Distinct B-Cell and T-Cell Lymphoproliferative Disease Prevalence among Dog Breeds Indicates Heritable Risk

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Abstract

Immunophenotypes in lymphoproliferative diseases (LPD) are prognostically significant, yet causative factors for these conditions, and specifically those associated with heritable risk, remain elusive. The full spectrum of LPD seen in humans occurs in dogs, but the incidence and lifetime risk of naturally occurring LPD differs among dog breeds. Taking advantage of the limited genetic heterogeneity that exists within dog breeds, we tested the hypothesis that the prevalence of LPD immunophenotypes would differ among different breeds. The sample population included 1,263 dogs representing 87 breeds. Immunophenotype was determined by the presence of clonal rearrangements of immunoglobulin heavy chain or T-cell receptor γ chain. The probability of observing the number of B-cell or T-cell tumors in a particular breed or breed group was compared with three reference populations. Significance was computed using χ^2 test, and logistic regression was used to confirm binomial predictions. The data show that, among 87 breeds tested, 15 showed significant differences from the prevalence of LPD immunophenotypes seen across the dog population as a whole. More significantly, elevated risk for T-cell LPD seems to have arisen ancestrally and is retained in related breed groups, whereas increased risk for B-cell disease may stem from different risk factors, or combinations of risk factors, arising during the process of breed derivation and selection. The data show that domestic dogs provide a unique and valuable resource to define factors that mediate risk as well as genes involved in the initiation of B-cell and T-cell LPD. (Cancer Res 2005; 65(13): 5654-61)

Introduction

Recent advances have improved our understanding of the genetic basis of cancer and the mechanisms of oncogenesis; yet, progress in the identification of genetic factors that define risk, as well as improvements in molecular diagnosis and classification of many cancers, has been less rapid. Causative factors, and specifically those associated with heritability, remain elusive, even for lymphoproliferative diseases (LPD) that have prognostically significant molecular signatures (1). This is due, at least in part, to the fact that discovery of cancer-associated genes in human populations is hampered by structural features that characterize most of these populations (i.e., they are outbred) have long generation times (averaging 15-25 years) and generally have <10 offspring per lifetime surviving to sexual maturity (2). These features result in the propagation of genes with weak penetrance, which often combine to form highly variable phenotypes. It is therefore not surprising that for complex diseases, such as LPD, there seems to be a broad spectrum of molecular diseases with considerable variation in natural history and response to therapy. Very few cases of familial lymphoma and leukemia are encountered that are not associated with established germ line mutations, such as the p53 mutation in Li-Fraumeni syndrome (3). The study of LPD in populations with restricted genetic heterogeneity is a strategy that should improve the likelihood of identifying factors that mediate risk and contribute to the pathogenesis of these conditions.

Domestic dogs offer a robust model to identify heritable factors for various diseases. Dogs are organized into >350 phenotypically distinct genetic isolates ("breeds") that are characterized by unique constellations of morphology, behavior, and susceptibility to specific diseases, including LPD (4). Humans and dogs have similar physiology, share extensive genome homology with a high degree of preserved gene order, and are exposed to the same environment (4). The limited level of genetic heterogeneity within dog breeds, combined with the fact that the incidence and lifetime risk of naturally occurring LPD differs among dog breeds, offers a unique opportunity to identify genetic risk factors that contribute to the pathogenesis of LPD.

The full spectrum of hematologic malignancies occurs in dogs, with features of clinical presentation, histology, and biology that closely parallel those of human malignancies (5, 6). Excluding breed as a discriminating criterion, B-cell and T-cell neoplasms occur at similar frequencies in dogs (5, 7–9) as they do in many human populations: non-Caucasians in the United States (10), indigenous Japanese [in human T lymphotropic virus type I (HTLV-I) nonendemic areas; ref. 11], Indians (12), and Chinese (11). In addition, LPD [and non-Hodgkin's lymphoma (NHL) in particular] occurs more frequently in dogs than in humans, and specific dog breeds have a distinct, significant, and reproducible predisposition for NHL (13, 14), suggesting that genetic risk (or protective) factors

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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for the disease have segregated with breed-specific traits. For example, when compared with the average risk of any dog to develop lymphoma, the risk for a Boxer is ~4-fold higher, whereas the risk for a Pomeranian is ~10-fold lower (13).

Familial clustering has been reported for canine NHL (15). A recent study showed that the lifetime risk for the disease in Golden Retrievers in the United States is ~1:8 (16) compared with a lifetime risk for people of ~1:50 (17). This suggests that breed barrier rules, which perpetuate inbreeding and line breeding, may have increased the homogeneity of alleles that contribute to risk and possibly even influenced the penetrance of these factors in the population. As importantly, the observation that breed type also influences response to therapy (18) suggests that genetic factors modulate disease progression and are thus prognostically significant.

Materials and Methods

Sample population. Samples were identified from a "volunteer" data set maintained by the Immunopathology Laboratory of the Colorado State Diagnostic Laboratory (DL-ImmLab). The DL-ImmLab provides a service for veterinarians who wish to submit samples for PCR-based antigen receptor gene rearrangement (PARR) testing. From this data set, we identified cases submitted sequentially that included a differential diagnosis of LPD (NHL, lymphoid leukemia), which were analyzed to assess clonality and molecular phenotype [immunoglobulin heavy chain (IgH) or T-cell receptor γ chain (TCRγ) rearrangements; ref. 19]. A histologic diagnosis was not specifically included for approximately half of the cases in the data set, but we have shown previously that the finding of a clonal population of lymphocytes using this assay in cases where LPD was not confirmed histologically is 92% specific for the diagnosis of lymphoid malignancy (19). All cases with a diagnosis distinct from LPD (e.g., nonlymphoid leukemias, inflammatory lesions, and infection with *Ehrlichia canis*) were excluded from the analyses. Samples where a clonal rearrangement could not be identified (n = 7) or that were not suitable for PCR analysis (n = 10) were only included when additional data (lymphadenopathy or monomorphic leukocytosis with dominant populations of B or T cells defined by flow cytometry and/or immunohistology) specifically indicated a diagnosis of B-cell or T-cell LPD. Diagnoses were based on routine clinical, histologic, and laboratory criteria as described previously (6, 8, 20, 21). Samples included 1,263 dogs representing 87 pure breeds. In samples where PARR and flow cytometry (n = 102) or PARR and immunohistology (n = 64) were done concurrently, the agreement was 97% for B-cell tumors and 93% for T-cell tumors. The discordant samples included cases where a clonal rearrangement was undetectable (two B-cell tumors and five T-cell tumors) and cases that harbored clonal rearrangements in both IgH and TCRγ (two T-cell tumors). Therefore, where a single clonal receptor rearrangement can be identified, the nature of the rearrangement can be used to unambiguously assign a phenotype to the tumor.

Breed confirmation and genotyping. The genetic derivation for 25 samples selected at random was examined by microsatellite mapping to ascertain the veracity of breed data as reported by owners or veterinarians in sample submission forms. Ninety-six microsatellite markers were genotyped on 25 random DNA samples as described previously (22). These genotypes were combined with a data set of 414 purebred dogs representing 85 breeds and 5 additional purebred dogs that did not belong to any of the 85 breeds in the data set (22) as controls. Breed membership was analyzed using two complementary methods. The first made use of the prior information from 85 breeds with a leave-one-out analysis implemented in the Doh assignment calculator (http://www2.biology.ualberta.ca/jbrzusto/Doh.php). The second, clustering analysis, used no prior population information to group the dogs in population clusters using *Structure* as described (22). Three samples were clustered with Lhasa Apso, Shih Tzu, and Pekingese, which are three breeds that are difficult to distinguish from one another (22). These three samples were then analyzed separately with only those breeds to determine which one was the most likely contributor. The assignment calculator identified 24 of 25 individuals as the reported breed. One dog was reported as a Shih Tzu, but assignment determined it was a Lhasa Apso. Clustering analysis supported the Lhasa Apso conclusion and also identified two dogs, a Rhodesian Ridgeback and a Beagle, as possible crosses (Supplementary Table S1). Using a correlation of 22 of 25 or 24 of 25 identical matches from a random sample of the population, the accuracy of self-reporting for breeds in this study is estimated at >87% and could be >95%.

Statistical analyses. We applied several methods to assess the distribution of LPD immunophenotypes among dog breeds. First, we used descriptive statistics to summarize the frequency of the variable occurrences. Specifically, we used the binomial distribution to calculate the probability of observing the number of B-cell tumors (and equivalently, the number of T-cell tumors) in a particular breed or group of breeds and compared this with three reference populations: (*a*) all other dogs in our data set, excluding the breed or group under analysis, (*b*) mixed-breed dogs, and (*c*) the rate identified in the literature, reporting of the *P*s of these occurrences if these comparison populations were truth. However, because these values assume that the comparative values were constant or true values, they would overestimate the significance of differences by not taking into account the variability in the reference populations. Thus, we also computed significance based on breed (or breed group) using a χ^2 test with 2×2 tables. Because of the exploratory nature of this study, we did not adjust for multiple comparisons. We note also that the power of these tests at the breed level was low, especially for breeds that included <20 animals, so the data may underestimate some breed-related differences to the reference populations. Finally, we used logistic regression to confirm binomial predictions regarding differences among aggregates of breeds grouped together by function (i.e., using American Kennel Club (AKC) breed groups ref. 23) or by genetic relatedness (22) and the reference groups.

Comparative genomic hybridization. To determine the presence of numerical chromosome aberrations, comparative genomic hybridization (CGH) analysis was done on 54 cases of canine lymphoma, comprising 38 B-cell cases and 16 T-cell cases. High molecular weight genomic DNA was isolated from tumor biopsy specimens and processed as described previously (24).

Results

The prevalence of B-cell and T-cell lymphoproliferative disease differs among dog breeds. Eighty-seven different pure breeds and a group of mixed-breed dogs (ranging from 1 individual for 18 breeds to 243 individuals for the mixed-breed group) were included in the analysis (Supplementary Table S2). The breed distribution and age at diagnosis were consistent both with breed popularity and with the reported relative risk for LPD (13), including >25 cases each from Golden Retrievers (n = 237), Labrador Retrievers (n = 105), Cocker Spaniels (n = 47), Rottweilers (n = 47), Boxers (n = 39), German Shepherd dogs (n = 34), and Doberman Pinschers (n = 27), whereas samples from others, such as Chihuahuas (n = 5) and Pomeranians (n = 1), were seen less frequently. There was a unimodal association between age and LPD, with a progressive increase that peaked at 11 years of age and then decreased (Fig. 1*A*). The mean age among all dogs was 9.1 years (SD, 3.2 years). The greatest number of cases were observed in the 10- to 11-year age group, with >90% of cases occurring in dogs over 5 years old (Supplementary Fig. S1).

Excluding cases that could not be definitively classified due to the concomitant presence of IgH and TCRγ rearrangements (3% or 37 of 1,263), the distribution of molecular immunophenotypes across the population under study was 61.4% B-cell tumors (753 of 1,226) and 38.6% T-cell tumors (473 of 1,226; Supplementary Table S2). The mean age did not differ between dogs

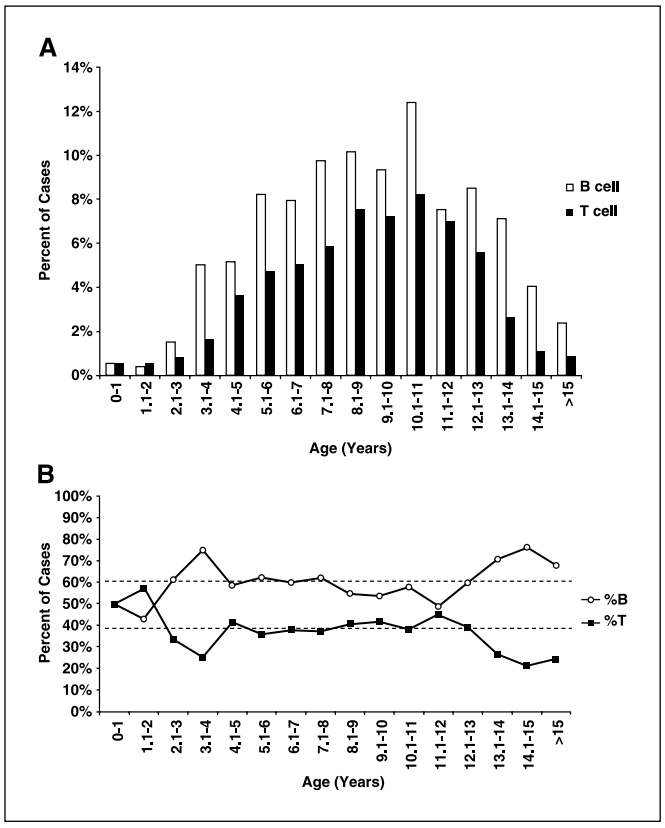

Figure 1. Phenotype distribution by age and cell type. *A,* LPD occurrences for each age group (1-year intervals) as a percentage of cases for each phenotype. *B,* frequency of B-cell and T-cell disease for each age group (1-year intervals). *Dashed lines,* average frequency of B-cell and T-cell LPD from all dogs of all breeds and ages included in the study.

immunophenotyped as B or T cell, but those typed as "B + T" were slightly older (mean age, 10.1 years; $P = 0.040$, compared with dogs with T-cell disease; $P = 0.067$, compared with dogs with B-cell disease). This distribution was not statistically significantly different from the combined prevalence reported in 642 dogs from 10 previous contemporary studies (mean, 66.8% B cells, 27.3% T cells; range, 55-82% B cells and 18-42% T cells, with an average of 4% classified as "null" cell or biphenotypic) done between 1984 and 1997 (5, 7–9, 20, 25–29).

The data showed persuasive breed-specific variability of LPD prevalence. We applied various statistical tests to the data to define the significance of these differences. The first type of analysis was to simply examine the frequency of occurrences in our population and compare them to a fixed reference, such as the number expected from previous reports in the literature, all dogs included in our data set (excluding the group under comparison), or mixed-breed dogs in our data set. The latter reference group was included not only because it contained the largest number of individuals in the data set, providing high power for comparisons, but also because it represents an "experiment of nature" that shows how interbreeding might affect disease predisposition across genetically restricted populations.

Table 1*A* shows breeds that were significantly different on B-cell versus T-cell disease from at least one of the reference populations based on a binomial distribution. Some breeds were significantly different from each reference population (e.g., Shih Tzu and Siberian Husky showed excess occurrences of T-cell LPD and Cocker Spaniel and Basset Hound showed excess occurrences of B-cell LPD). Several dog breeds (e.g., Rottweilers and Standard Schnauzers) were significantly different from "all other breeds" in our data set but not from mixed-breed dogs or from the frequency expected from previous reports in the literature. Finally, some breeds (e.g., Irish Wolfhounds, Airedale Terriers, and Chinese Shar-Peis) were significantly different from mixed-breed dogs and from dogs reported in the literature previously but not from "all other breeds" in this study. The converse was true for mixed breeds, which were different from a group, including all "all other breeds" in this study (i.e., pure-bred dogs considered as a single group) but not from dogs reported in the literature previously. The reason for these latter two observations is that the large number of Golden Retrievers in our data set introduces an overweighting into this reference population toward a larger representation of T-cell phenotypes.

There were other possible sources of bias in the data set. First, only 610 cases in the data set had a histologic diagnosis. Thus, given the specificity of the PARR assay, it is possible that as many as 52 of the remaining 656 cases might have been false positives. Second, 92 of these 610 (15%) cases were acute or chronic leukemias or multiple myelomas. To assess the potential impact of these sources of bias, we repeated the analysis by first excluding cases without a histologic (or cytologic) diagnosis ($n = 610$) and then by including only dogs with NHL ($n = 518$). Table 1*B* shows that when we only included dogs for which a histologic or cytologic diagnosis was available, the prevalence of B-cell and T-cell disease in the major breeds shown in Table 1*A* remained significantly different from control populations. Because of the reduced sample size, Irish Wolfhounds, Cavalier King Charles Spaniels, Yorkshire Terriers, Chinese Shar-Peis, and Basset Hounds were no longer different from controls. It is noteworthy, however, that the Basset Hound samples in this group included nine cases, of which eight were B-cell tumors. Similarly, Table 1*C* shows that, if we included only dogs with a histologic or cytologic diagnosis of NHL, the prevalence of B-cell and T-cell disease in the major breeds again remained significantly different from the reference populations. In this data set, differences in the prevalence of B-cell and T-cell tumors between Australian Shepherds and controls were not statistically significant, Corgis appeared as a breed with excess T-cell LPD, and Dobermans fell into the category with excess B-cell LPD.

The different prevalence rates for B-cell and T-cell lymphoproliferative disease are shared among closely related dog breeds. We recognize that comparing different populations using simple descriptive statistics could be misleading, because it does not account for the inherent variability in the reference populations and can overestimate the significance of observed differences by underestimating the natural variation. Thus, we also derived significance using a χ^2 test assuming both populations are estimates. Figure 2 shows the breeds that were significantly different from each of the reference populations based on this analysis. The main differences between the analyses in Table 1*A* and Fig. 2 is that, by the χ^2 test, Airedale Terriers are significantly different from all populations, whereas Doberman Pinschers are only different from all other dogs in the current data set. The observation that Dobermans also were different from dogs reported in the literature when we only included NHL in the analysis suggests that this breed has a small but significant excess of B-cell LPD when compared with the mean for all dog populations. Still, we cannot exclude that the difference between

Table 1. Breed-specific prevalence of B-cell and T-cell LPD

Breed	% B cells	% T cells	*n*	P (mixed-breed dogs)	*P* (other dogs in this set)	*P* (other dogs in literature)
(A) Breeds that differ from expected frequency of B-cell and T-cell LPD*						
Airedale Terrier	20.0	80.0	5	0.0460	0.0754	0.0444
Australian Shepherd	35.7	64.3	14	0.0180	0.0441	0.0169
Basset Hound	94.4	5.6	18	0.0066	0.0017	0.0070
Border Collie	90.9	9.1	11	0.0737	0.0357	0.0767
Boxer	44.7	55.3	39	0.0027	0.0149	0.0024
Cavalier King Charles Spaniel	20.0	80.0	5	0.0460	0.0754	0.0444
Chinese Shar-Pei	33.3	66.7	9	0.0433	0.0823	0.0414
Cocker Spaniel	93.2	6.8	44	<0.0001	<0.0001	<0.0001
Doberman Pinscher	84.6	15.4	26	0.0346	0.0085	0.0371
Golden Retriever	46.4	53.6	224	<0.0001	<0.0001	<0.0001
Irish Wolfhound	0	100	3	0.038	0.057	0.036
Rottweiler	75.0	25.0	47	0.1587	0.0368	0.1695
Scottish Terrier	87.5	12.5	16	0.0580	0.0225	0.0609
Shih Tzu	19.0	81.0	21	<0.0001	<0.0001	<0.0001
Siberian Husky	11.1	88.9	9	0.0010	0.0027	0.0009
Standard Schnauzer	85.7	14.3	14	0.1033	0.0476	0.1076
Yorkshire Terrier	20.0	80.0	5	0.0460	0.0754	0.0444
Mixed breed	66.5	33.5	233		0.0279	0.4851
All dogs (this study)	61.4	38.6	1,226			
All dogs (in the literature)†	66.8	27.3	615			
(B) Breeds that differ from expected frequency of B-cell and T-cell LPD (dogs with histologic diagnosis)*						
Airedale Terrier	0	100	2	<0.025	NS‡	<0.05
Akita	0	100	2	<0.025	NS	<0.05
Australian Shepherd	25.0	75.0	4	<0.05	NS	<0.05
Boxer	38.9	61.1	18	<0.01	<0.025	<0.01
Cocker Spaniel	95.2	4.8	21	<0.05	<0.01	<0.025
Flat-coated Retriever	25.0	75.0	4	<0.05	NS	<0.05
Golden Retriever	49.6	50.4	129	<0.001	<0.001	<0.001
Shih Tzu	12.5	87.5	8	<0.001	<0.001	<0.001
Siberian Husky	0	100	3	<0.01	<0.025	<0.01
Mixed breed	73.4	26.6	94		NS	NS
All dogs (this study)§	63.6	33.8	610			
All dogs (in the literature)†	66.8	27.3	615			
(C) Breeds that differ from expected frequency of B-cell and T-cell NHL*						
Boxer	35.3	64.7	17	<0.001	<0.01	<0.01
Cocker Spaniel	95.2	4.8	21	NS	<0.01	<0.025
Doberman Pinscher	100	0	11	NS	<0.025	<0.05
Golden Retriever	52.3	47.7	109	<0.001	<0.001	<0.001
Shih Tzu	20.0	80.0	5	<0.01	<0.025	<0.025
Siberian Husky	0	100	2	<0.01	<0.05	<0.05
Welsh Corgi	20.0	80.0	5	<0.01	<0.025	<0.025
Mixed breed	78.5	21.5	79		<0.05	NS
All dogs (this study)‖	67.4	30.9	518			
All dogs (in the literature) †	66.8	27.3	615			

*Individual breeds whose B-cell (and T-cell) prevalence differed from that expected across reference populations. Values for mixed-breed dogs and for all dogs in the study are shown for comparison.

†Twenty-seven dogs that did not have a definitive phenotype are excluded from this table.

‡NS, not significantly different ($P > 0.05$).

§Sixteen dogs that did not have a definitive phenotype are excluded from this table.

‖Nine dogs that did not have a definitive phenotype are excluded from this table.

Doberman Pinschers and "all other breeds" in our data set was partly driven by the large number of Golden Retrievers.

Sex and gonadal status (neutering) did not influence this distribution, and although there was a significant association between tumor type or location and molecular phenotype (i.e., extranodal lymphomas and most leukemias were of T-cell origin and all plasma cell tumors were of B-cell origin; Supplementary Table S3), this was not sufficient to explain the observed breed

predilections. The frequency of T-cell tumors was also greater than the average in dogs <3 years of age, and the frequency of B-cell tumors was greater than expected in dogs >14 years of age (Fig. 1*B*), but this similarly did not explain the observed breed predilections for B-cell and T-cell LPD.

Many breeds were underrepresented, thereby giving low power to detect differences among them and other breeds; therefore, we analyzed whether the distribution of B-cell and T-cell disease was conserved across similar breeds. Breed groupings based on the AKC group standards (23) showed that Toy breeds and Sporting dogs had an excess of T-cell LPD when compared with each of the control groups (Table 2*A*). However, when Shih Tzu dogs were excluded from the Toy breed population, the remaining Toy breeds were only different from the reference population consisting of 642 dogs reported previously in the literature (5, 7–9, 20, 25–29), and when Golden Retrievers were excluded from the Sporting dogs, the remaining breeds were not significantly different from any of the reference populations. Table 2*B* and *C* shows that the results for these groups were similar when the analyses were done on the populations restricted to dogs with a histologic diagnosis and dogs with NHL, respectively.

When dogs were grouped based on their genetic relatedness, an excess of T-cell LPD was present in Spitz breeds belonging to the oldest domestic dog group (that includes Akita, Basenji, Siberian Husky, Alaskan Malamute, and Chinese Shar-Pei as well as wolves) and in the Shih Tzu group (Shih Tzu, Lhasa Apso, and other Asian "lap" dogs; ref. 22) regardless of whether the analyses included all the dogs in the data set or only the restricted sample sets (Table 3).

One explanation for these results is that the risk factors predisposing for increased risk of T-cell tumors may have arisen ancestrally in these breeds, although Chow-Chow dogs, which belong to the Spitz group, did not share the predisposition to T-cell tumors. As was true for the AKC Sporting Group, the significant difference noted between group IV described by Parker et al. (ref. 22; European breeds of recent derivation) and the dogs reported previously in the literature was driven exclusively by the Golden Retrievers. In fact, the breeds that showed excess B-cell LPD were almost exclusively members of this group of recent European breeds.

These observations underscore the significant difference between Golden Retrievers and each of the reference groups we used in the study. Among the breeds examined, Boxers (Mastiff group) also showed increased risk to develop T-cell tumors, a finding that was recently confirmed in an independent population (30). The significance of breed to LPD phenotype was well illustrated by analyzing the phenotypes of chronic lymphocytic leukemia (CLL) cases in our study. Unlike people where CLL is almost exclusively a B-cell disease (31), CLL in the dog is most commonly (73%) a T-cell disease (32). In this study, we recorded 21 cases of CLL where 14 (66.7%) were T-cell tumors, 6 (28.6%) were B-cell tumors, and 1 was biphenotypic. The six dogs that had B-cell CLL were from breeds that show a preponderance (>75%) of B-cell tumors (Australian Cattle Dog, Chow-Chow, Doberman Pinscher, Poodle, and Standard Schnauzer), with one exception (a mixed-breed dog).

Outside the Spitz breeds and the Asian "lap" dogs, which seem to share common ancestral predilections to develop T-cell LPD, there seemed to be no relationship between breed groups and prevalence of LPD immunophenotype. This suggests that in non-Spitz breeds that have excess B-cell tumors or excess T-cell tumors, this peculiar risk probably stems from combinations of factors that arose during the process of selection imposed by strict breed barriers.

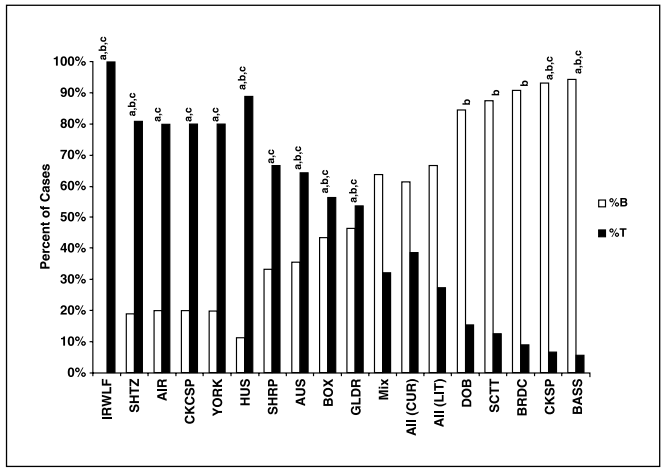

Figure 2. Phenotype distribution of breeds that are significantly different from reference populations. Frequency distribution of B-cell and T-cell immunophenotypes for LPD. Mixed-breed dogs and the reference populations for all dogs in the current data set and all dogs from previous reports in the literature are shown for comparison. *IRWLF,* Irish Wolfhound; *SHTZ,* Shih Tzu; *AIR,* Airedale Terrier; *CKCSP,* Cavalier King Charles Spaniel; *YORK,* Yorkshire Terrier; *HUS,* Siberian Husky; *SHRP,* Chinese Shar-Pei; *AUS,* Australian Shepherd; *BOX,* Boxer; *GLDR,* Golden Retriever; *DOB,* Doberman Pinscher; *SCTT,* Scottish Terrier; *BRDC,* Border Collie; *CKSP,* Cocker Spaniel; *BASS,* Basset Hound; *Mix,* mixed-breed dogs; *ALL (CUR),* all dogs in this study; *ALL (LIT),* all dogs reported previously in the literature. a, significantly different from mixed-breed dogs in current data set by χ^2 test; b, significantly different from all other dogs in current data set by χ^2 test; c, significantly different from all other dogs reported previously set by χ^2 test.

Recurrent cytogenetic abnormalities segregate with non-Hodgkin's lymphoma phenotypes in Golden Retrievers. To further validate the significance of our findings, we determined whether recurrent chromosomal abnormalities were significantly more common in NHL phenotypes within a specific breed. Previously, Thomas et al. (24) identified chromosome copy number aberrations that occurred in both B-cell and T-cell canine lymphoma [e.g., gain of *Canis familiaris* chromosome (CFA) 13]. Similarly, there were aberrations that were significantly more common in T-cell lymphoma (e.g., loss of CFA 11) and others that were significantly more common in B-cell lymphoma (e.g., gain of CFA 31). We thus hypothesized that, consistent with the findings reflecting different breed-specific prevalence of B-cell and T-cell LPD, we would identify recurrent changes in DNA copy number that would segregate with specific breeds. Including cases reported in ref. 24, 38 cases of B-cell NHL were evaluated by CGH analysis to identify recurrent abnormalities that segregated with the B-cell phenotype (Table 4). In addition to the recurrent chromosomal abnormalities mentioned above, we identified a deletion of chromosome 14 (del 14) with a minimum region of loss that extended from CFA 14q14-q22. This aberration was seen exclusively in diffuse B-cell lymphomas and occurred in 100% of Golden Retrievers from this group (7 of 7) but only in 13% of dogs from other breeds (4 of 31). These latter four dogs were from unrelated breeds (Lurcher, Cocker Spaniel, German Shepherd Dog, and Rottweiler). This shows that, although loss of chromosome 14 is not unique to Golden Retrievers, it is an aberration that is significantly more common in diffuse B-cell lymphoma of Golden Retrievers than all other breeds combined ($P < 0.001$). On the other hand, 16 cases of T-cell NHL were evaluated by CGH analysis (Table 4). We identified gain of CFA 36 and CFA 15 (15q24-q27) that were present exclusively in T-cell NHL. Gain of CFA 36 was present in 6

Table 2. Prevalence of B-cell and T-cell LPD in AKC breed groups

AKC Group	% B cells	% T cells	*n*
(A) Distribution of LPD immunophenotypes according to functional (AKC) breed groups*			
Nonsporting	67.6	32.4	71
Herding	65.6	34.4	122
Hound	64.4	35.6	73
Mixed breed	66.5	33.5	233
Terrier	65.3	34.7	72
Working	64.4	35.6	188
Sporting†	57.0	43.0	419
Toy†	31.7	68.3	41
(B) Distribution of LPD immunophenotypes according to AKC breed groups (dogs with histologic diagnosis)*			
Nonsporting	78.6	21.4	28
Herding	69.8	30.2	63
Hound	75.0	25.0	32
Mixed breed	73.4	26.6	94
Terrier	64.5	35.5	31
Working	67.0	33.0	100
Sporting†	59.8	40.2	229
Toy†	29.4	70.6	17
(C) Distribution of NHL immunophenotypes according to AKC breed groups*			
Nonsporting	78.6	21.4	28
Herding	73.6	26.4	53
Hound	76.6	23.3	30
Mixed breed	78.5	21.5	79
Terrier	70.8	29.2	24
Working	70.8	29.2	89
Sporting†	62.4	37.6	197
Toy†	35.7	64.3	14

*Mixed-breed dogs are not an AKC-recognized group and are shown for comparison.
†Significantly different from reference populations ($P < 0.05$) by χ^2 test and logistic regression. Note that these differences are driven by the inclusion of Golden Retrievers in the Sporting group and by Shih Tzu dogs in the Toy group.

of 16 samples, of which 2 were Golden Retrievers, and gain of chromosome 15 was seen in 3 of the 16 dogs, all of which were Golden Retrievers. Intriguingly, these two abnormalities appeared concurrently only in two Golden Retrievers, consistent with a trend for association with T-cell NHL of Golden Retrievers.

Discussion

The structural features of most human populations make it difficult to isolate heritable risk factors for complex conditions, such as LPDs (33). On the other hand, dogs provide numerous subpopulations (breeds) with restricted genetic heterogeneity and susceptibility to specific, naturally occurring diseases, including LPD. This, in turn, offers a robust and unparalleled model to study heritable influences (4, 22). For this study, we tested the hypothesis that heritable factors contribute to the risk of LPD immunophenotypes by exploring the relationship between breed and tumor immunophenotype (B or T cell) in 1,263 dogs representing 87 pure breeds and dogs of mixed breeding with a clinical or histologic diagnosis of LPD.

Our results and those of others indicate that T-cell diseases occur more frequently in dogs than in Caucasians living in Europe and North America, in which B-cell LPD (>90%) is most commonly encountered. However, the prevalence of canine T-cell LPD may approximate that seen in non-Caucasian people in the United States and in inhabitants of a subset of Far Eastern countries, such as Japan, India, and China (11). The elevated occurrence of T-cell LPD in Japan and other Far Eastern countries has in part been attributed to endemic tumor viruses, including HTLV-I, which causes adult T-cell leukemia/lymphoma, and EBV, which is associated with nasal T-cell lymphomas (34). Yet, despite sporadic reports of canine retroviruses (35–37), these agents do not seem to be causally related to most cases of canine LPD. Other environmental risk factors, such as phenoxy-acetic acid herbicides, insecticides, and organic solvents (38, 39), also do not fully account for the increased incidence of LPD (and especially NHL) over the past 10 years (17). Because humans and dogs are exposed to the same environment, because there is strong similarity in clinical presentation of LPD, and because of their extensively shared genomes, it is likely that similar genetic factors play key roles in LPD risk in both species. Therefore, the identification of genes that mediate heritable risk for LPD in dogs will be useful to identify

Table 3. Prevalence of B-cell and T-cell LPD in genetically-related breed groups

Group	% B cells	% T cells	*n*
(A) Distribution of LPD immunophenotypes according to genetic clusters or groups*			
I (Spitz)†	38.2	61.8	34
I/III (Shih Tzu)†	25.9	74.1	27
II (Mastiff)	64.5	35.5	152
III (Herding)	60.3	39.7	53
IV (Recent European)	61.3	38.7	574
Mixed breed	66.5	33.5	233
(B) Distribution of LPD immunophenotypes according to genetic clusters or groups (dogs with histologic diagnosis)*			
I (Spitz)†	40.0	60.0	15
I/III (Shih Tzu)†	20.0	80.0	10
II (Mastiff)	65.5	34.5	87
III (Herding)	82.6	17.4	23
IV (Recent European)	64.5	35.5	301
Mixed breed	73.4	26.6	94
(C) Distribution of NHL immunophenotypes according to genetic clusters or groups*			
I (Spitz)†	36.4	63.6	11
I/III (Shih Tzu)†	28.6	71.4	7
II (Mastiff)	68.4	31.6	79
III (Herding)	85.7	14.3	21
IV (Recent European)	67.7	32.3	257
Mixed breed	78.5	21.5	79

*Includes only dogs from 85 breeds assigned to the four major groups by Parker et al. (22). Mixed-breed dogs are shown for comparison.
†Significantly different from all reference populations ($P < 0.01$) by χ^2 test and logistic regression.

Table 4. Presence of recurrent cytogenetic aberrations in B-cell and T-cell NHL phenotypes in Golden Retrievers versus other dog breeds

Cytogenetic aberration	B cells		T cells	
	Golden Retrievers (affected/total)	Other breeds (affected/total)	Golden Retrievers (affected/total)	Other breeds (affected/total)
del 14	7/7*	4/31	0/8	0/8
+36	0/7	0/31	2/8†	4/8‡
+15	0/7	0/31	3/8†	0/8
+36;+15	0/7	0/31	2/8‡	0/8

*Significantly different from all other groups ($P < 0.001$, Fisher's exact test).
†Significantly different from the "other breeds" B-cell group ($P < 0.05$, Fisher's exact test).
‡Significantly different from both B-cell groups ($P < 0.05$, Fisher's exact test).

cancer-associated genes that have thus far been elusive in studies of human families, populations, and tumors.

Our results provide proof-of-principle for heritable origins of risk factors that predispose dogs to develop B-cell or T-cell malignancies (presumably genetic or epigenetic changes in genes that regulate lymphocyte development). Moreover, some risk factors may have arisen ancestrally in closely related breeds (Spitz-type dogs and Asian "lap" dogs, which are closely related to wolves and thus represent the oldest breeds derived because dogs were domesticated; ref. 22). On the other hand, some distinct risk-related factors seem to have developed independently in certain breeds during the process of selection imposed by strict breed barriers. Nevertheless, these factors are strongly embedded in the genome as illustrated by the example where B-cell CLL occurred mainly in breeds with an excess of B-cell tumors.

Our data also indicate that the relationships between breed and phenotypes are not incidental. Unique patterns of chromosomal gains and losses were identified that segregated specifically with B-cell tumors and T-cell tumors in Golden Retrievers, a breed that has a prevalence ratio of ~1:1 B-cell/T-cell LPD. Comparative cytogenetic data show that the minimal region of loss on CFA 14 is evolutionarily related to two distinct regions of *Homo sapiens* chromosome (HSA) 7, a region in the p-arm at 7p21-p15.1 and a region in the q-arm at 7q21-q21.3. Similar analysis shows that the gains of CFA 15q24-q27 and CFA 36 correspond to HSA 4q31.21-q32.3 and 2q24.1-q32.2, respectively. Deletions of HSA 7q21 have been documented in various human hematologic neoplasms, including acute myeloid leukemias with familial influence (3), but loss of HSA 7p21-p15 has only been reported for a single case of a gastric mucosa-associated lymphoid tissue lymphoma (40). Similarly, although a partial gain of HSA 4q (q13-q18) is a rare event in lymphocyte predominant Hodgkin disease (41), this does not generally include the region 4q31.21-4q32.3. Gain of HSA 2q has been reported rarely in transformation from follicular lymphoma to diffuse large B-cell lymphoma (42).

We used standard bioinformatic approaches to examine if there were known or predicted genes in these regions of the genome associated with human lymphoma. Among >160 genes in HSA 7p, diacylglycerol kinase-β and histone deacetylase-9 are two potentially "novel" tumor suppressor genes (43, 44). In the context of chromosome gains seen in canine T-cell lymphomas, candidates that may encode "novel" oncogenes include interleukin-15, FK506 binding protein-7, and histone acetyltransferase-1, which map to HSA 4q31, 2q31.3, and 2q31.2-33.1, respectively, and could initiate autocrine growth loops (45, 46), lower total calcineurin and NFATc2 activity (47), or counteract the activity of histone deacetylase (48).

These results indicate that recurrent genetic abnormalities that occur with significantly higher frequency in a single dog breed can assist in the identification of candidate genes that may be associated with the origin or progression of both canine and human cancers. Although a candidate gene approach may fail to pinpoint the precise genes that influence lymphomagenesis, these data offer opportunities to assemble gene expression arrays targeted to coding sequences in these regions as a means to identify other genes that show underexpression or overexpression. Similarly, they will eventually allow us to identify "modifier" genes that cosegregate under conditions of linkage disequilibrium and that may be prognostically significant or be associated with relative risk.

In summary, our results indicate not only that there is breed predilection for LPD in dogs but also that distinct breeds and breed types show unique susceptibility to develop B-cell or T-cell tumors. The newly completed canine genome sequence and the availability of optimized marker sets provide resources to undertake genome-wide scans to search for predisposition genes. Refinement and stratification of the data based on tumor types will provide greater statistical power to find genes important in disease susceptibility and progression. These features make domestic dogs a unique and valuable resource to define factors that mediate risk as well as genes involved in the initiation of B-cell and T-cell LPD in both dogs and humans.

Acknowledgments

Received 12/29/2004; revised 3/25/2005; accepted 4/21/2005.

Grant support: AKC Canine Health Foundation grants 1626, 2254, and 2214 (J.F. Modiano and M. Breen); Morris Animal Foundation grant D00CA-66 and American Veterinary Medical Foundation grant 81-98 (A.C. Avery); NIH grant K05 CA90754 and a Burroughs Wellcome Innovation Award (E.A. Ostrander); American Cancer Society grant RSG-02-173-01-LIB (J.F. Modiano); and Starlight Fund (AMC Cancer Center) and Monfort Family Foundation (University of Colorado Cancer Center).

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We thank Drs. Michelle Ritt, Cheryl London, and Gary Koretzky for helpful discussions and review of the article.

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