Journal of Heredity 2009:100(Supplement 1):S19–S27
doi:10.1093/jhered/esp039
Advance Access publication June 16, 2009

Epidemiology, Pathology, and Genetics of Histiocytic Sarcoma in the Bernese Mountain Dog Breed

Jérôme Abadie*, Benoit Hédan*, Edouard Cadieu, Clotilde De Brito, Patrick Devauchelle, Catherine Bourgain, Heidi G. Parker, Amaury Vaysse, Patricia Margaritte-Jeannin, Francis Galibert, Elaine A. Ostrander, and Catherine André

Unité d'Anatomie pathologique, Ecole Nationale Vétérinaire de Nantes, BP40706, 44307 Nantes cedex 3, France (Abadie); Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, 50 South Drive, Bethesda, MD (Cadieu, Parker, and Ostrander); Institut de Génétique et Développement, Une unité mixte de recherche 6061 Centre national de la recherche scientifique/Université de Rennes1, Faculté de Médecine, Rennes, France (Hédan, Brito, Vaysse, Galibert, André); Centre anti-cancereux, Ecole nationale vétérinaire d'Alfort 7 avenue du general de Gaulle 94700, Maisons-Alfort, France (Devauchelle); Institut Inserm U535—Genetique Epidemiologique et Structure des Populations humaines, Hôpital Paul Brousse Bâtiment Leriche B.P., Villejuif, France (Bourgain and Margaritte-Jeannin). Benoit Hédan is now at Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

*Authors equally contributed to the work.

Address correspondence to C. André at the address above, or e-mail: catherine.andre@univ-rennes1.fr.

Abstract

Histiocytic sarcoma (HS) refers to a highly aggressive and frequently disseminated neoplastic disease belonging to the class of canine histiocytic proliferative disorders. Disseminated HS (previously called malignant histiocytosis) is highly breed specific, with Bernese mountain dogs (BMDs), rottweilers, and retrievers having a high prevalence with a frequency of approximately 25% in the BMD breed. We collected DNA samples and clinical information from 800 BMDs, of which 200 are affected by HS. To better characterize the physiopathology and epidemiology, an in-depth analysis of 89 BMD cases has been performed. The mean age of onset was 6.5 years, males and females being equally affected. The clinical features, biochemical parameters, and pathological features have been determined. The life span after diagnosis has been estimated to be 49 days. A large BMD pedigree of 327 dogs, 121 of which are affected, was assembled. Using a subset of 160 BMDs, encompassing 21 complete sibships, we now propose an oligogenic transmission mode of the disease. Whole-genome linkage scans as well as association studies using a case/control analysis, in parallel with expression profiling of neoplastic versus normal histiocytes, are all underway. Altogether, these complementary approaches are expected to localize the genes for HS in the BMD, leading to advances in our knowledge of histiocyte diseases in dogs and humans.

Key words: *Bernese mountain dogs, cancer, dog, genetics, histiocytic sarcoma*

Canine histiocytic proliferative disorders are a group of heterogeneous diseases that include reactive disorders such as cutaneous and systemic histiocytosis and neoplasias such as cutaneous histiocytoma and localized or disseminated histiocytic sarcoma (HS), the latter previously called malignant histiocytosis (Moore 1984; Moore and Rosin 1986; Rosin et al. 1986; Affolter and Moore 2000; Affolter and Moore 2006). HS is a highly aggressive neoplasm that at diagnosis usually presents with disseminated neoplasm (Moore and Rosin 1986). Although reported clinical signs may vary, lethargy, anorexia, weight loss, as well as abnormalities of the respiratory system or liver and central nervous system (CNS) predominate in the initial diagnosis. Lungs and spleen are reported to be most frequent site of primary tumor (Rosin and Moore 1986; Affolter and Moore 2002). The clinical course of the disease is generally rapidly progressive with a poor prognosis. At death, the majority of dogs demonstrate high levels of neoplastic infiltration in the lungs, spleen, liver and lymph nodes, with frequent associated hematologic abnormalities such as thrombocytopenia and anemia (Moore and Rosin 1986). According to Affolter and Moore (2002), HS originates from the neoplastic proliferation of dendritic antigen-presenting cells,

hence resembling dendritic cell sarcomas and disseminated malignant Langerhans cell histiocytosis (LCH), described in humans (Schmitz and Favara 1998).

HS is a highly breed-specific disorder that has become of significant concern to Bernese mountain dog (BMD) owners throughout the world in recent years. The number of cases has expanded greatly in the last 10 years. In 1986, Moore and Rosin first described a series of 13 cases of "malignant histiocytosis," reported to originate from the same family (Moore 1984; Moore et al. 1986). This was the first description of a possible familial presentation or a related disease in dogs. In 1995, Padgett reported that 25.4% of tumors diagnosed in the BMD were "histiocytosis" (Padgett et al. 1995), and by analyzing the inheritance of "histiocytosis" in 127 affected BMDs, they reported that the disease was inherited in the BMD (Padgett et al. 1995). Previous segregation studies suggest a multigenic mode of inheritance (Moore and Rosin 1986; Padgett et al. 1995). Strikingly, it has been estimated that 80% of "histiocytosis" cases reported in France were diagnosed in the BMD breed (Devauchelle P, personal communication). Although HS is clearly overrepresented in the BMD, its mode of inheritance is not well understood, and the genes likely to be involved are unknown.

The aim of our research project is to identify the genetic causes of this highly aggressive cancer in dogs with consideration of the underlying comparative genetics. Malignant dendritic cell–related disorders are indeed rare in human beings, and their nosology, etiology, and pathogenesis remain largely undefined (Arico and Danesino 2001). A spontaneous animal model of malignant LCH is expected to enhance research efforts and may represent a powerful tool, particularly for exploring the role of genetic predisposition to the disease.

The domestic dog has emerged last years as an excellent model for genetic analysis of complex diseases, (Parker and Ostrander 2005; Galibert and André 2007; Wayne and Ostrander 2007; Ostrander et al. 2000) particularly cancer (Cadieu and Ostrander 2007). After availability of an assembled 7.5× sequence of the canine genome (Lindblad-Toh et al. 2005), single nucleotide polymorphism chips have recently been available, and genome-wide association studies (GWAS) are now possible in the dog (Karlsson et al. 2007; Jones et al. 2008). Studying such complex diseases as cancers in dogs allows access to large and well-documented pedigrees and numerous samples. Moreover, the genetic heterogeneity between cases and controls is limited inside a breed, thus facilitating the genetic analyses. Finally, because of close breeding pools and popular sire effect, rare causal variants may be concentrated and sometimes fixed in certain breeds, improving signal to noise ratios.

For these reasons, studying the epidemiology and genetics of HS in BMD appears to be of great benefit for human medicine. Towards this end, we undertook a collection of probands and their relatives, as well as unrelated cases and controls with rigorous diagnoses. In addition, with the plan of selecting the best population for genetic investigation, we have conducted a prospective study in order to collect an extensive pedigree and to define the epidemiological, clinical, and pathological features of HS in this BMD population. Finally, we were interested in gaining a better insight into diagnosis criteria used in current veterinary practice to define HS and to compare these data with those from human studies.

Material and Methods

Sample Collection

Two populations of BMDs are being sampled for genetic analyses: first, a large se of related dogs from France and close European countries were collected with the aim of assembling a large family. Second, a set of unrelated BMDs, including cases and controls, were collected from throughout the United States.

For the pedigree constitution, more than 800 blood and tissue samples were collected by a network including oncologist practitioners, the veterinary schools, veterinary pathology laboratories, and veterinarians throughout France and close European countries. All affected dogs presented with strong clinical evidence of HS, and, in most cases, the disease was confirmed by pathology reports, including histopathological reevaluation of biopsy samples by one author (J.A.). Diagnosis included immunohistochemical evaluation when required, using the classification scheme defined by Affolter and Moore (2002). Immunostaining was performed on formalin-fixed and paraffin-embedded tumor tissues, and a diagnosis of HS was confirmed when tumor cells display morphological features consistent with an HS and were CD18 positive (hematopoietic in origin) and CD3 and CD79 negative (excluding T-cell and B-cell lymphomas, respectively). Unaffected dogs were declared controls if they were older than 10 years and had never been diagnosed with any form of cancer. This population has been used in the present study for the evaluation of the epidemiology, pathology, and transmission mode of HS in the BMD breed.

For future case and control genetic analyses, more than 700 BMDs were collected in the United States for whole-genome association studies. Affected dogs (cases) are defined as less than 7.5 years of age at diagnosis with HS. Diagnosis must be made by a licensed veterinarian or veterinary oncologist. The unaffected (control) dogs are all older than 10 years and have never been diagnosed with any form of cancer. This strategy increases the likelihood of finding significant association. Cases are selected so that they do not share common grandparents, thus allowing us to interrogate a large portion of the historical contribution of the breed. Similarly controls are also selected so there are no shared grandparents within the control group.

Pedigree Analysis

A 21 146-dog multigenerational pedigree, connecting 327 BMD sampled dogs from France, with known phenotypes was constructed by investigations performed between 2001-01-01 and 2007-07-01. We used Cyrillic software v2.1 (33) (CyrillicSoftware) for genealogic and genetic data management. We calculated inbreeding coefficients within the pedigree, defining the probability that for any locus of an

individual both 2 alleles descend from the same ancestral genotype. It also equals the product of kinship coefficients of its sire and its dame. We used kinship algorithms programmed using R (http://cran.r-project.org) to calculate the inbreeding coefficient for all dogs belonging to the large extensive pedigree. All dogs for which parental DNA was available were tested for parental compatibility using a set of informative microsatellite markers.

Blood samples and the accompanying pedigree were collected by licenced veterinarians, and complementary information was obtained directly from the owners and breeders. All data were entered into a confidential database. Genomic DNA was extracted from 5 ml of blood collected into ethylene diamine tetraacetic acid using the nucleon BACC 3 kit (GE Healthcare Bio-Sciences Corp, Piscataway, NJ) and the Nucleospin kit (Macherey Nagel). A small number of samples with limiting amounts of DNA were whole genome amplified using the V1 genomiphi kit (GE Healthcare Bio-Sciences Corp).

Mode of Inheritance

To test whether a fully recessive model could explain the transmission of the disease, nuclear families with at least 2 phenotyped offspring, one of them being affected, were selected. Conditioning on the phenotyped parent, we computed for each family the expected number of affected offspring under a fully recessive model. In this calculation, we take into account the exact number of offspring in each family. When parental status was unknown, we considered them as affected with a probability pk and unaffected with a probability $1 - pk$ (pk is the estimation of the prevalence of the disease in the breed).

Epidemiologic Analyses

Among the 327 dogs sampled in France and Europe, 121 BMDs were affected by HS. Of those 121 dogs, 89 with pathological reports had been collected between 2005-01-01 and 2008-11-01. We next sent a questionnaire to each referring veterinarian. All dogs participating in the study were owned by private individuals. The questionnaire investigated epidemiological, clinical, and pathological features including identification of the dog (identity sex, age, etc.) anamnestic data and case history, previous illness, diseases, clinical presentation of HS at time of diagnosis, biological evaluations (including hematology, blood chemist, etc.), therapeutics, and follow-up. Quantitative epidemiological features are described as the mean ± 1 standard deviation. Differences between populations were analyzed statistically using Fisher's Exact test. Qualitative differences between populations were tested using the chi-square test with a significance level set at 5% for each analysis.

Results

Epidemiological Features of HS in BMD

Eighty-nine (89) BMDs affected by HS, for which we collected complete clinical information, were included in this study. Diagnosis of HS was made using strong clinical or pathological evidence of the disease and confirmed by histology or cytology in 79 cases (89%). Males (46 cases, 52%) were as likely to be affected as females (43 cases, 48%) with strong statistical evidence of absence of a sex predisposition ($P = 0.940$, chi square). The mean age at diagnosis was 6 years and 6 months (range = 2–11 years). A total of 70.5% of the cases had been diagnosed between 5 and 8 years of age. A total of 17.8% of the cases were diagnosed after the age of 8 years and only 3.4 after the age of 10 years, which highlights the importance of long-term follow-up for the clinical status of the controls. Mean age at diagnosis did not differ significantly between males and females (Fisher's Exact test).

Among the 89 affected BMDs, 58 (70%) have relatives (first or second degree) with a confirmed diagnosis of HS. Furthermore, 35 dogs (39%) have first- or second-degree relatives affected with malignant neoplastic diseases other than HS. Among them, 18 cases of malignant mast cell tumor (51%), 6 cases of malignant lymphoma (17%), 6 cases of mammary adenocarcinoma (17%), and 3 cases of malignant melanoma (9%) have been recorded. By comparison, only 2 cases have shown close relatives that were affected by reactive nonneoplastic histiocytosis.

Concerning environmental factors, which may have been involved in the neoplastic development, no particular factor has been underlined. A total of 46.5% of the affected dogs were living outdoors and 39.4% indoors, and presence of known or suspected carcinogens in the dog's environment was not identified, except for 2 cases in which toxic products (fertilizers and paint) were indicated.

Regarding anamnestic data, significant diseases were previously recorded for 57 dogs (64%). Fourteen dogs (20%) had a history of previous nonneoplastic dermatological disease. Of these, allergic dermatitis, atopy, recurrent pyoderma, and chronic otitis were the most commonly reported. These dermatological disorders are actually very common in the overall canine population, and the incidence was not excessive in our BMD population. Eleven dogs (16%) were reported to have experienced previous benign neoplastic diseases, including 7 cases of cutaneous histiocytoma, 1 melanocytoma, 1 benign mammary tumor, 1 lipoma, 1 case of multiple oral papillomatosis, and 1 vaginal fibroma. Furthermore, 10 cases of infectious diseases were recorded: 8 cases of babesiosis (11.5%), 1 case of localized leishmaniosis, and 1 case of positive serology for borreliosis.

Clinical and Pathological Features of HS in BMDs

Systemic signs have been recorded in all affected dogs. The most commonly reported clinical symptoms were anorexia (92% of the cases), asthenia and weakness (90%), weight loss (88%), and hyperthermia (43%). Abnormal pulmonary auscultation has been detected in 31% of the dogs, with polypnea (10 cases, 40%), rattle and abnormal pulmonary sounds (7 cases, 28%), dyspnea (6 cases, 24%), tachypnea (5 cases, 23%), shortness of breath (3 cases, 12%), and discordance (1 case, 4%). Coughing was recorded in 19% of affected dogs. Abnormal heart auscultation was observed in

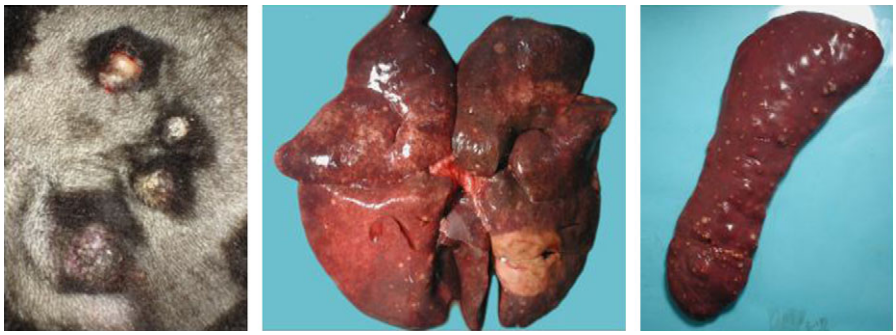

Figure 1. Pathological features of disseminated HS: multiple tumor masses affecting the skin, lung, and spleen.

16% of the dogs, with tachycardia (10 cases, 71% of the cardiac abnormalities), arrhythmia (2 cases, 14%), and decreased heart sound (2 cases, 14%).

Skin and subcutis involvement was reported in 16 affected dogs (19%), 10 of them presenting multicentric lesions (Figure 1). Lesions included nodules (12, 16%) associated or not with plaques (6, 6%). Skin of the thorax and forelegs was involved in 10 cases, head and neck in 6, and abdomen and hind legs in 6.

Cutaneous lesions were ulcerated in 25% of the cases, with pruritus in 50%. Dogs with previously reported skin diseases diagnosed before HS were not at increased risk to develop skin involvement when HS developed ($P = 0.4912$, Fisher's Exact test). Neurologic signs were recorded in 18 (22%) of the dogs, either involving the peripheral nervous system (12, 15%), the CNS (8, 10%), or both (2, 3%). Poorly specific digestive signs (anorexia, vomiting, diarrhea) were observed in 28% of the dogs. Clinical signs associated with other organ system involvement were not consistently reported. In 82% of the cases, there was involvement of internal organs, primarily, the spleen, the lungs and mediastinum, internal lymph nodes, and the liver, with one or several distinctive tumor mass(es) (Figure 1). Splenomegaly was present in 49% of the dogs (34 cases), hepatomegaly in 34% (24 cases), and lymphadenomegaly in 26% (16 cases). Involvement of multiple organ systems at the time of presentation was present in 55% of the dogs (37 cases). In these cases, the spleen was the most frequently involved organ (28 cases, 76%) followed by the lung and mediastinum (27 cases, 67%), the liver (24 cases, 65%), and the lymph nodes (16 cases, 43%).

Hematological investigations at time of diagnosis revealed anemia, which was listed as severe for 41 dogs (66%), and thrombocytopenia in 56% of the cases (22 dogs). Neutrophilia (40%), monocytosis (21%), and lymphopenia (13%) are inconstantly reported. The most frequently observed serum chemistry modifications was alanine-amino transferase and alkaline phosphatase increase (45% of the cases), indicative of liver-related issues.

The histopathological evaluation revealed tumor in poorly demarcated masses composed of a pleomorphic population of large round uni- or multinucleated neoplastic cells (Figure 2). Marked cellular atypias and a high mitotic index were present. Despite their variable morphology, the tumor cells displayed a consistent phenotype with expression of the leukocyte antigen CD18 and the absence of expression of the lymphocytic markers CD3 and CD79, respectively, specific of T-cell and B-cell lineages (Figure 2).

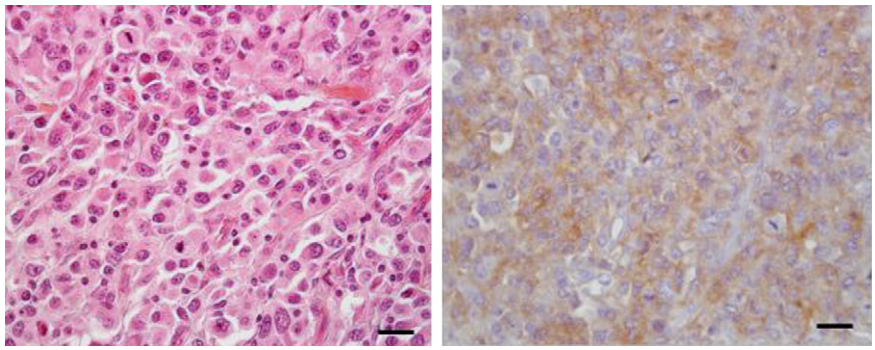

Figure 2. Histopathological and immunohistochemical features of HS. Left: neoplastic proliferation of a pleomorphic population of large round uni- or multinucleated cells, with marked atypia and high mitotic index; hemalun–eosin staining; bar = 30 μm. Right: neoplastic cells expressing the leukocyte CD18 marker (immunoperoxidase reaction, diaminobenzidine as chromogen, hematoxylin counterstaining); bar = 30 μm.

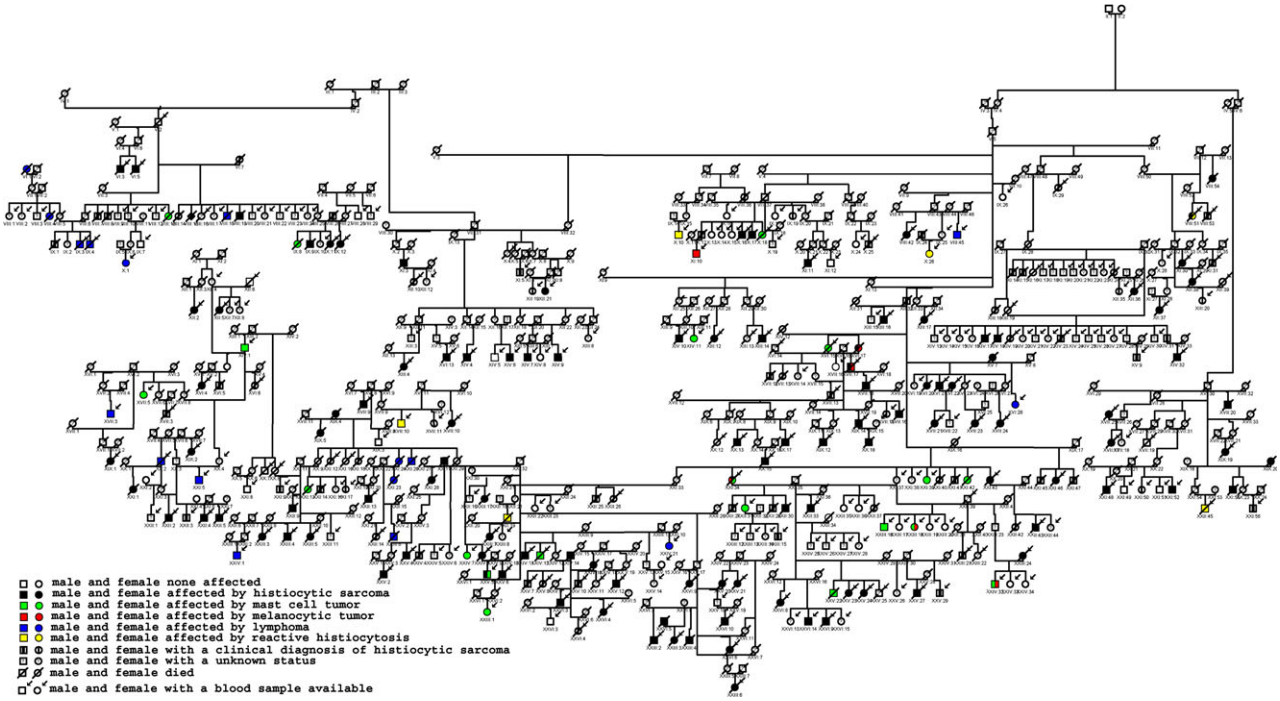

Figure 3. A portion of 650 dogs of the BMD family segregating HS.

Therapy and Biological Behavior

Follow-up and treatment information were available for all dogs. A surgical procedure was performed in 39 dogs, either for diagnosis purpose or as a therapeutic procedure. Among the 19 therapeutic surgeries, 13 (68%) consisted of splenectomy. In 35 cases, a palliative and supportive corticosteroid therapy (prednisone: 0.5–2 mg/kg) was performed. For 19 dogs, corticosteroid therapy was associated with antibiotics (cefalixin, quinolone, amoxicillin, neomycin, etc.) or anabolic steroid. Only 7 dogs received chemotherapy, with lomustine being the most commonly used drug. Clinical outcome reflected the very aggressive behavior and poor prognosis of this disease. Survival data were available for 77 affected BMDs. Mean survival time from diagnosis to death was 49 days, with the median time at 30 days (1 month). Less than 10% of the dogs lived longer than 4 months, and 61 dogs (79%) were euthanazied.

Pedigree Analysis

A pedigree of 327 BMDs (144 males, 183 females) was developed from the 800 French and European dogs (Figure 3). A total of 121 dogs (58 males and 63 females) had a clinical diagnosis of HS, and among those, 98 (47 males, 51 females) had a histopathology report. Among the remaining 206 dogs, 48 individuals were older than 10 years without evidence of HS or other cancers (20 males and 28 females) and were considered to be healthy controls. Other cancers observed in the sampled dogs included 13 lymphomas (7 males, 6 females), 18 mast cell tumors (7 males and 11 females), and 6 melanomas (5 males and 1 female). Because all the dogs used for the present study were collected in France and adjacent European countries, analysis of their pedigree structure revealed multiple common ancestors.

For each dog, pedigree data were collected on ancestors, and a family of 21 146 dogs (9989 males and 11 457 females) was constructed. The oldest dog was born in 1931, the youngest in 2007. The average time between 2 generations was 3.7 years with a standard deviation of 1.2 years. Pedigrees of sampled dogs were linked up, allowing the tracing of these dogs through several generations, the average being 20 generations. To minimize the effect of pedigree time depth, we calculated consanguinity average for dogs born between 1998 and 2004 to be 3.2%. This was close to values described by Calboli et al. (2008) (0.024–0.071) but is likely still an underestimation as the data is missing for many founders of this pedigree. The pedigree derives largely from a few male ancestors who were mated multiple times. At each generation, only 5.5 % (± 0.3%) of sires and 13.2 % (± 0.3%) of dams were used for reproduction, with only 0.78% (± 0.1%) of sires and 3% (± 0.3%) of dams producing more than the half of next generation (Figure 4). For example, among male dogs born between 2000 and 2002, 3 dogs produced more than 120 puppies.

Mode of Inheritance

Twenty-one nuclear families (including a total of 160 dogs) with at least 2 phenotyped offspring, one of them being

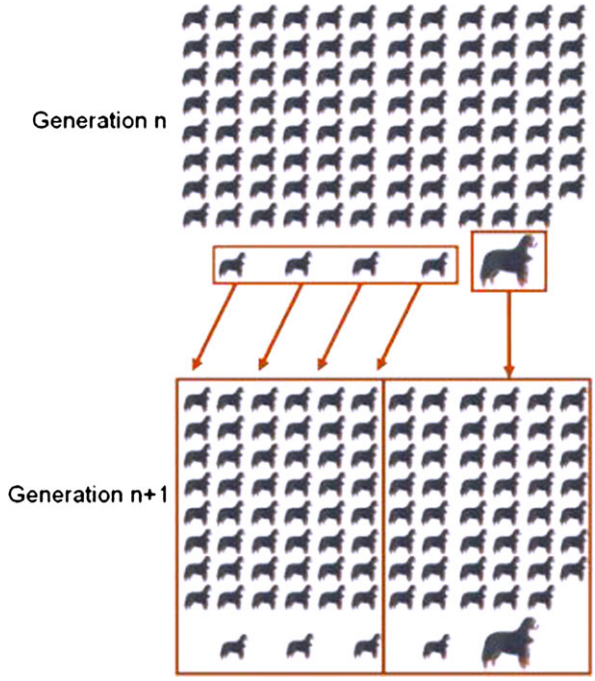

Figure 4. Illustration of the popular sire effect in the population of French BMDs: Only 5.4% of sires participate to the next generation (dogs in the rectangle), of which only 0.78% of sires (dog in the square) produce more than 50 % of the next generation (generation $n + 1$).

affected, could be identified from the larger pedigree. Parental status was available for 29 parents (69% of the 42 parents) with only 2 nuclear families having both parental status missing. Forty-six offspring were affected, representing 39% of the 118 offspring from the set. Dogs older than 10 years that have not been diagnosed with any other cancer were considered as healthy; the other unaffected dogs were considered as unknown status. The number of affected offspring expected in these 21 families under a recessive model was computed considering 3 different probabilities of being affected for parents of unknown status: $pk = 0.20$, $pk = 0.25$, and $pk = 0.3$. Results are presented in Table 1.

Table 1. Comparison of the expected proportion of affected offspring in the 21 nuclear families under a fully recessive model and the observed proportion (pk is the estimation of the prevalence of the disease in the breed).

	Frequency of the disease among parents of unknown status (pk)		
	0.20	0.25	0.30
Expected proportion of affected offspring under recessive model	0.46	0.47	0.48
P value**	0.10	0.06	0.04

**P value of the t-test comparing the expected proportion with the observed proportion (0.39).

The expected proportion of affected offspring under a fully recessive model is not very sensitive to the values of pk. It is clearly higher than the observed proportion in all cases, although the significance of the t-test comparing them is only strictly achieved (P value < 0.05) when $pk = 0.30$. These results show that the fully recessive model did not explain the segregation of the disease observed in these families. An oligogenic model was likely to be a better description of the genetic model underlying the disease.

Discussion

The long-term goal of our joint project is the identification of genetic factors contributing to HS in the BMD, particularly with comparative pathologic perspective. Dogs are indeed an exceptional model for studying the genetic bases of cancer (Cadieu and Ostrander 2007). With substantial recent progress in veterinary medicine, dogs live longer and are affected by many cancer types. Indeed, about 4 million new cancer diagnoses are recorded in dogs within the United States every year (Khanna et al. 2006). Naturally occurring cancers in pet dogs and humans have been shown to share many common features, including tumor genetics, molecular mechanisms of oncogenesis, histological appearance, and biological behavior. Studying dogs with naturally occurring cancers is likely to provide a valuable perspective different from that generated by the study of rodent cancers alone, often obtained after induction (Paoloni and Khanna 2007).

Histiocytic proliferative disorders are rare in humans, precluding the possibility of an efficient study of the genetic components of these diseases (Arico and Danesino 2001). Histiocytic disorders in human are classified according to the cell of origin as dendritic cell–related disorders and macrophage-related disorders (Schmitz and Favara 1998). Most HS cases in BMDs have been reported to be of dendritic antigen-presenting cell origin even though a distinctive neoplastic proliferation of macrophages has been recently described in dogs (Affolter and Moore 2002; Moore et al. 2006). The human equivalents of HS could encompass 2 types of aggressive proliferation of dendritic cells: disseminated LCH, which is an accumulation of tissue histiocytes in multiple organs and is generally associated with extensive multiorgan failure, and/or dendritic cell–related HS, which is a very rare malignancy featuring cells with a dendritic phenotype (Schmitz and Favara 1998). In France, with 50 recorded cases per year, the incidence is estimated to 1/200 000 children of less than 15 years per year (Donadieu et al. 1996). Due to the lack of familial cases in humans, and the low frequency of the disease overall, the physiopathology of LCH and its genetic components are still unknown. The observation, however, that up to 1% of all patients have a first-degree relative with LCH add considerable weight to the view that LCH patients may have an underlying "genetic predisposition" (Arico and Danesino 2001; Egeler et al. 2004). Identifying causative genes in dogs may help to highlight predisposing genes in human and/or

at least common pathways and genes. Therefore, the BMD breed represents a unique model to study genes involved in neoplastic transformation of histiocytes of the dendritic lineage, as well as genes more generally involved in other cancer types.

To be a powerful model in comparative pathology, the canine disease has to be characterized as precisely as possible and compared with the human counterpart. Such characterization is also useful for veterinary medicine in order to improve the diagnosis of the disease, as well as sample collection. For this characterization, our study included a large number of dogs. We have collected 800 BMDs of which 200 are affected by HS. A subset of 327 was selected to construct a pedigree segregating the disease. A subset of 21 nuclear families with full sibships (160 dogs) was retained to study the mode of inheritance of HS. Finally, 89 fully characterized and demonstrated cases of HS were used to describe the epidemiology as well as the clinical and pathological features of the disease. We found HS as a highly aggressive neoplastic disease of adult dogs (mean age at diagnosis 6.5 years) that affects males and females with an equal frequency. The absence of sex predisposition, also noticed by Padgett et al. (1995), allowed us to exclude the involvement of gene products related to sex chromosomes as determining factors.

We are currently unable to demonstrate or reject links between environmental exposures and HS. Indeed, information regarding canine exposure to carcinogenic substances is difficult to obtain. Interestingly, predisposing factors that have been suspected in humans, that is, vaccination for juvenile LCH and smoking for adult LCH (Donadieu et al. 1996; Arico and Danesino 2001) could not be postulated for dogs.

Moreover, pedigree analysis and clinical study showed the presence of other cancers in HS-affected dogs or relatives. More than 40% of cases displayed related cases of malignant neoplastic diseases other than HS, as close as 2 generations, as lymphoma, mast cell tumor, or melanoma. In the subset of 21 nuclear families, analyzed in detail for the segregation analysis, 12 mast cell tumors (7.5%), 5 lymphomas (frequency 3.1%), and 5 melanocytic tumors (frequency 3.1%) were found (data not shown). The frequency of mast cell tumors and lymphoma are increased in comparison to the frequency of these cancers in the general dog population reported to be, respectively, 90 per 100 000 dogs (0.09%) and 13–24 per 100 000 dogs (0.02%) (Jacobs et al. 2002; Gross et al. 2005). An increased number of hematopoietic cancers in BMDs was also found by Ramsey et al. (1996). Interestingly, in human, Egeler et al. (1993) reported associations of LCH and malignant neoplasms including malignant lymphoma or Hodgkins disease, as we observed in our population. It is possible that genes involved in the malignant neoplastic proliferation of dendritic cells in HS could also promote the neoplastic transformation of other hematopoietic cell lineages or that genes more generally involved in neoplastic transformation are fixed in the BMD breed.

The clinical course of HS was very similar in all affected dogs, but the most common clinical signs were nonspecific and consistent with a general illness (anorexia, weakness, weight loss, . . ., etc.). However, a common feature was the presence of internal masses at time of diagnosis (82% of the cases), commonly in the spleen, lungs, mediastinum, and lymph nodes, with frequent multiple organ involvement (55% of the cases).

In agreement with previous reports, the prognosis of BMDs with HS was very poor in our population as was response to treatment (Skorupski et al. 2007). There were, however, few reports of therapeutic trials and survival rates in dogs with HS. Most of the previously published studies refer to single case reports or to a series that includes mixing cases of localized and disseminated HS, often in different breeds of dogs (Rosin et al. 1986; Ramsey et al. 1996; Affolter and Moore 2002). Disseminated HS progresses rapidly to death or euthanasia, but localized HSs of the subcutis or joint are less devastating. They can be cured by early surgical excision. (Affolter and Moore 2002; Fidel 2006). In fact, 2 studies reported that appropriate treatment can improve survival. The first reported that flat coated retrievers with chemotherapy or radiation have a median survival of 182–185 days. But most of the HSs were localized, and, as expected, the presence of metastasis was associated with shorter survival times (Fidel et al. 2006). The second study reported responses to lomustine. The median survival time was 106 days for 59 dogs affected by HS who received treatment with lomustine. But the authors reported that anemia, thrombocytopenia, and splenic involvement were all associated with a poor prognosis. Dogs with anemia or thrombocytopenia have a median survival less than 30 days, instead of 163–165 days. Dogs with splenic involvement have a median survival of 58 days, rather than the more typical 165 days for dog without involvement (Skorupski et al. 2007). In our population, hematologic alterations and splenic involvement were frequent, which may partially explain the poor prognosis and short survival time observed. It seems indeed that this population of BMD was affected by a highly aggressive disseminated form of the disease, as compared with other breeds. Flat coated and golden retrievers, for example, are reported to be less frequently affected, and, in these 2 breeds, the sarcoma is generally localized and not disseminated as is observed in the BMD (Affolter and Moore 2002; Fidel et al. 2006). HS, in our BMD population, displayed reproducible presentation and clinical course. These homogenous features are probably related to common physiopathologic mechanisms of oncogenesis in most if not all the cases. These argue in favor of the use of the HS model in order to help to elucidate the complex mechanisms of the human LCH. This is also in favor of a major role of the genetic background of the BMD breed in the neoplastic transformation of dendritic cells. To get insight into these mechanisms, targeted gene expression analyses have been performed, excluding candidate genes such as *KIT*, *FLT3*, and *MET* (Zavodovskaya et al. 2006) as well as *TNFa*, *IL1a*, and *IL1b* (Soller et al. 2006).

However, to date, no susceptibility locus has been reported in the literature.

The BMD breed, as many others, was developed at the beginning of the 20th century with very few founders. Since that date, genetic diversity has been restricted by pedigree barrier and reduced by use of popular sires. The BMD population was originally developed from the Berne region of Switzerland. The club was founded in 1907 with 6 registered officially used for reproduction. Dogs were initially exported to other European countries in the 1920s and brought to the United States in 1926 (http://www.akc.org/breeds/bernese_mountain_dog/history.cfm), the present population being largely derived from the original set of 6 males. Analyses of the US and the European populations reveal common ancestors. The breed has since gained in popularity in several countries and was ranked 41st in 2006 with nearly 4000 new American Kennel Club (AKC) registrations per year. It is ranked 15th in 2006 with 2900 new registrations per year in France (http://www.scc.asso.fr). Despite the significant increase in registrations, particularly since the 1970s, BMDs are still regularly imported from Europe to the United States to enhance breeding programs. In France, a few champion males born in the 1980s have produced 400 puppies. If any of these males were affected or were carriers, it could easily explain the fact that the disease has spread so rapidly and in so few generations. Moreover, as in many dog breeds, the use of popular sires and inbreeding reduces diversity. These restrictive breeding practices reduce the effective population size. The intensive selection performed in breeds can lead to a loss of genetic diversity, as shown by Calboli at al. (2008) demonstrating that many canine breeds lose more than 90% of singleton variants in just 6 generations. In the BMD breed, small founding population and popular sire effects have enriched risk alleles for HS, where the estimated frequency of the risk allele is now 25%. In France, 80% of HS diagnoses occur in BMDs, yet the BMD is only the 13th most popular breed and represents 1.8% of dogs registered in 2007 in France (http://www.scc.asso.fr). In our population, epidemiological data and pedigree analysis showed that 78% of affected dogs have relatives with a diagnosis of HS. To study the mode of inheritance, we have used a subset of 160 dogs, and to avoid biases, only full sibships with known HS status were used. Although the expected proportion of affected offspring under a fully recessive model is not very sensitive to the values of HS prevalence, the significance of the *t*-test is only strictly achieved when the prevalence equals 30%. The expected prevalence of HS in BMD is 25%. Thus, the exclusion of a fully recessive model, although probable, is not significant (P value = 6%). Furthermore, the number of affected dogs in siblings with 1 or 2 affected parents is lower than expected for a monogenic transmission mode leading us to rule out a fully autosomal recessive mode of inheritance as proposed by Padgett et al. (1995). This could be explained by the involvement of several genes with environmental factors possibly acting on the age of onset and/or the severity of the disease. We thus favor an oligogenic model underlying the disease, rather than an autosomal recessive inheritance with incomplete penetrance. Moreover, the incidence of other hematopoietic neoplasia in the same families segregating HS could be suggestive of the involvement of several genes acting on general tumorigenic mechanisms.

In order to search for predisposing genes to this devastating disease, complementary genetic mapping projects are underway: 1) a genetic linkage study using the large multigenerational European BMD pedigree, 2) a whole-genome association study with matched cases and controls collected in the United States, and 3) a RNA profiling analysis on tumor versus healthy tissue samples. Altogether, these complementary approaches are expected to identify the genes for HS in the BMD, leading to a better knowledge of histiocytic associated diseases in dog and human.

Funding

AKC-Canine Health Foundation (grant 936b to B.H., E.C.); Italian Società Italiana Bovaro del Bernese club and Swiss Mountain Dogs International Working Group (Alberto Vittoni Award to B.H.); The Centre National de la Recherche Scientifique; National Human Genome Research Institute of the National Institutes of Health.

Acknowledgments

We thank the very cooperative French BMD Club association Française des Bouviers suisses, the Italian Società Italiana Bovaro del Bernese, and the Belgian and Swiss club of BMDs; the numerous veterinarians for providing us with clinical data and samples; and the many dog owners and breeders who provided samples and genealogical data of their dogs. We also thank the pathology laboratories who actively participated by sending us all pathology reports (particularly the LAPVSO, Toulouse, France, and Idexx Alfort laboratory, Alfortville, France).

References

Affolter VK, Moore PF. 2000. Canine cutaneous and systemic histiocytosis: reactive histiocytosis of dermal dendritic cells. Am J Dermatopathol. 22:40–48.

Affolter VK, Moore PF. 2002. Localized and disseminated histiocytic sarcoma of dendritic cell origin in dogs. Vet Pathol. 39:74–83.

Arico M, Danesino C. 2001. Langerhans' cell histiocytosis: is there a role for genetics? Haematologica. 86:1009–1014.

Cadieu E, Ostrander EA. 2007. Canine genetics offers new mechanisms for the study of human cancer. Cancer Epidemiol Biomarkers Prev. 16:2181–2183.

Calboli FC, Sampson J, Fretwell N, Balding DJ. 2008. Population structure and inbreeding from pedigree analysis of purebred dogs. Genetics. 179(1):593–601.

Donadieu J, Thomas C, Emile JF, Teillac-Hamel D, Landman-Parker J, Aubier F, Fagnoux C, Brousse N. 1996. Histiocytose langerhansienne: mise au point. Medecine Thérapeutique. 2:441–451.

Egeler RM, Neglia JP, Puccetti DM, Brennan CA, Nesbit ME. 1993. Association of Langerhans cell histiocytosis with malignant neoplasms. Cancer. 71:865–873.

Egeler RM, Annels NE, Hogendoorn PC. 2004. Langerhans cell histiocytosis: a pathologic combination of oncogenesis and immune 670 dysregulation. Pediatr Blood Cancer. 42:401–403.

Fidel J, Schiller I, Hauser B, Jausi Y, Rohrer-Bley C, Roos M, Kaser-Hotz B. 2006. Histiocytic sarcomas in flat-coated retrievers: a summary of 37 cases (November 1998–March 2005). Vet Comp Oncol. 4(2):63–74.

Galibert F, André C. 2007. The dog: a powerful model for studying genotype-phenotype relationships. In: Prunet PE, editor. New York.

Gross TL, Ihrke PJ, Walder EJ, Affolter VK. 2005. Mast cell tumors. Chapter 36. Skin diseases of the dog and cat. 2nd ed. Oxford: Blackwell publishing. p. 853–858.

Jacobs RM, Messick JB, Valli VE. 2002. Tumors of the hemolymphatic system. Chapter 3. In: Donald J Meuten, editor. Tumors in domestic animals. 4th ed. 119–198.

Jones P, Chase K, Martin A, Davern P, Ostrander EA, Lark KG. 2008. Single-nucleotide-polymorphism-based association mapping of dog stereotypes. Genetics. 179(2):1033–1044.

Karlsson EK, Baranowska I, Wade CM, Salmon Hillbertz NH, Zody MC, Anderson N, Biagi TM, Patterson N, Pielberg GR, Kulbokas EJ 3rd, et al. 2007. Efficient mapping of Mendelian traits in dogs through genome-wide association. Nat Genet. 39(11):1321–1328.

Khanna C, Lindblad-Toh K, Vail D, London C, Bergman P, Barber L, Breen M, Kitchell B, McNeil E, Modiano JF, et al. 2006. The dog as a cancer model. Nat Biotechnol. 24:1065–1066.

Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, Chang JL, Kulbokas EJ 3rd, Zody MC, et al. 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature. 438(7069):803–819.

Moore PF. 1984. Systemic histiocytosis of Bernese mountain dogs. Vet Pathol. 21:554–563.

Moore PF, Rosin A. 1986. Malignant histiocytosis of Bernese mountain dogs. Vet Pathol. 23:1–10.

Moore PF, Affolter VK, Vernau W. 2006. Canine hemophagocytic histiocytic sarcoma: a proliferative disorder of CD11dp macrophages. Vet Pathol. 43(5):632–645.

Ostrander EA, Galibert F, Patterson DF. 2000. Canine genetics comes of age. Trends Genet. 16:117–124.

Padgett GA, Madewell BR, Keller ET, Jodar L, Packard M. 1995. Inheritance of histiocytosis in Bernese mountain dogs. J Small Anim Pract. 36:93–98.

Parker HG, Ostrander EA. 2005. Canine genomics and genetics: running with the pack. PLoS Genet. 1:e58.

Paoloni MC, Khanna C. 2007. Comparative oncology today. Vet Clin N Am Small Anim Pract. 37(6):1023–1032.

Quignon P, Herbin L, Cadieu E, Kirkness EF, Hédan B, Mosher DS, Galibert F, André C, Ostrander EA, Hitte C. 2007. Canine population structure: assessment and impact of intra-breed stratification on SNP-based association studies. PLoS ONE. 2(12):e1324.

Ramsey IK, McKay JS, Rudorf H, Dobson JM. 1996. Malignant histiocytosis in three Bernese mountain dogs. Vet Rec. 138:440–444.

Rosin A, Moore P, Dubielzig R. 1986. Malignant histiocytosis in Bernese Mountain dogs. J Am Vet Med Assoc. 188:1041–1045.

Schmitz L, Favara BE. 1998. Nosology and pathology of Langerhans Cell Histiocytosis. Hematol Oncol Clin N Am. 12:221–246.

Soller JT, Murua Escobar H, Janssen M, Fork M, Bullerdiek J, Nolte I. 2006. Cytokine genes single nucleotide polymorphism (SNP) screening analyses in canine malignant histiocytosis. Anticancer Res. 26(5A):3417–3420.

Skorupski KA, Clifford CA, Paoloni MC, Lara-Garcia A, Barber L, Kent MS, LeBlanc AK, Sabhlok A, Mauldin EA, Shofer FS, et al. 2007. CCNU for the treatment of dogs with histiocytic sarcoma. J Vet Intern Med. 21(1):121–126.

Wayne RK, Ostrander EA. 2007. Lessons learned from the dog genome. Trends Genet. 23(11):557–567.

Zavodovskaya R, Liao AT, Jones CL, Yip B, Chien MB, Moore PF, London CA. 2006. Evaluation of dysregulation of the receptor tyrosine kinases Kit, Flt3, and Met in histiocytic sarcomas of dogs. Am J Vet Res. 67(4):633–641.

Received November 25, 2008; Revised April 8, 2009; Accepted May 15, 2009

Corresponding Editor: Dr Marilyn Menotti-Raymond