

Investigation into the genetic background of malignant histiocytic tumors in Bernese Mountain Dogs

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In the 1980s it was discovered that Bernese Mountain Dogs were sometimes affected with a widespread malignant histiocytic cancer, called malignant histiocytosis (MH) and a familial predisposition was shown. Shortly thereafter, collection of data regarding the disease began in the Netherlands (NL) and in other countries. Some animals, it was found, developed a localized form with high metastatic propensity that was often referred to as histiocytic sarcoma (HS). A few years ago an international consortium was established to collaborate in the scientific investigation of the genetic background of MH/HS. Our research involves analysis of the genome of affected animals and healthy veteran controls, through the use of high density genetic markers. NL is currently participating in a breed-wide association study, which does not depend upon family background.

Of 250 BMD that were reported to the UUCA suspected to be affected with MH in the past 10 years, insufficient data leading to a positive diagnosis or lack of biological material excluded 100 from entry into the association study. In each of these cases, either no blood had been collected, or the diagnosis had been based on clinical signs and radiographic examination without examination of the tumor tissue. Of those dogs where cytological and/or histological examination was possible, 15 did not have cancer, and 35 dogs with masses in bone or near the joint (HS), or in thorax or abdomen (sometimes central nervous system) had a variety of other cancers, such as multiple myeloma, osteosarcoma, bronchial carcinoma, liver or ovarian carcinoma and malignant lymphoma, not MH. Of these tumor types, the latter (malignant lymphoma) is most often differentiated early on because access is easy and immunohistochemistry can identify the true histogenetic type. Of the 80 unaffected control dogs that donated blood at > 8 years of age, subsequent follow-up found that 8 died of unknown causes, 10 died of tumor or immune disease with suspicion of HS/MH, but without verification, and 12 developed either HS/MH (with transport into the MH/HS group) or another malignant tumor that necessitated exclusion from the control group.

From this experience we found that, for an effective genetic study, proper identification of the tumor type by laboratory analysis, with central revision, and constant follow up regarding health status is essential.

Dr. Ostrander's group at the NIH has recently completed a scan using 500 microsatellite markers spanning the entire canine genome in 350 Bernese mountain dogs, both affected and unaffected, coming from the USA, France, NL and several other European countries. Focusing on a subset of 55 Bernese diagnosed before the age of 8 and 125 unaffected Bernese over the age of 10, we have identified four regions of the genome that are likely to contain MH susceptibility genes. The combination of our findings and the results obtained by Dr. André et al. at the University of Rennes based on an extended family of Bernese highlights two highly significant regions that are currently under investigation. We are working on fine mapping in these regions in order to find the specific genes that are responsible for the disease while at the same time applying new SNP genotyping technologies to our population samples in order to verify our findings and identify additional loci.

Furthermore, tumors that are rapidly frozen (or 3 mm particles put in a vials containing a special solution: RNA later) are being examined by Dr. Breen et al. at North Carolina State University using the high-tech cytogenetic technique, comparative genomic hybridization (CGH). With dozens of tumors thus

analyzed, common patterns, such as genomic amplification or translocation or deletion of specific chromosomal areas, can be deciphered. Identification of such regions may help both in the localization of important genes and also in discovering the mechanisms behind cancer development. From NL a shipment of about 35 samples will be shipped to NCSU next month for extension of the CGH analysis.

In addition, combination of data from the above NL cases with data from archives from collaborating laboratories from the past 15 years, will be used to assemble a group of > 300 MH/HS cases, to be examined for possible differences of disease manifestation (MH versus HS) or age of occurrence between families.

In the coming years, the collaborating scientists will call on societies, breeders and owners to continue to inform other BMD owners to report dogs suspected of developing malignancies and to provide the study with blood and tissue. Great urgency also exists with respect to the entry of veteran BMD into the control group. Unaffected dogs can enter the study as controls by providing data on their health together with a blood sample.

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