigung dieser Ergebnisse werden größere Stichproben von Pferden benötigt.

Genomweite Suche nach quantitativen Merkmalsgenorten (QTL) für radiologische Veränderungen am Strahlbein beim Hannoverschen Warmblut

Familienmaterial

Die Familienanalyse umfasste 132 direkte Nachkommen und zwölf Enkel von 17 Hengsten. Blut- und Haarproben waren von zwölf Hengsten, allen 144 Nachkommen bzw. Enkeln und von 36 Müttern der Nachkommen vorhanden. Die Anzahl der Nachkommen und Enkel je Hengst variierte von drei bis 23 Tieren, mit einer mittleren Anzahl von 8,47 Tieren je Hengst. Die Nachkommen und Enkel sowie die Mütter wurden röntgenologisch auf Strahlbeinveränderungen untersucht. Die meisten Pferde (68,02 %) hatten bei der der Untersuchung ein Alter zwischen zwei und sechs Jahren (Mittel: 3,77 Jahre); 17 Tiere waren zwischen vier und neun Monaten alt. Die radiologischen Befunde dieser 17 Fohlen wurden aufgrund des unklaren Manifestationsalter radiologisch erfassbarer Strahlbeinveränderungen nicht in die Analyse einbezogen und nur deren Genotypen wurden genutzt. Zusätzlich wurden die Untersuchungsbefunde von drei Müttern in die Auswertungen mit einfließen, Blutproben waren von ihnen aber nicht vorhanden. Die insgesamt 39 Mütter waren bei der radiologischen Untersuchung zwischen vier und 19 Jahren alt (Mittel 9,5 Jahre).

Merkmale

Die nach der Oxspringmethode (dorsoproximal-palmarodistaler Strahlengang) angefertigten Röntgenbilder der Zehen beider Vordergliedmaßen wurden nach dem Schema von BRUNKEN (1986) (Abbildungen 1-3) ausgewertet. Die Merkmale Canales sesamoidales, Kontur und Struktur des Strahlbeins wurden anschließend in binäre Merkmale umcodiert. Für die Canales sesamoidales wurden Befunde mit $\geq$ C3a, für die Kontur $\geq$ K2 und für die Struktur $\geq$ S2a als pathologisch verändert bewertet. Alle anderen Tiere wurden als nicht betroffen von Strahlbeinveränderungen betrachtet. Unter allen Nachkommen (n = 127) mit Untersuchungsergebnissen wiesen 37,80 % deformierte Canales sesamoidales (DCS), 21,26 % radiologische Veränderungen in der Strahlbeinkontur (RAC) und 15.75 % Veränderungen der Strahlbeinstruktur (RAS). Wenn ein Tier dabei mehr als einen Befund aufwies, wurde jeder Befund jeweils separat analysiert.

Auswahl der Mikrosatellitenmarker

Für einen ersten Genomscan wurden 161 hochpolymorphe Mikrosatellitenmarker ausgewählt. Ihre Position und Anordnung wurde den bekannten Karten für das Pferdegenom

(SWINBURNE et al. 2000a, CHOWDHARY et al. 2003, PENEDO et al. 2005, SWINBURNE et al. 2006) und der HORSEMAP *database* (http://locus.jouy.inra.fr) entnommen.

Der *Polymorphism information content* (PIC) des Markersets lag im Mittel bei 60,08 %. Einen PIC ≥ 50% besaßen 127 Mikrosatellitenmarker (78,9 %), während vier Marker (2,5 %) einen PIC ≤ 25 % aufwiesen. Die Heterozygosität (HET) lag im Mittel bei 66,07 %, der mittlere Abstand der Marker betrug 17,2 cM (7,9 cM auf dem Pferdechromosom (ECA) 24 bis 38,2 cM auf ECA14). Im Mittel besaßen die Marker 6,44 Allele.

In einem zweiten Schritt wurden 52 neue Mikrosatellitenmarker ausgewählt um die Markerdichte an chromosomenweit signifikanten ($p \leq 0,05$) QTL auf ECA2, ECA3, ECA10 und ECA15 zu erhöhen.

DNA-Isolierung und Genotypisierung der Mikrosatellitenmarker

Aus allen 190 Blut- und Haarproben wurden mit dem QIAamp® 96 DNA Blood Kit (QIAGEN, Hilden, Germany) DNA isoliert. Die erhaltene DNA wurde als template in eine PCR Reaktion eingesetzt (Denaturierung bei 94 °C für 4 min., 37 Zyklen von je 30 s 94 °C Denaturierung, 60 s Annealing bei je Primer-spezifischer Temperatur, 30 s 72 °C Elongation, abschließendes Kühlen für 10 min bei 4 °C). Je PCR-Ansatz wurden bis zu vier Primerpaare gepoolt. In Anwesenheit von hitzestabiler DNA-Polymerase amplifiziert. Die PCR-Produkte wurden anschließend mit Formamid-Ladepuffer verdünnt und die einzelnen Allele elektrophoretisch auf 4 %igen- oder 6 %igen Polyacrylamidgelen auf den automatischen Sequenziergeräten Li-COR 4200/S-2 und 4300 (Li-COR,Lincoln, NE, USA) aufgetrennt und der Genotyp der Marker mit Hilfe eines Längenstandards visuell bestimmt.

Auswertung

Die Ergebnisse der Genotypisierung in Kombination mit den Pedigree- und Phänotypdaten der Familienmitglieder wurden über eine nichtparametrische Kopplungsanalyse (NPL) mit Hilfe der Merlin software (multipoint engine for rapid likelihood inference, version 0.10.2, ABECASIS et al. 2002) analysiert. Die Kopplung zwischen den verschiedenen Merkmalen für Strahlbeinveränderungen und den Mikrosatelliten wurde durch den gemeinsamen Anteil der Allele basierend auf dem „identical-by-descent (IBD-Verfahren)“ in chromosomenweiten Mehrpunktanalysen ermittelt (WHITTEMORE und HALPERN 1994, KRUGLYAK et al. 1996, KONG und COX 1997). Die einer Normalverteilung folgenden Teststatistik für den Anteil von „identical-by-descent“ (IBD) Markerallelen (Zmean) und ein daraus linear

abgeleiteter LOD-Score wurden berechnet. Die Irrtumswahrscheinlichkeit für eine signifikante Kopplung wurde auf $p \leq 0,05$ festgelegt. Um eine genomweite signifikante Kopplung zu ermitteln, wurden die Irrtumswahrscheinlichkeiten von chromosomenweit signifikanten Markern nach der Formel von Bonferroni ($P_{genomweit} = 1 - (1 - P_{chromosomweit})^{1/r}$ (r = Chromosomenlänge/Genomlänge (2772 cM)) korrigiert, um eine genomweite Kopplung für $p \leq 0,05$ zu ermitteln.

Ergebnisse

Chromosomenweit signifikante Merkmalsgenorte (QTL) wurden auf 17 verschiedenen Chromosomen nachgewiesen. Spezifisch für die jeweiligen Merkmale ergaben sich chromosomenweit signifikante QTL auf 5 (DCS), 10 (RAC) bzw. 6 Chromosomen (RAS). Die chromosomale Region von 45,50 cM bis 49,80 cM auf ECA10 erreichte den signifikanten Schwellenwert der genomweiten Irrtumswahrscheinlichkeit von $p \leq 0,05$ für das Merkmal DCS. Der Mikrosatellitenmarker UCD380 bei 48,00 cM auf ECA2 erreichte ebenfalls eine genomweite Irrtumswahrscheinlichkeit von $p \leq 0,05$ für das Merkmal RAC.

Diskussion

Die große Anzahl an gefundenen signifikanten Merkmalsgenorten für die einzelnen Merkmale weist auf einen Einfluss mehrerer Gene. Einige dieser QTL könnten auch falsch positiv sein. Um dies auszuschließen, müsste die Markerdichte in diesen Regionen weiter erhöht werden.

Die QTL auf den Chromosomen ECA2, 3, 10 und 15, welche im ersten Genom-Scan mit einer Markerdichte von 17,2 cM gefunden wurden, zeigten große Zmeans und LOD-Scores und kleine Irrtumswahrscheinlichkeiten und wurden deshalb durch eine Erhöhung der Markerdichte weiter eingegrenzt und bestätigt. Die anderen signifikanten QTL müssen noch genauer untersucht werden. Falls keine informativen Mikrosatellitenmarker vorhanden sind, können neue Marker über *Single Nucleotide Polymorphisms* (SNPs) entwickelt werden.

Es wurden drei verschiedene Merkmale (DCS, RAC, RAS) auf der Grundlage des Beurteilungsschemas nach BRUNKEN (1986) ausgewertet. Es wurden Merkmalsgenorte auf 17 verschiedenen Chromosomen gefunden. Diese QTL waren zwischen den Merkmalen sehr unterschiedlich verteilt.

Die Merkmale DCS, RAC und RAS besitzen alle QTL auf ECA4, welche jedoch unterschiedlich auf dem Chromosom lokalisiert sind. Das Gleiche gilt für Kontur- und Strukturveränderungen auf ECA7 und ECA26. Ein Grund dafür kann ein unterschiedlicher

Informationsgehalt für die jeweiligen Marker in den verschiedenen Familien sein. Mit einer Erhöhung der Markerdichte könnten diese QTL verfeinert werden. DCS und RAC besitzen einen gemeinsamen QTL auf ECA3 bei 30,20 cM. Dieser QTL beherbergt möglicherweise Gene, welche an der Ausprägung beider Veränderungen involviert sind oder es handelt sich um unterschiedliche Gene, die sehr nah beieinander liegen.

Gemäß SVALASTOGA and SMITH (1983), SVALASTOGA et al. (1983), SVALASTOGA (1983) ähnelt Podotrochlose in vielen Aspekten der menschlichen Osteoarthritis. Parallelitäten ergeben sich z. B. für den subchondralen Knochendruck, Veränderungen des fibrillären Knorpels und mikrovaskuläre Veränderungen.

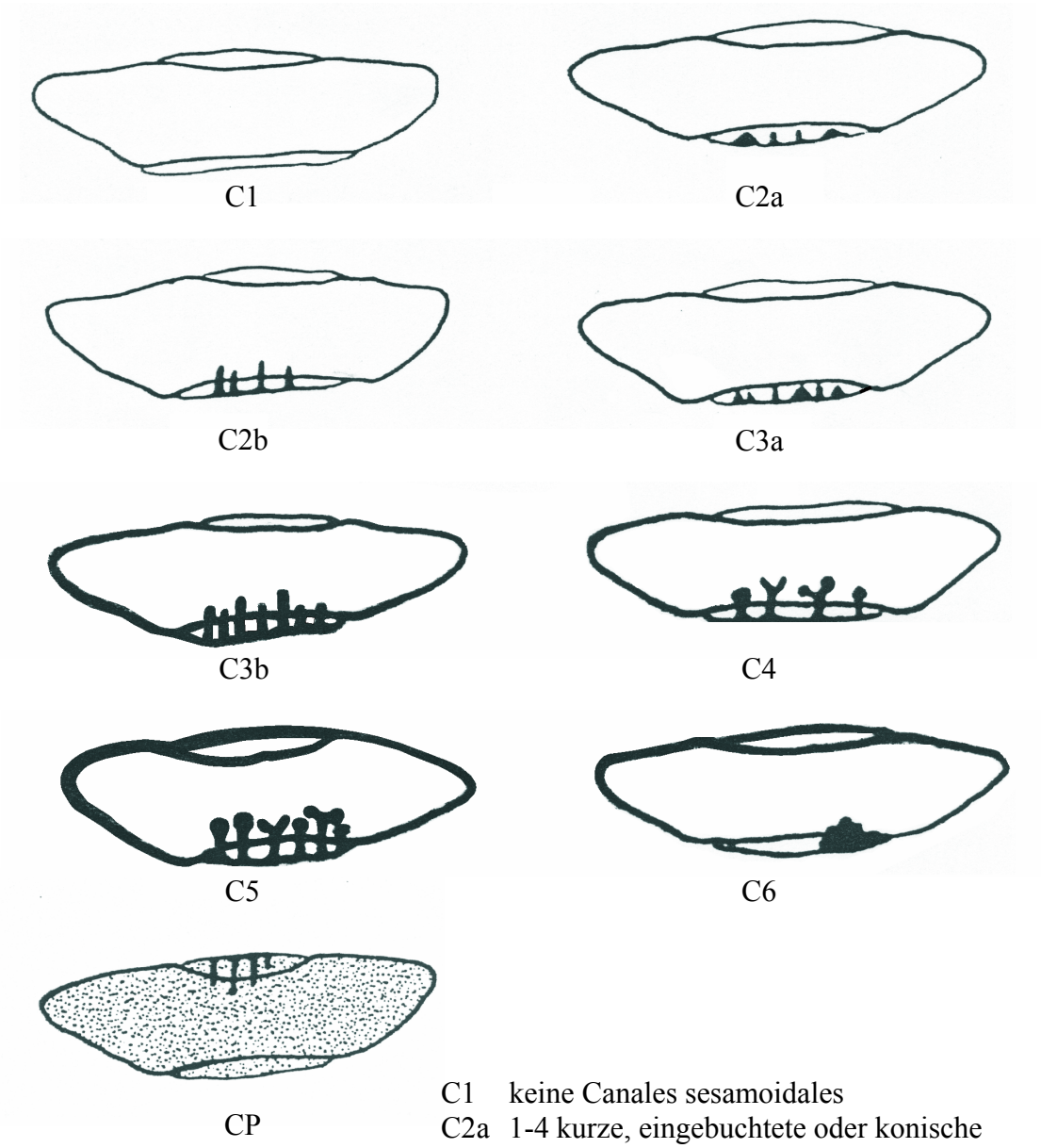
Einige für Strahlbeinveränderungen gefundene signifikante Merkmalsgenorte befinden sich in syntänen humanen Chromosomenabschnitten, die positionelle Kandidatengene aufweisen, welche beim Menschen bereits als Kandidatengene für Osteoarthritis beschrieben wurden. Da Podotrochlose keine humane Erkrankung darstellt, ist noch nicht nachgewiesen worden, ob Osteoarthritis verursachende Gene als Kandidatengene für Podotrochlose in Frage kommen.

Schlussfolgerungen

Die Merkmale haben eine erbliche Komponente. Die geschätzten Heritabilitäten betrugen für DCS $h^2 = 0,63 \pm 0,19$, für RAC $h^2 = 0,24 \pm 0,13$ und RAS $h^2 = 0,27 \pm 0,14$. Strahlbeinveränderungen sind signifikant mit Merkmalsgenorten gekoppelt.

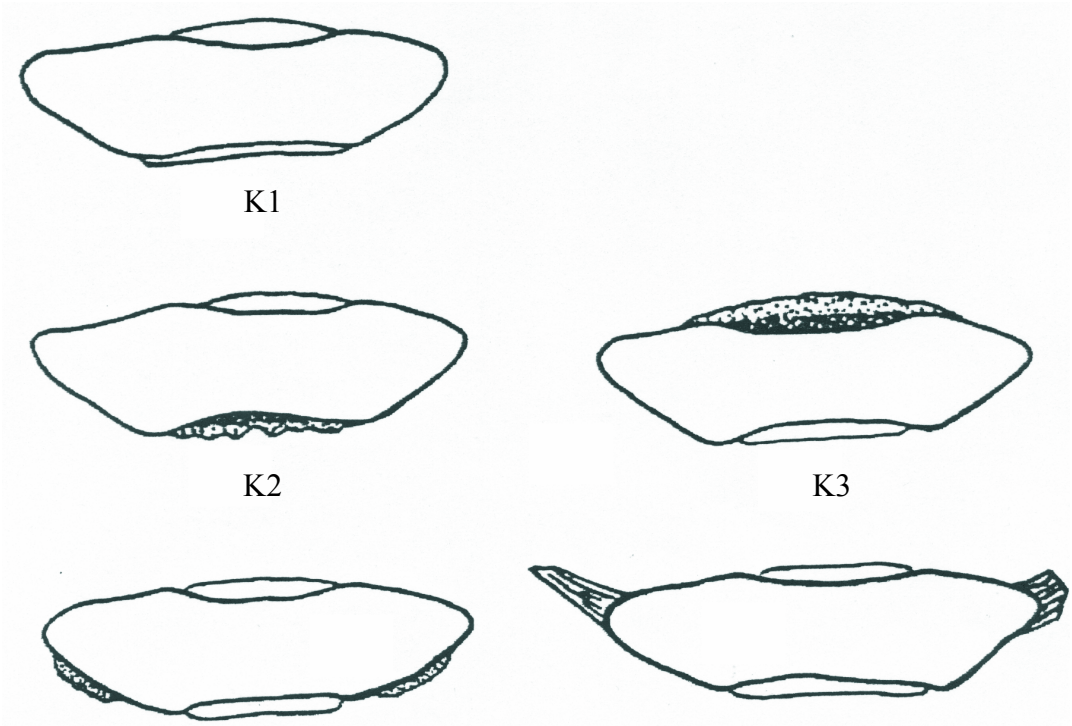
An der Entstehung der unterschiedlichen Strahlbeinveränderungen sind vermutlich verschiedene Gene beteiligt. Mittlere positive additiv-genetische Korrelationen wurden für DCS und RAC ($r_g = 0,38 \pm 0,08$) und für DCS und RAS ($r_g = 0,27 \pm 0,14$) geschätzt, während RAC und RAS negativ korreliert waren ($r_g = - 0,79 \pm 0,10$). Die gefundenen signifikanten QTL verteilten sich unterschiedlich auf die einzelnen Merkmale.

Strahlbeinveränderungen scheinen auch genetisch eng verwandt mit humaner Osteoarthritis zu sein. Die gefundenen QTL für Strahlbeinveränderungen befanden sich in homologen humanen Genomregionen, in denen eine Kopplung zu verschiedenen menschlichen Osteoarthritisformen gefunden wurde.



C1 keine Canales sesamoidales
C2a 1-4 kurze, eingebuchtete oder konische Canales sesamoidales
C2b 1-4 lange, tiefe, schmale und gerade Canales sesamoidales
C3a ≥ 5 kurze, eingebuchtete oder konische Canales sesamoidales
C3b ≥ 5 lange, tiefe, schmale und gerade Canales sesamoidales
C4 1-4 einfach oder verzweigt endständig kolbige oder endständig verzweigte Canales sesamoidales
C5 ≥ 5 einfach oder verzweigt endständig kolbige oder endständig verzweigte Canales sesamoidales
C6 größere Strukturauflösungen am Margo distalis, Canales sesamoidales nicht sichtbar
CP Canales sesamoidales proximales

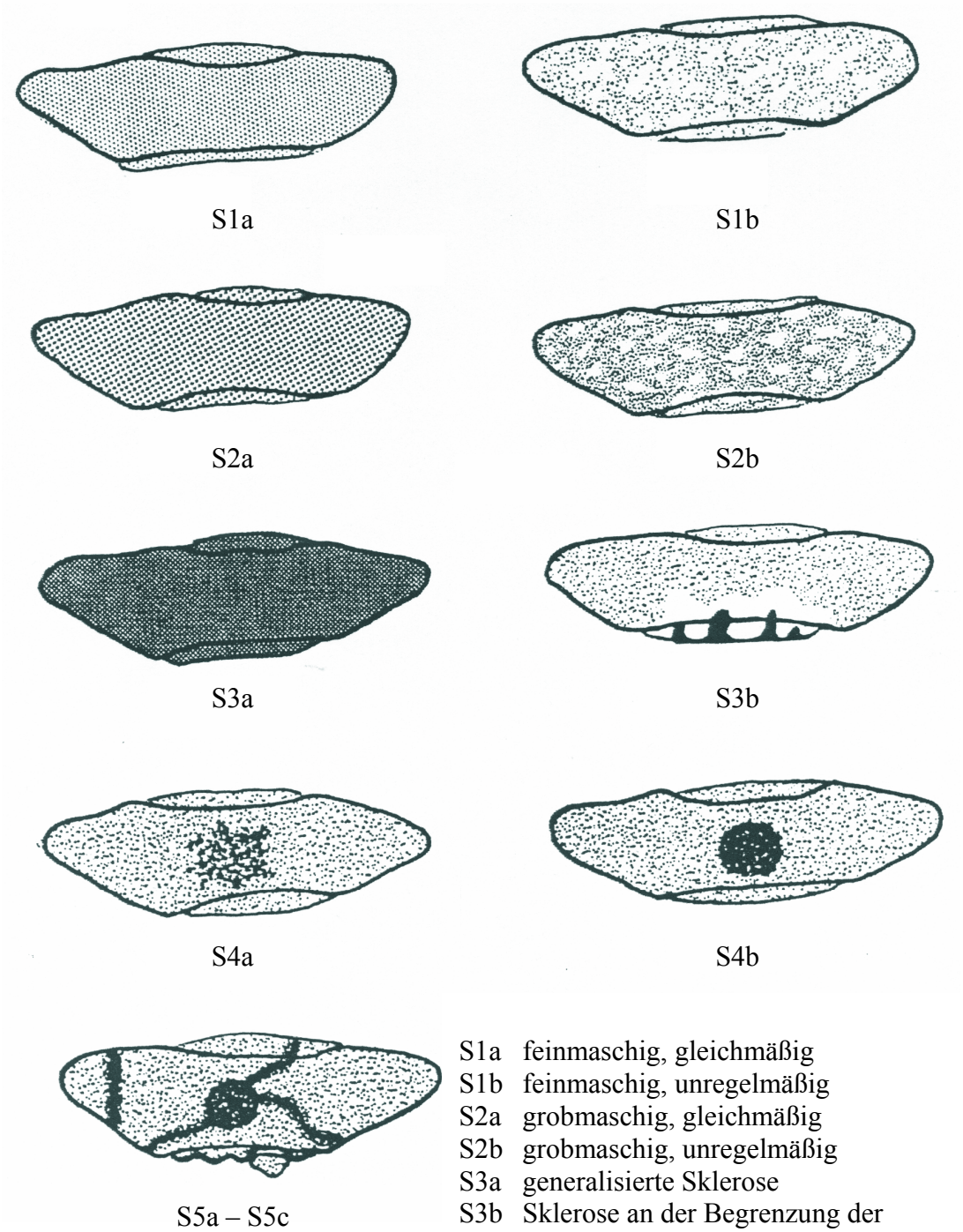
Abbildung 1 Schematische Darstellung der Röntgenbefunde nach BRUNKEN (1986) Canales sesamoidales



K1 ohne Konturveränderungen
K2 Zubildungen am Margo distalis, zentral
K3 Zubildungen am Margo proximalis
K4 Zubildungen am Margo distalis, Seitenteile
K5 Zubildungen an den Seitenenden, eckig oder spitz

Abbildung 2 Schematische Darstellung der Röntgenbefunde nach BRUNKEN (1986)

Strahlbeinkontur



S1a feinmaschig, gleichmäßig
S1b feinmaschig, unregelmäßig
S2a grobmaschig, gleichmäßig
S2b grobmaschig, unregelmäßig
S3a generalisierte Sklerose
S3b Sklerose an der Begrenzung der Canales sesamoidales
S4a zentrale Aufhellung mit diffuser Begrenzung
S4b zentrale Aufhellung mit scharfer Begrenzung
S5a Fraktur am Margo distalis (Chipfraktur)
S5b Sagittalfraktur
S5c Pathologische Fraktur

Abbildung 3 Schematische Darstellung der Röntgenbefunde nach BRUNKEN (1986) Strahlbeinstruktur

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Appendix 1: Pedigreestructure of family 1 - 17

female, male affected by deformed canales sesamoidales (DCS ≥ C3a)

female, male affected by radiological alterations in the contour (RAC ≥ K2)

female, male affected by radiological alterations in the structure (RAS ≥ S2a)

female, male affected by DCS ≥ C3a and RAS ≥ S2a

female, male affected by DCS ≥ C3a and RAC ≥ K2

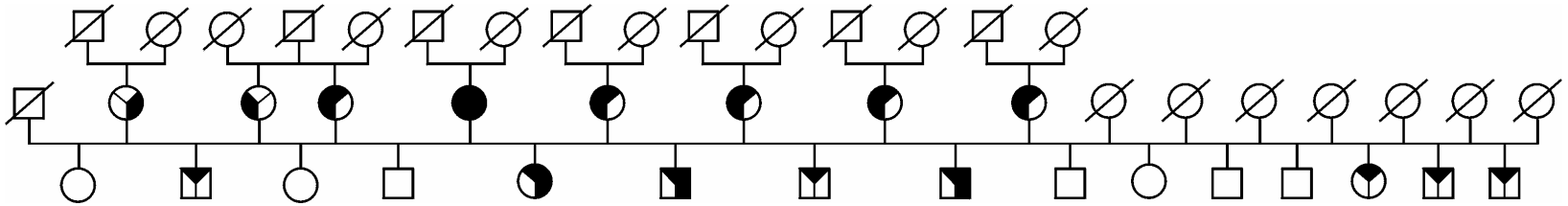
female, male affected by RAC ≥ K2 and RAS ≥ S2a

female, male affected by DCS ≥ C3a, RAC ≥ K2 and RAS ≥ S2a

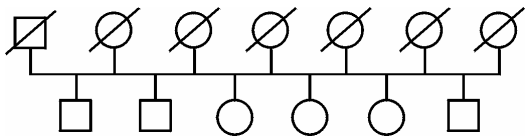
female, male unaffected

female, male with unknown status

Figure 1 Legend for the pedigrees following on the next pages



Pedigree of family 1



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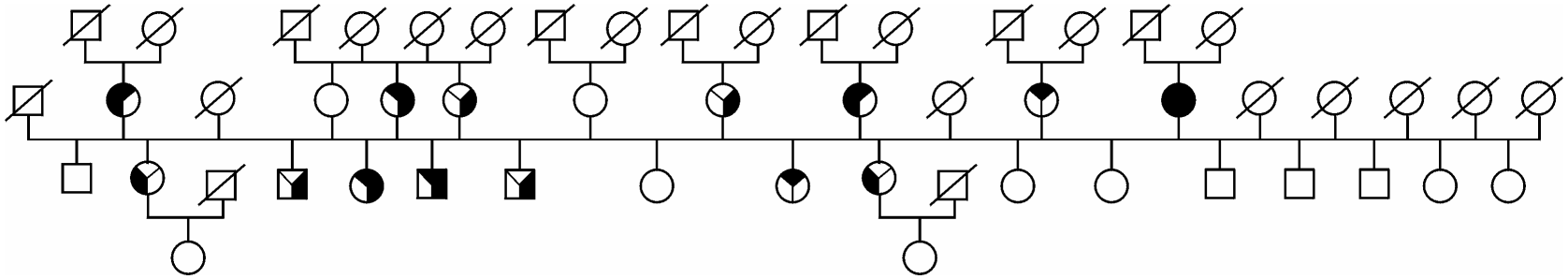
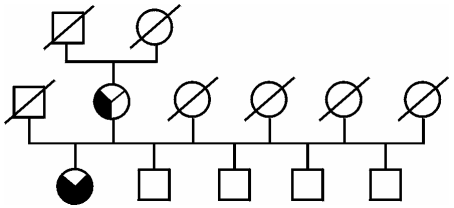


Pedigree of family 3

Figure 1 Pedigrees of familie 1 to 17



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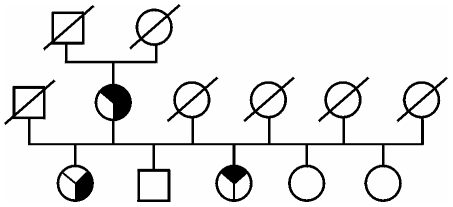
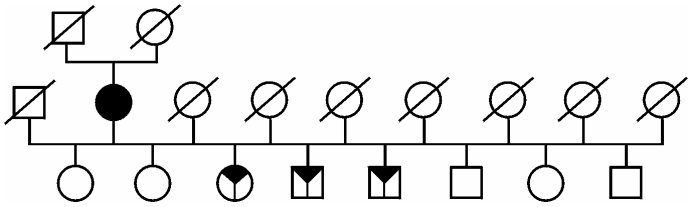
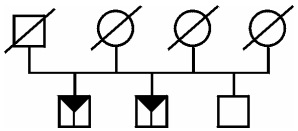


Pedigree of family 5



Pedigree of family 6



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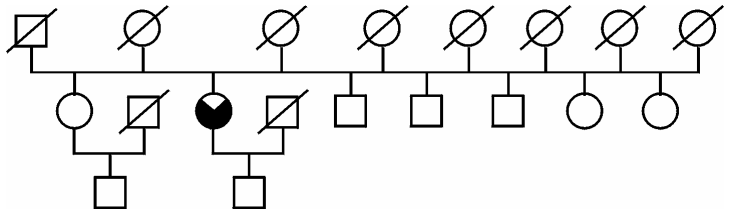
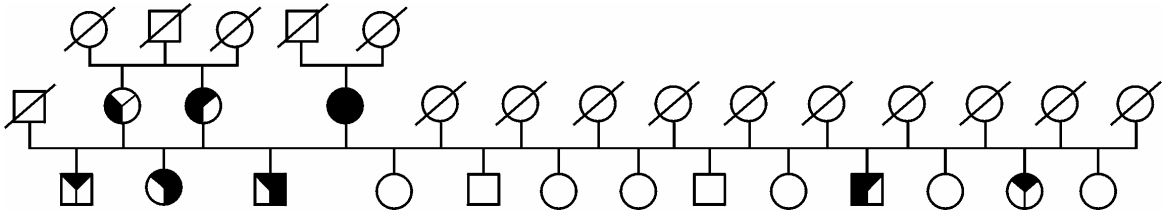
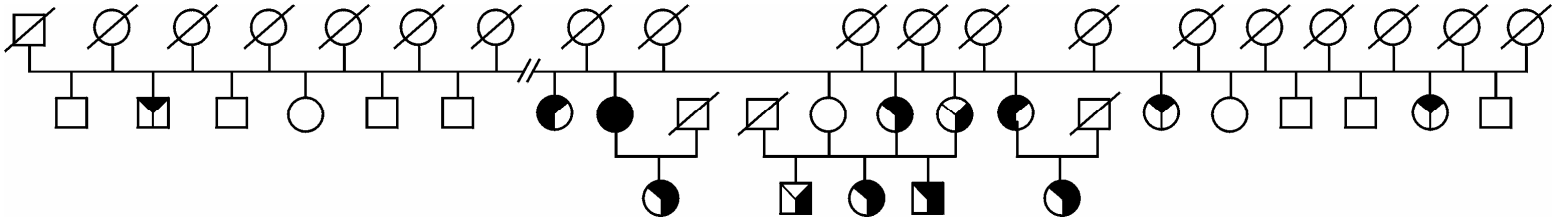


Pedigree of family 8

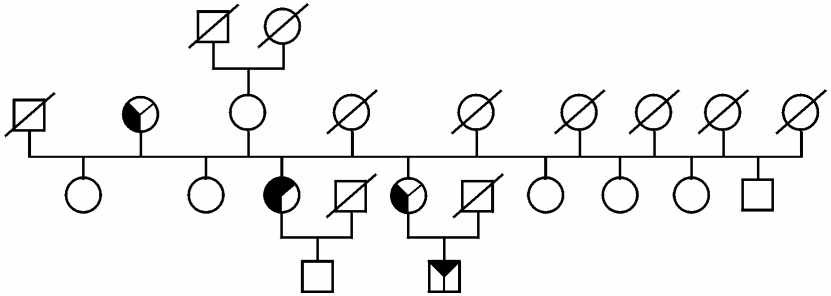


Pedigree of family 9

Figure 1 continued



Pedigree of family 10



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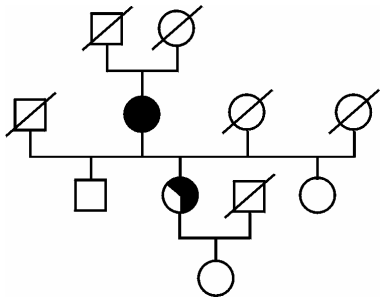

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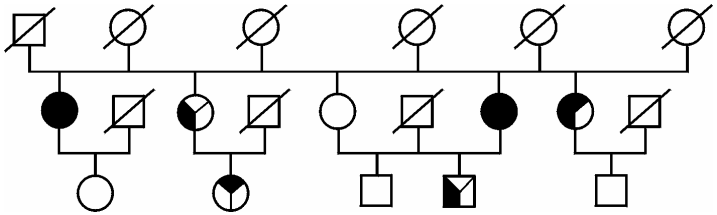

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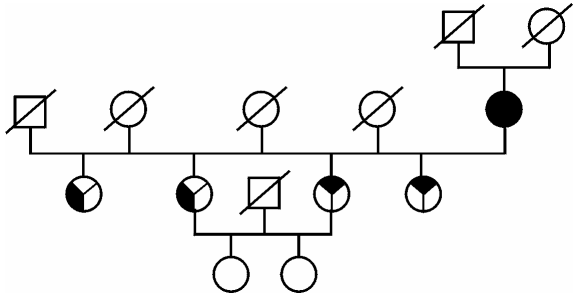
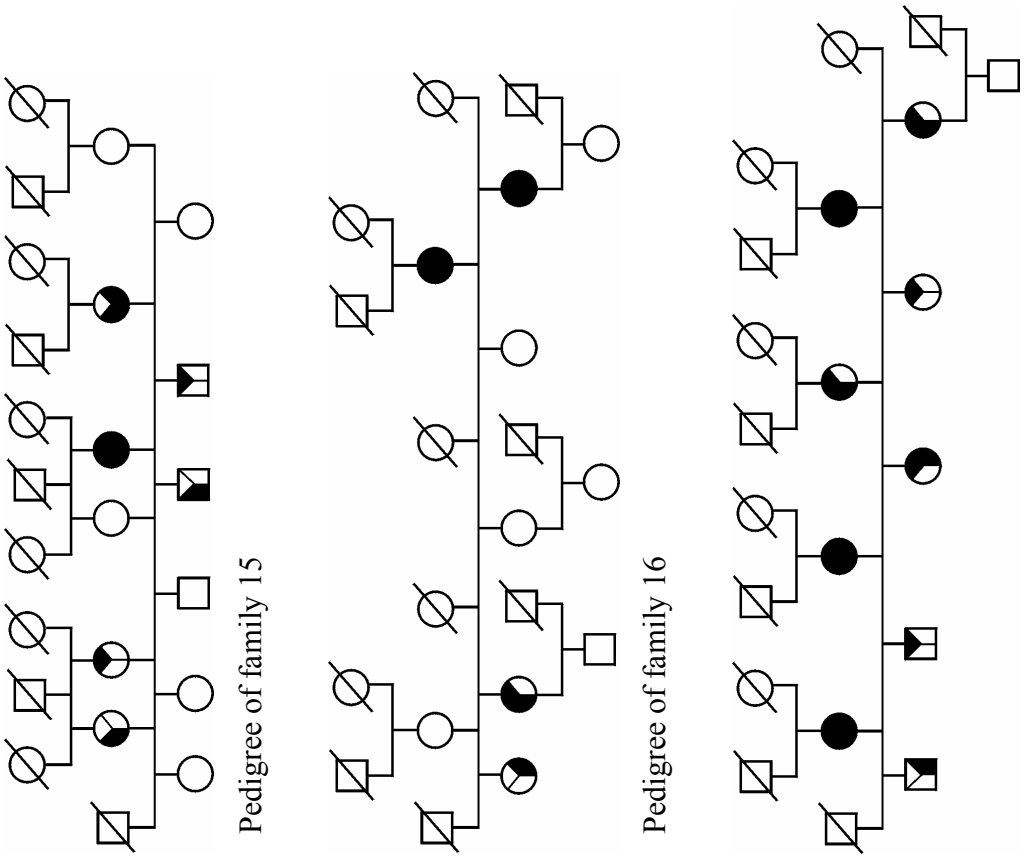


Pedigree of family 14

Figure1 continued



Pedigree of family 15

Pedigree of family 16

Pedigree of family 17

Figure 1 continued

Appendix 2: Characteristics of the 213 microsatellite markers

Table 1 Characteristics of the 213 microsatellite markers used in this study

ECA	Marker	Ta (°C)	Forward/Reversed Primer Sequence	Reference	Alleles (n)	Allele size (bp)	HET	PIC	Primer (pmol)
01	COR054	58	F: CAAGCAAAACAAGAAATCCC R: CTTTGTACACGTTGCAGTGG	Ruth et al. (1999)	5	229 - 243	51.9	47.1	2.5
01	ASB041	60	F: AAAGTTCACTTAGTCCTTGG R: CCACCTGTTTGCACTTGC	Irvin et al. (1998)	7	129 - 159	51.1	50.1	5.0
01	LEX020	55	F: GGAATAGGTGGGGGTCTGTT R: AGGGTACTAGCCAAGTGACTGC	Coogle et al. 1996	9	192 - 213	65.8	63.5	7.0
01	1CA12	60	F: GGGAGTGGTGATTACTTCTTGC R: TAGCCGTGAGAAGGTGTGTG	Swinburne et al. (2000a)	5	101 - 109	78.8	70.8	8.0
01	COR079	58	F: TGCTGCCAGATCTTCTGAAT R: TGGAGAGCGTGAAATTAACC	Tallmadge et al. (1999b)	3	204 - 212	63.2	58.0	3.0
01	ASB012	60	F: TCAGCAATAGAAGCCAGCTCC R: TCCTATGGAGGTGACCTTCCC	Breen et al. (1997)	6	168 - 182	69.9	53.1	8.0
01	AHT021	60	F: TCCAAGTTGCTGAATGGATC R: ACGGCCTGATTCTCTCTTTG	Swinburne et al. (1997)	7	199 - 215	79.9	76.1	6.0
01	1CA20	60	F: CTAAGCAGGTTCCCTATCATGG R: TCCACTACACAGGAAAACGAA	Swinburne et al. (2000a)	3	112 - 122	44.9	40.7	3.0
01	HTG012	60	F: CACTAGAGTCAGGGGGGGTGGGCT R: TTGGAGTACTCTTTCTCCCTTCCC	Marklund et al. (1994)	5	109 - 119	49.5	38.3	2.0
01	HMS015	60	F: ATATCTCTTGCTGTCCTACTTTCC R: AATGTGACACGTAAGATAGGCCTC	Guérin and Bertaud (1996)	6	214 - 234	64.6	61.5	5.0
01	HMS007	60	F: CAGGAAACTCATGTTGATACCATC R: TGTTGTTGAAACATACCTTGACTGT	Guérin et al. (1994)	6	170 - 182	74.1	71.7	8.0
01	COR053	58	F: AATTGACTGTGGAAGCCTTG R: GGCTGAGGAGTAAGCTGAAAG	Ruth et al. (1999)	6	171 - 197	57.0	52.9	2.0
02	COR065	58	F: CAAAAGCACACACAAAGTGC R: TCCGGAAAGTGCAAAGTTAG	Tallmadge et al. (1999b)	7	266 - 284	70.7	59.1	4.0
02	COR041	60	F: CAACTTGGGTTCTCGCTTAG R: TCCGGAAAGTGCAAAGTTAG	Ruth et al. (1999)	7	226 - 244	70.3	58.6	5.0
02	ASB018	60	F: TGCAGACAAAGCTGGACACTC R: CTGCTGAGAAAGCTTCTGC	Breen et al. (1997)	7	190 - 206	70.3	61.7	6.0
02	HTG019	58	F: GTATGTGCTGTACCTTCTGC R: ATGAGAAAGACGATAGATGATAT	Lindgren (2000)	3	143 -153	35.1	29.1	7.0
02	UMNe323	60	F: GATCCTGCAGGAAAGCATGT R: CCGCTCGGAATATTTCATTG	Wagner et al. (2004a)	11	154 - 210	75.0	70.5	5.0
02	COR037	58	F: GAGCAATTTCTGGGTCTGAG R: CACCCCTCTCTTTGTTTGTC	Murphie et al. (1999)	4	238 - 244	65.0	52.6	3.0
02	TKY0003	60	F: GGTTCACACAGGAGTCAGGGA R: CCTTCTGGTTTGCCTCGTCTC	Tozaki et al. (1995)	5	162 - 170	55.1	54.1	3.0
02	COR090	62	F: GGTTTGTCTCTTTGAGGTGTG R: TGCTCATATCTTCACCCTGC	Tallmadge et al. (1999a)	5	91 - 101	60.6	54.9	11.0
02	UMNe513	60	F: AGAATCTCACTTAGCTCCGCC R: TGCTGTTCTTTACAGCGTGG	Wagner et al. (2004b)	8	232 - 254	63.2	55.2	3.0
02	AHT035	60	F: TGACTTAGAGCTTTTGCTCCC R: CCAGAAGTCCAGGCATTTGT	Swinburne et al. (2000)	9	123 - 145	71.2	67.7	3.0
02	HMS054	58	F: AATGAACGGAGAAGCAGAAAGGC R: ACTGCGGTGCAGGTTGGAGG	Mariat et al. (2001)	4	112 - 122	59.3	48.4	3.0
02	ASB017	60	F: GAGGGCGGTACCTTTGTACC R: ACCAGTCAGGATCTCCACCG	Breen et al. (1997)	10	89 - 113	84.4	78.2	5.0
02	AHT067	60	F: CGGAGTTGGTATTTAACACACG R: ACGCACCAACTTCTGAAAGG	Swinburne et al. (2003)	6	234 - 252	67.7	66.1	4.0
02	AHT073	60	F: AACAGGACTGCAGAGACTCCA R: CTCCCTCACTAAACTGTGACCC	Swinburne et al. (2003)	7	145 - 157	78.1	70.1	3.0
02	UM007	60	F: GGGAATAGAGAAAGGTGAAG R: TTAGAGTTCCTGCTCCTCC	Meyer et al. (1997)	9	119 - 157	69.0	62.2	10.0
02	HMS051	58	F: GCTCCATGTTTCCTGACAGG R: GAAAAGAAAGTGTCAGACTGCC	Mariat et.al (2001)	4	164 - 178	37.1	30.7	5.0
02	UCD380	58	F: GTGGGATGGGGTAGGAAC R: ACTGGGAAGTGCTGCAAA	Eggleston-Stott et al. (1999)	4	115 - 135	58.8	50.7	5.0

HET: mean observed heterozygosity; PIC: mean polymorphism information conten

Table 1 continued

ECA	Marker	Ta (°C)	Forward/Reversed Primer Sequence	Reference	Alleles (n)	Allele size (bp)	HET	PIC	Primer (pmol)
02	AHT012	60	F: ACCCAAAGTCATGGGAATCA R: TTGTTGCCGACAACATGC	Swinburne et al. (1997)	6	102 - 114	69.2	64.7	6.0
02	A-14	60	F: CAGCTGGGTGACACAGAGAG R: GTCATCACTACTCCCTACAC	Marti et al. (1998)	8	208 - 236	69.2	59.7	11.0
02	ASB013	60	F: CTCTGAAAGAGCAGGATTGG R: GTCTTCTAAGTGGTAAGAGCC	Breen et al. (1997)	5	122 - 132	70.4	60.8	5.0
02	HMS016	60	F: AGTGTAATCAATGGATGAGTGGAC R: TGTTGTCGCAAATGGCAGGCATC	Godard et al. (1997)	7	142 - 154	46.5	43.4	7.0
02	UMNe076	60	F: CCCTCAGGTTGAGGACTCAG R: AGGTGACAACCTGGATTTGC	Roberts et al. (2000)	4	100 - 120	48.0	38.8	6.0
02	100G3_T7_MS	65	F: GGGTGAACAGTAGGGGAAAC R: CTGTTGTAGAGAGGGGGCTC	Leeb and Böneker (2005)	5	185 - 197	66.1	61.3	5.0
02	COR026	55	F: GGCGTCCAACGTAAAGTAGA R: CCTCTTCGGAAACTCTGACA	Murphie et al. (1999)	2	228 - 230	20.9	17.9	3.0
02	COR043	55	F: ACGCTCATTTCATAGGCTCTGAC R: GCAGGAGTGGTGTTTGTTTCAC	Ruth et al. (1999)	2	132 - 134	15.3	13.2	6.0
02	TKY0784	60	F: GATCAGTACTTTGCAAATGGATAAC R: GTAACTCCAAGGCTACGTTC	Tozaki et al. (2004)	7	200 -214	74.5	69.1	5.0
02	TKY0352	58	F: TCTGCTAAGTTCAATGGGTC R: TTCTTACTTAACAACCATCG	Tozaki et al. (2001)	5	77 - 95	64.4	58.2	5.0
02	TKY0474	60	F: TGTCCTACTTCCCAGCTACG R: CCTGCCTTTGCAGTTCAGTG	Tozaki et al. (2004)	4	153 - 159	38.2	30.7	5.0
02	TKY0605	60	F: AGTGCCATCTTGAATTGCTG R: CGATGAAATTGAGGATTTATGGA	Tozaki et al. (2004)	4	157 - 165	54.7	40.4	5.0
02	TKY0340	58	F: CAGGCTTTGGGTTTCATTATG R: TCAGTCTCCATTATAGGAGG	Tozaki et al. (2001)	5	132 - 154	66.0	60.2	5.0
02	TKY0358	60	F: GAAGCAGTGCCTCTTATGTG R: CAGAACAGTCAGGACTTZGAC	Tozaki et al. (2001)	5	154 - 164	61.3	54.5	5.0
03	AHT036	60	F: TGCTGCTCCAGTGTCCT R: TAGATTTCACAGGCGGGTG	Swinburne et al. (2000b)	8	134 - 148	81.5	74.7	5.0
03	COR028	60	F: TAAAGAGGAAGGCAATGGAC R: ACCTTTTGTGCTAGGCACTG	Murphie et al. (1999)	8	229 - 243	74.3	70.9	7.0
03	COR033	58	F: CCTCCCCTACTTCCTCTCTG R: CATTTTCTTTCCAGGTTCCC	Murphie et al. (1999)	10	213 - 245	67.5	61.2	2.5
03	SG18	58	F: TGGGGAAGAGGGATTCAT R: AAATGCCAAGCCTATCTATGCR	Godard et al. (1997)	5	151 - 167	52.2	48.9	5.0
03	UMNe158	55	F: AATTGAGAGCCAAGATGACACC R: GGCACCATTTGAGGAAGATG	Wagner et al. (2004a)	9	125 - 149	84.8	80.1	5.0
03	AHT022	58	F: AAGCACAATGTGGGGGTTAG R: TCCACGTTCACACATACCTCA	Swinburne et al. (1997)	6	189 - 201	50.6	47.8	6.0
03	LEX057	55	F: TGGTCCCCTAATCAAATCAGA R: ACGGCATCCCACATAAAATAG	Coogle and Bailey (1997)	6	157 - 171	25.0	23.6	4.0
03	HMS061	55	F: TGCATCTGACCTAATGTGGC R: CGTCAAACCATGCTGTCACG	Mariat et al. (2001)	6	190 - 204	29.2	26.3	4.0
03	UCD437	60	F: CTGTTCTGGGCAGGCTTCTCTA R: TTGCTGGCTTGGCTGGTC	Eggleston-Stott et al. (1997)	7	165 - 187	66.2	60.3	6.0
03	HTG002	55	F: GATTGGCAACAGATGTTAACTCGG R: CCCCATGAGAACTAACAATGTTAG	Ellegren et al. (1992)	2	99 - 103	10.4	11.7	3.0
03	LEX007	55	F: GGTAGGGCTCTGGGATGA R: AACACTGGGGAAAAGTCAG	Coogle et al. (1996c)	4	192 - 200	73.2	64.4	8.0
03	AHT097	58	F: AGTTCGGTAACTTGCCCATG R: GTTCATGGGCAGAATGGC	Swinburne et al. (2003)	7	149 - 163	78.8	70.9	5.0
03	AHT092	55	F: TGAGCATCTTGAAGATGAGCA R: CAACAGTTGTTAGCTCAGGTGC	Swinburne et al. (2003)	17	254 - 296	89.4	84.7	5.0
03	TKY0450	60	F: GTTCTTCCTTCCCAGCACTT R: CATTGTAAGTCGGGGACCAT	Tozaki et al. (2004)	9	94 - 114	84.9	78.8	5.0
03	TKY0937	58	F: TCCTGCGGAAATACATTAGG R: AGTTCAAAGTGGTCCCATAG	Tozaki et al. (2004)	8	120 - 150	77.9	73.4	5.0
04	AHT043	60	F: ACACAAGTGACAGGAGCGTG R: TGGAAGCATGCAAGAGGTC	Swinburne et al. (2000b)	11	156 - 190	84.5	77.0	7.0
04	HMS006	60	F: GAAGCTGCCAGTATTCAACCATTG R: CTCCATCTTGTGAAGTGTAACTCA	Guérin et al. (1994)	6	157 - 167	75.1	66.7	5.0
04	ASB003	60	F: AATTCATCTCAGTGCTCTACCAGC R: TTCATTTTCTACATGCACTACAGC	Breen et al. (1997)	6	196 - 208	58.4	55.0	2.5
04	ASB022	62	F: AGGAATGTGAAATACAGGAGG R: TTTGTGGTCTTCCGTGCACC	Breen et al. (1997)	7	155 - 167	70.7	65.4	5.0

HET: mean observed heterozygosity; PIC: mean polymorphism information content

ECA	Marker	Ta (°C)	Forward/Reversed Primer Sequence	Reference	Alleles (n)	Allele size (bp)	HET	PIC	Primer (pmol)
04	LEX050	55	F: ATAGTCTGGGGTTAGGTAAGG R: TCTAGCCCAATGTAAATGC	Coogle and Bailey (1997)	5	112 - 124	49.8	46.5	6.5
04	HTG009	55	F: TGTGGGAAGAGTGTCAATAGCTGT R: AGGCATCTGGTTTGCTGCAATTTC	Marklund et al. (1994)	6	118 - 138	63.3	53.3	5.0
04	SG23	58	F: GGCTTAAGATATGGGTGAGTAAGG R: GCCCACCCTCTTACTTTTCTCAA	Godard et al. (1997)	14	198 – 236	87.1	84.5	18.0
05	UCD304	55	F: CGCTTTCCTGCTGTCACC R: GAGGGACTGTGGGGGAGGT	Eggleston-Stott et al. (1999)	5	95 - 113	60.2	56.5	4.0
05	LEX034	55	F: GCGGAGGTAAGAAGTGGTAG R: GGCCTAAGATGAGGGTGAA	Coogle et al. (1997)	4	243 - 253	70.5	63.4	6.0
05	LEX014	55	F: CCTTACTCACTGGGGAATAAA R: AGACTGAACACCTAACTATGA	Coogle et al. (1996a)	4	390 - 400	74.9	60.3	2.0
06	HTG031	55	F: CTCTGTAACCCTTATATCCTTA R: TGTTGATTGCTCCTCCCCT	Lindgren et al. (1999)	5	135 – 145	53.9	55.9	5.0
06	NV082	60	F: TGTGGCAGCATCCCACAAAC R: CCTCCATTTTTGTCGGTTAGCG	Bjornstad et al. (2000)	6	123 – 137	56.7	54.2	5.0
06	UM015	55	F: AGTCTGGCTGAGGATACTG R: GGTGAGAAAGGAGATAAATG	Meyer et al. (1997)	7	298 - 312	78.1	67.1	2.5
06	COR070	62	F: CATCTGTTCCGTGGCATTA R: TTCAGGTGTGGGTTTTGAATC	Tallmadge et al. (1999b)	8	273 - 299	89.5	80.5	11.0
06	TKY0284	62	F: CTGGACTAGAGTCAGATTGC R: AACAGGATTCCCCCAATGCC	Tozaki et al. (2000)	6	157 - 171	66.8	68.6	3.0
06	TKY0028	55	F: TTCAGCAGGGTCTCATGCCAC R: TTCGGCTCTGGTTCAAGAGG	Kakoi et al. (2000)	10	271 - 359	82.5	77.2	6.0
07	HTG020	55	F: CTGGTTTACCTTCCCTACAG R: CCAATGGTTCCTCTGAGAAG	Lindgren et al. (1999)	7	142 - 156	54.4	59.1	3.0
07	LEX038	58	F: CTGCATTCCCATCATCACAT R: TGCCTTGCCTCTTTCTGTTTA	Coogle et al. (1997)	3	133 - 143	59.9	52.2	2.0
07	COR095	58	F: TACCTCTGGTGGTGATGCTT R: CCCACACTTACTCCCATCAC	Tallmadge et al. (1999a)	8	206 - 320	59.4	57.6	5.0
07	SG28	62	F: CTGTGGCAGCTGTCATCTTGG R: CCCAATTCCAGCCCAGCTTGC	Godard et al. (1997)	5	149 - 165	64.7	56.0	3.0
07	AHT019	62	F: CATTTCTCTGGTGTATCTCCCA R: GGAATAGTCATAGTCCACGACC	Swinburne et al. (1997)	3	137 - 145	36.9	34.3	7.0
08	COR097	58	F: GGGATTTCTGAGATGCTGAA R: ATGGCTGGCTAGAGTTTGTG	Tallmadge et al. (1999a)	5	236 - 244	57.4	45.1	8.5
08	UCD046	60	F: GCCAAACGCTGGAGGGTT R: CCACATTCACACACATGCACAC	Eggleston-Stott et al. (1996)	4	228 - 234	68.	63.5	12.0
08	COR012	58	F: TCTAGGAAAGACCCATCACG R: AGTAAGTGGAGGCCAAGGAT	Hopman et al. (1999)	6	166 - 180	77.0	69.7	2.5
08	COR003	58	F: TAGGGAAACTCCTCAAAGCC R: GAAACCAAAACCTTCATCCA	Hopman et al. (1999)	7	192 - 208	66.7	59.9	5.0
08	COR056	58	F: AGATTCCAGGCATTAGGACC R: TCAGGGACAATCTTCCTCAAG	Ruth et al. (1999)	9	190 - 212	78.2	72.2	5.0
09	HMS003	58	F: CCAACTCTTTGTCACATAACAAGA R: CCATCCTCACTTTTTCACTTTGTT	Guérin et al. (1994)	7	149 - 167	83.6	73.9	5.5
09	HTG004	55	F: CTATCTCAGTCTTCATTGCAGGAC R: CTCCCTCCCTCCCTCTGTTCTC	Ellegren et al. (1992)	6	127 - 137	61.2	52.5	1.5
09	HTG008	56	F: CAGGCCGTAGATGACTACCAATGA R: TTTTCAGAGTTAATTGGTATCACA	Marklund et al. (1994)	6	178 - 190	76.2	67.9	5.0
09	COR098	55	F: GCAACAGATGTTGGCTCAG R: GGAGATGTCCTTGACCACAG	Tallmadge et al. (1999a)	5	233 - 251	54.0	43.4	7.0
09	ASB004	56	F: TAAATTGTAAAAGCTGGAGCCG R: GCAAATAGTAGTTAAGTCCTC	Breen et al. (1997)	10	120 - 144	55.6	52.8	10.0
09	ASB005	62	F: TCGAGGAGCTCATGACCTGG R: TTGTACAACTCTCCACCATAGC	Breen et al. (1997)	4	105 - 117	69.7	61.3	4.0
10	HMS023	60	F: GATCCAATATTGTAAACCCCGCC R: CCTTCATAACCCTTATTGCAGCC	Godard et al. (1997)	4	82 - 94	29.8	29.0	2.5
10	COR020	60	F: TCTCTACCGCAAGTGAAACC R: CTGAATTGTAGGACATCCCG	Hopman et al. (1999)	8	146 - 162	81.2	80.0	5.0
10	UCD482	58	F: CACAGCCCTGACCACTGA R: CCAAAACAGCCCTGGACT	Eggleston-Stott et al. (1999)	4	114 - 126	38.4	36.4	4.0
10	COR048	58	F: GATTGGGATGCAAAGATGAG R: CAAGAGGATTGGGAACAAAGG	Ruth et al. (1999)	5	167 - 187	64.5	62.0	3.0

HET: mean observed heterozygosity; PIC: mean polymorphism information content

Table 1 continued

ECA	Marker	Ta (°C)	Forward/Reversed Primer Sequence	Reference	Alleles (n)	Allele size (bp)	HET	PIC	Primer (pmol)
10	AHT015	58	F: GGGTCCTGAGCAGGTCTTTT R: GAAGTAATGGCGGTCATGCT	Swinburne et al. (1997)	7	137 - 159	69.8	60.1	4.0
10	ASB006	60	F: GGCACAGATGTTAGCTCAGC R: ATGGAACCAGCCTGGATTGC	Breen et al. (1997)	5	190 - 204	75.2	64.7	2.0
10	NV018	64	F: GGAGGAGACAGTGGCCCCAGTC R: GCTGAGCTCTCCCATCCCATCG	Roed et al. (1997)	10	112 - 168	76.5	71.7	5.0
10	SG30	62	F: ACTGGAGGGGTGAAACAGATTCAGA R: GGAAGGGAGGTCATCAGAA	Godard et al. (1997)	6	156 - 166	81.3	76.2	6.0
10	COR015	58	F: GGTGTGGAAACATTCCGTAT R: ACTGCATGTGTGGGAGAGAT	Hopman et al. (1999)	9	215 - 231	79.4	75.7	2.5
10	LEX008	55	F: AAACTGTCACAACGGTTAGGAC R: CGAAAAAGCCACTTGAGGTC	Coogle et al. (1996)	7	403 - 421	80.7	75.1	8.0
10	LEX066	62	F: GCTCTCAGTAACCTCGATGTT R: ATTAAGGAGAAGGTGGAAAAGAC	Coogle & Bailey (1999)	3	207- 211	49.8	36.9	6.0
10	NV007	62	F: TCAGCGACAATCTTCCTCATACA R: ATTCACACCTCCCCCAGAAAT	Roed et al. (1997)	4	217 - 223	29.3	25.8	5.0
10	ASB009	62	F: GTGCGCATGTATGTGCGTGCC R: ATTTCCACAAGGGACATGAGG	Breen et al. (1997)	8	86 - 102	84.7	75.8	5.0
10	HMS002	60	F: ACGGTGGCAACTGCCAAGGAAG R: CTTGCAGTCGAATGTGTATTAAATG	Guérin et al. (1994)	8	220 - 240	74.4	65.6	5.0
10	LEX009	55	F: AAAGCCGTAAGATTGGGACA R: TCCATTGTGAGGGTGTAACA	Coogle et al. (1996)	5	366 - 378	61.2	49.2	5.0
10	NV067	58	F: GCTCACTCAACTCCCAGAG R: GGATTAGATTACCCAGACAACT	Roed et al. (2000)	2	212 - 220	41.4	31.6	5.0
10	AHT086	60	F: CCCAATGAAGTCCAAGATGG R: GAAATCTCTAGCAAGACCCAGG	Swinburne et al. (2003)	9	185 - 217	67.0	57.8	2.0
10	LEX017	58	F: CCTGCCCAAGAAGAACTCAGA R: AGCAGTGTATTTTTGAAACAT	Coogle et al. (1996a)	6	136 - 156	59.5	61.4	5.0
10	TKY0537	60	F: TCAGGGGTTCCTCTTCAGTG R: TTGCCTGGTGTCTAGGTTCC	Tozaki et al. (2004)	6	139 - 149	72.1	66.0	5.0
10	TKY0592	60	F: TGCAGTGGGTACGTGTGTAA R: GGGCAGACTTCCAAACAGTC	Tozaki et al. (2004)	6	177 - 191	57.8	43.3	5.0
10	TKY0867	58	F: AGCTAATGTCAGTAGGTTGG R: TTCCAAGCATCTTAAGGAGG	Tozaki et al. (2004)	7	202 - 226	68.7	62.8	5.0
11	LEX068	50	F: AAATCCCGAGCTAAAATGTA R: TAGGAAGATAGGATCACAAGG	Coogle and Bailey (1999)	7	154 - 168	79.4	74.2	4.0
11	SG24	58	F: CTACCATTGAAGAGGGGTGGC R: GAAACGAGCAGGAAGTGAATCTCC	Godard et al. (1997)	10	107 - 127	77.4	72.2	8.0
11	SG13	60	F: GGACTAAAGCCCAACCATCCAGC R: CTCACCAGTAAGGGGTTATGGGGC	Godard et al. (1997)	6	163 - 189	64.8	62.1	3.0
11	UCD457	60	F: GGGGCGTGAGCATAAAGG R: CGCTGGATGAGTGAGGGA	Eggleston-Stott et al. (1999)	9	73 - 95	72.8	67.7	10.0
12	SG10	60	F: CATCCATCCTTTCCAGCTCGATATTC R: CAAGACCGTAACTCAGGAGCCC	Godard et al. (1997)	6	173 - 183	60.4	52.7	6.0
12	SG08	58	F: GAGTTCATTCTTTTTCGTGGCTG R: GGAAACACCCTAAGTGTCCCTTG	Godard et al. (1997)	8	121 - 135	85.0	77.1	10.0
12	COR058	58	F: GGGAAGGACGATGAGTGAC R: CACCAGGCTAAGTAGCCAAAG	Ruth et al. (1999)	11	208 - 230	80.8	79.8	8.0
13	COR069	58	F: AGCCACCAGTCTGTTCTCTG R: AATGTCCTTTGGTGGATGAAC	Tallmadge et al. (1999b)	7	265 - 279	72.4	67.1	7.0
13	LEX041	55	F: TATTTTCTGAATGCTTCTGTGC R: CTCTACACCCAATGCCTGAT	Coogle et al. (1997)	6	143 - 161	69.2	62.6	3.5
13	VHL047	55	F: GTTTGCTGTGGTTACCAGGCAGA R: GCAAATTGAATATTTGAAGTTGAGAC	van Haeringen et al. (1998)	5	126 - 142	68.4	58.2	14.0
13	ASB001	60	F: AGCAGAAACCCACTCAAGCC R: GCATAATACCCTCAAGGTC	Breen et al. (1997)	5	153 - 167	71.7	62.7	3.0
14	AHT029	60	F: ACTCATTCATTCACAAATCCCC R: AGAAAATTCCCTCCTGTCCC	Swinburne et al. (2000b)	4	264 - 284	51.6	39.9	3.0
14	LEX043	58	F: CATTAAGCAACAAAAAGCATC R: GGAAAAGCATGACAAGACACT	Coogle et al. (1997)	5	224 - 244	50.5	44.8	3.0
14	UM010	56	F: TACAGCCATTGGAAATCTAC R: CACCATTACATTTTCCCAG	Meyer et al. (1997)	7	106 - 120	81.2	73.3	5.0
14	LEX078	58	F: AATGTGCGCATTTAACCACTGTG R: CAAGCCATGCTGTGGAAACG	Bailey et al. (2000)	3	160 - 164	40.7	33.2	3.5

HET: mean observed heterozygosity; PIC: mean polymorphism information content

Table 1 continued

ECA	Marker	Ta (°C)	Forward/Reversed Primer Sequence	Reference	Alleles (n)	Allele size (bp)	HET	PIC	Primer (pmol)
15	LEX046	58	F: ATAAGCCAATCCACTTTTCC R: ATTACCACCCCATTTCCTT	Coogle et al. (1997)	8	115 - 129	78.7	75.2	7.0
15	B-8	60	F: TCCTCAGTCCTTTCTCATGC R: AGCTGAAGGCAATCTGTACC	Marti et al. (1998)	10	79 - 101	75.1	66.3	5.0
15	LEX051	58	F: CCTACGTGTCTCTTTCTCTTT R: GTAACGCAATAATACAGCACT	Coogle et al. (1997)	6	130 - 160	64.7	52.8	5.0
15	TKY011	58	F: ATGAGAGAGGTCACCAAAAT R: CCCTGCCAACAAAAACCTTG	Hirota et al. (2001)	6	126 - 136	71.1	64.4	10.0
15	ASB015	64	F: GTCCCAAAGGGACTCAGGAAGG R: TGGATGCCAGTGCATAGACAG	Breen et al. (1997)	10	121 - 145	67.1	63.9	7.0
15	SG06	60	F: GGGCCTGGTTTTCCTTCTAA R: GCATTTGTGGCCTGTGTCATA	Godard et al. (1997)	2	165 - 167	39.1	30.3	5.0
15	ASB019	64	F: GAGTTGGAGCTCAAGTCTGTC R: GTTTAGCAACTACAGCGTAGG	Breen et al. (1997)	9	160 - 190	52.6	48.4	5.0
15	ASB002	60	F: CCTTCCGTAGTTTAAGCTTCTG R: CACAACTGAGTTCTCTGATAGG	Breen et al. (1997)	9	234 - 254	79.6	75.9	10.0
15	COR077	56	F: AGGGAGCATTTGAACCAAGT R: CAATGGTGGTAGAAGCAAGG	Tallmadge et al. (1999)	2	152 - 154	54.9	35.6	3.0
15	HTG006	55	F: CCTGCTTGGAGGCTGTGATAAGAT R: GTTCACTGAATGTCAAATTCTGCT	Ellegren et al. (1992)	5	82 - 104	75.0	65.9	3.0
15	COR075	55	F: GCCCTAGTTAGCAACCAACA R: AAGATTGATTCCTCAGCACG	Tallmadge et al. (1999b)	9	192 - 210	87.2	80.1	2.0
15	HMS001	60	F: CATCACTCTTCATGTCTGCTTGG R: TTGACATAAATGCTTATCCTATGGC	Guérin et al. (1994)	5	170 - 180	66.0	54.2	5.0
15	COR014	58	F: CTATCATGTCAGGGACCAGG R: CTGCCCTAGTTAGCAACCAA	Hopman et al. (1999)	9	143 - 161	87.0	79.7	8.0
15	COR042	62	F: CAAATGTGCTCCATCTCCAAC R: GCCAGCTCCCTTACTCTGTA	Ruth et al. (1999)	2	153 - 155	15.5	13.3	5.0
16	HTG003	55	F: TAACCTGGGTGCAAAGCCACCCAT R: TCAGGGCCAATCTTCCTCAC	Ellegren et al. (1992)	5	114 - 124	75.4	71.2	6.0
16	LEX059	55	F: TGAAATGTCACCTTCTCAGAG R: GTGGACACTTGCCNTCAT	Coogle and Bailey (1997)	3	227 - 231	27.4	26.7	3.0
16	LEX056	55	F: GACCTACAGGCCACTCATCAA R: GGCAGTTTCCTCCATCCTTA	Coogle and Bailey (1997)	8	211 - 227	88.7	81.6	3.0
16	COR064	58	F: TCCATACATGTGTGAGGGC R: AAGATGGCTTACAAGGATTATG	Tallmadge et al. (1999b)	4	192 - 202	65.8	55.6	2.5
16	AHT091	62	F: TAGCTGTCTGCAAAGGCTCA R: CCAGTGTTCCACATGCCTC	Swinburne et al. (2003)	4	108 - 126	29.8	27.1	7.0
17	COR105	60	F: TTTCCTCATTGCTTCCTGAG R: CCCAAGGTCTGTCTTGCTCTC	Swinburne et al. (2000a)	7	174 - 192	81.1	76.3	5.0
17	UCD014	60	F: GCATTTGCTCACTGGCTAC R: ACTCCTCCACTCCCACCTA	Eggleston-Stott et al. (1996)	4	128 - 134	57.5	54.2	6.0
17	COR032	58	F: GCCCTCTTAGAGCATTTTCC R: CAGAGATGGCTGGAGTAAGG	Murphie et al. (1999)	4	249 - 255	48.4	41.3	2.0
17	HMS025	60	F: CAAACATAAAATATGCATGTCCATGT R: CTTTTGGATATGTAAGGCTTGAGG	Godard et al. (1997)	3	124 - 128	51.7	44.9	12.0
18	UCD136	60	F: CTTTGGGCCTTTCCTCCAT R: CGAGCCTGGGAGTGATAC	Eggleston-Stott et al. (1997)	5	111 - 119	69.0	64.1	2.5
18	TKY019	56	F: CTTCTGCTGATTCCTGAATG R: GGATCTCCTTAAATGGAACA	Kakoi et al. (1999)	7	144 - 164	85.5	76.9	8.0
18	LEX054	55	F: TGCATGAGCCAATTCCTTAT R: TGGACAGATGACAGCAGTTC	Coogle and Bailey (1997)	7	164 - 180	72.7	67.6	6.0
18	HMS046	60	F: GTCTCAGCCAAAAGGTATTCAAGC R: TGGCACCAATATAGGTCACCTGG	Godard et al. (1997)	6	122 - 134	71.2	63.3	5.0
18	COR096	58	F: CCCCTCTTTTGCTTGAGAAT R: GCGTGTATGTGAGGATTGAAG	Tallmadge et al. (1999a)	6	307 - 321	63.9	58.0	3.0
18	TKY0101	55	F: TCTGAAATACCGTGTGCCT R: TTCTGCCTCCCTCCAACTTT	Mashima et al. (1999)	8	197 - 217	80.7	74.6	4.0
18	UCD387	60	F: ACCCCCGCCCCAGCAC R: TGCCCCGTCATTCTGC	Eggleston-Stott et al. (1999)	7	76 - 88	57.6	51.6	1.5
19	COR062	58	F: GTCATCCAGTGACGAACACA R: AGGAAGTGCGCAGTAGAGAA	Tallmadge et al. (1999b)	9	208 - 236	83.8	75.1	8.0
19	AHT094	60	F: CACCTCCATCACATTGGTCA R: GGCTGGAGTCAGCTGACATT	Swinburne et al. (2003)	5	232 - 240	61.8	55.4	2.0

HET: mean observed heterozygosity; PIC: mean polymorphism information content

Table 1 continued

ECA	Marker	Ta (°C)	Forward/Reversed Primer Sequence	Reference	Alleles (n)	Allele size (bp)	HET	PIC	Primer (pmol)
19	LEX036	55	F: ATCAGCCCAGCCTCTTCA R: AACAACCGGCNAAATAGTGC	Coogle et al. (1997)	8	137 - 161	50.0	49.6	3.0
19	HMS008	58	F: GGTGAGGAATTATCTCTTTGAAGG R: GCAGGTAGGATTGGATAGGTACAT	Guérin et al. (1994)	5	207 - 215	59.2	58.7	10.0
19	NV011	60	F: GGCCCCACCCACTAAATATCACTG R: CGGGGTCTTGGAAATTTATGAAGG	Roed et al. (1997)	5	120 - 130	54.7	45.8	10.0
19	AHT055	60	F: TGAAAATACACCCAGCTACGC R: GGGAGATATTTCTTGGCTTGC	Swinburne et al. (2003)	5	147 - 159	46.6	39.8	6.0
20	HTG005	55	F: TGCTAAGCCTCAGCACATACA R: TGGAAATAAGGTTAGCAGGGATGC	Ellegren et al. (1992)	6	79 - 89	29.3	29.4	7.0
20	LEX064	50	F: ACCCTTTCCGCAGACAA R: CACATCAGAGCCCATCTTCTC	Coogle and Bailey (1999)	6	192 - 207	67.7	65.7	5.0
20	UM011	56	F: TGAAAGTAGAAAGGGATGTGG R: TCTCAGAGCAGAAGTCCCTG	Meyer et al. (1997)	10	160 - 180	76.8	76.7	2.5
20	COR050	58	F: TCTGTTGCCTTTATCCACAA R: ATGAAAACCCTGGGAATAGC	Ruth et al. (1999)	5	287 - 297	64.2	55.6	2.0
20	HMS042	55	F: TAGATTTCTTAAGTGCCAATAGTGG R: GAACTGCTATAGATATACCTAACTC	Godard et al. (1998)	5	111 - 133	71.0	64.3	5.0
21	SG16	55	F: AATTCTCAAATGGTTCAGTGA R: CTCCCTCCCTTCCTTCTA	Godard et al. (1997)	7	146 - 192	70.7	69.2	9.0
21	UMNe229	60	F: CTTCTCTGGACAAAGGGGTG R: CATGAATTTGCCAGTTTGATG	Mickelson et al. (2003)	2	122 - 124	23.4	19.8	3.0
21	HTG010	55	F: CAATTCCCGCCCCACCCCCGGCA R: TTTTTATTCTGATCTGTCACATTT	Marklund et al. (1994)	8	93 - 113	83.9	81.4	6.0
21	COR073	58	F: GCCAAGACATGGAAACAATC R: GTTCTCAAGGTGCATCCCTA	Tallmadge et al. (1999)	7	180 – 198	82.4	79.9	2.0
21	COR068	60	F: AACCAATTGTGAGATTTTTGCT R: GGCTAGTCCTGGATCATGTG	Tallmadge et al. (1999)	6	146 – 156	81.0	73.3	4.0
21	LEX031	58	F: CCCATTAAGAACTTTTCATCCTG R: GGCAAGCCCCACAAAATTAT	Coogle et al. (1996b)	4	252 – 258	55.7	40.9	5.0
21	LEX037	55	F: GGATTCCTCAACCTCCTAAA R: AGGGATAAGTGACCACCAC	Coogle et al. (1997)	4	193 – 199	35.0	33.7	3.0
22	HTG014	55	F: CCAGTCTAAGTTTGTTGGCTAGAA R: CAAAGGTGAGTGATGGATGGAAGC	Marklund et al. (1994)	5	131 – 147	69.7	60.8	3.0
22	HTG021	58	F: ATTACTTCCTCCAGGTATCTCAG R: AGGCAGGGCTGGGAGACGT	Lindgren et al. (1999)	6	124 – 134	59.5	55.7	5.0
22	COR016	58	F: CAGCTCAGTAGATGATTGTCCA R: GCAAAGACAAGGAGGTTAAGTT	Hopman et al. (1999)	7	172 – 202	50.8	46.6	10.0
22	HMS047	60	F: CCTGCTGAGGACCTTGGAAGCT R: ATGTATTTTCAAGTCTAATATCTGCC	Godard et al. (1997)	4	196 – 202	27.6	25.3	8.0
22	SG19	58	F: GCCCCCACCTGCTCCACC R: GGGGCAAAGTGGAAATCC	Godard et al. (1997)	6	139 - 149	50.3	41.3	1.0
23	COR055	58	F: TAGTGACGCCTACGGATTTC R: CCCAAGAGGGCTTAGAAAGAG	Ruth et al. (1999)	6	228 – 256	64.3	53.5	7.0
23	UM019	55	F: TACTGCCAGCACTTGTACC R: TCTCTCAGTTTCTCTCTCTGTC	Meyer et al. (1997)	8	154 – 168	61.1	55.7	10.0
23	ASB039	60	F: ACAGCTGCCTGGATATGTGG R: GCAGAGAGAAATAGAGATGC	Irvin et al. (1998)	6	154 - 172	61.9	51.8	8.0
23	LEX053	55	F: TTATTCCTGCTTCGTANATGA R: ACACACTTGGGTTCAAATC	Coogle and Bailey (1997)	6	123 - 133	72.7	72.9	5.0
23	SG04	60	F: CGACGCCTCCTCCTAAAC R: CAGCTGTGTGCCTTTGATTAT	Lindgren et al. (1998)	6	203 - 213	44.0	39.8	1.0
24	LEX042	55	F: ACATACAAACCTGCTCAACAT R: CCTACACATCGCTCATCAA	Coogle et al. (1997)	5	212 - 224	35.8	28.2	5.0
24	AHT004	60	F: AACCGCCTGAGCAAGGAAGT R: CCCAGAGAGTTTACCCT	Binns et al. (1995)	9	148 - 164	74.9	64.7	7.0
24	EA2C4	50	F: ATGTATCTTCGAGGGATGAT R: GGCAGTTAATGGTGAGTAAG	Gralak et al. (1994)	5	142 - 162	68.1	62.4	10.0
24	LEX032	55	F: CGTAGTAGGGTTTTGGGTCC R: TTGCGTTTCAATTTTTAATGAC	Coogle et al. (1996)	7	249 - 261	76.4	71.9	5.0
24	COR024	58	F: CAAAAGTGATTGCCTTCGAT R: TTGGAAGCTGGGTGATTG	Murphie et al. (1999)	7	205 - 217	67.9	72.2	5.0
24	COR025	58	F: ACAGAGCTGACTGCCTATGG R: TCCTCTTCTCAGGGAGACCT	Murphie et al. (1999)	3	172 - 178	21.4	21.9	9.0

HET: mean observed heterozygosity; PIC: mean polymorphism information content

Table 1 continued

ECA	Marker	Ta (°C)	Forward/Reversed Primer Sequence	Reference	Alleles (n)	Allele size (bp)	HET	PIC	Primer (pmol)
25	NV043	60	F: TGACACAAGATAAAAGCCCCAGG R: GATTGGGAAAAGAGCACAGCC	Roed et al. (1998)	7	142 - 158	71.8	65.7	15.0
25	UCD405	60	F: ACCTCGTCTGGCTGTTGTAAG R: ACTTGCTGTGCGACTCTG	Eggleston-Stott et al. (1997)	7	252 - 270	72.3	64.2	4.0
25	COR018	58	F: AGTCTGGCAATATTGAGGATGT R: AGCAGCTACCCTTTGAATACTG	Hopman et al. (1999)	6	253 - 275	48.2	46.1	5.0
26	COR071	58	F: CTTGGGCTACAACAGGGAATA R: CTGCTATTTCAAACACTTGGA	Tallmadge et al. (1999b)	8	180 - 208	78.6	71.5	7.5
26	UM005	56	F: CCCTACCTGAAATGAGAATTG R: GGCAAAAGATCAGGCCAT	Meyer et al. (1997)	7	212 - 224	68.1	66.0	5.0
27	COR031	58	F: CAATTGCCATTTGTTCCAGTG R: GCTTAAGAAACACCAGGCAG	Murphie et al. (1999)	4	202 - 214	73.1	62.4	5.0
27	UCD005	56	F: AGCGGAAGTGCTGCGAAAG R: CCAGCATCTCTGGGCAGG	Eggleston-Stott et al. (1996)	8	226 - 240	81.6	74.9	13.0
27	LEX005	55	F: AAGGCAATGCTTATCAAATGC R: TTACCCGCAGTGACTTCTATT	Coogle et al. (1996c)	7	243 - 263	42.0	38.8	6.0
27	COR017	58	F: GAAGGCCTGAAGCATTTACA R: CGTAATGTTGACCAAACTTCA	Hopman et al. (1999)	7	239 - 253	77.0	72.1	2.5
28	UM003	56	F: GGAGGGACGATAGAGAGTAAG R: GCAGAGATAACGGACATGG	Meyer et al. (1997)	4	149 - 155	57.8	45.2	5.0
28	UCD425	55	F: AGCTGCCTCGTTAATTCA R: CTCATGTCCGCTTGTCTC	Eggleston-Stott et al. (1997)	7	233 - 247	65.4	55.3	7.0
29	LEX018	60	F: TTTCATCACTTTCTGCTTCC R: TTCTCTTCCTTTGCTCATCCT	Coogle et al. (1996a)	8	228 - 246	69.8	62.2	5.0
29	COR027	58	F: CAGCTCTGCAATTTCTCCTC R: AATGACCAAGGCATTGAAAG	Murphie et al. (1999)	8	229 - 245	79.7	67.4	8.0
29	ASB043	60	F: TCACTTAGTAGGGGCATGC R: GTGTTTGTCCTTGACTCTCC	Irvin et al. (1998)	6	85 - 99	74.3	63.9	10.0
30	LEX025	55	F: CAATCGTGGCCCGGTAAC R: TTCACTCCAATCCTCAGTCA	Coogle et al. (1996b)	7	141 - 157	69.7	63.3	5.0
30	VHL020	60	F: CAAGTCCTCTTACTTGAAGACTAG R: AACTCAGGGAGAATCTTCCTCAG	van Haeringen et al. (1994)	8	88 - 106	82.2	72.4	8.0
30	LEX075	55	F: TGAAAAGTTGCAGTTTGAGA R: CAACCTCTTGCTACCAGAATA	Bailey et al. (2000)	5	150 - 160	59.1	49.6	5.0
31	AHT033	58	F: CTGAGGGCGTAAGTCGAGTC R: GTTAATAGGAGCGGTTGTTTGG	Swinburne et al. (2000b)	8	145 - 165	74.9	69.1	9.0
31	AHT034	60	F: CTCAGGGCGAATGTTCCTC R: CCCCACCATGAGTCAAAAAC	Swinburne et al. (2000b)	7	121 - 141	77.5	69.3	5.0
X	LEX027	56	F: ACCACTGGGAAACTGTGTAA R: GCCCAGAATCCGAACC	Coogle et al. (1996b)	7	187 - 201	80.7	71.3	10.0
X	AHT028	60	F: CCTGGCTTATAGATGGCTGC R: ATTTGGAGATGGGGGTCTTT	Swinburne et al. (2000b)	12	178 - 222	83.3	81.6	6.0
X	LEX024	55	F: GGGGGTAGAGGGAAAAAGAG R: TTGTTGGCAGATCCCAGG	Coogle et al. (1996a)	10	132 - 150	80.2	80.8	8.0
X	LEX003	55	F: ACATCTAACCAGTGCTGAGACT R: GAAGGAAAAAAAGGAGGAAGAC	Coogle et al. (1996c)	9	143 - 163	78.4	75.7	7.0

HET: mean observed heterozygosity; PIC: mean polymorphism information content

Appendix 3: Laboratory paraphenalia

Chemicals

Boric acid ≥ 99.8 %, p.a.
DMSO ≥ 99.5 %, p.a.
EDTA ≥ 99 %, p.a.
Formamide ≥ 99.5 %, p.a.
rotiphorese®Gel40
TEMED 99 %, p.a.
Tris PUFFERAN® ≥ 99.9 %, p.a.
Urea ≥ 99.5 %, p.a.
Carl Roth GmbH & Co, Karlsruhe, Germany

Bromophenol blue
Paraffin
Merck KgaA, Darmstadt, Germany

Ammonium persulfate (APS) ≥ 98 %
SIGMA-ALDRICH CHEMIE GmbH, Steinheim, Germany

All water used was taken from the water purification system Milli-Q®

Enzymes

Taq-DNA-Polymerase 5 U/µl
Incubation Mix T.Pol with $MgCl_2$ [1.5 mM]
Qbiogene GmbH, Heidelberg, Germany

The polymerase was always used in the presence of incubation Mix T.Pol buffer.

Kits

QIAamp 96 DNA Blood Kit
QIAGEN, Hilden, Germany

Primers, dNTP's, size standard

The primers used have been produced by MWG-BIOTECH AG, Ebersberg, Germany

dATP, dCTP, dGTP, dTTP > 98 %
Carl Roth GmbH & Co, Karlsruhe, Germany

Size Standard IRDye™ 700 or 800
LI-COR, Lincoln, USA

Reagents and buffers

TBE-buffer (10x)
108 g Tris PUFFERAN® [121.14 M]
55 g boric acid [61.83 M]
7.44 g EDTA [372.24 M]
H_2O ad 1000 ml
pH 8.0

TBE-buffer (1x)
100 ml TBE-buffer (10x)
900 ml H_2O

APS solution (10 %)
1 g APS
10 ml H_2O

Urea/TBE solution (6 %)
425 g urea [60.06 M]
250 ml H_2O
100 ml TBE-buffer (10x)
solubilise in a water bath at 65°C
H_2O ad 850 ml

Urea/TBE solution (4 %)
425 g urea [60.06 M]
300 ml H_2O
100 ml TBE-buffer (10x)
solubilise in a water bath at 65°C
H_2O ad 900 ml

Bromophenol blue solution
0.5 g bromophenol blue
10 ml 0.5 M EDTA solution
H_2O ad 50 ml

Loading buffer for gel electrophoresis
2 ml bromophenol blue solution
20 ml formamide

Gel solution 6%
12.75 ml urea/TBE solution (6%)
2.25 ml rotiphorese® Gel 40
95 µl APS solution (10 %)
9.5 µl TEMED

Gel solution 4%
13.5 ml urea/TBE solution (4%)
1.5 ml rotiphorese® Gel 40
95 µl APS solution (10 %)
9.5 µl TEMED

dNTP solution
100 µl dATP [100 mM]
100 µl dCTP [100 mM]
100 µl dGTP [100 mM]
100 µl dTTP [100 mM]
1600 µl H_2O
The concentration of each dNTP in the ready-to-use solution is 5 mM.

Consumables

Reaction tubes 1.5 ml
nerbe plus GmbH, Winsen/Luhe, Germany

Thermo-fast 96 well plate, skirted
ABgene, Hamburg, Germany

Combitips® plus
Eppendorf AG, Hamburg, Germany

Pipette tips
0.1 – 10 µl (K138.1)
0.1 – 10 µl (A407.1)
5 – 200 µl (7058.1)
Carl Roth GmbH & Co, Karlsruhe, Germany
0.1 – 10 µl (7600)
Matrix Technologies Corporation, Lowell, USA

Pipettes

Multipette® plus
Eppendorf AG, Hamburg, Germany

pipetus®-akku
Hirschmann® Laborgeräte GmbH & Co.KG, Eberstadt, Germany

Pipetman® (P2, P10, P20, P100, P200, P1000)
Gilson Medical Electronics S.A., Villiers-le-bel, France

Pipettor, Multi 12 Channel (0.1 – 10 µl)
Micronic® systems, Lelystad, The Netherlands

8-Channel, gel loading syringe
Hamilton Bonaduz AG, Bonaduz, Switzerland

Equipment

Milli-Q® water purification system
Millipore GmbH, Eschborn, Germany

Sigma centrifuge 4-15
QIAGEN, Hilden, Germany

Thermocycler:
PTC-100™ Programmable Thermal Controller
PTC 100™ Peltier thermal Cycler
PTC 200™ Peltier thermal Cycler
MJ Research, Watertown, USA

Automated sequencer:
LI-COR 4200/S-2, LI-COR 4300
Lincoln, USA

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