The origin of the Ridge and Associated Anomalies in Rhodesian Ridgebacks

Nicolette Salmon Hillbertz
Faculty of Veterinary Medicine and Animal Science
Department of Animal Breeding and Genetics
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2007

Acta Universitatis Agriculturae Sueciae
2007:133

ISSN: 1652-6880
ISBN: 978-91-85913-32-9

Cover illustration: Li Gessbo
Tryck: SLU Service/Repro, Uppsala 2007

Abstract

Salmon Hilbertz, N.H.C. The origin of the ridge and associated anomalies in Rhodesian Ridgebacks. Doctor's dissertation.
ISSN: 1652-6880, ISBN: 978-91-85913-32-9

The thesis presents studies on the inheritance of the dorsal hair ridge and Dermoid Sinus (DS) in Rhodesian Ridgeback dogs. DS is classified as a neural tube defect in humans. Thus, the dog is proven to be an excellent comparative model regarding neural tube defects. It was shown that the hair ridge is caused by an autosomal dominant mutation that predisposes for DS. Collection of material from DS-affected Rhodesian Ridgeback puppies, their parental animals and littermates was performed. Evaluation by histopathology to confirm the presence of DS was conducted. Results revealed that DS (in dogs) were located in the cervical region and that a novel skin lesion (previously referred to as DS), denoted Lipoma of the terminal filum (with skin-dimple and extra-spinal connection) (LTF) was located in the sacral region. A common genetic origin between DS and LTF was suggested. It was proposed that different types of DS and LTF may be caused by differences in FGF levels in combination with different genetic backgrounds and environmental interactions. Samples from eleven DS-affected Rhodesian Ridgebacks and nine ridgeless Rhodesian Ridgebacks were genotyped by a dog-specific genome-wide association analysis utilizing an array of 26.500 SNPs. Association between a 750 kb region and the ridge phenotype was identified. The region contained five genes *FGF3*, *FGF4*, *FGF19*, *ORAOV1* and the 3'-end of *CCND1*. Further fine-mapping of the identified region, utilizing the recently developed multiple ligation-dependent genome amplification (MLGA) technique, enabled identification of the mutation causing the ridge. It was shown that the dorsal hair ridge in ridgeback dogs is caused by a 133 kb duplication of three fibroblast growth factor genes *FGF3, FGF4* and *FGF19* and the *ORAOV1* gene. Dogs homozygous for this copy number variation mutation have an increased risk of developing DS, a neural tube-like defect. The hair ridge and development of DS is most likely caused by a gene dosage effect of increased FGF expression during a critical phase of dermal development. Nucleotide sequence analysis of the internal breakpoint of the duplication further showed that the ridge mutation was identical in Rhodesian- and Thai Ridgebacks, revealing a common origin of the mutation in the two breeds. Ridgebacks homozygote for the ridge mutation, have an increased susceptibility to develop DS and/or LTF. Further studies regarding the genetic complexity of DS and LTF will shed light on the biological complexity of these dermal lesions.

Keywords: dermoid sinus, neural tube defect, lipoma of the terminal filum with skin-dimple and extra-spinal connection, genome-wide association analysis, duplication, MLGA.

Author's address: Nicolette Salmon Hillbertz, Department of Animal Breeding and Genetics, SLU. BMC, Box 597, S-751 24 UPPSALA, Sweden. Email: Nicolette.hillbertz@hgen.slu.se.

To my husband Anders and
the Rhodesian Ridgeback puppies that were autopsied.

Contents

Appendix

This thesis is based upon the following papers, which are referred to in the text by their Roman numerals:

I. Hillbertz, N.H.C. 2005. Inheritance of dermoid sinus in the Rhodesian ridgeback. *Journal of Small Animal Practice 46(2)*:71-4.

II. Hillbertz, N.H.C & Andersson, G. 2006. Autosomal dominant mutation causing the dorsal ridge predisposes for dermoid sinus in Rhodesian ridgeback dogs. *Journal of Small Animal Practice 47(4)*:184-8.

III. Salmon Hillbertz, N.H.C., Wikström, N., Hedhammar, Å., Andersson, L., Andersson, G. & Hellmén, E. The ridge mutation in ridgeback dogs is associated with two types of congenital lesions, cervical Dermoid sinus and sacral lipoma of the terminal filum. *Manuscript*.

IV. Karlsson, E.K., Baranowska, I., Wade, C.M., Salmon Hillbertz, N.H., Zody, M.C., Anderson, N., Biagi, T.M., Patterson, N., Pielberg, G.R., Kulbokas, E.J. 3rd., Comstock, K.E., Keller, E.T., Mesirov, J.P., von Euler, H., Kämpe, O., Hedhammar, Å, Lander, E.S., Andersson, G., Andersson, L. & Lindblad-Toh, K. 2007. Efficient mapping of mendelian traits in dogs through genome-wide association. *Nature Genetics 39 (11)*:1321-8.

V. Salmon Hillbertz, N.H., Isaksson, M., Karlsson, E.K., Hellmén, E., Pielberg, G.R., Savolainen, P., Wade, C.M., von Euler, H., Gustafson, U., Hedhammar, Å., Nilsson, M., Lindblad-Toh, K., Andersson, L. & Andersson, G. 2007. Duplication of *FGF3*, *FGF4*, *FGF19* and *ORAOV1* causes hair ridge and predisposition to dermoid sinus in Ridgeback dogs. *Nature Genetics 39 (11)*:1318-20.

Abbreviations

bp	Base pairs
CCND1	Cyclin D1
cDNA	Complementary DNA
C_t	Cycle threshold
DNA	Deoxyribonucleic acid
DS	Dermoid sinus
FGF3	Fibroblast growth factor 3
FGF4	Fibroblast growth factor 4
FGF19	Fibroblast growth factor 19
GWAA	Genome-wide association analysis
LEF	Lymphoid enhancer factor
LTF	Lipoma of the terminal filum with skin-dimple and extra-spinal connection

mtDNA	Mitochondrial DNA
mRNA	Messenger RNA
NTD	Neural tube defect
ORAOV1	Oral Cancer Over-expressed Gene 1
PCR	Polymerase chain reaction
RTQ-PCR	Real-time quantitative PCR
RNA	Ribonucleic acid
rr	Homozygote ridgeless
Rr	Heterozygote ridged
RR	Homozygote ridged
SNP	Single Nucleotide Polymorphism
TCF	T-cell factor

Introduction

The ridge phenotype and breed history of the Rhodesian Ridgeback dog

The dorsal hair ridge is found in two dog breeds registered by the The Fédération Cynologique Internationale – The Rhodesian Ridgeback and the Thai Ridgeback. The ridge, in the Rhodesian Ridgeback constitutes of three components; two symmetrical crowns, a box and a tail. The hair of the tail grows in the opposite direction of the normal coat, the left crown rotates counter-clockwise and the right crown rotates clockwise (Fig. 1).

The ridge phenotype is congenital, *i.e*. no changes regarding ridge phenotype occur in the growing dog, and should be dorsally located between withers and the hips according to breed standard for Rhodesian Ridgeback dogs. A breed standard is often a very detailed description set to ensure that dogs are bred according to the specifics of the breed. Individuals deviating from the breed standard are classified as faulty, which in many instances results in exclusion from breeding. Thus, the most common faulty ridges found in Rhodesian Ridgebacks are the ones displaying three crowns (Salmon Hillbertz, data not shown). Ridgeless individuals are produced, thus less common are the ridges in which four or more crowns are present (Fig. 2). The ridge, according to the 1926 breed standard, was a fiddle ridge (3-4 crowns). Further, it was stated that dogs; *"without a clearly defined ridge"* were not recognized. During 1948, the breed standard was rephrased in some aspects, whereas one was the first mentioning of two symmetrical crowns; "*should contain two identical crowns opposite each other*" (Murray, 1989).

A dorsal ridge may infrequently be found in other breeds, such as the Boxer and the Bavarian Mountain scent hound, thus the ridges are most commonly located in the cranial/cervical region (Fig. 3). The historical origin of the ridge has been largely debated. Gwatkin (1934) hypothesized that the ridge originated from Asia, *i.e*. the extinct Phu-Quoc dog. Hubbard (1948) hypothesized that the ridge was of African origin, *i.e*. the extinct Hottentot Khoi dog, which was involved in the creation of the modern Rhodesian Ridgeback. Epstein (1937) discussed the possibility of parallel mutations which could have occurred in both Asia and Africa. Available English literature regarding the origin of the Thai Ridgeback is extremely scarce. Around year 1660, "*powerful dogs*" were imported to South Africa by the Europeans from Java to protect the fenced cattle (Theal, 1900). In the early 1860, hound dogs (Bloodhounds such as Cuban Bloodhound, and Schweisshunden such as the Bavarian and Hanoverian types, Staghound, Greyhound, Deerhound and Foxhound), Great Dane, Bulldogs, Terriers, Mastiffs, Labradors and Pointers were found at European military posts in South Africa (Hubbard, 1948; Hawley, 1984; Helgesen, 1991). The typical Boer farm dogs (some carried the ridge, others were ridgeless) were steekbaard- (stick beard) and vuilbaardhonde (dirty beard) (Hawley, 1984) which originated from the Boerhond. It is most likely that several of the European dog breeds were bred to the indigenous Hottentot Khoi dog, as many European dogs were not as adaptable to the African environment as the indigenous dogs (Helgesen, 1991).

During the 1930ies, two reports emerged from England and South Africa, which described a congenital skin-condition identified in Rhodesian Ridgeback families. The condition was described as tubular growths with openings on the skin surface (Hare, 1932; Steyn *et al*., 1939). The congenital nature and its clinical appearance suggested a developmental origin of the skin lesion.

Extensive registries of the Swedish population of Rhodesian Ridgeback dogs

An extremely successful long-term collaboration between the Swedish Kennel Club and dog breeders/owners has resulted in an impressive register containing extensive pedigrees, veterinary information (such as Hip- and elbow status, dog show results, field trial results and behavioural test results). All information is available to the public. Until 1989, neutering/spaying of dogs was exclusively allowed due to specific medical conditions according to Swedish law, which has resulted in a Swedish dog population where intact dogs are more common than sterilized dogs. The Swedish Rhodesian Ridgeback Club keeps a complementary and more comprehensive register (1964 to current date) as the Swedish Kennel Club register, thus it is far more informative in aspects such as number of stillbirths, number diseased and euthanized puppies, phenotypic deviation, gender distribution in born litters and tooth status of parental animals.

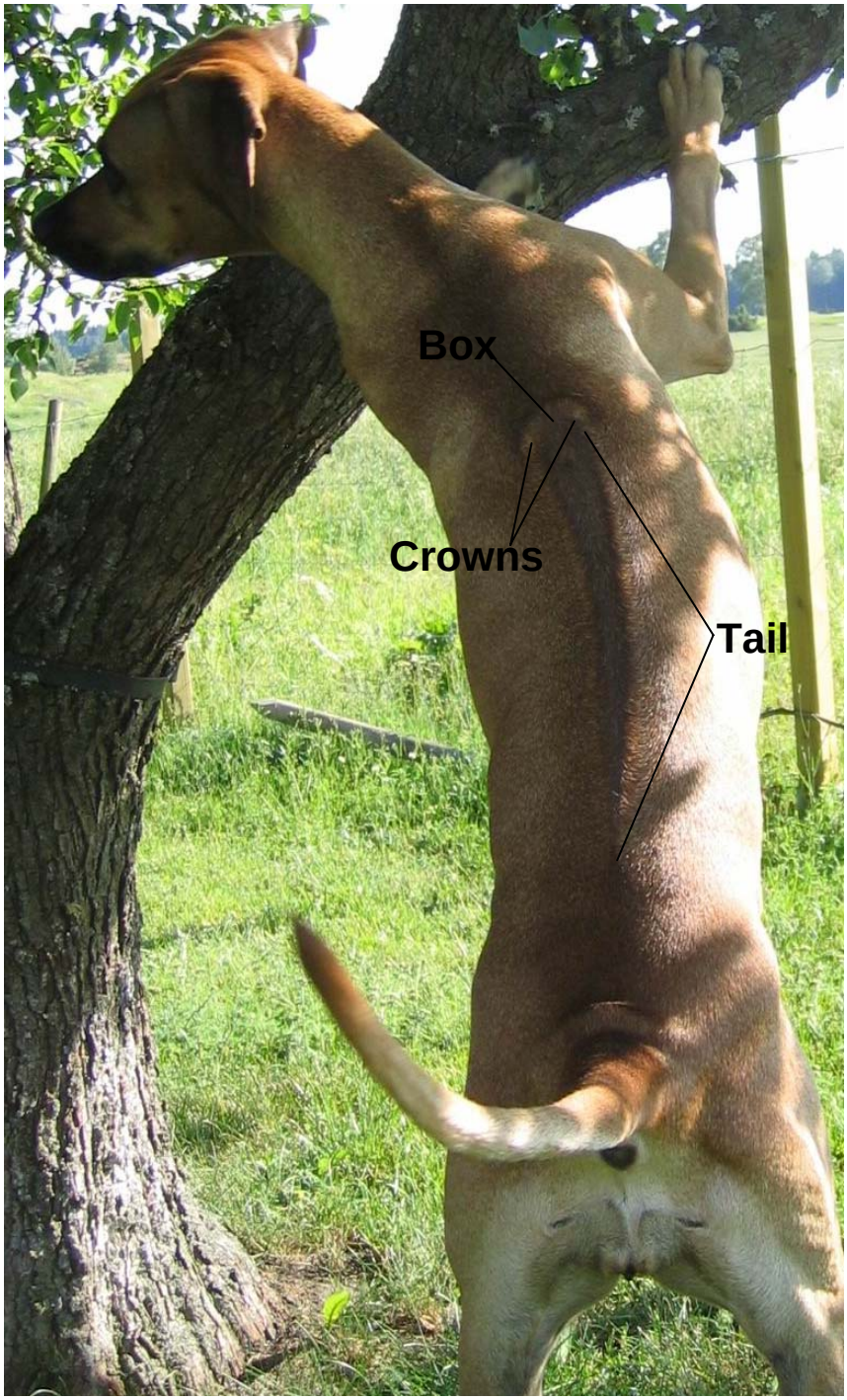


Figure 1a. The dorsal ridge of a Rhodesian Ridgeback. The ridge constitutes of three parts, 1) the box, 2) the two crowns of which one rotates counter-clockwise (left) and the other rotates clockwise (right) and 3) the tail. Image by Salmon Hillbertz.

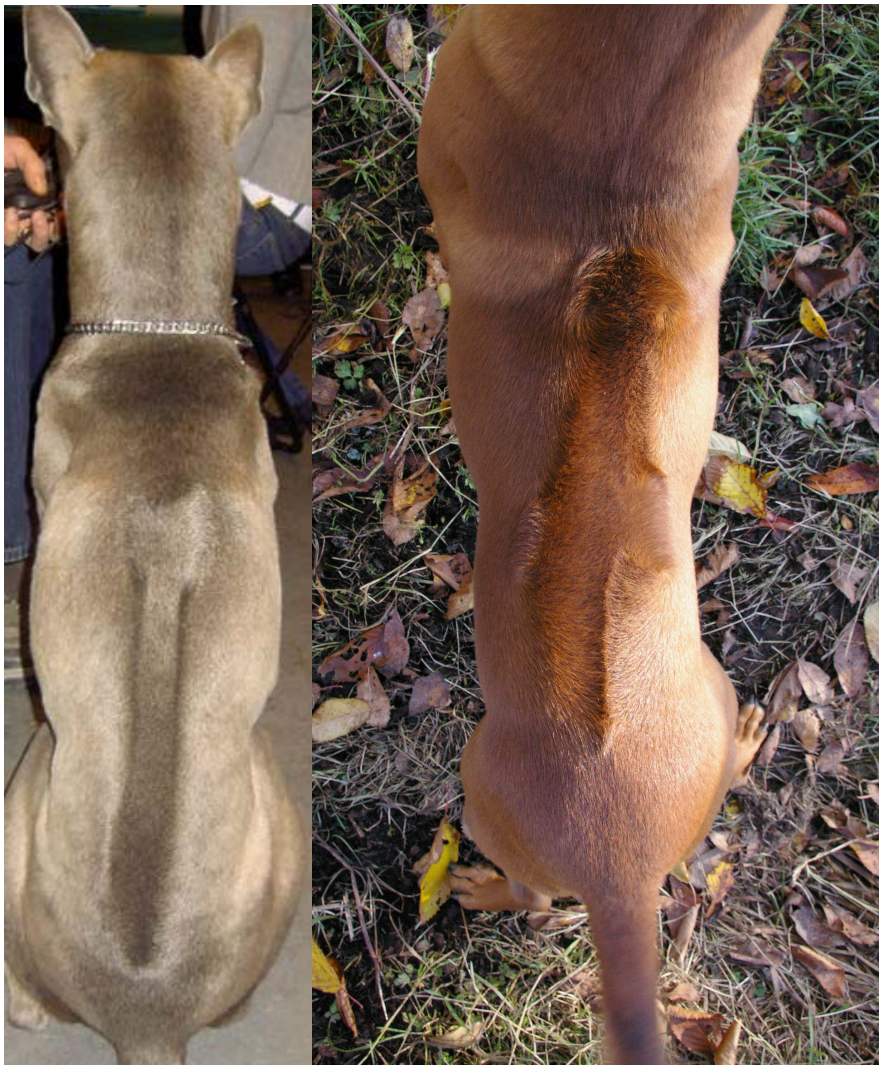


Figure 1b. Dorsal ridge in a male (left) and female (right) Thai Ridgeback. Variation in ridge phenotype is acceptable according to Thai Ridgeback breed standard. Images by Salmon Hillbertz (left) and Selin M (right).

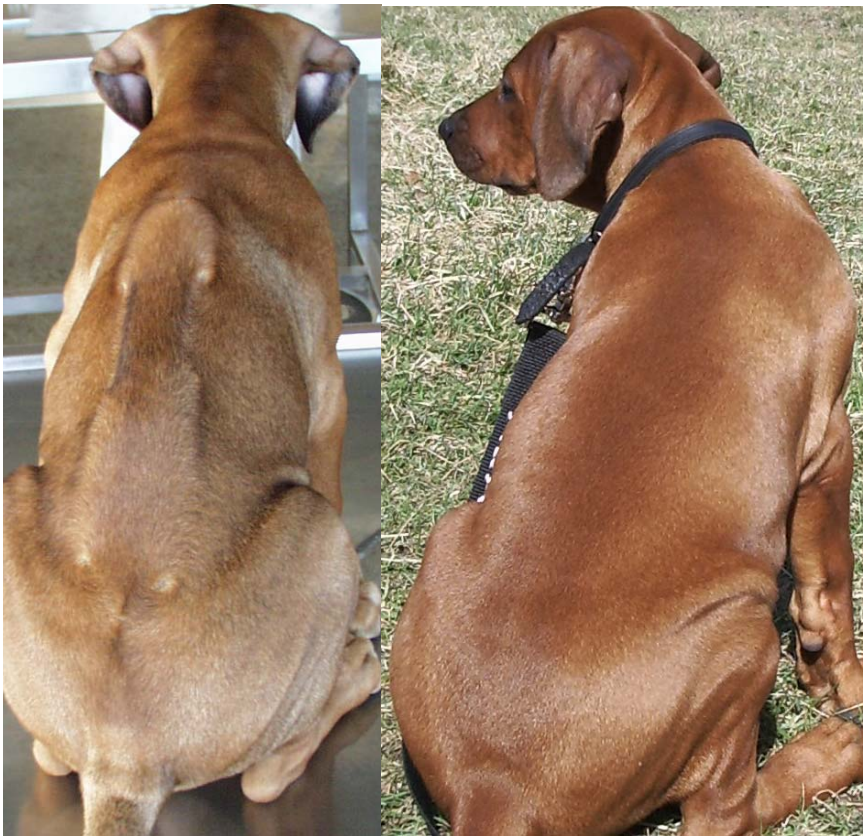


Figure 2. According to the Rhodesian Ridgeback breed standard, more than two crowns are classified as a faulty ridge. In the left image, a female Rhodesian Ridgeback displays six crowns. Ridgeless Rhodesian Ridgebacks are also produced (right image). Images by Salmon Hillbertz.

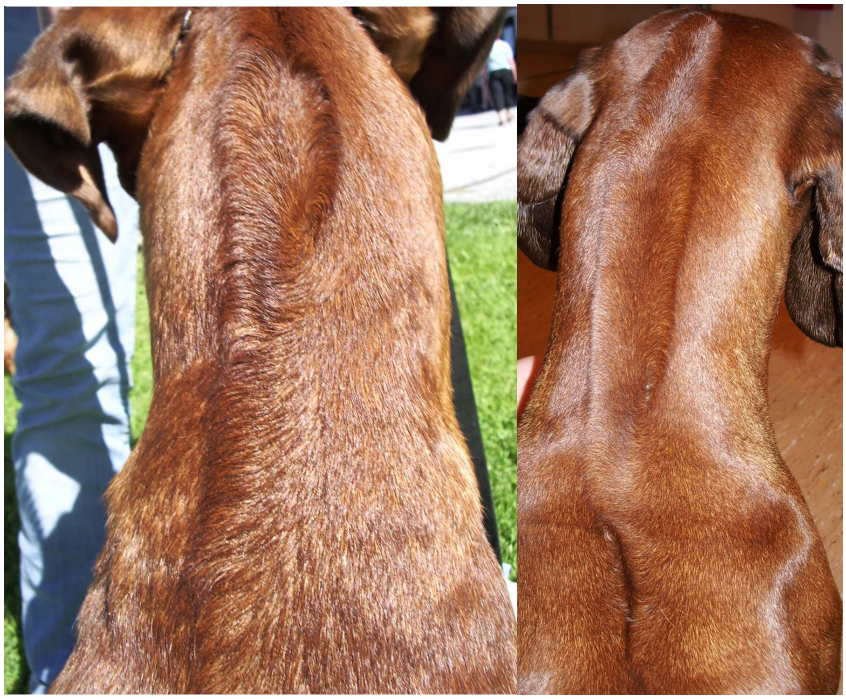


Figure 3. Ridges identified in Boxer and Bavarian Mountain Scenthound (right). In these breeds, the ridges are normally found in the dorsal cranial/cervical region. Images by Salmon Hillbertz.

Canine development

The nature of the ridge phenotype implies a mutation that influence the embryonic development of the skin and hair follicles, both which originate from the neural tube of the ectoderm. Here I will briefly discuss the gestation and embryonic development in dogs.

Normal embryogenesis and neurulation

The gestation period of dogs is normally 63 days, but with variation (Willis, 1989). Four days after mating, the fertilized egg has divided into two cells and by the 8-9th day the morula (early embryo) is transported, via the uterine tube, to the uterus (Fig. 4). On the 20-21st day, the embryo is implanted to the epithelium of the uterine horns (Evans & White, 1997; Concannon, 2000).

Neurulation is the process in which the neural plate develops and forms the neural tube. The formation of the neural tube is initiated as the surrounding cells of the neural plate signal proliferation, invagination, fusion and detaching leading to the formation of a cylinder. Between days 21 and 28, the formations of the brain, spinal cord and skin occur (ectoderm) in dogs (Evans & White, 1997). The mesoderm (which contributes to the formation of connective tissue, bone, muscle) and the endoderm (giving rise to the epithelium lining of the digestive, urinary- and respiratory systems as well as the liver, pancreas and thyroid glands) are

developed (Sjaastad *et al*., 2004; Wolpert *et al*., 2004). The three germ layers give rise to all the additional tissues/organs besides those stated above. Van der Put *et al*. (2001) described multiple neural tube closure sites in man. As similar closing sites have been suggested to occur in other vertebrates (Golden & Chernoff, 1993; Peeters *et al*., 1998), the sites may be applicable to dogs. Primary closure of the neural tube has been suggested to occur initially in the high cervical region in a caudal and cranial direction. A secondary closure point occurs bi-directionally in the fore- and midbrain region, followed by the third closure site initiated at the primitive mouth with a caudal direction to convene to the second closure site. The fourth closure site (cranial direction) occurs between the first and second closure site (Fig. 5). Van der Put *et al*. (2001) indicate a fifth closure site, occurring in the sacral region (secondary neurulation). In dogs, the spinal cord ends at lumbar disc 6/7. Below the cauda equine (sacral and caudal region) the remaining spinal nerve roots are collected (Kainer & McCracken, 2003).

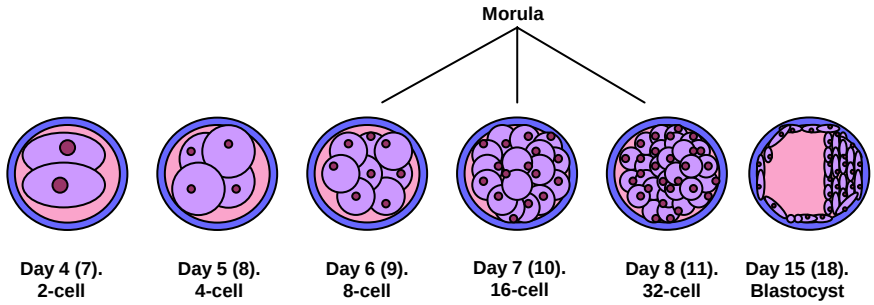


Figure 4. The development of a fertilized egg of a dog. Fifteen days after fertilization, the blastocyst is free-floating in the uterus and by the twentieth to twenty-first day the egg is implanted to the wall of the uterus horn. Image by Salmon Hillbertz.

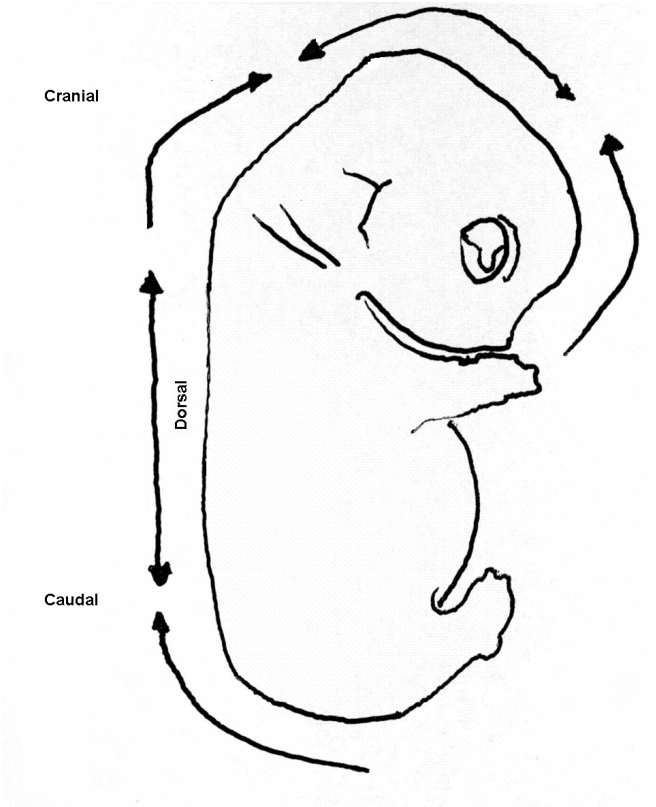


Figure 5. Five closure sites of the neural tube, according to van der Put *et al*. (2003). Arrows indicate the directions of closures and directional terms show functional changes in positions. Image by Salmon Hillbertz.

Neural tube defects

Neural tube defects (NTD) are a wide range of congenital malformations which occur due to incomplete closure of the neural tube during early embryogenesis. NTD's are suggested to occur between the third and fourth week after conception in human (Martinez-Lage *et al*., 1995) which correlates to week three during dog embryonic development according to Evans & White (1997). NTD's are classified as *i*) open (spina bifida aperta (Fig. 6) and spina bifida cystica) - and *ii*) closed defects (spina bifida occulta, a vertebrae bone fusion) (Chesney, 1973; Clayton, 1983; van den Broek *et al*., 1991). Another anomaly classified as a NTD is Dermoid sinus (DS). DS occurs due to incomplete separation between de cutaneous ectoderm and the neuroectoderm, resulting in isolation of cutaneous elements (Booth, 1998). DSs may be associated with spina bifida occulta or exist as sole entities (Hofmeyer, 1963; Chesney, 1973; Fatone *et al*., 1995).

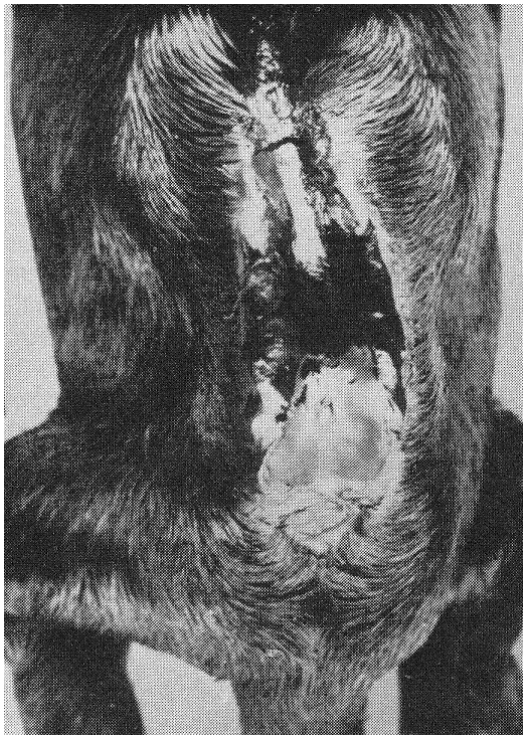


Figure 6. A stillborn German shepherd puppy affected by an open neural tube defect. Note the "ridge" associated with the congenital defect. Image from Clayton (1983) with permission from *Veterinary Records*.

Dermoid sinus

Dermoid sinus (DS) is as discussed above a disease affecting both the Rhodesian and Thai Ridgeback dogs. DS frequencies in breeds other than Ridgeback dogs are currently unknown, as many breeders/veterinarians only associate DS occurrence with Ridgeback dogs. Therefore, the number of euthanized dogs (across breeds) due to incorrect/failed diagnosis is currently unidentified.

The term dermoid sinus, also known as dermal sinus/sacral dimple/dermoid cyst, in Rhodesian Ridgebacks was originally used by Steyn *et al*. (1939), followed by Hofmeyer (1963) regarding Rhodesian Ridgeback crosses. Both authors described the identical skin disorder as originally described by Hare (1932). Five types of DS have been described (Lord *et al*., 1957; Mann & Stratton, 1966; Booth, 1998; Tshamala & Moens, 2000; Pratt *et al*., 2000), classified according to their association to the nuchal/supraspinous ligament or dura mater. The nuchal ligament overlays the cervical vertebrae continuing over the thoracic and sacral vertebrae as the supraspinous ligament (Goody, 2002). Three layers of protective membranes which cover the spinal cord and brain compose the meninges; *i*) the outer and most fibrous membrane, dura mater *ii*) the arachnoid mater and *iii*) the vascular pia mater (inner membrane) located closest to the neuroaxis (Kainer & McCracken, 2003). DSs occur along the extremes of the midline axis (dorsal). The anatomical locations correspond to the regions of late neural tube closure (Fig. 7). Skin palpation is the most widely used diagnosis method to detect DS in young

puppies. The procedure is mainly performed by dog breeders. The most common clinical diagnosis is one or multiple skin-openings. Skin-dimples (lacking a skin-opening) located by the root of the tail have also been classified as DSs. Currently, conclusive clinical diagnosis of DS requires histopathological examination (Lord *et al*., 1957; Antin, 1970; Gammie, 1986; Marks *et al*., 1993; Penrith, 1994; Cornegliani *et al*., 2001). The inheritance pattern has been described as either autosomal recessive, dominant or complex (Mann & Stratton, 1966; Gammie, 1986; Angarano & Swain, 1993; Scott *et al*., 1995, Lord *et al*., 1957). The high incidence of DS in Rhodesian Ridgebacks leaves no doubt as to its heritable nature, but there is still some uncertainty concerning the mode of inheritance as well as the underlying genetics (Hathcock *et al*., 1979; Kàsa *et al*., 1992). The uncertainty of reaching a conclusive definition of the mode of inheritance is influenced by the difficulty to stringently phenotype DS. Finally, genetic interactions between the major disease causing mutation and the genetic background of multiple genes with minor effects are likely to influence the DS phenotype.

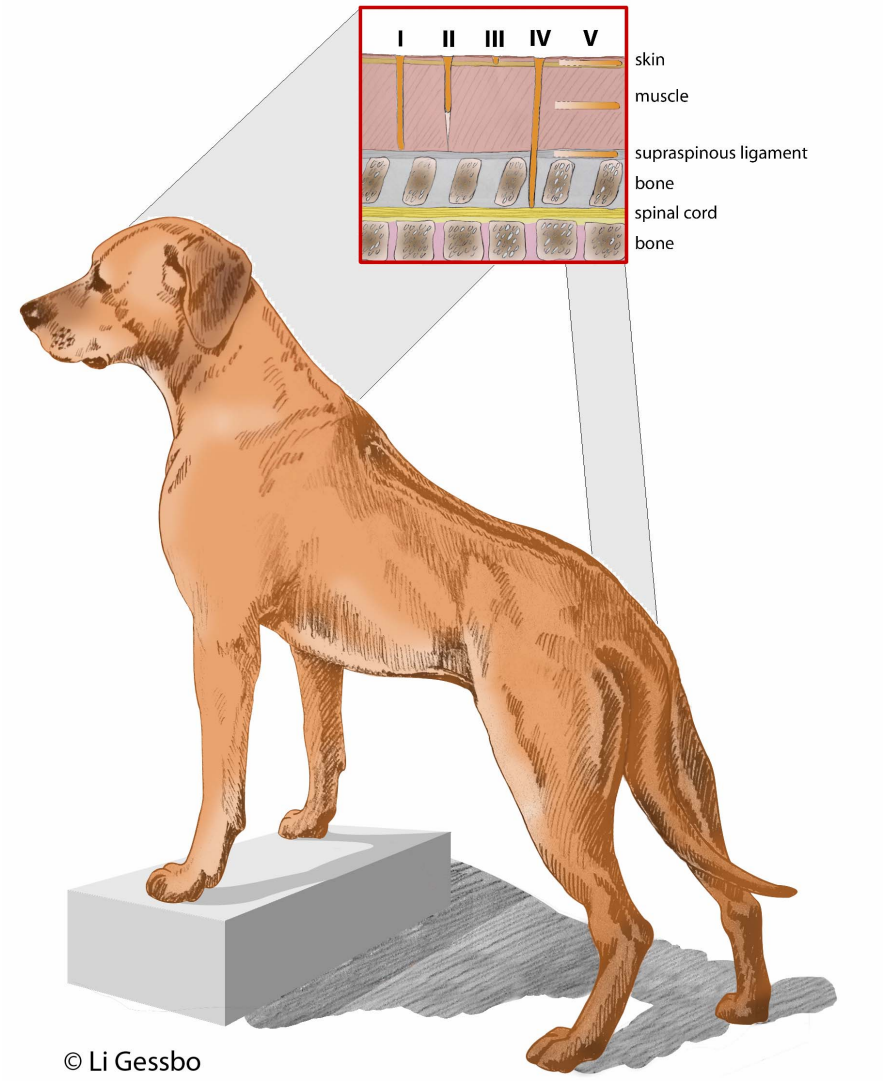


Figure 7. Five different types of Dermoid sinus have been reported. The types are classified according to vertebrae association and extension, anatomical location (dorsal) and if a skin-opening is present or not. Image by Gessbo, with permisson.

Features of canine genomics and its role in comparative studies

The domestic dog (*Canis familiaris)* originated form wolves 10,000-15,000 years ago (Vilà *et al*., 1997; Savolainen *et al*., 2002) and the long history of human/dog companionship has resulted in various modern dog breeds, which display vast phenotypic diversity (compare the Pug and the Great Dane for example). The breeding of pure-bred dogs for specific traits has resulted in highly inbred dog breeds with high frequency of genetic disease. In fact, high frequency of particular diseases that are specific for some or a few breeds is very common in the dog.

Therefore, the dog is an excellent model species for identifying genes underlying genetic disease (see below).

In an effort to breed healthier dogs and to use the dog as a model for genetic disease in human, the structure and organization of the dog genome has been of interest since the 1990ies resulting in an international collaboration denoted the DogMap (Binns *et al*., 1998; Breen *et al*., 1999; Breen *et al*., 2001). The DogMap research teams established the platform for future canine research. The National Human Genome Research Institute decided in 2002 to sequence the complete dog genome. The main reason for the choice of sequencing the dog, prior to commercially important domestic species such as cattle (*Bos taurus*), was the realisation of the dog as an excellent model for genetic diseases, which also affect humans.

In 2003, Kirkness *et al*. presented 1.5-fold coverage of the poodle genome sequence. Parker *et al*. (2004) identified sequence variation in 120 dogs (60 breeds) via a ~2 kb non-contiguous genome sequence analysis; they found 14 breed-specific Single Nucleotide Polymorphisms (SNPs). To further investigate genetic variation within and between breeds, a microsatellite approach was conducted utilizing 414 dogs representing 85 breeds. Each breed was represented by four to five individuals that were unrelated (3^{rd} generation). The study presented two distinctive clusters of dog groups consisting of *i*) ancient breeds (including the grey wolf, *Canis lupus*) and *ii*) more recently founded breeds (such as herding and hunting breeds). Two months after the Parker *et. al*., publication (July 2004), a ~7.5-fold coverage of the dog (CanFam1.0) genome became public to the scientific community. The choice of breed was Boxer and the draft was assembled by a group, headed by Kerstin Lindblad-Toh, at the Broad Institute of MIT and Harvard (Cambridge, MA, USA) and Agencourt Bioscience Corp (USA), enabling a variety of comparative studies between dog/human and thus initiating the major transformation regarding dog research project planning, technology development and methodological choices currently available and applied. By 2005, an improved assembly (CanFam2.0) was launched covering 99% of the dog genome (Lindblad-Toh *et al*., 2005). The International HapMap (human) Consortium (2005) provided ample evidence of efficacy regarding a recently developed high-throughput genomic method; a SNP-based genome-wide association array. During this time-frame, Lindblad-Toh *et al*. (2005) presented a SNP map (http://www.broad.mit.edu/mammals/dog/snp/), which contained more than 2.5 million unique SNPs, representing coverage of ~1 SNP/kb. With such a dense SNP coverage available, in combination with extensive linkage disequilibrium found within dog breeds, the development of a dog-specific SNP array became possible.

Many dog breeds are predisposed for specific diseases, *i.e.* one breed may be severely affected by a specific disease whilst another breed may not. Not only DS but also several other diseases seen in dogs resemble those in man *i.e.* Atopic dermatitis, heart diseases such as dilated cardiomyopathies, autoimmune diseases such as lymphocytic thyroiditis and several forms of cancers. Purebred dogs have been subjected to several bottlenecks such as small founder populations, high inbreeding for specific phenotypic traits and also the first and second world wars.

Diminishing access to breeding-stocks and/or the quest of attaining representative breeding animals would most likely have influenced distribution of disease-causing mutations due to indirect selection, *i.e.* breeding animals displaying desired phenotypes may have carried disease-causing mutations. Purebred dogs have for many generations also been subjected to different levels of inbreeding, resulting in relatively low genetic variation within breed (SNP rate ~1/1600 base pairs (bp)) and higher genetic variation (SNP rate ~1/900 bp) between breeds (Lindblad-Toh *et al*., 2005). Another explanation of breed-specific disease segregation is that only 10-20% of a dog population is used for breeding purposes of which the majority of the breeding animals are selected due to successful show-ring results. Historic breeding strategies have resulted in long haplotypes (0.5-1Mb) as well as high degree of linkage disequilibrium within dog breeds, far more extensive than reported in humans (Sutter *et al*., 2004; Lindblad-Toh *et al*., 2005). Note that LD-pattern between breeds resembles that of human populations (Lindblad-Toh *et al*., 2005). Mutations being identical by descent (disease genes originates from a common ancestor) therefore enable scientists to trace mutation-segregation throughout several generations. Relatively short generation intervals, fairly large litters (breed-specific), shared environments with humans, access to extended pedigrees and phenotypic data are aspects of consideration regarding genetic research. Following, a study in which the Rhodesian Ridgeback was utilized, is presented. The study provides ample evidence of the grandness of using the dog regarding disease-genetic studies.

Aims of the thesis

- to indicate the mode of inheritance for DS (paper I)

- to establish the inheritance for the Ridge and indicate its relation to DS (paper II)

- to improve the diagnostic classification of Dermoid sinus (paper III)

- to reveal mutation(s) underlying the Ridge and DS phenotypes (paper IV & V)

Summary of investigations

The summary of investigations provides a broad overview, discussion and comments of the work included in this thesis. The coat-colour study presented in paper VI will not be discussed herein. Detailed information may be found in each of the separate papers included in this thesis.

Materials & Main methods

Dogs and collection of material

The collection procedures of Rhodesian Ridgeback material were:

- collection of whole blood and tissue from DS-affected (identified by breeders) puppies aged 1-56 days as identified by breeders.
- whole-blood from their parental animals and littermates.
- collection of DS-tissue and whole-blood from individuals where the DSs were surgically removed.
- collection of material from ridgeless dogs, their parental animals and littermates.
- collection of whole-blood or cheek-swabs from Norwegian-, Danish-, German-, English-, South African-, North American- and Brazilian Rhodesian Ridgeback families in which DS-affected puppies were identified as well as random collection of healthy individuals.
- collection of whole-blood or cheek-swabs of DS-affected or healthy Thai Ridgeback individuals from Europe and Thailand.
- collection of a family of cervical ridged Bavarian Mountain scent hound
- collection of ~20 Boxers with crowns located at the scapula.

All collections of dog material were conducted in accordance with the animal ethics regulations of the Swedish National Board for Laboratory Animals and the Swedish Board of Agriculture. The DS-affected puppies were brought to the Swedish University of Agricultural Sciences (SLU), where they were subjected to euthanazation followed by diagnostic classifications, whole-blood extraction and blunt autopsy. For the majority of the Ridgeback samples, pedigrees are available as well as information regarding the ridge phenotypes.

Statistical analysis of inheritance

To identify if DS and the ridge followed Mendelian inheritance patterns, a Chi-Square test was employed. DS was tested to evaluate an autosomal recessive mode of inheritance where the expected ratios would be 1:2:1 (paper I). The dorsal ridge was tested for an autosomal dominant inheritance pattern where the ratios 3:1 would be expected (paper II). For paper I, data from a total of 372 litters was selected from the Swedish Rhodesian Ridgeback Club register, of which 243 litters were excluded to avoid bias (such as breeders not palpating all stillborn/destroyed puppies for DS, lack of information). The degree of freedom was one, as two classes were of interest; DS-affected *vs.* healthy individuals. In paper II, data from a total of 508 litters was selected for the segregation analyses. Lack of information from 106 of the selected litters, resulted in exclusion from the statistical test. Two classes were to be evaluated; ridged *vs*. ridgeless individuals. To avoid bias in segregation ratios, correction of estimated data was employed. Bias may occur as observed ratios not always correlate with expected ratios. Further, in paper II a possible association between DS and the ridge was evaluated.

Histopathology

Extracted tissues (blunt dissection) were fixated in 4% buffered formalin and paraffin-embedded according to standard procedures. Cut sections were stained with hematoxylin and eosin. An E1000 microscope (Nikon Eclipse) was used for digital light micrograph images.

Genome-wide association analysis

A SNP array is a chip with a large number of minute wells (features) attached to a coated quartz surface and is used to simultaneously analyze different genotypes in a single individual. Each feature contains covalently immobilized SNP-specific oligonucleotides (probes). PCR amplifications of genomic DNA with fluorescent marked primers are performed. The PCR amplicons are digested and denatured (fragmentation step to allow increased hybridization efficacy), followed by hybridization of the fluorescent-marked strands to the probes located in features on the array. The hybridization signal intensities are detected and recorded, resulting in a vast number of base calls for further computational analysis. Approximately 26.500 informative SNPs distributed across the chromosomes were selected for the genome-wide association study with the aim of proving the

principle that only relatively small amounts of genetic markers as well as cases and controls are necessary to obtain significant results.

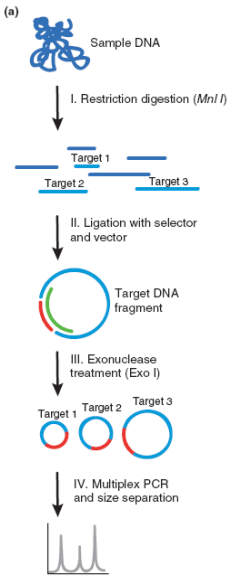
Pyrosequencing

Pyrosequencing is a cost-efficient sequencing method based on synthesis of a complementary strand to a single stranded DNA template by the addition of nucleotides (real-time pyrophosphate detection). As the complementary synthesis elongates, chemical enzymatic reactions occur resulting in light signals which are detected and analyzed. Signal intensity reveals the number of added nucleotides (Nordfors *et al*., 2002). The high-throughput method is often used for SNP genotyping. The SNP of interest was SNP_51,399,353 utilizing primer-pairs (of which one primer was biotinylated) in the near vicinity of the SNP.

Multiple ligation-dependent genome amplification

The recently developed multiple ligation-dependent genome amplification (MLGA) technique uses the spelling of the base pairs to determine if portions of chromosomes or genes are changed or mutated, by amplifying gDNA instead of probe molecules (Isaksson *et al*., 2007). A set of restriction fragments are generated by using a restriction enzyme. Single fragments for each target sequence are then chosen in such a way that fragments in each pool are between 100 & 400 nucleotides in length (each fragment with different lengths) with a minimum size difference of six nucleotides (Fig 8, Step 1). The selector technique is employed regarding probe and vector designs (Fig 8, Step 2). Each selector consists of two synthetic oligonucleotides (selector constructs); a target-specific selector probe and a vector oligonucleotide. The vector is designed with target-specific ends flanking the motif of interest. The central part of each selector probe is complementary to the vector oligonucleotide so that hybridization between the two generates the recognition sequence for the *Hind*III restriction enzyme and a universal primer pair site for parallel PCR amplification. The ends of the selector probes each have sequences complementary to the ends of the restriction fragments targeted for selection.

Step 1.



Step 2.

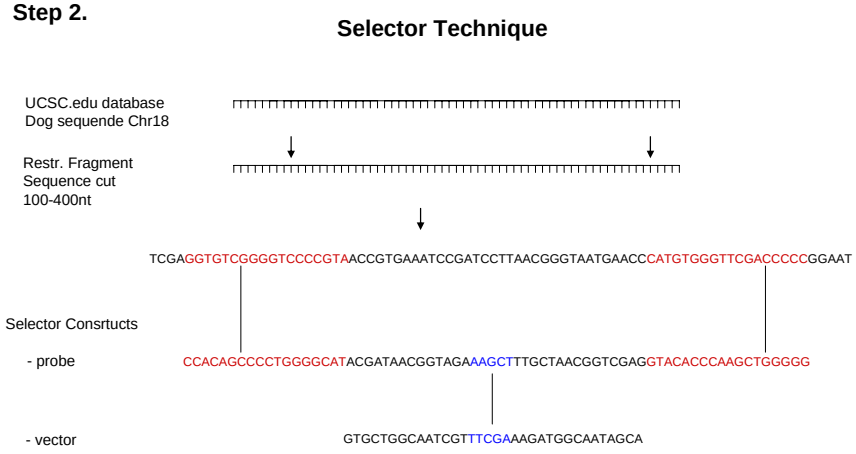


Figure 8. Step 1; Principle of MLGA methodology (image by Isaksson *et al*., 2007, with permission from the authors). Step 2; The selector technique is applied regarding the selector constructs (image by Salmon Hillbertz).

Real-Time Quantitative PCR

To evaluate gene expression, the real-time quantitative PCR (RTQ-PCR) technique is applied. RTQ-PCR is a sensitive technique used for identification of gene expression and copy number variation for which a reporter (attached the 5'-end) and quencher (attached to the 3'-end) dyed probe is used. The probe is designed to be complementary to the target sequence and is flanked by forward and reverse primers. Upon hybridization, the probe and complementary DNA template bind. When polymerization reaches the site of probe, the polymerase degrades the probe resulting in the release of the reporter dye, followed by single nucleotide releases and the release of the quencher dye. Due to the release of the reporter- and quencher dye detectable light signals are generated. If no signals are detected, the gene is not expressed in the tissue type from which mRNA was extracted and cDNA synthesized. Detected light signals generated during the exponential phase of the run are plotted against cycle numbers on a logarithmic scale and a detection threshold is determined. During a cycle when the light signal from an experimental sample crosses the threshold (cycle threshold, C_t) the gene of interest is expressed. Each experimental sample (specific genotype or tissue type) per gene is run in duplicate or triplicate to obtain a mean C_t value per experimental sample. For quantification purposes, the mean C_t value of each experimental sample/gene is divided by the mean C_t value of a control gene (often a house-keeping gene).

Results and discussion

Mode of inheritance

Segregation analysis did not support a simple autosomal recessive inheritance pattern regarding Dermoid sinus (DS), thus suggesting a more complex mode of inheritance (paper I). However, statistical support was provided regarding an autosomal dominant inheritance of the dorsal ridge as well as a significant association between ridge and DS (paper II). Frequency estimations regarding DS- and ridgeless prevalence in produced litters (Swedish Rhodesian Ridgeback population) were 8-10% and 5.6%, respectively.

Segregation analyses regarding DS, the ridge and ridge/DS association were only possible due to the impressive register kept by Swedish Rhodesian Ridgeback club register. Thus, even though the register contains information regarding produced litters from 1964 to the current date, full breeder participation did not occur until the beginning of 1981. However, a drawback regarding information from the 1980ies was the lack of reports of DS-affected puppies. Non-reporting of DS-affected individuals occasionally still takes place. Further, anatomical locations of DS (cervical *vs*. caudal locations) and phenotypic recordings (skin-opening/skin-dimple) were most often not available. Information regarding caudally located DSs was available from merely fourteen litters (between 1964 and 2006 a total of seven hundred sixty-seven litters were reported).

The majority of DS-affected offspring are euthanized, according to breed-club recommendations and no autopsies are performed. DS examinations of stillborn and ridgeless Rhodesian Ridgebacks which are euthanized are normally not conducted. The frequency of DS type V within the Swedish Rhodesian Ridgeback population is currently not identified and only two scientific case-reports describing DS type V are available (Booth, 1998; Tshamala & Moens, 2000). Even though no reports are available regarding ridgeless Rhodesian Ridgebacks affected by DS, it may theoretically occur as a stochastic event. The Thai Ridgebacks share not only the ridge phenotype but also an increased frequency of DS occurrence with the Rhodesian Ridgeback. Strangely, DS occurrence in the Thai Ridgeback has previously not been scientifically reported. DS frequency in the Thai Ridgeback is yet unknown. The evidence of clear association between the ridge and DS proved to be a novel finding.

Histopathological evaluation

During autopsies morphological differences were observed between DS type I-IV and DS type VI, which were histopathologically confirmed. Thus, similarities between partially pigmented DS with skin-openings and DS type VI were also observed. Based on histopathology, a new definition of DS type VI was suggested; Lipoma of the terminal filum with skin-dimple and extra-spinal connection (LTF). In figure 9, a DS-type II is shown and in Figure 10, a LTF. Criteria of LTFs were the presence of skin-dimple and mesoderm tissue (adipose-, connective and muscle tissue).

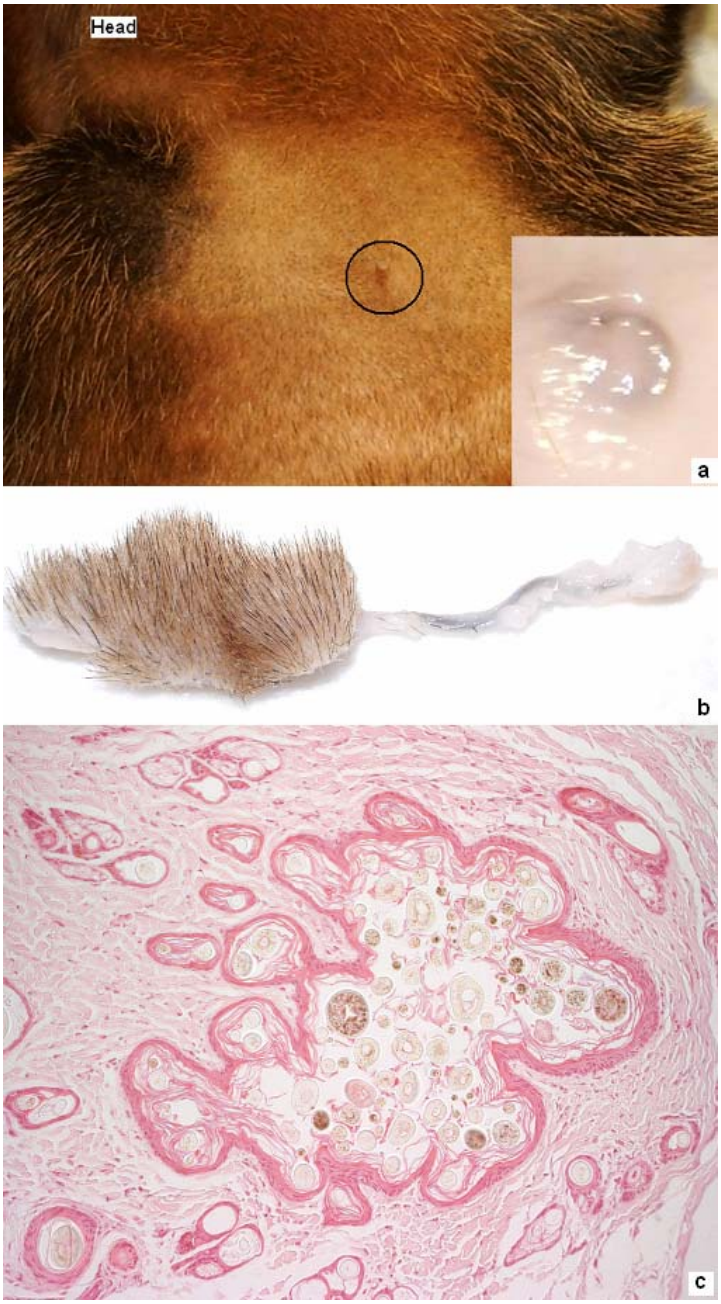


Figure 9. a) Dermoid sinus (DS) in a three weeks old Rhodesian Ridgeback male, where a skin-opening is marked (circle). Inserted is the DS morphology in subcutis. b) The extracted DS in full length. The DS was classified as a type II. c) A cross-section of the DS. The lumen is lined by stratified squamous keratinized epithelium and contains keratin and hair debris. Hematoxylin and eosin stain, objective lens x 20. Image 10a-b by Salmon Hillbertz. 10c by Hellmén E.

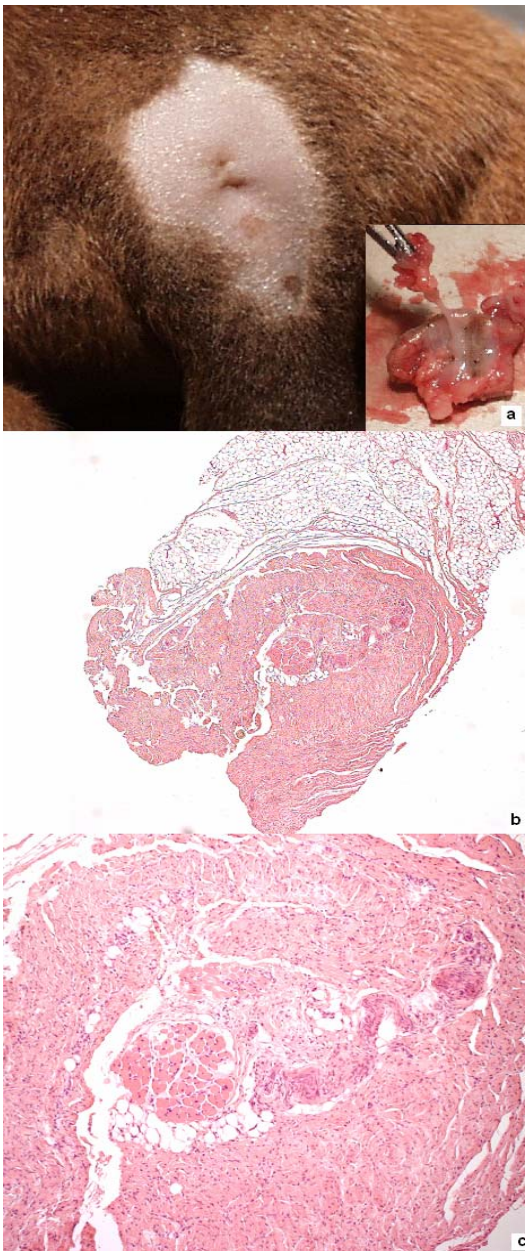


Figure 10. a) Skin-dimples at the root of the tail in a two weeks old Rhodesian Ridgeback male. Upon *In toto* removal morphological appearance displayed a non-pigmented funnel-like structure. b & c) Histopathological results revealed presence of muscle-, connective- and adipose tissues. Histopathology and the anatomical location of the condition in Rhodesian Ridgebacks resulted in a new definition; Lipoma of the terminal filum with skin-dimple and extraspinal connection (LTF). In figure b, objective lens x 4 was used and in c) objective lens x 10 was used. Image a by Salmon Hillbertz and images b and c by Hellmén E, objective lens x 4.

Extraction of DS/LTF tissue was not successful in four cases thus anatomical locations, phenotypic criteria and autopsy observations supported DS/LTF appearance. Major emphasis was made on quick and efficient extraction followed by instant tissue freezing in liquid nitrogen in an attempt to limit tissue degradation, as the samples were prepared for gene expression studies. Unfortunately, Computer tomography and Magnetic resonance imaging technologies were not available. Therefore, identification of possible Spina Bifida Occulta in the euthanized puppies was not achievable. According to the dog owners, abnormal movement patterns were not observed. An interesting observation during autopsy was that none of the observed DSs were identical in regards of growth direction, DS extension *vs.* age of the puppy and morphology. In contrast, the majority of the observed LTFs showed very low divergence in morphology and length. Autopsy observations in combination with morphological differences and histopathological analysis of extracted tissues, led to the conclusion that DSs and LTFs ought to be distinguished, hence the suggested descriptive denotation "Lipoma of Termile Filum with skin-dimple and extra-spinal connection". Anatomical locations of DS/LTF in Rhodesian Ridgebacks seem to correlate with primary neurulation respectively secondary neurulation development described in human studies. Further, classification, anatomical locations, skin markings and histopathological results regarding DS and LTF identified in dogs seem to be in concordance with findings presented in human literature. Finn & Walker (2007) mentioned a possible genetic factor regarding LTFs. Even though the Swedish Rhodesian Ridgeback club-register cannot provide the quantity of data required for statistical analysis, a heritable nature of LTF is generally accepted (hence the lay definition "tail-DS") by breeders. A three generation pedigree including six of fourteen litters, produced over a twelve year period, highlights litters in which both ridgeless and DSs with cervical/caudal locations were produced (Fig. 11). The high resemblance of skin lesion presentations between two species (dog/human) presents additional motivation regarding selection of utilizing the dog as model organism in human disease research.

The findings presented in paper III, led to the conclusions that histopathology must be employed to confirm *bona fide* DS prior incorporation of samples to molecular genetic approaches. Further, the combination of DS anatomical locations, extensions, morphology and histopathological information will most likely play an important role regarding the enablement of genetically distinguishing between different DS types. Association between DS and LTF has previously been discussed in human literature as both lesions originate during neurulation. Thus, the suggestion provided herein, a shared genetic origin between DS and LTF is most likely original and is supported by the three-generation pedigree provided herein.

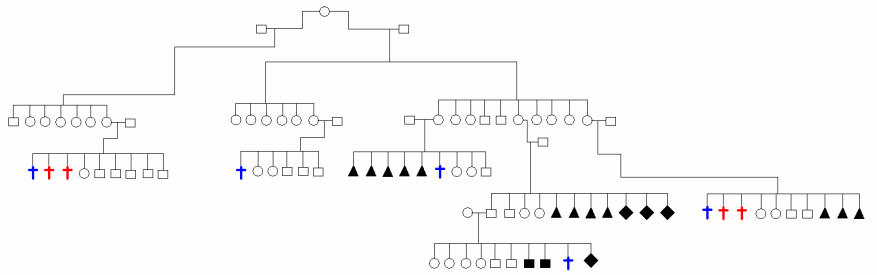


Figure 11. Pedigree of three generations of Rhodesian Ridgebacks displaying litters in which ridgeless and Dermoid sinus affected puppies were produced. Red crosses (✝) are cervical DS, blue crosses (✝) are caudal DS, triangles (▲) are ridgeless puppies (gender unknown) and squares (◆) indicate euthanized puppies (reason and gender unknown). All information regarding litter outcome were reported by breeders to the Swedish Rhodesian Ridgeback Club. Image by Salmon Hillbertz.

Genome-wide association analysis

The ridgeless-locus was mapped to a 750-kb region followed by identification of a haplotype defined by three SNPs in which ten (n=11) DS-affected Rhodesian Ridgebacks were homozygous. The haploblock was not present in ridgeless Rhodesian Ridgebacks. Within the identified haploblock, five genes were located; *FGF3* (fibroblast growth factor 3), *FGF4* (fibroblast growth factor 4), *FGF19* (fibroblast growth factor 19), *ORAOV1* (oral cancer over-expressed gene1) and *CCND1* (cyclin D1).

The GWAA approach proved to extremely successful, utilizing only 11 DS-affected and nine ridgeless Rhodesian Ridgebacks. The mapped locus on chromosome 18 left no doubt regarding the presence of a region associated with the ridge phenotype. The region contained five genes *FGF3*, *FGF4*, *FGF19*, *ORAOV1* and *CCND1* of which all are involved during early development (Table 1).

The power of the GWAA approach will from now on have a large impact regarding future molecular genetic studies. The time consuming and costly days of sample collections of entire families will most likely be replaced by the GWAA approach as only cases and controls are necessary for association analysis. The low number of animals necessary to obtain reliable results is remarkable; monogenic traits, ten cases and ten controls and polygenic traits, predicted 100 cases and 100 controls. This, in combination with the genetic makeup of purebred dogs (low genetic diversity within breed, high genetic diversity between breeds) will lead to identification of loci, associated with phenotypes of interest, in a more rapid mode than anyone could have expected just a few years ago.

Table 1. Description of the role of *FGF3*, *FGF4*, *FGF19*, *ORAOV1* and *CCND1*. The genes were included in the loci which was associated with the dorsal hair ridge in Ridgeback dogs by Genome wide association analysis.

Gene	Role	Reference
FGF3	Cerebellum, hindbrain and forebrain, tail/retina/tooth and inner ear development, Extra-embryonic endoderm,	Wilkinson *et al*. (1988)/(1989); Murakami *et al*., (2004); Galdemard *et al*., (2000); Pasqualetti *et al*., (2001); Tekin *et al*. (2007)
FGF4	Limb development, inner cell mass proliferation, promote neural stem cell proliferation and neuronal differentiation in the postnatal brain	Zúñiga *et al*. (1999); Feldman *et al*., (1995); Kosaka *et al*., (2006); Zhong *et al*., (2006)
FGF19 (Mouse *FGF15*)	Fetal brain development, fetal cartilage, skin, and retina bile acid synthesis and gallbladder filling, mesoderm expression during neural groove closure, involvement in steroid hormone activity	Nishimura *et al*., (1999); Xie *et al*., (1999); Inagaki *et al*., (2005); Choi *et al*. (2006); Ladher *et al*. (2000); Fu *et al*. (2004).
ORAOV1	Unknown	Huang *et al*., (2002)
CCND1	Regulates G1 (gap) phase of cell division cycle, developing brain, involved in mitochodirial function and nuclear DNA synthesis	Sherr & Roberts, (1999); Ishibashi & McMahon, (2002); Wang *et al*., (2006)

Fine-mapping

One SNP (SNP_51,399,353) located within the identified haploblock, revealed significant deviations ($p<0.001$) from Hardy-Weinberg expectations as additional ridged individuals were genotyped. Via MLGA, ~87% of the DS-affected ridged Ridgebacks (n=15) were scored as homozygote, ~66% of the healthy ridged individuals (n=29) were scored as heterozygote and all ridgeless individuals homozygote wild-type.

Genotyping, utilizing Pyrosequencing (SNP_51,399,353), results confirmed the presence of a duplication (Table 2).Via MLGA, a ~133 kb region was identified as the duplication breakpoints (Fig. 11). *CCND1* was initially (via GWAA) a candidate gene regarding the ridge phenotype. Further fine-mapping analysis (MLGA) revealed the only the 3'-end of *CCND1* was included in the duplication, suggesting that *CCND1* most likely does not contribute to the ridge phenotype. Utilization of additional probes located in the near vicinity of the internal breakpoint revealed the internal breakpoint. Sequencing results of 700 bp up- respectively downstream of the internal breakpoint showed complete identity between the two Ridgeback breeds compared to the available Boxer sequence. Several purebred dogs were sequenced, from which results confirmed the duplication as Ridgeback-specific. Gene expression (RTQ-PCR) was not detectable in post-natal tissue regarding the three *FGF*-genes of interest. However,

two-fold and one-and-a-half fold *ORAOV1* mRNA expression was identified in homozygote and heterozygote ridged individuals compared to ridgeless dogs. The control gene (*β-actin*) was expressed in all analysed tissue-types. Histopathological analysis of dorsal skin derived from ridged and ridgeless Rhodesian Ridgebacks, confirmed phenotypic differences regarding hair bulb orientation. Further, it was identified that the hair bulbs in ridged Rhodesian Ridgebacks were located in a more distal mode (deeper into dermis) than those of a ridgeless Rhodesian Ridgeback. mtDNA analysis did not reveal a close association between Rhodesian and Thai Ridgebacks, *i.e.* the divergence between the breeds indicated an ancient history of the ridge mutation.

Table 2. SNP genotyping by pyrosequencing. The SNP of interest (SNP_51,399,353) was found to be heterozygote in DS-affected Rhodesian Ridgeback and homozygote in Thai Ridgebacks.

	No. Individuals	**Genotype**	**Ratio**
DS+/*RR* Rhodesian Ridgeback	22	A C	1 1
DS+/*RR* Rhodesian Ridgeback	5	A C	1 2
DS+/*RR* Rhodesian Ridgeback	1	A C	1 3
DS+/*RR* Thai Ridgeback	3	C	0 1
DS+/*rr* Bloodhound	1	C	0 1
DS+/*RR* Cambodian Local	1	C	0 1
Ridgeless (*rr*) Rhodesian Ridgeback	19	C	0 1
Ridgeless (*rr*) Rhodesian Ridgeback	4	A	1 0
Ridgeless (*rr*) Rhodesian Ridgeback*	1	A C	1 1
Ridgeless (*rr*) Rhodesian Ridgeback*	1	A C	1 2
Ridged Rhodesian Ridgeback	6	A C	1 1
Ridged Rhodesian Ridgeback	9	A C	1 2
Ridged Rhodesian Ridgeback	1	A C	1 3
Ridged Thai Ridgeback	11	C	0 1

* Phenotyped by breeder

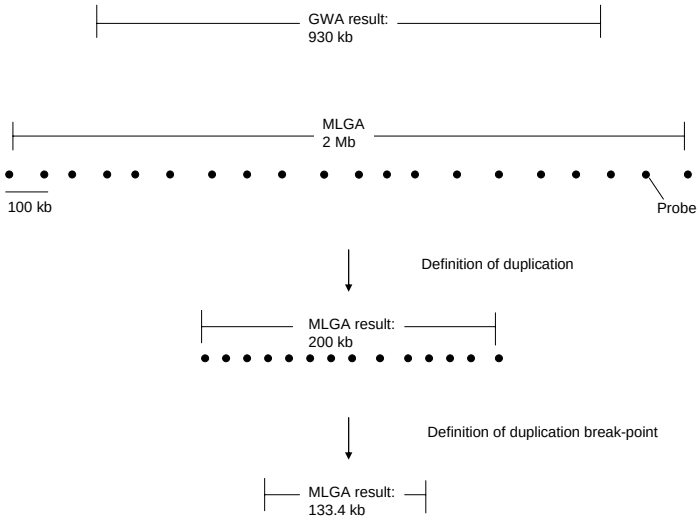


Figure 11. Results from MLGA technique.

The molecular mechanism of *FGF3* is yet to be further investigated, thus Miyoshi *et al*. (2002) implicated involvement with several signalling pathways, including the WNT signalling pathway. Transcription of the *FGF4* gene is activated by a β-catenin/TCF/LEF complex, which forms in the presence of a WNT signal (Ishibashi & McMahon, 2002; Kratochwil *et al*., 2002; Miyoshi *et al*., 2002). Four FGF receptor (*FGFR1-4*) genes have been identified, thus alternative splicing of *FGFR1-3* mRNAs generate additional receptor isoforms (Murakami *et al*., 1999; Zhang *et al*., 2006). The *FGF3* ligand binds to *FGFR1b* and *FGFR2b* (Ornitz *et al*., 1996). Both "b" splice forms are involved in epithelial lineages (Zhang *et al*., 2006). Kawano *et al*. (2005) mention *FGFR2b* as keratinocyte-specific. The FGF4 ligand binds to *FGFR1c*, *FGFR2c* and *FGFR3c* (Ornitz *et al*., 1996). The "c" receptor splice forms are involved in mesenchymal lineages (Zhang *et al*., 2006). *FGF19* has been suggested to be a component in the WNT pathway (Ohyama *et al*., 2006) and binds to the *FGFR4* receptor (Harmer *et al*., 2004; Tamimi *et al*., 2006; Abraira *et al*., 2007), which has been associated with endoderm tissue development (Jung *et al*., 1999).

The WNT- and FGF-signalling pathways have been suggested to play crucial roles during different stages of development (Böttcher & Niehrs, 2005; Katoh & Katoh, 2006). Merrill *et al*. (2001) discussed *TCF* (T-cell factor), *LEF* (lymphoid enhancer factor) and *β-catenin* involvement regarding epidermis, sebaceous gland and hair follicle development. *LEF*/*TCF*s are transcription factors which form complexes to activate target genes (Jamora *et al*., 2003; de Lau & Clevers, 2001). *β-catenin* is involved in intercellular junction formation and regulates transcription (such as by binding to *LEF*/*TCF*s) in the WNT-signalling pathway (Merrill *et al*.,

2001). *ORAOV1* has been associated with oral cancer and Huang *et al*. (2002) reported *ORAOV1* expression in several types of tissue. The expression levels correlated to CNV. Herein, *OROAV1* mRNA expression was identified in adult skin derived from Rhodesian Ridgeback dogs, which has previously not been reported.

Over-expression of many FGF genes has been associated with different cancer forms (Tai *et al*., 2005; Xie *et al*., 1999; Nishimura *et al*. 1999; Sherr & Roberts 1999). Tekin *et al*. (2007) reported hereditary deafness in humans associated with mutations in *FGF3*. The *FGF3-FGF4-FGF19-ORAOV1-CCND1* locus identified in dogs corroborate with findings reported across species. Katoh & Katoh (2003) reported that mouse *FGF15* was the ortholog of human *FGF19* and that the *FGF4-FGF19-OROAV1-CCND1* locus is evolutionary conserved between human, zebra fish and rodents. Additional comparative studies showed that *FGF3* was positioned upstream of *FGF4* (Katoh & Katoh, 2005). The *FGF3* enhancer (located upstream of *FGF3*) was not included in the duplicated region reported herein.

The proteins encoded by the *Fz* (Frizzled) genes function as WNT receptors. Guo *et al*. (2004) presented results in which *Fz6*$^{-/-}$ mice displayed alterations in hair patterning, such as additional hair whorls. The patterns identified in the Fz6 knockout mice, highly resemble the ridge phenotype identified in dogs. Knockout of *Fz3*, a gene involved in the developing CNS and neural tube closure, and *Fz6* displayed failure in neural tube closure and abnormal tail development (Wang *et al*., 2006).

Twenty two FGF genes have previously been described in humans. *FGF3*, *FGF4* and *FGF19* are only expressed during embryological development (Ornitz & Itoh, 2001), which confirms the absence of identifiable gene expression in postnatal tissue described herein. Hypothetically, there might be a common factor between the FGF- and WNT-signalling cascades downstream of *β-catenin* which very well may explain the phenotypic deviations in hair patterning and deficiency in neural tube closure reported by Guo *et al*. (2004) and Wang *et al*. (2006) as well as the hair ridge and DS in Ridgeback dogs. To investigate the hypothesis, knock-out mice models could be utilized in an effort to identify the common gene/genes involved in the development of NTDs. Furthermore, gene-environment interactions may also play a role in NTD development, as discussed in human literature (Johnston, 2008) as well as interactions between several loci.

Based on the findings herein, there is little doubt that homozygote ridged Ridgebacks are subjected to a highly increased risk of developing DS/LTF. The situation becomes complicated for breeders, as the ridge is the trademark of the breed. When the genetic complexity of DS/LTF has been identified, a DNA-test could be developed. Such a test would aid breeders as parental animals that carry the DS/LTF-causing mutation could be identified. To avoid decrease in genetic variation (*i.e.* decreasing the number of breeding animals), a long-term breeding program could be established with the aim of diluting the DS/LTF-causing mutation over several generations. This approach is only applicable if there is an open communication between breeders on a global aspect. Another approach would be the introduction of ridgeless individuals as breeding animals during

some generations (with careful monitoring of the produced offspring). Thus, if breeders wish to maintain the ridge in the Ridgebacks, this approach also requires open communication between breeders. In both scenarios, all results from DNA-testing (future DS/LTF specific DNA-test or ridge-specific DNA-test) of individuals must be available to the public.
Certain important aspects ought to be considered such as selection of cases/controls within breed and selection within geographically distinct populations. For fine-mapping approaches, the availability of other breeds, which do and do not display similar phenotypes, is necessary upon identifying ancestral haplotype blocks associated with phenotypes of interest. Therefore, an important issue to address is the awareness of the origin of breeds. Many breeds are not more than 200-150 years old and during breed creations, multiple breeds (whereas some are now extinct) were commonly used. This may most likely have an important impact for dog research from two aspects; *i*) selection of control breeds, ***i.e.*** breeds that do not display phenotypes of interest and are genotypically as diverse as possible and *ii*) selection of control breeds in which mutations of interest would be expected to be present, ***i.e.*** lower genotypic diversity between breeds.

Herein, only Rhodesian Ridgebacks derived from the Swedish population were utilized for the GWAA approach to avoid population stratification. All cases (homozygote ridged and DS-affected) were histopathologically confirmed to avoid bias and all controls were defined as ridgeless. Initially, exclusively Swedish Rhodesian Ridgebacks were used for identification of the 133 kb duplicated region (MLGA). Subsequently, additional Rhodesian Ridgeback cases/controls and their parental animals were used as well as ridged Thai Ridgebacks. Screening of homozygote ridged (DS-affected) Ridgebacks was performed regarding internal breakpoint identification, which enabled detection of the ancient ridge-causing mutation present in both Rhodesian- and Thai Ridgebacks, as both breeds showed identical nucleotide sequences in the near vicinity of the internal duplication breakpoint and nucleotide sequence diversity 5' and 3' of the 133 kb duplicated region. Multiple non-ridged breeds were utilized to precisely define/confirm the ancient haplotype block associated with the ridge phenotype. Rhodesian Ridgeback family material (parental animals and littermates) was used for segregation analyses to confirm obtained results regarding the ridge-causing mutation.

The geographical origin of the ridge present in Ridgeback dogs is still a mystery, thus solid proof has now been provided that the ridge mutation in Ridgeback dogs is identical by descent and the likelihood of parallel mutations occurring in Asia and Africa (Epstein, 1937) can therefore be rejected. Unravelling the mystery of the ridge's geographic origin, may shed new light on the pathway of dog domestication and distribution. Further, identification of the DS and LTF complexity may also reveal a previously unknown ancient background.

As both DS and LTF-affected Ridgeback puppies may be present within a produced litter, several possible explanations are plausible regarding the genetic complexity of DS/LTF origin. One explanation could be CNV where the number of duplicated copies could correlate to different types of DS or LTF. Another explanation could be allelic variants within the duplication (genetic heterogeneity)

which was elegantly shown by Pielberg *et al*. (2002). Within the near future, the enigma of the occurrence of DS versus LTF will be unravelled and a new era of knowledge regarding genes involved during development will be initiated.

Conclusions

- The mode of inheritance for DS was evaluated (paper I).

- Inheritance of the Ridge was defined. Further, it was shown that the Ridge predisposes to DS (Paper II).

- The diagnostic classification was shown to be improved by histopathology and a new terminology "Lipoma of the terminal filum with skin-dimple and extra-spinal connection was suggested (paper III).

- The mutation underlying the Ridge phenotype was identified (paper IV & V).

The knowledge gained from histopathological analysis of extracted tissues via blunt dissection revealed the necessity of correct disease diagnosis which became evident as results from the GWAA were obtained and analyzed.

The proof-of-principle study was proven to be extremely cost- and time-efficient.
The combinations of *i*) high linkage disequilibrium and long haplotypes, *i.e.* high degree of inbreeding within breeds (low genetic diversity), *ii*) high genetic diversity between breeds (up to three-fold higher than described in human populations according to main reference IV), *iii*) access to genealogical material, *iv*) shared environmental factors and *v*) similar disease morphology and diagnosis clearly provide support for the notion of the supremacy of dogs as model organism for human oriented research fields.
The overall impact of the proof-of-principle approach described herein, will most likely have a major influence regarding future project designs for genome-wide studies, *i.e.* previously family-based approaches will most likely be substituted by case/control approaches.

Future prospects

- Further elaboration on the genetic complexity of DS and LTF origin in Ridgeback dogs.

- Screen other breeds which display cervical ridges, such as Boxers and Bavarian Mountain scent hounds, to identify if the ridge mutation is identical or variable to that found in the Ridgebacks.

- Studies on transcription regulatory pathways associated with the phenotypes identified in *Fz6*$^{-/-}$/*Fz3*$^{-/-}$ mice (Guo *et al*., 2004; Wang *et al*., 2006) and Ridgeback dogs reported herein, utilizing knock-out mice.

Populärvetenskaplig sammanfattning

Denna avhandling presenterar studier avseende nedärvningsmodellen av den hårkam (ridge) vilken löper på ryggen hos Rhodesian Ridgeback hundar samt sjukdomen Dermoid sinus (DS) vilken är associerad med ridgen. DS är klassificerat som en defekt som uppstår under utvecklingen av centrala nervsystemet hos människa. Därmed, har hunden påvisats vara en utmärkt modell för jämförande studier. Det visades att ridgen orsakas av en autosomal dominant mutation som också ökar risken för DS. Insamling av material från Rhodesian Ridgeback valpar drabbade av DS, deras föräldradjur och kullsyskon utfördes. Histopatologiska analyser utfördes för att bekräfta DS. Resultat visade att DS var lokaliserad i nackregionen och att en ny hud defekt (tidigare kallad DS), som hos människa benämns Lipom av terminal filum (LTF), fanns lokaliserad vid svansroten hos hund. Ett gemensamt genetiskt ursprung mellan DS och LTF föreslogs, samt att olika typer av DS och LTF skulle kunna orsakas av skillnader i uttryck av tillväxtfaktorer viktiga för fosterutvecklingen (s.k. fibroblast tillväxtfaktorer). Förhöjda FGF nivåer i kombination med olika genetiska bakgrunder och miljöfaktorer är sannolikt viktiga för utvecklandet av DS. Elva DS drabbade, samt nio ridgelösa, Rhodesian Ridgeback hundar analyserades via ~26.500 genetiska markörer distribuerade över hundens arvsmassa. Samband mellan en specifik region på kromosom 18 och förekomsten av ridgen identifierades. Regionen innehöll fem gener *FGF3*, *FGF4*, *FGF19*, *ORAOV1* och *CCND1* radade efter varandra. Vidare analyser av regionen tillät identifiering av mutationen vilken ger upphov till ridgen. Det visades att ridgen orsakades av en dubbel kopia av de tre *FGF*- generna *FGF3, FGF4* och *FGF19,* samt genen *ORAOV1*. Ordningen var följande *FGF3*, *FGF4*, *FGF19*, *ORAOV1* (kopia ett) direkt följt av *FGF3*, *FGF4*, *FGF19*, *ORAOV1* (kopia två). Hundar som får mutationen från båda föräldradjuren har en ökad risk att drabbas av DS och eller LTF. Ridgen och DS orsakas troligtvis av ökade nivåer av FGF under en kritisk period av hudens utveckling. Analys av den exakta gränsen (brytpunkten) mellan kopia ett och kopia två (vilka tillsammans utgör ridge mutationen), visade att mutationen var identisk hos Rhodesian- och Thai Ridgeback hundar. Detta visade att båda hundraserna troligen har en gemensam anfader. Ridgeback hundar som har fått mutationen från båda föräldradjuren får en ökad risk att drabbas av DS och/eller LTF. Dessa resultat kan leda till ökad förståelse av liknande sjukdomar hos människa och kan leda till avel av friskare Ridgebackhundar. Fortsatta studier gällande den genetiska komplexiteten av DS och LTF pågår för närvarande.

References

Abraira, V.E., Hyun, N., Tucker, A.F., Coling, D.E., Brown, M.C., Lu, C., Hoffman, G.R. & Goodrich, L.V. 2007. Changes in Sef levels influence auditory brainstem development and function. *Journal of Neuroscience. 27(16)*, 4273-82.

Angarano, D.W. & Swain, S.F. 1993. *Disease Mechanisms in Small Animal Surgery*. 2nd ed. Eds: Bojrab, M.J. Lea & Febiger, Philadelphia, USA. 178-183 pp.

Antin, I.P. 1970. Dermoid sinus in a Rhodesian Ridgeback Dog. *Journal of the American Veterinary Medical Association 157 (7)*, 961-962.

Binns, M., Holmes, N. & Breen, M. 1998. The Dog Gene Map. Institute for *Laboratory Animal Research Journal 39(2-3)*, 177-181.

Booth, M.J. 1998. Atypical dermoid sinus in a chow chow dog. *Journal of South African Veterinary Association. 69 (3)*, 102-104.

Böttcher, R.T. & Niehrs, C. 2005. Fibroblast growth factor signaling during early vertebrate development. *Endocrine Reviews 26(1)*, 63-77.

Breen, M., Langford, C.F., Carter, N.P., Holmes, N.G., Dickens, H.F., Thomas, R., Suter, N., Ryder, E.J., Pope, M. & Binns, M.M. 1999. FISH mapping and identification of canine chromosomes. *Journal of Heredity 90(1)*, 27-30.

Breen, M., Jouquand, S., Renier, C., Mellersh, C.S., Hitte, C., Holmes, N.G., Chéron, A., Suter, N., Vignaux, F., Bristow, A.E., Priat, C., McCann, E., André, C., Boundy, S., Gitsham, P., Thomas, R., Bridge, W.L., Spriggs, H.F., Ryder, E.J., Curson, A., Sampson, J., Ostrander, E.A., Binns, M.M. & Galibert, F. 2001. Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. *Genome Research 11(10)*, 1784-95.

Chesney, C.J. 1973. A case of spina bifida in a Chihuahua. *Veterinary Records 93 (5)*, 120-121.

Choi, M., Moschetta, A., Bookout, A.L., Peng, L., Umetani, M., Holmstrom, S.R., Suino-Powell, K., Xu, E.H., Richardsson, J.A., Gerard, R.D., Mangelsdorf, D.J. & Kliewer, S.A. 2006. Indentification of a hormonal basis for gallbladder filling. *Nature Medicine 12 (11)*: 1253-1255.

Clayton, H.M. 1983. Spina bifida in a German shepherd puppy. *Veterinary Records 112*, 13-15.

Concannon, P.W. 2000. *Recent advances in Small Animal Reproduction*. Canine Pregnancy: Predicting Parturition and Timing Events of Gestation. Eds: Verstegen J. International Veterinary Information Service (www.ivis.org).

Cornegliani, L., Jommi, E. & Vercelli, A. 2001. Dermoid sinus in a golden Retriever. *Journal of Small Animal Practice 42,* 514-516.

de Lau, W. & Clevers, H. 2001. LEF1 turns over a new leaf. *Nature Genetics 28,* 3-4.

Epstein, H. 1937. Animal Husbandry of the Hottentots. *Onderstepoort Journal of Veterinary Science and Animal Industry 9 (2)*, 645-647.

Evans, J.M. & White, K. 1997. *Book of the Bitch A Complete Guide to Understanding and Caring for Bitches*. Ringpress Books Ltd, Gloucestershire, England. 71-75 pp.

Fatone, G., Brunetti, A., Lamagna, F. & Potena, A. 1995. Dermoid sinus and spinal malformations in a Yorkshire Terrier: Diagnosis and follow-up. *Journal of Small Animal Practice 36*, 178-180.

Feldman, B., Poueymirou, W., Papaioannou, V.E., DeChiara, T.M. & Goldfarb, M. 1995. Requirement of FGF-4 for Postimplantation Mouse Development. *Science 267*: 246-249.

Finn, M.A. & Walker, M.L. 2007. Spinal lipomas: clinical spectrum, embryology, and treatment. *Neurosurgery Focus 23 (2)*, 1-12.

Fu, L., John, L.M., Adams, S.H., Yu, X.X., Tomlinson, E., Renz, M., Williams, P.M., Soriano, R., Corpuz, R., Moffat, B., Vandlen, R., Simmons, L., Foster, J., Stephan, J.P., Tsai, S.P. & Stewart, T.A. 2004. Fibroblast Growth Factor 19 Increases Metabolic Rate and Reverses Dietary and Leptin-Deficient Diabetes. *Endocrinology 145(6)*:2594-603.

Galdemard, C., Yamagata, H., Brison, O. & Laviall, C. 2000. Regulation of FGF-3 gene expression in tumorigenic and non-tumorigenic clones of a human colon carcinoma cell line. *Journal of Biological Chemistry 275(23)*:17364-73.
Gammie, J.S. 1986. Dermoid sinus removal in a Rhodesian Ridgeback dog. *Canadian Veterinary Journal 27*, 250-251.
Golden, J.A. & Chernoff, G.F. 1993. Intermittent pattern of neural tube closure in two strains of mice. *Teratology 47 (1)*, 73-80.
Goody, P.C. 2002. *Dog Anatomy – A Pictorial Approach to Canine Structures*. J. A. Allen; London, UK. 26-27 pp.
Guo, N., Hawkins, C. & Nathans, J. 2004. Frizzled6 controls hair patterning in mice. *Proceedings of the National Academy of Sciences of the United States of America 101(25)*, 9277-81.
Gwatkin, R.D.S. 1934. Dogs and Human Migrations. *Journal of the South African Veterinary Medical Association 5 (1)*, 37-40.
Hare, T. 1932. A congenital abnormality of hair follicles in dogs resembling trichostasis spinulosa. *The journal of pathology and bacteriology 35*, 569-571.
Harmer, N.J., Pellegrini, L., Chirgadze, D., Fernandez-Recio, J. & Blundell, T.L. 2004. The crystal structure of fibroblast growth factor (FGF) 19 reveals novel features of the FGF family and offers a structural basis for its unusual receptor affinity. *Biochemistry 43 (3)*, 629-40.
Hathcock, T.J., Clampett, G.E. & Broadstone, V.R. 1979. Dermoid sinus in a Rhodesian Ridgeback. *Veterinary medicine & small animal clinician 74 (1)*, 53-56.
Hawley, T.C. 1984. *The Rhodesian Ridgeback The Origin, History and Standard*. 4th ed. N. G. Sendingpers Bloemfontein, South Africa.
Helgesen, D.H. 1991. *The definitive Rhodesian Ridgeback*. 2nd ed (Revised). Anglo-American Communication Consultants, Pitt Meadows, Canada.
Hofmeyer, C.F.B. 1963. Dermoid sinus in the Ridgeback dog. *Journal of Small Animal Practice 4*, 5–8.
Huang, X., Gollin,S.M., Raja,S. & Godfrey, T.E. 2002. High-resolution mapping of the 11q13 amplicon and identification of a gene, TAOS1, that is amplified and overexpressed in oral cancer cells. *Proceedings of the National Academy of Sciences of the United States of America 99*, 11369-11374.
Hubbard, C.L.B. 1948. *Dogs in Britain A Description of All Native Breeds and Most Foreign Breeds in Britain*. Macmillan and Co. Ltd, London, England. 372-376 pp.
Inagaki, T., Choi, M., Moschetta, A., Peng, L., Cummins, C.L., McDonald, J.G., Luo, G., Jones, S.A., Goodwin, B., Richardson, J.A., Gerard, R.D., Repa, J.J., Mangelsdorf, D.J. & Kliewer, S.A. 2005. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metababolism 4*:217-25.
International HapMap Consortium. 2005. A haplotype map of the human genome. *Nature 437 (7063)*, 1299-320.
Isaksson, M., Stenberg, J., Dahl, .F, Thuresson, A-C., Bondesson, M-L. & Nilsson, M. 2007. MLGA- a rapid and cost-efficient assay for gene copy-number analysis. *Nucleic Acids Research* 35 (17). doi:10.1093/nar/gkm651.
Ishibashi, M. & McMahon, A.P. 2002. A sonic hedgehog-dependent signaling relay regulates growth of diencephalic and mesencephalic primordia in the early mouse embryo. *Development 129 (20)*, 4807-19.
Jamora, C., DasGupta, R., Kocieniewski, P. & Fuchs, E. 2003. Links between signal transduction, transcription and adhesion in epithelial bud development. *Nature 422 (6929)*, 317-22.
Johnston, R.B. Jr. 2008. Will Increasing Folic Acid in Fortified Grain Products Further Reduce Neural Tube Defects without Causing Harm? Consideration of the Evidence. Pediatric Research 63 (1): 1-3.
Jung, J., Zheng, M., Goldfarb, M. & Zaret, K.S. 1999. Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science 284 (5422)*, 1998-2003.
Kainer, R.A. & McCracken, T.O. 2003. *Dog anatomy – A coloring atlas*. Eds: Cann, C.C., Hunsberger, S.L. & Giandomenico, N. Teton NewMedia, Jackson, Wyoming, USA.

Kása, F., Kása, G. & Kussinger, S. 1992. Dermoid sinus in a Rhodesian ridgeback. Case report. *Tierarztliche Praxis 20 (6)*, 628-31.

Katoh, M. & Katoh, M. 2003. Evolutionary conservation of CCND1-ORAOV1-FGF19-FGF4 locus from zebrafish to human. *International Journal of Molecular Medicine 12 (1)*, 45-50.

Katoh, M. & Katoh, M. 2005. Comparative genomics on mammalian Fgf3-Fgf4 locus. *International Journal of Oncology 27 (1)*, 281-5.

Katoh, M. & Katoh, M. 2006. WNT and FGF gene clusters (review). International *Journal of Oncology 21 (6)*, 1269-73.

Kawano, M., Komi-Kuramochi, A., Asada, M., Suzuki, M., Oki, J., Jiang, J. & Imamura, T. 2005. Comprehensive Analysis of FGF and FGFR Expression in Skin: FGF18 Is Highly Expressed in Hair Follicles and Capable of Inducing Anagen from Telogen Stage Hair Follicles. *Journal of Investigative Dermatology 124*, 877-885.

Kirkness, E.F., Bafna, V., Halpern, A.L., Levy, S., Remington, K., Rusch, D.B., Delcher, A.L., Pop, M., Wang, W., Fraser, C.M. & Venter, J.C. 2003. The dog genome: survey sequencing and comparative analysis. *Science 301 (5641)*, 1898-903.

Kosaka, N., Kodama, M., Sasaki, H., Yamamoto, Y., Takeshita, F., Takahama, Y., Sakamoto, H., Kato, T., Terada, M. & Ochiya, T. 2006. FGF-4 regulates neural progenitor cell proliferation and neuronal differentiation. *Federation of American Societies for Experimental Biology 20*:1484-1485.

Kratochwil, K., Galceran, J., Tontsch, S., Roth, S., Roth, W. & Grosschedl, R. 2002. FGF4, a direct target of LEF1 and Wnt signalling, can rescue the arrest of tooth organogenesis in *Lef1*$^{-/-}$ mice. *Genes & Development 16*, 3173-3185.

Ladher, R.K., Anakwe, K.U., Gurney, A.L., Schoenwolf, G.C. & Francis-West, P.H. 2000. Identification of Synergistic Signals Initating Inner Ear Development. *Science* 290: 1965-1967.

Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson,E.K., Jaffe, D.B., Kamal, M., Clamp, M., Chang, J.L., Kulbokas, E.J., Zody, M.C., Mauceli, E., Xie, X., Breen, M., Wayne, R.K., Ostrander, E.A., Ponting, C.P., Galibert, F., Smith, D.R., deJong, P.J., Kirkness, E., Alvarez, P., Biagi, T., Brockman, W., Butler, J., Chin, C-W., Cook, A., Cuff, J., Daly, M.J., DeCaprio, D., Gnerre, S., Grabherr, M., Kellis, M., Kleber, M., Bardeleben, C., Goodstadt, L., Heger, A., Hitte, C., Kim, L., Koepfli, K-P., Parker, H.G., Pollinger,J.P., Searle, S.M.J., Sutter, N.B., Thomas, R., Webber, C. & Lander, E.S. 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* 438, 803-819.

Lord, L.H., Cawley, A.J. & Gilray, J. 1957. Mid-dorsal dermoid sinuses in Rhodesian Ridgeback dogs – a case report. *Journal of American Veterinary Medical Association 131*, 515-518.

Mann, G.E. & Stratton J. 1966. Dermoid Sinus in the Rhodesian Ridgeback. *Journal of Small Animal Practice 7*, 631-642.

Marks, S.L., Harari, J. & Dernell, W.S. 1993. Dermoid sinus in a Rhodesian Ridgeback. *Journal of Small Animal Practice 34*, 356-358.

Martinez-Lage, J.F., Esteban, J.A., Poza, M. & Casas, C. 1995. Congenital dermal sinus with an abscessed intramedullary epidermoid cyst in a child. Case report and review of the literature. *Child's nervous system 11*, 301-5.

Merrill, B.J., Gat, U., DasGupta, R. & Fuchs, E. 2001. Tcf3 and Lef1 regulate lineage differentiation of multipotent stem cells in skin. *Genes Development 15 (13)*, 1688-705.

Miyoshi, K., Rosner, A., Nozawa, M., Byrd, C., Morgan, F., Landesman-Bollag, E., Xu, X., Seldin, D.C., Schmidt, E.V., Taketo, M.M., Robinson, G.W., Cardiff, R.D. & Hennighausen, L. 2002. Activation of different Wnt/beta-catenin signaling components in mammary epithelium induces transdifferentiation and the formation of pilar tumors. *Oncogene 21 (36)*, 5548-56.

Murakami A., Thurlow J & Dickson C. 1999. Retinoic acid-regulated expression of fibroblast growth factor 3 requires the interaction between a novel transcription factor and GATA- 4. *Journal of Biological Chemistry 274*, 17242–17248.

Murakami, A., Shen, H., Ishida, S. & Dickson, C. 2004. SOX7 and GATA-4 are competitive activators of Fgf-3 transcription. *Journal of Biological Chemistry 279 (27)*, 28564-73.

Murray, J.N. 1989. *The Rhodesian Ridgeback Indaba*. J N Murray, 5 Melbourne road, Yea Victoria 3717, Australia.

Nishimura, T., Utsunomiya, Y., Hoshikawa, M., Ohuchi, H. & Itoh, N. 1999. Structure and expression of a novel human FGF, FGF-19, expressed in the fetal brain. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression 1444*, 148-151.

Nordfors, L., Jansson, M., Sandberg, G., Lavebratt, C., Sengul, S., Schalling, M. & Arner, P. 2002. Large-Scale Genotyping of Single Nucleotide Polymorphisms by Pyrosequencing™ and Validation Against the 5'Nuclease (TaqMan®) Assay. *Hum Mutatation 19*, 395-401.

Ohyama, T., Mohamed, O.A., Taketo, M.M., Dufort, D. & Groves, A.K. 2006. Wnt signals mediate a fate decision between otic placode and epidermis. *Development 133 (5)*, 865-75.

Ornitz, D.M., Xu, J., Colvin, J.S., McEwen, D.G., MacArthur, C.A., Coulier, F., Gao, G., Goldfarb, M. 1996. Receptor specificity of the fibroblast growth factor family. *Journal of Biological Chemistry 271 (25)*, 15292-7.

Ornitz, D.M. & Itoh, N. 2001. Fibroblast growth factors. *Genome Biology 2(3)*, doi:10.1186/gb-2001-2-3-reviews3005.

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

Pasqualetti, M., Neun, R., Davenne, M. & Rijli, F.M. 2001. Retinoic acid rescues inner ear defects in Hoxa1 deficient mice. *Nature Genetics* 29*(1)*:34-9.

Peeters, M.C.E., Viebahn, C., Hekking, J.W.M. & Van Straaten, H.W.M. 1998. Neurulation in the rabbit embryo. *Anatomy and Embryology 197***,** 167–175.

Penrith, M-L. & Van Shouwenburg, S. 1994. Dermoid sinus in a Boerboel bitch. *Journal of the South African Veterinary Medical Association 65 (2)*, 38-39.

Pielberg, G., Olsson, C., Syvänen, A.C. & Andersson, L. 2002. Unexpectedly high allelic diversity at the KIT locus causing dominant white color in the domestic pig. *Genetics 160(1)*:305-11.

Pierre-Kahn, A., Zerah, M., Renier, D., Cinalli, G., Sainte-Rose, C., Lellouch-Tubiana, A., Brunelle, F., Le Merrer, M., Giudicelli, Y., Pichon, J., Kleinknecht, B. & Nataf, F. 1997. Congenital lumbosacral lipomas. *Child's nervous system 13 (6)*, 298-334; discussion 335.

Pratt, J.N.J., Knottenbelt, C.M. & Welsh, E.M. 2000. Dermoid sinus at the lumbosacral junction in an English Springer Spaniel. *Journal of Small Animal Practice 41*, 24-26.

Savolainen, P., Zhang, Y., Luo, J., Lundeberg, J. & Leitner, T. 2002. Genetic Evidence for an East Asian Origin of Domestic Dogs. *Science 298*, 1610-1613.

Scott, D.W., Miller, W.H. & Griffin, G.E. 1995. *Small Animal Dermatology*. 5th ed. Eds: Scott, D.W., Miller W.H, & Griffin, G.E. W.B. Saunders, Philadelphia, USA. 736-805 pp.

Sherr, C.J. & Roberts, J.M. 1999. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Development 13 (12)*, 1501-12.

Sjaastad, Ø.V., Hove, K. & Sand, O. 2004. *Physiology of Domestic Animals*. Eds: Steel C. Scandinavian Veterinary Press, Oslo, Norway. 110-117 pp.

Steyn, H.P., Quinlan, J. & Jackson, C. 1939. A skin condition seen in Rhodesian Ridgeback dogs: report on two cases. *Journal of the South African Veterinary Medical Association X (4)*, 170-174.

Sutter, N.B., Eberle, M.A., Parker, H.G., Pullar, B.J., Kirkness, E.F., Kruglyak, L. & Ostrander, E.A. 2004. Extensive and breed-specific linkage disequilibrium in *Canis Familiaris*. *Genome Research 14*, 2388-2396.

Tai, A.L., Sham, J.S., Xie, D., Fang, Y., Wu, Y.L., Hu, L., Deng, W., Tsao, G.S., Qiao, G.B., Cheung, A.L. & Guan, X.Y. 2005. Co-overexpression of fibroblast growth factor 3 and epidermal growth factor receptor is correlated with the development of nonsmall cell lung carcinoma. *Cancer 106 (1)*, 146-55.

Tamimi, Y., Skarie, J.M., Footz, T., Berry, F.B., Link, B.A. & Walter, M.A. 2006. FGF19 is a target for FOXC1 regulation in ciliary body-derived cells. *Human Molecular Genetics 15 (21)*, 3229-40.

Tekin, M., Hişmi, B.O., Fitoz, S., Ozdağ, H., Cengiz, F.B., Sirmaci, A., Aslan, I., Inceoğlu, B., Yüksel-Konuk, E.B., Yilmaz, S.T., Yasun, O. & Akar, N. 2007. Homozygous mutations in fibroblast growth factor 3 are associated with a new form of syndromic deafness characterized by inner ear agenesis, microtia, and microdontia. *American journal of human genetics 80 (2)*, 338-44.

Theal, G.M. 1900. *The Story of the Nations - South Africa*. 5th ed. T. Fisher Unwin, London. 2-4 pp.

Tshamala, M. & Moens, Y. 2000. True dermoid cyst in a Rhodesian Ridgeback. *Journal of Small Animal Practice 41*, 352-353.

van den Broek, A.H.M., Else, R.W., Abercromby, R. & France, M. 1991. Spinal dysraphism in the weimaraner. *Journal of Small Animal Practice 32 (5)*, 258-260.

van der Put, M.N.J., van Straaten, H.W.M., Trijbels, F.J.M. & Blom, H.J. 2001. Folate, Homocysteine and Neural Tube Defects: An Overview. *Experimental Biology and Medicine 226*, 243-270.

Vilà, C., Savolainen, P., Maldonado, J.E., Amorim, I.R., Rice, J.E., Honeycutt, R.L., Crandall, K.A., Lundeberg, J. & Wayne, R.K. 1997. Multiple and ancient origins of the domestic dog. *Science 276 (5319)*, 1687-9.

Wang, Y., Guo, N. & Nathans, J. 2006. The role of Frizzled3 and Frizzled6 in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *The journal of neuroscience 26 (8)*, 2147-56.

Wilkinson, D.G., Peters, G., Dickson, C., McMahon, A.P. 1988. Expression of the FGF-related proto-oncogene int-2 during gastrulation and neurulation in the mouse. *European molecular biology organization 7(3)*: 691-5.

Wilkinson, D.G., Bhatt, S. & McMahon, A.P. 1989. Expression pattern of the FGF-related proto-oncogene int-2 suggests multiple roles in fetal development. *Development 105(1)*:131-6.

Willis, M.B. 1989. *Genetics of the Dog*. Howell Book House, New York, USA.

Wolpert, L., Beddington, R., Jessel, T., Lawrence, P., Meyerowitz, E. & Smith, J. 2004. *Principles of Development.* 2nd ed. Oxford University Press, Oxford, UK. 278-284 pp.

Xie, M.H., Holcomb, I., Deuel, B., Dowd, P., Huang, A., Vagts, A., Foster, J., Liang, J., Brush, J., Gu, Q., Hillan, K., Goddard, A. & Gurney, A.L. 1999. FGF-19, a novel fibroblast growth factor with unique specificity for FGFR4. *Cytokine* 11 (10), 729-35.

Zhang, X., Ibrahimi, O.A., Olsen, S.K., Umemori, H., Mohammadi, M. & Ornitz, D.M. 2006. Receptor Specificity of the Fibroblast Growth Factor Family. THE COMPLETE MAMMALIAN FGF FAMILY. *Journal of Biological Chemistry 281 (23)*, 15694-15700.

Zhong, W., Wang, Q.T., Sun, T., Wang, F., Liu, J., Leach, R., Johnson, A., Puscheck, E.E. & Rappolee, D.A. 2006. FGF ligand family mRNA expression profile for mouse preimplantation embryos, early gestation human placenta, and mouse trophoblast stem cells. *Molecular Reproduction and Development 73*:540-50.

Zúñiga, A., Haramis, A.P., McMahon, A.P. & Zeller, R. 1999. Signal relay by BMP antagonism controls the SHH/FGF4 feedback loop in vertebrate limb buds. *Nature 401(6753)*:598-602.

Acknowledgements

Anders Hillbertz, my husband. This project would never been a reality without your dedication and support. I am so proud of you and expect that we can sit on the porch when get older and relax.

My family, your support has been invaluable throughout the years and I would not have come this far without you.

Göran Andersson, my main supervisor and very close friend, you have always given me support and the freedom and respect to hypothesise and be creative as a student. For this you have my utter most respect and I will always cherish you! I thank you from the bottom of my heart for the years, dedication and opportunities you have given me. It has been an honour.

Åke Hedhammar, my co-supervisor. You always told me that I should focus on my studies. During the past two years I have understood the actual meaning of your message! You are the person who introduced me to the Swedish dog community, from which I learnt so much more than expected. I appreciate all your support, enthusiasm and commitment.

Leif Andersson, my co-supervisor. Your skills are absolutely amazing regarding pinpointing genetic features as well as writing manuscripts. Thank you for all the time and effort you have employed in these studies and I consider it an honour to have had the opportunity to work with you.

Kerstin Lindblad-Toh. Your support and commitment is amazing and I am grateful for every moment we spent together. Thank you for believing in me.

Gerli Pielberg Rosengren. You have been my best friend throughout the years in the laboratory. Further, you have also been my mentor during my PhD-studies regarding laboratory work! Everything I have learned in the laboratory is due to your supervision and guidance! You are absolutely brilliant!

Elinor Karlsson, **Claire Wade** and **Mike Zody**. It was fantastic to have the opportunity to work with you! I am very glad for the time we spent together during May 2007 at the Broad Insitute.

Eva Hellmén. You were the one that taught me everything about Dermoid sinus, for this I am grateful. Our collaboration has been ongoing for many years and I appreciate your dedication. We did indeed have many good laughs.

Mats Nilsson & Magnus Isaksson. Thank you both for all the efforts!

Anne-Sophie van Laere and **Emmanuelle Bourneuf**. We have shared many joyful moments and laughter's. Hope to meet you soon again.

Jonas Eriksson, thanks for your participating in Dermoid sinus studies.

Per Wahlberg, there will be many more barbeques in the future.

Ulla Gustafson and **Gudrun Wieslander**, the back-bones of the group. What would I have done without you?

To the other members of our group. I am grateful for all the help and support you have provided throughout the years.

Patricio Rivera, my close friend and colleague. Boston was great!

Kerstin Olsson, you have been my close friend for many years now! You have always been available and supportive and I am proud of our friendship!

Ulla Thedin, my confidant regarding the Rhodesian Ridgeback breed. You have assisted my regarding many questions associated with the Ridgebacks during five study years. Your knowledge has, in many instances, been crucial regarding the Ridgeback studies. I am proud that we have had the opportunity to work together during the past years.

To the Rhodesian- and Thai Ridgeback communities. This study is a result of a successful cooperation between us. I am proud of your commitments to the breeds.

Neale Fretwell, I really appreciate your commitment and everything you have done.

Christine Knudsen, my close friend. We have spent countless hours together searching for and sampling dogs necessary for my research projects. I enjoyed every minute.

Kennel Clubs and veterinarians. Thank you for supporting the project.

Professor Olle Kämpe, this has been a journey and I am glad you participated.

Olafur Gudjonsson. Thank you for everything you have done!