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Inherited Polyneuropathy in Leonberger dogs

1. State of research in the field

1.1 The canine genome resources

The dog, as a favored companion of humans, is unique among animal species in providing new insights into human genetic diseases. The major advantages dogs offer for comparative genetic studies are the high degree of medical surveillance of the dog by veterinary specialists, the structure of dog populations consisting of >300 partially inbred genetic isolates (breeds) with genetic disorders predominantly or exclusively in one or a few breeds and the excellent arsenal of dog genome resources (Ostrander *et al.* 2000). After the first meiotic linkage map of the whole dog genome was published (Mellersh *et al.* 1997) considerable effort in the canine mapping was made by the development of an integrated high quality radiation hybrid map (Guyon *et al.* 2003) and a high density resolution dog-human chromosomal comparative map (Breen *et al.* 2004). Finally, after the availability of a 1.5x Poodle sequence (Kirkness *et al.* 2003), the first high-quality draft (7.5x) sequence of the Boxer dog was made publicly available in July of 2004 (Lindblad-Toh *et al.* 2005). These outstanding dog genome resources together with the well characterized relationship between the human and canine genomes currently facilitate analyses of well-characterized canine inherited diseases. Due to the recent genetic bottlenecks within dog breeds, linkage disequilibrium typically extends by an order of magnitude farther than in humans. The known haplotype structure of the dog suggests that genome-wide association mapping within dog breeds requires just 30,000 single nucleotide polymorphisms (SNP) (Lindblad-Toh *et al.* 2005) and recently an appropriate mapping tool (SNP chip) for whole genome association mapping in dogs became available.

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1.2 Description of the phenotype

The inherited polyneuropathy (IPN) of Leonberger dogs is characterized by a distal symmetrical degenerative peripheral polyneuropathy (Shelton *et al.* 2003; Riche 2006). Most dogs are affected at a young age (age of onset at 1 to 3 years) and show severe clinical signs and pathological abnormalities. However, a later age of onset (3 to 5 years) associated with milder clinical signs was identified in some cases (Shelton *et al.* 2003; Riche 2006). First clinical signs of disease onset in Leonberger dogs are commonly respiratory symptoms (dyspnea) mostly accompanied by locomotion troubles and marked atrophy of distal limb muscles (Riche 2006). The most common abnormalities include laryngeal paresis or paralysis, distal muscle atrophy, high-steppage gait and depressed spinal reflexes; less common findings are facial nerve paralysis, and a decreased gag reflex (Shelton *et al.* 2003). Electrophysiological studies of affected Leonberger dogs revealed abnormalities consistent with denervation and a polyneuropathy (Shelton *et al.* 2003; Riche 2006). The neuropathy, however, could not be characterized because in most dogs the amplitude of the motor response to tibial nerve stimulation was so reduced that latency measurements could not be made (Shelton *et al.* 2003). In affected dogs breathing abnormalities or swallowing disturbances (dysphagia and aspiration) can become very severe leading to the death of the patient (Shelton *et al.* 2003). Therefore a surgical correction of laryngeal paresis by lateralization of the arytenoid cartilage is performed in affected dogs, resulting in improved respiratory function.
Muscle biopsies demonstrated a pattern of severe atrophy and fatty replacement of muscle fibers, indicative of denervation and axonal loss (Shelton *et al.* 2003; Riche 2006). Consistent with these findings, decreased myelinated fiber density and endoneurial fibrosis, suggestive of chronic nerve fiber loss, was present in peripheral nerve biopsies (Shelton *et al.* 2003; Riche 2006).
The IPN disease of Leonberger dogs represents an example of a breed-associated polyneuropathy (Coates and O'Brien 2004). Isolated cases of a similar disease were reported for Rottweilers and Alaskan Malamutes (Braund *et al.* 1994; 1997). In general, inherited degenerative neuropathies in dogs are extremely rare (Sargan 2004; http://server.vet.cam.ac.uk/index.html).

1.3 IPN genetics

During the last 20 years breeders of Leonberger dogs worldwide recognized a familial accumulation of affected dogs. These observations gave first evidence for a genetic component. In the recently reported Leonberger dogs with polyneuropathy, neither parent was affected in most instances, indicating a possible recessive mode of inheritance (Shelton *et al.* 2003; Riche 2006). Based on the results of the pedigree analysis, Shelton *et al.* (2003) postulated a X-linked recessive inheritance as the most likely mode of inheritance for IPN in Leonberger dogs. Among 15 IPN affected dogs of a single pedigree 14 were male and only one female. The analysis of Leonberger dog families from the French population suggested an autosomal recessive transmission (Riche 2006). In the French study a large inbred pedigree including 20 IPN affected dogs of ten litters with one to five affected littermates was reported. As both sexes (12 females and 8 males) were roughly equally affected within this dog family this observation confirms reports of Leonberger dog breeders from different countries that the true mode of inheritance is monogenic autosomal recessive.

2. Relevance of the project

The discovery of the gene responsible for canine IPN could lead to a DNA based test for the eradication of the disease in this dog breed. Furthermore it could provide better understanding of the pathophysiological mechanisms, which lead to the development of polyneuropathies in dog and also possibly in human. In addition, identification of the mutations responsible for canine diseases may lead to the discovery of new causes of similar human cases for which the genetic bases remain to be determined.

Charcot-Marie-Tooth (CMT) is the most common inherited human disorder of the peripheral nervous system. Soon after the original description by J.M. Charcot, P. Marie and H.H. Tooth in 1886, it became apparent that this syndrome is clinically and genetically heterogeneous. Historically, two classes of CMT have been differentiated: Demyelinating forms of CMT (CMT1), in which nerve conduction velocities are decreased, and the axonal CMT2 forms, in which nerve conduction velocities are preserved. Several subtypes of inherited peripheral neuropathies were delineated and classified as hereditary motor and sensory neuropathies (HMSN), hereditary motor neuropathies (HMN) and hereditary sensory (and autonomic) neuropathies (HSAN). All these types are further subdivided in several subtypes. CMT patients suffer from slowly progressive, distally pronounced, symmetric muscle atrophy and less prominent sensory loss have an estimated prevalence of one to four in 10'000 (Shy *et al.* 2005). Mutations in 24 human genes are currently known to cause CMT (http://www.molgen.ua.ac.be/CMTMutations). All modes of inheritance have been reported in humans with CMT, but CMT is most commonly transmitted as an autosomal dominant trait.

The most striking similarities of IPN affected Leonberger dogs to the CMT syndrome in humans are the common presence of a high-steppage gait, laryngeal weakness and paralysis, and pharyngeal and bilateral facial weakness (Shelton *et al.* 2003). The clinical, electrophysiological, and pathological changes in these dogs point to an axonal variant with similarities to a mixed or intermediate form of CMT (Shelton *et al.* 2003). Therefore canine IPN has the potential to serve as model for the analysis and treatment of analogous human CMT. In general, animal models might open up new perspectives on the pathomechanisms and possible treatment strategies of inherited neuropathies (Meyer zu Hörste and Nave 2006). Naturally occurring domestic animal models are particularly valuable because they are larger and more closely related to human than rodents. Their longer life expectancy allows for investigations or treatments over a longer time period. Moreover, a high level of expertise in reproductive technology and veterinary care is available for dogs.

3. Detailed research plan

3.1 Samples and phenotype

As IPN in Leonberger probably follows a monogenic autosomal recessive mode of inheritance, we aim to collect at least 20 cases of IPN affected Leonberger dogs representing both sexes

and 20 controls from unrelated healthy Leonberger dogs. We will record the pedigrees for at least three generations to ensure that animals are not closely related. Samples will mostly be collected in Switzerland but Dutch samples have already been included as well to build the sample cohorts.
There is an active collaboration with Prof. A. Jaggy, expert in small animal neurology at the Clinic for Small Animals, Vetsuisse Faculty, University of Berne, to assist in phenotype evaluation of uncertain cases from Switzerland. An essential diagnostic tool to evaluate the disease will be the histological analysis of muscle/nerve biopsies which can be performed at the collaborating lab of Prof. Thomas Bilzer, Institute of neuropathology of the University Düsseldorf, Germany.
EDTA blood samples (2 ml) for genomic DNA isolation and genetic analysis will be taken from all dogs in the study and stored at –20 °C.
At the Institute of Genetics the collection of material from Leonberger dogs has already been initiated. Currently, 119 EDTA blood samples including 17 suspicious dogs or diagnosed cases are available. At the beginning of the study the missing blood samples should be collected to reach the envisioned cohort sizes. In addition, the recruitment of samples from healthy control dogs through the hospital populations will be started.

3.2 Genotyping and analysis plan

Step 1
(Costs of about CHF 20'000, duration of about 4 months; chance of success 80%)
Once the DNA samples are isolated we will genotype 22'000 SNP using a dog specific microarray. We expect that this analysis will result in the identification of a single genomic interval of 500 kb at most, which is associated with IPN and probably contains the functionally relevant mutation.

Step 2
(Costs of about CHF 20 to 100'000, duration of about 2 months to 2 years; chance of success 80%)
Once the candidate interval has been defined we will select a panel of dogs (cases + controls) representing all major haplotypes as deduced from the whole genome SNP association analysis to sequence their DNA with the final goal of identification the causative mutation. The outcome of this study will enable us to develop a gene test for selection purposes.

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