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CURRENT GENETICS OF OSTEOCHONDROSIS (OCD) IN EQUINES
(A REVIEW OF LITERATURE)

Diploma work

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1. INTRODUCTION

1.1. Genetic disorders of horses

Genetic disorders are seen in many different horse breeds and are caused by negative mutation of one or multiple genes. Genetic disorders can be strictly genetic mean while some disorders results form a combination of environmental factors and genetics, such as in osteochondrosis. The genetic disorders can be either congenital or have a later onset in life. The occurrence of genetic disorders seams to bee lower in horses compared to other domestic animals, this might be due to the late domestication of the horse (BOWLING and RUVINSKY, 2000). Identification of genetic disorders in the horse has been negatively effected due to their long gestation period, single births, and sometimes incomplete recordings since horses often changes owners. Genetic disorders are unwanted from both a health and welfare point of view, as well as from an economical view. The life quality, value and performance possibilities are reduced in effected horses (FINNO et al, 2008).

Genetic disorders of ***the respiratory system*** are *guttural pouch tympany* and *roaring.* Gutteral pouch tympany is still under investigation, but recent studies suggest that it is a sex linked and a polygenic trait. It is 4 times more often seen in female horses. Tympany is caused by trapped air due to the malformation of the pharyngeal opening of the Eustachian tube. *Roaring* might bee of recessive inheritance, affected horses have a loud and painful breathing during exercise.

Genetic disorders affecting the ***cardiovascular system*** in horses are; Equine neonatal isoerythrolysis, fragile blood vessels, haemophilia A and ventricular septal defects. *Equine neonatal isoerythrolysis* is seen in foals which are born healthy, but after feeding from the mares colostrums develop a possibly life-threatening heamolytic anaemia due to a hupersensitivity reaction between the mares antibodies and the inherited antigens from the stallion that are found on the surface of the red blood cells of the foal. *Fragile blood vessels* is a recessive inheritance affected horses will easily rapture blood vessels. It is typical seen in the nasal cavity, this condition could be lethal. *Haemophilia A* is a sex linked inherited trait caused by a deficiency of blood clotting factor VIII, it lead to development of hematomas which can develop on various parts of the body. *Ventricular septal defects*, is a specially seen in miniature horses.

Genetic disorders seen in the ***brain and spinal cord*** are; cerebellar abiotrophy, hydrocephalus, juvenile epilepsy syndrome, occipito-atlantoaxial malformation and wobbler syndrome.

Cerebellar abiotrophy is recessive inherited and found in almost only in Arab horses. Clinical signs include; ataxia, head-tremor, wide-based stance, exaggerated action of the forelegs and lack of menace reflex. Cerebellum is smaller than normal and disorganized because of post-natal degeneration of Purkinje cells and associated granular neurons. *Hydrocephalus* is possibly dominant inherited. Hydrocephalus is caused by accumulation of cerebrospinal fluid in the ventricular system, resulting in dilated ventricles and decreased size of the brain
Juvenile epilepsy syndrome occurs in Arab horses. It is not known how it is inherited. Affected foals are born normal and are normal in between the seizures; they usually outgrow this condition around the age of one. It is also referred to as benign or idiopathic epilepsy.
Occipito-atlantoaxial malformation (OAAM) is a possible autosomal recessive disorder. The cervical vertebra and the base of the skull are fused together causing compression of the spinal cord. It is seen in foals one month old, clinical signs include coordination problems, and even paralysis of both front and hind limbs.
Wobbler syndrome is especially seen in thoroughbreds, and occurs more frequently in males than in female. incoordination, unstable gait and muscular weakness is seen in horses with wobbler. Wobbler syndrome is a chronic disorder.
Genetic disorders of the ***eyes*** are aniridia, congenital cataract and congenital stationary night blindness. *Aniridia* is dominant inherited found in rocky mountain horses. Iris is absence in both eyes, leading into unresponsive pupils and cataract.
Congenital cataract is a recessive disorder which is not always inherited, it could also occur due to developmental defects.
Congenital stationary night blindness (CSNB) is a decreased or absent night visions. It is recessive inherited, but the disorder is still under investigation. It has been shown that Appaloosa horses that are homozygous for leopard gene have higher incidence for congenital stationary night blindness.
Some of the Genetic disorders of the ***musculoskeletal system*** are, contracted flexor tendons, dwarfism, glycogen branching enzyme deficiency (GBED), hyperkalemic periodic paralysis (HYPP), malignant hyperthermia, patella luxation, Polysaccharide storage myopathy (PSSM), Recurrent exertional rhabdomyolysis (RER) and umbilical hernia.
Contracted flexor tendons is a recessive inherited disorder. It affects the pastern, fetlock and carpal joints of the forelimbs. It is characterised with flexed forelimbs due to shortening of the deep digital flexors tendon and associated muscles. The foetus position during gestation may also affect the degree of flexion.

Dwarfism is a recessive inherited disorder and is seen in miniature horse as they have been selected for there small size. They could be both proportionate and disproportionate, disproportionate horses have immature musculoskeletal system, a large head, silky hair coat, floppy ears and are presented with mandibular brachygnathia.

*Glycogen branching enzyme deficiency (GBED***)** is an autosomal recessive disorder found in Quarter horses. It is caused by a mutation in the glycogen branching enzyme gene, causing an insufficient storage and utilization of glycogen. It can be lethal in foals and also cause abortion.

Hyperkalemic periodic paralysis (HYPP) is a dominant disorder in Quarter horses, Appaloosa and paint horses. Clinical sign are; sporadic attacks of uncontrollable muscle twitching, weakness, paralysis, it is a lethal disorder, due to heart failure or paralysis of respiratory muscles.

Malignant hyperthermia (MH) is an autosomal dominant disorder, caused by a mutation in the ryanodine receptors found in skeletal muscles. The disorder is only present in 1% or less in Quarter horses.

Patellar luxation is *recessive* inherited which is most commonly seen in miniature horses and Shetland ponies. A lateral luxation is seen due to the hypoplasia of the femoral trochlea and a shallow intertrochlear groove.

*Polysaccharide storage myopathy (PSSM***)** is *dominant* disorder caused by large amount of glycogen accumulation in skeletal muscles.

Recurrent exertional rhabdomyolysis (RER) is an autosomal dominant inherited disorder. The cause of RER is still unknown but clinical signs include recurrent periods of tying up.

Umbilical hernia is most frequently seen in standardbreds and draft horses. The aetiology of umbilical hernia is still not clear and other component may effect the development such as cutting the umbilical cord too close to the abdominal wall or heavy forces on an oversized foetus.

Some of the genetic disorders of the ***skin and integument*** are, albinism, naked foal syndrome, hereditary equine regional dermal asthenia (HERDA)/ hyperelastosis cutis**,** Lavender foal syndrome/Coat colour dilution lethal (CCDL), and vitiligo.

Albinism is a recessive disorder causing lack of pigment. Albino horses lack the enzyme tyrosinase with is needed to produce melanin leading into amelanotic skin, hair and mucous membranes. Some of the effected horses may also present a depigmented irides and photophobia.

Hairless/naked foal syndrome is a recessive disorder found in Akhal Teke. Foals born with the disorder have no hair coat, mane or tail..
Hereditary equine regional dermal asthenia (HERDA)/hyperelastosis cutis is a recessive inherited disorder most commonly seen in Quarter horses. It causes a thin and fragile skin, which easily gets wounds.
Lavender foal syndrome/Coat colour dilution lethal (CCDL) is an autosomal recessive disorder found in foals of Arab horses, especially in Egyptian Arabs. It is a neurological disorder, but characterised with a diluted hair coat colour as well. The coat can be lavender, pale pink or silvery coloured in affected horses. Foals are typically larger than normal at birth, other clinical signs seen are; seizure, opistohonus, paddling, recumbence and abnormal eye movement. It is a lethal disorder.
Vitiligo is an acquired genetically programmed depigmentation, but the aetiology is still unclear. Vitiligo is most frequently seen in young, grey Arabian horses. Areas of depigmentation can bee seen on lips, muzzle and eyelids other places are occasionally involved. Affected horses will sometimes get their pigment back within a year.
Severe combined immunodeficiency (SCID) is an autoimmune disorder in Arab horses. It is a recessive disease with a fatal outcome. Foals born with SCID suffer from a weakened immune system leading into secondary infections and eventually causing death.
Cryptorchidism, hermaphrodite and pseudohermaphrodite are ***genital and sex-determination*** genetically disorders in horses. Cryptorchidism is the retention of testis in the abdomen, either unilateral or bilateral. Its aetiology is not completely understood, and is thought to involve genetics, hormonal and mechanical factors. Horses with both testis retained in abdomen are sterile.
Hermaphrodite and pseudohermaphrodite are intersex disorders meaning that individuals harbour both male and female characteristics and it is often a result of abnormality in the sex chromosomes. Disorders included are; *gonadal hypoplasia, gonadal dysgenesis, XY sex reversal syndrome/XY female type, Swyer syndrome, Sry-XX hermaphroditism, and XX male pseudohermaphroditism.*

1.2. Osteochondrosis in horses

Osteochondrosis (OC) is one of the most common orthopedic development disorders in growing horses. It is a lesion caused by disturbance in the endochondral ossification process.

Disturbance in this process is seen in young growing horses. The disorder is commonly seen in trotters, coldblood and warmblood horse breeds.
The lesion is commonly seen in different joint of horses most frequently in the hock, stifle, and fetlock joint. OC in horses is considered to be a disorder of polygenic origin. Growth rate, nutrition, body size, mineral imbalance, endocrinological dysfunction, biomechanical trauma, and hereditary factors may be included in the causes of OC (LÖHRING, 2003). It affects many breeds and has also been shown to have a genetic influence which plays an important role (SCHOUGAARD et al, 1990; PHILIPSSON et al, 1993; VAN WEEREN, 2006)
The heritability estimated in different publications vary largely, the reason for the big variations may be due to the method of estimation, the number of horses included in the examination (WITTWER, 2007)
The frequency of OC varies greatly between reports, it depends on the number of horses in the population, the scale of the measurement, the number of joints studied what definition of OC that is given, and the age of the horses in the report.
The horse genome has been explored during the last years it has made it possible to investigate many of the quantitative traits for hereditary disorders in horses, OC is one of these.This review is an overview of the current genetic background to OC in horse.

1.3. Material and methods

Aim of my study is to provide an overview of the genetic background of osteochondrosis (OC) and osteochondrosis dissecans (OCD) in equines, since it is a common and important joint disorder in our sport horses. I have focus on main features of this disorder, mostly from a molecular point of view, but also giving a short introduction of the disorders pathology, aetiology and heritability. New possibilities to manage breeding selection have developed when new molecular method have been explored, these include techniques such as whole genome scans, candidate gene analysis and SNP microarrays.
Data for this study have mostly been collected from various books, articles and internet sources.

2. OSTEOCHONDROSIS IN HORSES

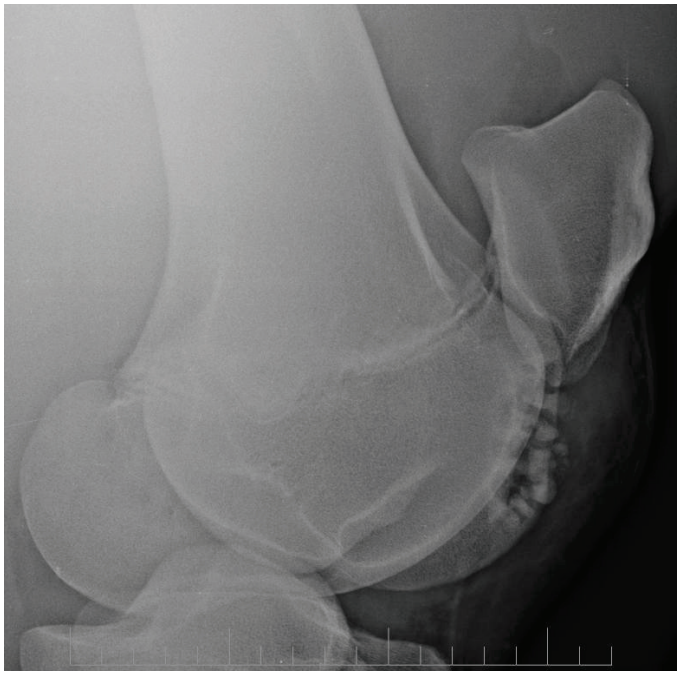
2.1. Definition of osteochondrosis

The first definition for OC was mentioned in the late 1800s as osteochondritis, but it was not used in veterinarian medicine until the 1960s. It is now a day general accepted that it is not primary caused by an infection, and it is named as osteochondrosis. In spite of its long history many aspects of OCD is still unexplained.

OC is a disease caused by disturbance of the normal endochondral ossification, the process of cartilage developing into bone. Affected parts will be degenerated and necrotized, eventually leading into formation of cartilage flaps. The cartilage flaps develop into loose bodies called joint mice, when joint mice are found the disorder is called osteochondrosis dissecans OCD. This is a lesion that's not caused by trauma or primary arthritis it's a developmental disturbance in the locomotors system (LAMPE et al, 2009)

OC is found in several joints, most commonly in the hook, stifle and fetlock joint. These lesions can develop into chronic degenerative joint diseases such as osteoarthrosis or arthropathy.

The disorder is frequently diagnosed by macroscopic inspection of an x-ray. Severity of the lesion is classified in a scoring system (BRUNS, 2005)



http://www.mallorequina.com/en/services/List/show/osteocondrosis-240

2.2. Pathogenesis of osteochondrosis

Osteochondrosis is seen in young animals with growing cartilage, and is characterized by focal or multifocal failure of endochondral ossification. OC is a common lesion seen in several animal species, such as in pigs, dogs, horses, cattle, poultry and rats.
Endochondral ossification is the process when cartilage develops into bone (JEFFCOTT, 1996) Endochondral ossification includes several processes, such as; cell proliferation, extracellular matrix synthesis, cellular hypertrophy, matrix mineralization and vascular invasion (LEFEBVRE and SMITS, 2005).
The endochondral process is essential for the process of bone to grow and elongate. Failure in the endochondral process leads to local areas of necrosis, when vascular invasion or matrix calcification fails (EKMAN and CARLSON, 1998).
The primary lesion develops due to focal necrosis and starts at the articular/epiphyseal surface. Primary lesion can progress and develop into secondary lesions when the cartilage channels regress and affects the nutrition of deeper layers leading to necrosis. Mechanical forces on the lesions causes bone bodies, cartilage flaps and subchondral cysts to develop (LAMPE et al, 2009). Cartilage flaps fractures and end up free in the joint leading into the development of "joint mice". OCD can cause pain, joint effusions and nonspecific secondary lymphoplasmocytic synovitis. OC/OCD may develop in any synovial joint, including those of the facets of the vertebral column.
The most common site OC in horses are the stifle, hock joint and fetlock joint (WITTWER et al, 2007). In some animals the joints with osteochondrosis might never heal and can develop into degenerative joint disease.
In warmblood horses lesions has been found on x-ray as early as in one month old foals. Lesions found in such young horses are able to regress, since the extracellular matrix of articular cartilage undergoes a rapid remodeling in young animals (VAN WEEREN, 2006). Osteochondric lesions may heal as long as the chondrocytes metabolism is active, this differs in between joints. Different joints will show lesion at different ages.

2.3. Aetiology of osteochondrosis

The cause of OC is still not fully understood, but it is regarded as a disorder of multifactorial origin (JEFFCOTT et al, 1991; WITTWER et al, 2007). Nutritional factors, growth rate, anatomic characteristics, exercise, and heritability are discussed as the most important causes

of the development of OC. Most published material can not prove the direct role of increase growth rate. There is little proof that body weigh could be the cause of OC, but it might increase the risk of developing osteoarthritis from osteochondrosis. Trauma seems to promote the development of OC into OCD, but it's not the cause of the primary lesion. The most important contributors to OC seem to be hereditary and anatomical factors (LAMPE, 2009). Inherited defected joint conformation can cause a temporary stress on blood vessels supplying cartilage channels leading into OC; this might be one of the main causes of OC.

Estimated heritability vary widely, perhaps due to the different material and methods used among the different studies (GREVENHOF et al, 2009). Heritability were highest for the hock OCD and lowest for stifle OC/OCD.

In foals licking fences with Zink based white paint, lesions of OCD has been seen without predisposing dysplasia. Zinc excess leads to Copper deficiency and Copper is need as cofactor for the enzymes required in the cross linkage between tropocollagen molecules.

3. GENETICS OF OSTEOCHONDROSIS IN HORSES

3.1. Current development in equine genetics

The horse genome project started in 2007 and is an international project involving over a 100 scientist in 20 countries to explore the equine genome of the domestic horse; Equus Caballus. With this information it is possible to understand the genetic aspects of equine physiology and diseases. (http://www.uky.edu/Ag/Horsemap/welcome.html). The first step against identifying the genes causing OC in horses was the whole genome scans. The Equine genome consists of approximately 3000 megabases (Mb) of DNA found on 31 autosomes and a X and Y chromosomes (MURRAY and BOLWING, 2000). Genetic maps have been developed and are used to identify the location of genes and markers on the chromosomes. Maps that has been developed are; the radiation hybrid map (RH) (CHOWDHARY et al, 2003), comprehensive genetic linkage maps (SWINEBURNE et al, 2000. 2006) and a medium-density horse gene map (PERROCHEAU et al, 2006). There are two types of markers used to identify genetic traits in horses, microsatellites and single-nucleotide polymorphisms (SNPs) Microsatellites are polymorphic and more informative than SNPs, SNPs on the other hand are more common and can provide denser maps which are more informative than microsatellites maps (LAMPE et al, 2009).

Genome map can be used to identify genes regulating different phenotypic traits, such as OC. "Many hereditary diseases in horses are of quantitative genetic nature, which means that the influences of many genes combine to contribute to a particular phenotype" (LÖHRING et al, 2003). The whole genome scan was the first step in the investigation of genomic areas holding genes for osteochondrosis (OCD). During the last years several studies has been done regarding the genetic background of OC in horses. In some reports it has already been proven that it's a disorder carried on many different genes (WITTWER et al, 2008).

3.2. Heritability

Breeds prone to OC are shown to be thoroughbred, standarbred and other sporthorse breeds, in the articles analysed for this study warmblood trotters, Hanoverian warmblood and South German coldblood are included. Heritability of OC has been estimated in a number of horse populations, different joints have been studied, and data have been analysed with either linear models or threshold models.

In a study by Philipson Swedish standarbred trotter stallions were used for studying OC in hock joint, fetlock and palmar/plantar osteochondral fragment (POF). The heritability was estimated to be 0.08-0.09 on the threshold scale for both OC and POF traits, corresponding to 0.24-0.27 on the underlying quantitative scale (PHILIPSON et al, 1993).
Van Grevenhof did a study in Dutch Warmblood horses, and the overall heritability for OC was 0.23. Heritability was highest in the fetlock and POF, heritability was lowest for the stifle. Genetic correlation between fetlock and stifle were shown to be strong meanwhile correlation between POF and the other joints were moderate (VAN GREVENHOF et al, 2009).
In a study of Swedish Warmblood horses, heritability for OC was shown to be 0.05 on the visible binomial scale, corresponding heritability for OC in stifle was; 0.03 and hock 0.08. On the underlying quantitative scale these values correspond to 0.09-0.38. (JÖNSSON et al, 2011) The majorities of estimates for OC are within the range 0.08-0.27 and transformed into the underlying quantitative scale within the range 0.2-0.40. Estimation difference for heritance seems to be higher for different joints than for different breeds. Breeds and joints examined are not the only parameter responsible for the different results, in the studies horses of various ages and various experiences is used, leading to different results. Heritability for OCD seams to increase when older horses are included in the study, heritability grew from 0.37 to 0.46 when not only young horses were examined (DOROTA LEWSZUK AND AGNIESZKA KORWIN-KOSSAKOWSKA, 2012). Different scales are used for estimations of heritability, for example the VAN GREVENHOF study showed that computations based on a wider scale gave better results as the heritability was higher. In the Swedish study of Warmblood horses, the fetlock joint reached an unusually high value. Fetlock joint has usually shown low value of heritance and have there for in many countries not been considered by selection against OC. Most countries in Europe select against OCD as early as possible and breeding strategies against OC is almost the same, even though the procedures and scales are not exactly the same. Stallions are radiographically examined before doing their own performance test. They have to be OC free to start their performance test. Only stallions are tested, and selection has been based on phenotypic results, but breeders are encouraged to test their breeding mares as well. (DOROTA LEWSZUK AND AGNIESZKA KORWIN-KOSSAKOWSKA, 2012).

3.3. Candidate genes for osteochondrosis

It is important to pick out the candidate genes in the horse genome, because there physiological products are linked to OC. Candidate genes are found in the specific region on the chromosome were an expression for the trait of OC is suspected to be found. Candidate genes are genes which code for hormones, enzymes, metabolic factors and/ or their receptors involved in cartilage differentiation and maturation. The Equine Articular Cartilage cDNA Library is often used to choose candidate genes which are expressed in cartilage. Today there are 13,964 equine articular expressed sequence tag (EST) which can be found at NCBIs nucleotide database (http://www.ncbi.nlm.nih.gov/sites/entrez). It is also useful to use candidate genes causing osteoarthritis in other species, to explore the candidate gene linked to OCD (LAMPE et al, 2009).

Hormones controlling skeletal growth and development are such as insulin, thyroxin, growth hormone, parathyroid hormone and calcitonin. The *Transforming growth factor B* (TGF-B) has a significant role in the growth of cartilage, especially in differentiation of chondrocytes (HENSON et al, 1997; JEFFCOTT and HENSON, 1998).

In 1997 HENSON et al distinguished a reduced expression of TGF-B in focal lesion of damaged cartilage, but a strong expression in the surrounding lesion; this might show that TGF-B has a role in the pathogenesis of OC. In 2001 SEMEVOLOS et al, discovered that there was a higher expression of TGF-B in areas with OC. TGF-B is located on chromosome 30 and maybe a potential candidate gene for OC in horses.

Insulin-like-growth factor (IGF-1) is a gene found on equine chromosome 28 and has an important role in cartilage metabolism and growth. Foals suffering from OC showed significant lower concentration of IGF-1 than healthy foals did. It is suggested that reduced chondrocyte differentiation caused by lover plasma IGF-1 concentration may lead into the development of OC (LAMPE et al, 2009). Positional candidate genes for the QTL regions can be located using comparative analysis with humans (DIERKS et al, 2006; SWINEBURNE et al, 2006). Candidate genes could be easier identified and located on the equine maps by using comparative human-equine maps.

There are candidate genes in syntenic region reported to cause similar condition in humans as osteochondrosis dose in equines. *Frizzeled related protein* is one of them; this gene plays a role in osteoarthritis is humans and is also found to do so in horses. One other gene discovered using comparative human equine maps is the *Activin A receptor type 1.*

A result from WITTWER et al, 2009 indicated that the gene- *Xin actin-binding repeat containing 2 (ZIRP2)* has an influence in both OC/OCD in fetlock and hock joints of coldblood horses. For equine chromosome 4 a better human-equine comparative map made it possible to compare the candidate genes of osteoarthritis in humans with the QTLs regions identified in horses. *Acyloxyacyl hydrolase* genes were found by a significant associated with OCD in the fetlock joint (WITTWER et al, 2008), but the role in development of OC is still unclear. A result from LAMPE in 2009, showed that the candidate genes; *hyaluronon*glucosaminidase is located on equine chromosome 16.
When QTLs for chromosome 2 were localized, the *Matrilin 1 gene* and the *collagen, type IX, alpha gene* were selected for these regions (LEWCZUK and KORWIN-KOSSAKOWSKA, 2012).

3.4. Single nucleotide polymorphism microarrays

SNPs are used as genetic markers for large scale genetic mapping projects and have been used with good result to identify chromosome regions associated with polygenic human and animal diseases. In 2006 an international team started to sequence and assemble the horse genome, the collaboration involved scientist from 20 different universities all around the world. In 2008 a commercial SNPs microarray became available, the Equine SNP50 Genotyping BeadChip. It makes it possible to do, genome-wide analyses, quantitative trait loci identification and validation. The chip was developed by International Equine Genome Mapping Workshop and the Morris Animal Foundation's Equine Genome Consortium. The SNPs chip makes it possible to identify those genes causing genetic disease. The BeadChip makes it possible to development a DNA test that can determine a horse's genetic risk for a specific genetic disease. Horse breeding programs would be improved since it enables the development of new diagnostic methods to upgrade equine health and welfare. (http://www.morrisanimalfoundation.org/blog/category/horse/international-effort-on.html)
With SNPs it is now easier to perform genome-wide association analysis, and make it possible to map many more genes responsible for traits and diseases. It also easier to collect DNA directly from the specific animal, than to collect DNA from large families (BANNASCH, 2008). Equine SNPs is used to detect and identify both single genes and multigenic diseases such as; musculoskeletal, neuromuscular, cardiovascular and respiratory disorders (SWINEBURNE et al, 2006).

In 2010 KOMM did a whole genome scan with SNPs in a Hanoverian warmblood horse to approve detected QTLs of OC. Known QTLs on equine chromosomes 2, 4 and 16 were valid. The study also resulted in the detection of new potential QTLs on equine chromosome 3, 7, 19, 20, 22, 26 and 29, and this revealed new potential candidate genes (K0MM 2010)
The use of microarrays makes it possible to reveal the genes for equine OC, and it aids in the development of marker test for OC.

4. ARTICLES REVIEWED REGARDING THE CURRENT GENOMIC SITUATION FOR OSTEOCHONDROSIS.

4.1. GENOME SCAN FOR QUANTITATIVE TRAIT LOCI FOR OSTEOCHONDROSIS IN HANOVERIAN WARMBLOOD HORSES USING AN OPTIMIZED MICROSATELLITE MARKER SET. LÖHRING ET AL, 2003

The aim of this study was to identify Quantitative trait loci for osteochondrosis and osteochondrosis dissecans, by using an optimized microsatellite marker set.
In the study a total of 123 half sib foals were used, 66 female and 57 males. All foals used in the study had been radiographically evaluated and proven affected with osteochondrosis, osteochondrosis dissecans or both conditions simultaneously. At the examination the foals had the average age 6, 7 month. All mares to the foals were genotyped, and the phenotype of the stallions remained unknown.
All horses were genotyped by using a genome scan panel consisting of 157 microsatellites markers. The markers scanned the 31 autosomes and the x chromosomes.
Separate analysis for done for the different traits; OC (fetlock and/or hock joint) OCD (fetlock and/or hock joint), OC fetlock, OCD fetlock, OC hock joint and OCD hock joint. Female, males and both sexes together were also analyzed separately.
Results: the linkage analysis based on IBD (identical by descent) mapping QTL was found on 13 chromosomes, these regions were further analyzed. In total 27 chromosome-wide QTL were mapped for the different traits for OC and OCD on the 13 chromosomes. Twelve QTL were found in females, nine in male and six in both sexes. QTL were found on the equine chromosomes; 2, 3, 4, 5, 6, 10, 15, 16, 19, 21, 23, 24 and 25. On equine chromosome 21 a single QTL showed a genome-wide significant error probability. The QTL for fetlock OC/OCD and hock joint OC/OCD was found on different chromosomes, indicating that fetlock OC/OCD and hock OC/OCD may be independently inherited as different traits. QTL for fetlock OC/OCD were found on equine chromosomes; 2, 3, 16, 19 and 24. For hock OC/OCD QTLs were found on equine chromosomes; 4, 5, 15 and 21.

4.2. GENOME WIDE SEARCH FOR MARKERS ASSOCIATED WITH OSTEOCHONDROSIS IN HANOVERIAN WARMBLOOD HOSES. DIERKS ET AL, 2007

The aim of this study was to detect quantitative trait locus for osteochondrosis and osteochondrosis dissecans in horses.

Randomly sampled Hanoverian warmblood horses from the stud book were examined for OC and OCD in the limbs. 211 Hanoverian warmblood horses was used, these where of 14 paternal half-sib families. Foals used were both those showing radiographic signs of OC/ OCD and those considered free of OC/OCD. Of the horses examined 73,1% showed signs of OC, 45,2 % signs of OCD and 26,9 % didn't show any radiographic signs of OC/OCD in the joints. They were examined twice, at the second time the average age was 24, 4 month. The predilection site was examined by x-ray examination, and it was shown that for fetlock OC the most frequently site was the sagittal ridge of the third metacarpal/metatarsal bone. Foals with signs at this specific location were therefore used in the study, even if other predilection sites are found. For hock joint OC/OCD two sites were considered, intermediate ridge of the distal tibia and lateral trochlea of talus. Radioluceny, irregular bone margin, new bone formation, or osseous fragments were considered as signs of OC or OCD at the predilection sites.

The traits used where osteochondrosis in fetlock and/or hock joints, osteochondrosis dissecans in fetlock and/or hock joints, osteochondrosis in fetlock, osteochondrosis dissecans in fetlock, osteochondrosis in hock joint and osteochondrosis dissecans in hock joint.

For the study 260 microsatellites where used as markers. In the first genome scan 172 microsatellite markers from equine linkage maps was used. The map positions of the microsatellites on the chromosomes were taken from the latest sex-averaged genetic map (SWINEBURNE et al, 2006), but not all microsatellites were on this map and for these the comprehensive male linkage map and equine radiation hybrid map was used. Foals and mares were genotyped. To show linkage with OC multipoint nonparametric linkage tests were used. In the second step of the study the chromosomes with the highest linkage test statistics indication QTLs were scanned again. In the second scan 88 microsatellite markers were used to refine the QTLs found in the first scan. The result showed that linkage analysis based on IBD (identical by descent) mapping reached the chromosome-wide significance level on eight different equine chromosomes; 2, 3, 4, 5, 15, 16, 19 and 21. The traits for fetlock and hock may be genetically related, due to the QTLs for theses traits were partly overlapping on the same chromosome.

QTLs for fetlock OC/OCD were located on five different chromosomes; equine chromosome 2, 3, 4, 5 and 16. QTL for hock joint were located on four of these chromosomes as well (equine chromosome; 2, 4, 5, and 16). On three chromosomes there were significant QTLs for either fetlock OC (equine chromosome 3) or hock OC (equine chromosome 15 and 21). This genome scan was the first step in identifying the genes causing osteochondrosis in equine, for a further investigation these QTL can be refined using denser markers.

4.3. MAPPING QUANTITATIVE TRAIT LOCI FOR OSTEOCHONDROSIS IN FETLOCK AND HOCK JOINTS AND PALMAR/PLANTAR OSSEUS FRAGMENTS IN FETLOCK JOINTS OF SOUTH GERMAN COLDBLOOD HORSES BY WITTWER ET AL, 2007

WITTWER et al preformed a whole genome scan in South German coldblood horses to identify QTLs for osteochondrosis in palmar/plantar osseous fragments (POF) in the fetlock joint. The scan included 117 half –sib South German coldblood horses, with an average age of 17 month. The horses were diagnosed by radiographic examination. Predilections site for fetlock and hock joint OC/OCD included; sagittal ridge of the third metacarpal/metatarsal bone, intermediate ridge of the distal tibia, the lateral/medial trochlear ridge of the talus and the lateral/medial malleolus of the tibia. Typical signs at these sites include variable radiopacity, irregular bone margin, new bone formation and osseous fragments. In the study 18% did not show any signs of OC, 96% were affected with OC and 39% were affected with OCD in the fetlock.

157 microsatellites were homogenously spread of 31 autosomes and X chromosomes at an average distance of 17, 7 cM. The microsatellites were taken from latest genetic linkage map for equine chromosomes made by SWINBURNE et al, 2006, the radiation hybrid map and the horse map database at the INRA biotechnology laboratories home page. In the second step 16 chromosomes which contained supposed QTL were further analyzed using 93 additional markers.

17 QTLs were found on 17 chromosomes, they were located on equine chromosomes; 1, 4, 5, 8, 12, 13, 15, 16, 17, 18, 22, 23, 25, 26, 27, 28 and 31. QTLs on equine chromosomes; 5, 15, 17, 22, 23, 25, and 28 were found for OC in hock and fetlock joints. For OCD in fetlock joints significant QTLs on equine chromosome 1, 13, 18, 22 and 25 were identified. For fetlock OC significant QTL on equine chromosomes 1, 5, 15, 16,17,23,25 27, and 28 were found. For hock OC significant QTLs were identified on equine chromosome 15, 18 and 31. Since there weren't enough horses with hock OCD in the study, no QTLs for hock were identified.

On equine chromosome 4 significantly associated SNPs in the acyloxyacyl hydrolase (AOAH) could be identified (WITTWER et al,.2008) on equine chromosome 18 significantly associated intronic SNPs in the xin actin-binding repeat containing 2 (XIRP2) gene could be identified (WITTWER et al,.2009).

4.4. FINE MAPPING OF QUANTITATIVE TRAIT LOCI (QTL) FOR OSTEOCHONDROSIS IN HANOVERIAN WARMBLOOD HORSES BY LAMPE ET AL, 2009.

At Hanoverian university LAMPE et al did a study for fine mapping of identified QTLs for OC. The whole genome scan of horses together with previous studies by DIERK and LÖHRING had lead to identification of several QTLs. The aim of this study were to refine mapping of identified QTLs on equine chromosome 5, 16, 18 and 21 using microsatellites and single nucleotide polymorphisms (SNPs). This will help in the development of genetic tests based on gene-associated markers. Furthermore the aim was to identify new potential candidate genes that might influence the development of OC.

New genetic tools that has been developed, thanks to the horse genome sequencing aids in a more efficient search for associated genetic polymorphism. LAMPE et al preformed a whole genome association analysis of SNPs with the aim to further refine the already known QTLs and to detect new potential QTLs for OC in Hanoverian warmblood horses.

The genome-wide significant QTLs on equine chromosome 5,16 and 21 were as earlier mentioned for fine mapping, together with the release of the horse genome assembly EquCab2 it was made possible to identify new microsatellites. All QTLs on theses chromosomes were confirmed. Equine chromosome 18 was previously only investigated in South German coldblood horses in a linkage study for OC (WITTWER et al, 2007), and it was now confirmed in Hanoverian warmblood horses. A new QTLs was identified on chromosome 18, thanks to the new microsatellites which made a more evenly and denser distribution. On all chromosomes investigated in the study QTL were overlapping for hock OC and OCD, respectively for fetlock OC and OCD. This gave an assumption that the same genes will cause both OC and OCD. The genetic influences of the development of fetlock OC and hock OC did not show any similarity, due the hock QTL did not map at the fetlock QTL. This was assumed likely as the genetic correlation between fetlock OC and hock OC were close to zero in trotter horses (GRØNDAHL and DOLVIK, 1993) and even negative in Hanoverian warmblood horses (STOCK et al, 2005).

Candidate genes: Putative candidate genes were also identified, the QTL on equine chromosome 5 included collagen type *XXIV, alpha* 1. Collagen type *XXIV* is a marker for embryonic bone formation and might play a role in the regulation of type 1 collagen fibrillogenesis (KOCH et al, 2003) it is also stated by MATSUO et el 2008 that collagen type *XXIV* also is expressed in trabecular bone and periosteum of newborn mice, due to these functions it seams likely that this is a suitable functional candidate gene for OC. Candidate genes on QTL on equine chromosome 16 could include several hyaluronaglucosaminidase genes. Hyaluronan is one of the important glycosaminoglycans of the extracellular matrix, hyaluronan is an important integral structure component of articular cartilage and act as a lubricant in the joint. Candidate gene on QTL on equine chromosome 18 includes a gene which encodes the parathyroid hormone 2 receptor, the exact function of this in unknown. But, since parathyroid hormone is one of most important regulator for calcium metabolism this gene might play a role in the development of OC. On equine chromosome 21 a gene which encodes for the phoshoinositide-3-kinase regulatory subunit 1 among other things is a candidate gene for osteoporosis this is also a genes that seam to be a functional candidate gene for OC. For these candidate genes further studies need to be done to proof their actual function in the development of OC.

Single nucleotide polymorphisms: Since 2008 a commercial SNP microarray became available, containing about 57000 SNPs. The Equine SNP50 Genotyping BeadChip, makes it possible to do genome-wide analyses, quantitative trait loci identification and validation. A refinement of QTL and an identification of new genomic regions for OC were done in Hanoverian warmblood horses.

The analysis resulted in a huge number of associated SNPs distributed over the already known QTL region. Many of the SNPs were found in intergenic regions, therefore the detection of mutations causing OC could not be done with SNP analysis. Other types of investigations were necessary, and statistical calculations were done to refine the QTL regions. Halotype association and variance analyses were preformed. It was possible to show with halotypes in the QTL regions which were associated with the phenotypic traits for OC which could demarcate the genomic regions harboring QTLs for osteochondrosis. LAMPE identified additional regions for the different phenotypic traits on ten different chromosomes.

4.5. FINE MAPPING OF QUANTITATIVE TRAIT LOCI (QTL) FOR OSTEOCHONDROSIS IN HANOVERIAN WARMBLOOD HORSES BY KOMM, 2010

The aim of KOMM's study was to make a refine mapping of the already identified QTL on equine chromosome 2 and 4 in Hanoverian warmblood horses using dense marker sets, and also identify new potential candidate genes.
Previous scans in Hanoverian warmblood horses had revealed QTLs for OC on equine chromosomes 2,4,5,16,18 and 21.
The horse genome assembly EquCab2 made it possible to develop new microsatellites, which lead to a decreased marker distanced in significant regions.
104 Hanoverian warmblood horses from 14 paternal half-sib families were used in the study. The same phenotypic traits as in previous studies were analyzed this includes; OC (fetlock and/or hock joints), OCD (fetlock and/or hock joints), fetlock OC, fetlock OCD, hock OC, and hock OCD. On equine chromosome 2; 37 new microsatellites and 24 SNPs were genotyped. The already identified QTL were confirmed and the significant regions could be narrowed down for both OC/OCD and fetlock OC and hock OC. On equine chromosome 4; 41 new microsatellites and 11 SNPs were genotyped marker distanced were decreased.
Another aim of KOMM's study was to confirm already identified QTLs and to detect new potential QTL by doing a whole genome scan with SNPs using the newly developed equine SNP50 BeadChip. 154 unrelated Hanoverian warmblood horses were used in the whole genome scan. 313 significant associated SNPs for the different phenotypic traits were observed. The QTL on equine chromosome 2 was confirmed at 17.55Mb and on equine chromosome 4 two QTL were revealed (at 7.61Mb and 39, 26 MB) these two QTL were located within the same QTL as identified before. In total ten new QTL were detected on equine chromosome 3, 5, 7, 16, 19, 20 22, 26 and 29.
The genome scan and the fine mapping are important first steps towards the identification of regions harbouring potential candidate genes for osteochondrosis. The equine BeadChip is a great progress in molecular genetic research and made it possible to confirm the identified QTL on equine chromosome 2 and 4. The BeadChip microarray also made it possible to identify new QTL that where previously unknown.

5. DISCUSSION AND CONCLUSION

Genetic diseases in horses are a big concern and we are far away from reaching elimination of any of the genetic diseases mention in the introductory chapter. In theory it seams relatively easy to eliminate genetic diseases, since we today know the causative mutations of many of them and especially easy for those we know are of dominant inherited. But, why does genetic disease still occur? The terms "popular-sire syndrome" and "the founder's effect" might help to explain the reason for still breeding horses with genetic disease.

Breeders tend to breed on stallions which are top- winners in their category, these stallions genes will quickly spread though out the gene pool causing the "popular-sire syndrome". If one stallion gives many offspring his mutated gene will spread quickly through the horse population leading into breed-related genetic diseases through the so called founder's effect. There seems to be remaining attitude amoung breeders to value performance over health although this attitude is waning. One example of this is in *Hyperkalemic periodic paralysis* for example were effected horse seems to have been preferred in breeding due to their good muscle development and some claim that show judges support this way of thinking by selecting *Hyperkalemic periodic paralysis* affected horses as superior halter horses. Other examples of the popular-sire syndrome are *hereditary equine regional dermal asthenia* and *glycogen branching enzyme deficiency* both originally originating from Quarter stallion King who has had large influence in the breed. There is also a theory that *severe combined immunodeficiency* originated from a popular Arab horse, leading into a widespread disorder today. Paint horses are selected for their popular color pattern increasing the risk for spread of LWOS if not bred cautiously. These are a few examples of how humans have selected mutation with desirable phenotypic traits.

Genetic diseases are comparatively rare compared to acquired diseases, owner and breeders may think this will not effect there horses specifically. It's important that it's easy to find information for owners and breeders about their breed's genetic disease, to prevent and control the spread of the disease.

The cost and availability of diagnostic tests are also of big importance, to make owner and breeders willing to voluntary test their horses. Most research and diagnostic laboratories are situated in USA today, which makes it harder for other countries to easily test and diagnose there horses.

Furthermore the horse is not a very good candidate for genetic research, because of their large body, expensive maintenance, long gestation period and their few offspring.

Genetic research is more favorable to perform on e.g. rodents which have short gestation periods with many offspring produced each time.

Preventative measure to avoid occurrence of genetic diseases in horses:

The horse Genome project completed the mapping of the horse genome in 2007. This was a major step for research on hereditary disease, many of them are today known and molecular test for diagnosis is developed, but this is not enough to prevent genetic disease to spread. The health of future horse is dependent on different breeding associations making clear rules and regulation for which horse that can be used as breeding animals. Breeders and owner should be able to easily find information about the genetic disease so they can make good decision when breeding their animals.

The breeding association in USA, such as American Quarter horse association AQHA, , APHA and WAHO (world Arab horse association) has developed policy and education program for breeding. In most European countries the American breeding association policy are followed. It seams like most breeding association still does not aid the exclusion of carriers of genetic disease from their breeding registries. One argument used is the risk of losing a unique trait when excluding a carrier from the breed. But, the only way to eliminate a genetic disease from a breed is to dilute the genes over time. To decrease amount of genetic disease carries should never be bred with other carriers or horses that are homozygous for the mutated gene, a carrier should only be aloud too bred with horses that are homozygous negative for the given mutation. I believe it is important to reveal horses carrier status in there certificate so that it is easy for breeders to make a correct decision to the benefit of the breed. This is only possible to apply for recessive diseases, as breeding with dominant carriers will in best case effect 50% of the offspring's.

Today most genetic research is done in USA were breeding associations provide financial support for the development of new knowledge and laboratorial work. Genetic testing need to be possible in a wider geographical area, to obtain this a greater financial support for genetic research is needed. In Norway and Sweden there were some positive changes in this area during 2009, they decided to improve the situation and become one of the leading equine researchers. Veterinarians has an important role in equine genetic diseases, they are to be well educated and up to date regarding genetic disease to provide good information and advice to owners and breeders.

Conclusion from articles analyzed:

OC in a common and important joint disease in many horse breeds, the causes of OC is multifactorial. Genetics plays an important role in the development of OC. Thanks to the

possibility to identify quantitative trait loci (QTL) in Hanoverian warmblood and South German coldblood the genetic research have enable a search for potential and functional candidate genes influencing development of OC.

Since the start of the "horse genome project" much seams to have happened. Equine genetic maps have developed, making it possible to localize QTLs for different phenotypes. In 2003 LÖHRING identified totally 27 chromosome-wide QTL that were mapped for the different traits for OC and OCD on 13 chromosomes. The QTLs were found on the equine chromosomes; 2, 3, 4, 5, 6, 10, 15, 16, 19, 21, 23, 24 and 25. The QTL for fetlock OC/OCD and hock joint OC/OCD was found on different chromosomes, indicating that fetlock OC/OCD and hock OC/OCD may be independently inherited as different traits. QTL for fetlock OC/OCD were found on equine chromosomes; 2, 3, 16, 19 and 24. For hock OC/OCD QTL were found on equine chromosomes; 4, 5, 15 and 21.

In 2007 DIERKS continued the search for QTL for OC. DIERKS found chromosome-wide significance level on eight different equine chromosomes; 2, 3, 4, 5, 15, 16, 19 and 21. The traits for fetlock and hock may be genetically related, due to the QTLs for these traits were partly overlapping on the same chromosome. QTLs for fetlock OC/OCD were located on five chromosomes; equine chromosomes 2, 3, 4, 5 and 16. QTL for hock joint were located on four of these chromosomes as well; equine chromosome; 2, 4, 5 and 16. On three chromosomes there were significant QTLs for either fetlock OC (equine chromosome 3) or hock OC (equine chromosome 15 and 21). This genome scan was a first step in revealing the candidate genes responsible for OC, but further studies were necessary to refine the regions harboring QTLs.

WITTWER preformed a whole genome scan in South German coldblood horses to identify QTLs for OC. 17 QTLs were found on 17 chromosomes, they were located on equine chromosome; 1, 4, 5, 8, 12, 13, 15, 16, 17, 18, 22, 23, 25, 26, 27, 28 and 31. QTLs on equine chromosome; 5, 15, 17, 22, 23, 25, and 28 were found for OC in hock and fetlock joints. For OCD in fetlock joints significant QTLs on equine chromosome 1, 13, 18, 22 and 25 were identified. For fetlock OC significant QTL on equine chromosomes 1, 5, 15, 16, 17, 23, 25, 27, and 28 were found. For hock OC there were only three markers that were significant on equine chromosome 15, 18 and 31. Since there weren't enough horses with hock OCD in the study, no QTLs for hock were identified

At Hanoverian university LAMPE et al did a study for fine mapping of identified QTLs. The whole genome scan of horses together with previous studies by DIERK and LÖHRING had lead to identification of several QTLs. The aim of this study was to refine mapping of

identified QTLs on equine chromosome 5, 16, 18 and 21 using microsatellites and single nucleotide polymorphisms (SNPs) making a denser and more evenly distributed marker set. LAMPE et al preformed a whole genome association analysis of SNPs with the aim to further refine the already known QTLs and to detect new potential QTLs for OC in Hanoverian warmblood horses.

The genome-wide significant QTLs on equine chromosome 5, 16 and 21 were as earlier mentioned for fine mapping, together with the release of the horse genome assembly EquCab2 it was made possible to identify new microsatellites. All QTLs on theses chromosomes were confirmed. Equine chromosome 18 was investigated as well, earlier QTLs on this chromosome was only confirmed in South German coldblood horses in a linkage study for osteochondrosis (WITTWER et al, 2007), and now a further investigation was done in Hanoverian warmblood horses. New QTLs was identified on chromosome 18, thanks to the new microsatellites.

Breeding for OCD-free horses, which is included in some breeding programs seams to be complicated. It's difficult to internationally select for a trait that is not clearly defined, a stallion defined as OCD-free could in another country be defined as an OCD affected horse. Different scale for estimating hereditary is used; even if they don't vary widely its still makes a different in evaluating results.

DNA analysis for OCD has some limitations as a large number of genes seams to be involved in this genetic disease. Equine breeding is a need of healthy horses free of OCD, so all method for excluding OCD needs to be used.

New potential candidate genes located on identified chromosomes makes it possible to select healthy horses for breeding.

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SUMMARY

The aim of the study was to perform a review study regarding the current genetic background of OC in the domestic horse. OC is a commonly seen locomotor disorder in young horses (WITTWER et al, 2007). The cause of OC is still not clear, even though it is assumed that it is of multifactorial origin, affecting many breeds and with an important genetic aspect. The whole equine genome scan was the first step in the investigation of genomic areas holding genes for OC and OCD. During the last years several studies has been done regarding the genetic background of OC in horses. In some reports it has already been proven that it's a disorder carried on many different genes (WITTWER et al, 2008).
The articles analyzed for QTL mapping were mostly studies from the University of Hannover done between the years 2000-2010. The first article was the; Genome scan for Quantitative Trait Loci for OC in Hanoverian Warmblood horses using an optimized microsatellite marker set by LÖHRING (2003). LÖHRING revealed; 27 chromosome-wide QTLs on thirteen equine chromosomes for the OC trait in Hanoverian warmblood horses. In Dierks study 19 chromosome-wide significant QTLs were reveled on 17 equine chromosomes. They were found on equine chromosome; 2, 3, 4, 5, 7, 8, 9, 13, 14, 15, 16, 18, 19, 21, 22, 24 and 30.
In 2009 Lampe did a complete genome scan in Hanoverian Warmblood horses and refined the QTLs for OC on chromosome 5, 16, 18 and 21. The genome-wide significant QTLs on equine chromosome 5, 16 and 21 were as earlier mentioned for fine mapping, together with the release of the horse genome assembly EquCab2 it was possible to identify new microsatellites. All QTLs on theses chromosomes were confirmed. Equine chromosome 18 was investigated as well, earlier QTLs on this chromosome was only confirmed in South German Coldblood horses in a linkage study for OC (WITTWER et al, 2007), and now a further investigation was done in Hanoverian warmblood horses. A new QTLs was identified on chromosome 18, thanks to the new microsatellites which made a more evenly and denser distribution marker set. The genetic influences of the development of fetlock OC and hock OC did not show any similarity, due the hock QTL did not map at the fetlock QTL. This was assumed likely as the genetic correlation between fetlock OC and hock OC were close to zero in trotter horses (GRØNDAHL and DOLVIK 1993) and even negative in Hanoverian warmblood horses (STOCK et al, 2005)

WITTWER preformed a whole genome scan to confirm the QTLs identified in Hanoverian warmblood horses in South German Coldblood. A scan was preformed in 216 coldblood

using 250 polymorphic microsatellite markers. WITTWER identified 17 putative QTLs on 17 equine chromosomes for the OC / OCD traits. The aim of WITTWER's study was to confirm the QTLs by using single nucleotide polymorphisms (SNPs) of these genomic regions.
SNPs are used as genetic markers for large scale genetic mapping projects and have been used with good result to identify chromosome regions associated with polygenic human and animal diseases. In 2008 a commercial SNPs microarray became available, the Equine SNP50 Genotyping BeadChip. It makes it possible to do, genome-wide analyses, quantitative trait loci identification and validation. It enables the development of a DNA test that can determine a horse's genetic risk for susceptibility to a genetic disease, improving horse breeding programs since it enables the development of new diagnostic methods to upgrade equine health and welfare.
The aim of KOMM's study was to make a refine mapping of the already identified QTL on equine chromosome 2 and 4 in Hanoverian warmblood horses using dense marker sets, and also identify new potential candidate genes. Another aim of KOMM's study was to confirm already identified QTLs and to detect new potential QTL by doing a whole genome scan with SNPs using the newly developed equine SNP50 BeadChip. 154 unrelated Hanoverian warmblood horses were used in the whole genome scan. 313 significant associated SNPs for the different phenotypic traits were observed. The QTL on equine chromosome 2 was confirmed at 17.55Mb and on equine chromosome 4 two QTL were revealed (at 7.61Mb and 39, 26 MB) these two QTL were located within the same QTL as identified before. In total ten new QTL were detected on equine chromosome 3, 5, 7, 16, 19, 20, 22, 26 and 29.

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