A Review of Canine Inherited Bleeding Disorders: Biochemical and Molecular Strategies for Disease Characterization and Carrier Detection

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Many different inherited bleeding disorders have been identified in dogs, defined on the basis of quantitative, functional, or structural defects in specific hemostatic proteins or pathways. Most of these disorders are caused by single-gene defects and biochemical assays provide an accurate measure of disease phenotype. Phenotypic disease classifications, however, are often genetically heterogeneous. Protein-based carrier detection assays are fast, inexpensive, and do not require specific identification of causative mutations. The limitations of these tests arise from variable "overlap" regions between carrier and clear dogs, influencing positive and negative predictive values of carrier detection tests within breed populations. Molecular diagnostic techniques enhance the accuracy of carrier detection, providing their clinical application takes into account the molecular heterogeneity underlying naturally occurring hemostatic defects in dogs.

The process of hemostasis is an intricate system designed to maintain blood flow within injured blood vessels. Inhibitors and activators of hemostasis act to maintain physiologic balance and prevent abnormal hemorrhage, thrombosis, or fibrinolysis (Colman et al. 1994). Most inherited bleeding disorders are caused by a defect or breakdown in a single component of one hemostatic pathway (Forbes and Madhok 1991).

On the basis of clinical signs and screening tests, canine acquired and inherited bleeding diatheses are broadly categorized as defects of primary hemostasis (failure of platelet plug formation) or defects of secondary hemostasis (failure of fibrin clot formation). Specific functional, quantitative, and structural assays are required to characterize the precise protein or metabolic pathway abnormality responsible for an observed phenotype (Table 1). Acquired bleeding disorders are common and different heritable defects occur within a single breed (Dodds 1989). Evaluation of dogs expressing a bleeding tendency therefore requires an accurate definition of disease phenotype before any familial or breed screening programs are undertaken.

Inherited Defects of Primary Hemostasis

The initial or primary phase of hemostasis consists of a series of interactions between platelets and the site of endothelial cell disruption, culminating in platelet plug formation. Exposure of subendothelium triggers conformation changes in von Willebrand factor (vWF), an adhesive plasma protein which acts to link platelets to the site of vessel injury (Turitto et al. 1985). After platelets adhere to subendothelium, they undergo shape change, form intraplatelet bridges, and release numerous agonist compounds from storage organelles. Ultimately platelet aggregates accumulate and cover the zone of vascular damage to form a hemostatic plug (Lefkovits et al. 1994).

Clinical signs of primary hemostatic defects typically consist of bleeding from mucosal surfaces, cutaneous bruising, prolonged hemorrhage following trauma or surgery, and long in vivo bleeding time. An initial determination of plasma vWF concentration differentiates dogs affected with von Willebrand disease (vWD) from those having intrinsic platelet defects. All of the primary hemostatic defects identified in dogs appear to be inherited as autosomal traits.

Von Willebrand disease is the most common canine (and human) heritable bleeding disorder. Three classifications (types 1, 2, and 3) have been defined on the basis of quantity and multimeric structure of plasma vWF in affected human beings (Holmberg and Nilsson 1992). These classifications are applicable to dogs, and

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Table 1. Specific tests of hemostatic proteins and pathways

Platelet tests	von Willebrand factor tests	Coagulation factor tests
Functional assays	Functional assays	Functional assays
Clot retraction	Cofactor assays (vWF-dependent platelet agglutination)	Coagulant activity assays (factors II, V, VII, VIII, IX, X, XI, XII)
Aggregation response: collagen, ADP, epinephrine arachidonic acid, Ca ionophore	—Botrocetin cofactor	—Clotting time tests = fibrin endpoint
Secretion studies: serotonin, ADP, ATP ADP/ATP ratio	—Ristocetin factor	—Chromogenic assays = colorimetric endpoint
		Fibrinogen assays (factor I)
		—Clottable (Clauss) fibrinogen
		—Reptilase time
Structural assays	Structural assays	Structural assays
Membrane glycoproteins: GPIb, GPIIb, GPIIIa	vWF multimer composition: protein electrophoresis/western blot	Protein electrophoresis/western blot
Ultrastructure: electron microscopy		
	Concentration assays	Concentration assays
	von Willebrand factor antigen (vWF:Ag)	Individual factor antigen (factors I–XII)
	—ELISA	—ELISA
	—Laurell immunoelecrophoresis	—IRMA
		Fibrinogen-heat precipitable

within each affected breed a single type predominates (Table 2). The prevalence and clinical severity of vWD vary widely between different breeds (Brooks et al. 1992; Dodds 1982; Johnson et al. 1988).

Type 1 vWD is characterized by a low concentration of structurally normal vWF and relatively mild clinical signs. Type 1 vWD is by far the most common form in dogs and people. Doberman pinschers have the highest reported breed prevalence of type 1 vWD, with many clinical descriptions characterizing the bleeding diathesis (Brooks et al. 1992; Dodds 1982; Johnson et al. 1985). In breeds affected with type 1 vWD, dogs having the most severe deficiency of vWF are most likely to express a clinically relevant bleeding tendency. The inheritance pattern of type 1 vWD in most human kindreds is autosomal dominant. There is some evidence for both autosomal dominant and recessive inheritance patterns of type 1 vWD in dogs (Dodds 1982; Moser et al. 1996).

Type 2 vWD accounts for up to 20% of all vWD cases in human beings, but is uncommon in dogs, having been reported only in German shorthaired and German wirehaired pointers (Brooks et al. 1996a; Johnson et al. 1988). The characteristic findings of type 2 vWD include a specific deficiency of high molecular weight vWF multimers and a disproportionate reduction of vWF activity compared to vWF concentration. A cluster of mutations causing type 2 vWD have been identified in exon 28 of the human vWF gene (Sadler et al. 1995). This exon encodes the A domains of vWF protein, regions involved in vWF platelet interactions and vWF multimer assembly and stability.

Type 3 vWD is the most severe form. Affected patients invariably express a bleeding diathesis and have no or only trace amounts of plasma vWF. Type 3 vWD is inherited as an autosomal recessive trait in most human kindreds. The type 3 phenotype has been found in association with homozygous and compound heterozygous mutations in the vWF gene. These mutations include repeated de novo mutations in "hot spots" and a variety of large and small gene deletions (Sadler et al. 1995; Schneppenheim et al. 1994). Two breeds, Scottish terrier and Shetland sheepdog, have relatively high prevalence of this severe form of vWD (Brooks et al. 1992). However, sporadic cases of type 3 vWD occur in many different breeds and in mixed breed dogs (Table 2).

Many different mutations and polymorphisms have been reported in the human vWF gene (Ginsburg and Sadler 1993; Sadler et al. 1995), and it is likely that there is similar molecular heterogeneity in canine vWD. Compound heterozygosity in affected kindreds and mutations occurring in unlinked vWF regulatory elements complicate an understanding of the genetic basis of vWD (Mohlke et al. 1996; Sadler et al. 1995). The recent finding of two different homozygous vWF mutations in type 3 vWD-affected Dutch kooiker dogs (Rieger et al. 1998) further illustrates the complex molecular pathogenesis of vWD. Different mutations believed to be causative for vWD have been identified in other breeds

Table 2. Canine von Willebrand disease

Classification	Plasma vWF concentration/multimer structure	Clinical severity	Breeds
Type 1	Low concentration/proportional decrease all multimer forms	Variable	Airedale,[a,b] akita,[c] dachshund,[b,d] Doberman,[a–c,e–h] German shepherd,[i] golden retriever,[b,c,e] greyhound,[b] Irish wolfhound,[i] Manchester terrier,[e] schnauzer,[a,e] Pembroke Welsh corgi,[c,e,f] poodle,[c,e,f] Shetland sheepdog[e–g,k]
Type 2	Low concentration/absence of high molecular weight multimers	Severe	German shorthaired pointer,[a,c] German wirehaired pointer[l]
Type 3	Plasma vWF absent	Severe	Familial:
			Chesapeake Bay retriever,[a,f,m] Dutch Kooikder,[n] Scottish terrier,[a–g] Shetland sheepdog[g,k]
			Sporadic:
			Border collie,[o] bull terrier,[c] cocker spaniel,[c] Labrador retriever,[o] mixed breed,[o] Pomeranian[c]

[a] Johnson et al. 1988.
[b] Stokol and Parry 1993.
[c] Brooks 1991.
[d] Woods et al. 1995.
[e] Dodds et al. 1981.
[f] Dodds 1982.
[g] Brooks et al. 1992.
[h] Johnson et al. 1985.
[i] Dodds 1975.
[j] Clark and Parry 1995.
[k] Raymond et al. 1990.
[l] Brooks et al. 1996b.
[m] Johnson et al. 1980.
[n] Rieger et al. 1997.
[o] Brooks MB, case records, Comparative Coagulation Laboratory.

Table 3. Canine inherited platelet function defects

Classification	Characteristic laboratory findings	Breeds
Membrane glycoprotein defect (Glanzmann's-type thrombasthenia)	Abnormal clot retraction and adhesion Absent or trace aggregation response Absent or reduced $GPII_bIII_a$ complex	Otterhound,[a,b] Great Pyrenees[c]
Delta storage pool disorder	Abnormal ADP storage and secretion Abnormal aggregation to ADP and collagen	American cocker spaniel[d]
Signal transduction defect	Abnormal adhesion Absent or trace aggregation response Abnormal cAMP metabolism	Basset hound[e]
	Abnormal adhesion Absent or trace aggregation response Abnormal signal pathway	Spitz[f]
Storage pool and signal transduction defect	Normal aggregation response to ADP Abnormal aggregation to other agonists Defective uptake and storage of serotonin Abnormal platelet protein phosphorylation	Collie[g]

[a] Raymond and Dodds 1979.
[b] Catalfamo and Dodds 1988.
[c] Boudreaux et al. 1996.
[d] Callan et al. 1995.
[e] Catalfamo et al. 1986.
[f] Boudreaux et al. 1994.
[g] Lothrop et al. 1991.

(Brewer et al. 1998), but details of these mutations have not been published.

Platelet function defects are broadly classified as abnormalities of membrane receptors (integrins), deficiencies of storage granule compounds, or defects of intracellular signal transduction. Inherited platelet function defects have been identified in relatively few canine breeds, and some reports describe a single family (Table 3). Because of the special sampling and assay procedures involved in platelet function testing, it is likely that these disorders are underdiagnosed in dogs.

Specific deficiency or absence of platelet membrane glycoproteins cause failure of adhesion (glycoprotein I_b-IX) or failure of aggregation (glycoprotein II_bIII_a). The disease phenotype, Glanzmann's thrombasthenia, is caused by lack of glycoprotein II_bIII_a (Lanza et al. 1992). Canine thrombasthenia has been described in two different breeds of dog (Boudreaux et al. 1994; Raymond and Dodds 1979). It is likely that the molecular defects underlying the similar phenotypes are different in these breeds, as is the case in different ethnic groups affected with Glanzmann's thrombasthenia (Lanza et al. 1992; Russell et al. 1988).

Platelet storage pool disorders are characterized by a lack or diminution of the secretable substances stored in platelet granules (Weiss 1994). Storage pool diseases are heterogeneous; selectively involving compounds found in alpha granules (platelet-derived growth factor, platelet factor 4, beta-thromboglobulin, fibrinogen), delta granules (ADP, ATP, serotonin, calcium), or nonspecifically affecting compounds found in both types of granules. The characterization of storage pool disorders in human beings and dogs includes platelet uptake and secretion studies, and visualization of platelet organelles using electron microscopy (Catalfamo and Dodds 1988; Weiss 1994).

The third broad classification of platelet dysfunction comprises abnormalities of postreceptor signal transduction and secretion. The cellular and/or biochemical defects causing these disorders are not well defined. They are differentiated on the basis of measurements of intermediaries and end points of platelet metabolism (Weiss 1994).

The biochemical and molecular characterization of platelet function defects in human beings has been most successful for those defects involving surface integrins, where the disease phenotype and candidate genes are well defined (Bray and Shuman 1990; Lanza et al. 1992). Canine models of platelet dysfunction may prove to be most valuable for discovering platelet metabolic pathway defects and their associated causative mutations.

Inherited Defects of Secondary Hemostasis

The pathways of secondary hemostasis comprise a series of enzymatic reactions resulting in formation of a stable, cross-linked fibrin clot (Colman et al. 1994). All coagulation factors and cofactors circulate in an inactive form but are rapidly converted to their active forms at the site of vessel injury. Most of the clotting factors are serine protease enzymes, with similar C-terminal catalytic domains, but unique N-terminal sequences that determine the specialized biochemical properties of each factor (Furie and Furie 1992).

The classic signs of coagulation factor deficiency include hemorrhage into joints, chest, or abdomen, and subcutaneous or intramuscular hematoma formation. Signs common to both primary and secondary hemostatic defects include excessive bleeding from sites of surgery or trauma and from sites of tooth eruption. Although a deficiency of any single factor will cause prolongation of in vitro clotting time, the clinical severity associated with heritable factor deficiencies varies between factors (Forbes and Madhok 1991). Most of the heritable factor deficiencies identified in human beings have been identified in dogs, including disorders widespread within breeds and defects described in only a single individual (Tables 4–7). Dogs express the same "bleeder" phenotypes as human patients affected with these disorders and show a similar response to transfusion of homologous factors (Dodds 1989; Fogh and Fogh 1988).

The human genes for all coagulation factors have been cloned (Furie and Furie 1992). Fibrinogen, or factor I, consists of three tightly linked genes; all other factors are encoded by a single gene. The molecular defects underlying factor deficiencies are heterogeneous, including large and small gene deletions, insertions, and point mutations (Thompson 1994).

Hemophilia

Hemophilia is the most common inherited coagulation disorder in human beings and dogs. It is composed of two distinct defects: hemophilia A, caused by specific deficiency of coagulation factor VIII, and hemophilia B, caused by specific deficiency of coagulation factor IX (Thompson 1994). The inheritance of hemophilia A and B is X-linked recessive. The genes coding for these factors are located on the long arm of the X chromosome, but they are not linked (factor VIII at X_q28; factor IX at $X_q26.3$). The clinical severity of hemophilia ranges from mild to severe, and in vitro measures of factor activities are predictive of clinical expression. Molecular analyses of case series of human patients reveal that de novo mutations occur frequently and that recurrent observations of a mutation in unrelated individuals are likely to be of independent origin (Furie and Furie

Table 4. Summary of canine hemophilia A: case records of comparative coagulation section (1990–1996)

Breed	FVIII deficiency[a]	Incidence pattern[b]	Breed	FVIII deficiency	Incidence pattern
Akita	Moderate	Familial	Labrador retriever	Severe	Sporadic
Australian shepherd	Moderate	Familial		Moderate	Familial
Basenji	Severe	Sporadic	Lhasa apso	Severe	Familial
Basset hound	Moderate	Sporadic	Manchester terrier	Severe	Sporadic
Beagle	Severe	Familial	Miniature dachshund	Moderate	Familial
Bichon frise	Severe	Sporadic	Miniature schnauzer	Severe	Familial
Blue heeler	Moderate	Sporadic	Mixed breed dogs	Mild	Sporadic
Boxer	Severe	Familial		Moderate	Sporadic
Boykin spaniel	Severe	Familial		Severe	Sporadic
Brittany spaniel	Severe	Sporadic	Pit bull terrier	Severe	Sporadic
Cairn terrier	Mild	Sporadic	PW Corgi	Severe	Sporadic
Chihuahua	Moderate	Sporadic	Portuguese water dog	Severe	Familial
Chow	Severe	Sporadic	Rottweiler	Severe	Familial
Cocker spaniel	Severe	Familial	Rough collie	Moderate	Familial
	Moderate	Familial	Scottish terrier	Severe	Familial
English bulldog	Severe	Familial		Moderate	Familial
English Springer spaniel	Severe	Familial	Shar pei	Moderate	Familial
German shepherd	Moderate	Familial	Shetland sheepdog	Severe	Sporadic
	Mild	Familial	Shiba Inu	Severe	Sporadic
German short-haired pointer	Moderate	Familial	Shih Tzu	Severe	Familial
Golden retriever	Mild	Familial	Toy poodle	Severe	Sporadic
Husky	Moderate	Familial	Vizsla	Severe	Familial
Irish setter	Severe	Sporadic	West Highland white terrier	Severe	Sporadic
			Yorkshire terrier	Severe	Sporadic

[a] Factor VIII deficiency: severe = factor VIII coagulant activity <1%; moderate = factor VIII coagulant activity 1–10%; mild = factor VIII coagulant activity >10%.

[b] Incidence pattern: sporadic = single individual or single litter; familial = cases in more than one generation of pedigree and/or new cases in 3 or more years.

1990). Examination of the factor IX gene in hemophilia B patients has revealed apparent mutation "hot spots" at CpG dinucleotides (Giannelli et al. 1996). A partial inversion of the factor VIII gene, resulting from recombination between a factor VIII intron gene and homologous upstream sequences, is believed to be the mutation mechanism underlying approximately half of severe hemophilia A cases (Millar et al. 1994).

Hemophilia A and B have been identified in many different canine breeds and in unrelated mixed-breed dogs (Tables 4 and 5) (Dodds 1989; Fogh and Fogh 1988). The incidence of hemophilia A appears to be about three to four times that of hemophilia B, similar to the ratio found in human populations. There is a bias toward diagnosis of the most clinically severe forms of canine hemophilia, however, widespread propagation of relatively mild forms of hemophilia has occurred in at least two breeds, German shepherd (Fogh and Fogh 1988; Hein 1986) and German wirehaired pointer (Brooks MB, unpublished data), where the defect can be traced through many generations to a single founder male. Reports of molecular analyses of canine hemophilia B have revealed at least three unique causative mutations, including a missense mutation, a 5 bp deletion and transition, and a large gene deletion spanning the entire coding sequence (Brooks et al. 1997; Evans et al. 1989; Mauser et al. 1996). Ongoing studies combining biochemical and molecular analyses of canine hemophilia B pedigrees indicate that the defect within each family is likely to represent an independent mutation and that more than a single mutation may be responsible for hemophilia B within a single breed (Brooks et al. 1998). There have been no reports identifying the molecular defect(s) responsible for canine hemophilia A. The variability in clinical severity and in vitro factor VIII activity and the large number of affected breeds suggest that the canine disease is similar to its human counterpart in molecular heterogeneity.

Table 5. Summary of canine hemophilia B: case records of comparative coagulation section (1990–1996)

Breed	FIX deficiency[a]	Incidence pattern[b]	Breed	FIX deficiency	Incidence pattern
Airedale terrier	Severe	Familial	Maltese	Severe	Familial
Beagle	Severe	Sporadic	Mixed breed dogs	Severe	Sporadic
Bichon frise	Severe	Sporadic		Moderate	Sporadic
Chow	Moderate	Sporadic		Mild	Sporadic
Doberman pinscher	Moderate	Sporadic	Pit bull terrier	Severe	Sporadic
German shepherd	Severe	Familial	Rottweiler	Moderate	Familial
GWH pointer	Mild	Familial	Saint Bernard	Moderate	Familial
Golden retriever	Severe	Sporadic	Scottish terrier	Moderate	Sporadic
Jack Russell terrier	Moderate	Sporadic	Sealyham terrier	Moderate	Sporadic
Labrador retriever	Severe	Familial	Shih Tzu	Moderate	Familial
	Moderate	Familial	Weimaraner	Severe	Sporadic
			Wirehaired fox terrier	Moderate	Sporadic

[a] Factor IX deficiency: severe = factor IX coagulant activity <1%; moderate = factor IX coagulant activity 1–10%; mild = factor IX coagulant activity >10%.

[b] Incidence pattern: sporadic = single individual or single litter; familial = cases in more than one generation of pedigree and/or new cases in 3 or more years.

Autosomal Factor Deficiencies

Deficiencies of the autosomal coagulation factors (factors I, II, V, VII, X, XI, XII) are less common than hemophilia (Fogh and Fogh 1988; Thompson 1994). Most are inherited as recessive traits, with clinical hemorrhage and/or in vitro prolongation of clotting time seen in association with either quantitative factor deficiency or dysfunctional forms of the clotting factor. Fibrinogen (factor I) defects are heterogeneous, including deficiencies (afibrinogenemia, hypofibrinogenemia) and a wide variety of dysfunctional forms (dysfibrinogenemias) demonstrating abnormal cleavage, polymerization, or degradation. Clinical signs of the various forms of dysfibrinogenemia range from severe hemorrhage to thrombosis, and in some forms hemorrhage and thrombosis occur (McDonagh et al. 1994). Hemorrhage in association with afibrinogenemia and hypofibrinogenemia has been reported in dogs (Dodds 1989; Kammerman et al. 1971).

The severity of hemorrhage associated with deficiencies of factors II, V, VII, and X tends to correlate with in vitro clotting factor activity (Furie and Furie 1990). In contrast, even marked reduction in factor XII activity has not been associated with a bleeding tendency (Fuhrer et al. 1990). Factor XI deficiency is the most clinically variable factor deficiency in dogs and human beings. Spontaneous bleeding episodes are rare and hemorrhage typically occurs h after surgery or trauma. Clinical severity of hemorrhage may not correlate with in vitro determination of factor XI activity, and even for an individual patient,

Table 6. Canine autosomal inherited coagulation factor deficiencies

Factor deficiency	Characteristics	Breeds
Factor I (fibrinogen)	Low concentration, severe signs	Bernese mountain dog[a]
	Abnormal function, mild signs	Borzoi, collie, vizsla[b]
Factor II (prothrombin)	Low activity, variable signs	Boxer[c]
	Low activity, severe signs	Cocker spaniel[d]
Factor VII (proconvertin)	Low activity and low antigen	Beagle[e–g]
	Mild signs, common in laboratory colonies	
	Low activity, mild clinical signs	Bernese mountain dog,[g] miniature schnauzer,[g] malamute[g,h]
Factor X	Low or absent activity, severe signs	Cocker spaniel[i]
(Stuart–Prower factor)	Low activity, severe signs	Jack Russell terrier[j]
Factor XI	Moderate to low activity, signs of	English springer spaniel[k]
(plasma thromboplastin antecedent)	hemorrhage after injury	Kerry blue terrier[l]
Factor XII (Hageman factor)	Low activity, mild or no clinical signs	Miniature poodle[m]
		Shar Pei[n]

[a] Kammerman et al. 1971.
[b] Dodds 1989.
[c] Dodds 1979.
[d] Hill et al. 1982.
[e] Spurling et al. 1972.
[f] Spurling 1988.
[g] Brooks MB, case records, Comparative Coagulation Laboratory.
[h] Mills et al. 1997.
[i] Dodds 1973.
[j] Cook et al. 1983.
[k] Dodds and Kull 1971.
[l] Knowler et al. 1994.
[m] Randolph et al. 1986.
[n] Otto et al. 1991.

the response to similar hemostatic challenge is highly variable (Knowler et al. 1994; Ragni et al. 1985).

Strategies for Carrier Detection of Inherited Bleeding Disorders

Biochemical tests measuring the concentration or function of specific hemostatic proteins provide rapid, inexpensive, and direct methods to screen for von Willebrand disease, hemophilia, and individual clotting factor deficiencies in dogs (Dodds 1989). A small-volume (1 ml) plasma sample provides sufficient material to screen for all of these defects, with assays complete within a few h. In applying these tests for carrier detection, the interpretation of an individual dog's test value depends on the inheritance and expression pattern of the defect and the prevalence of that disease in the population (Brooks et al. 1996a; Dodds 1982). In general, plasma concentration or activity of the various hemostatic proteins represent a continuum. Dogs expressing a bleeding tendency have very low values, and carriers have values intermediate between affected dogs and those genetically normal. While protein-based assays have proven clinical utility, there is a variable "overlap region" between carrier and clear dogs when screening large-breed populations (Brooks et al. 1996b; Dodds 1982; Johnson et al. 1988). Progeny tests provide a means for further clarifying genetic status, but the addition of molecular genetic testing could definitively classify dogs having indeterminate protein values and in the process lead to better understanding of the molecular pathology underlying various disease phenotypes.

The characteristics of the disease gene of interest and the molecular heterogeneity of the defect in the population to be screened are critical features for designing effective molecular diagnostic test strategies (Thompson 1994). Regardless of phenotypic disease classification, the basic choice for DNA-based carrier detection is between linkage studies or direct detection of causative mutations (Knobloch et al. 1993; Sadler 1990). Linkage studies depend on the availability of intragenic or extragenic markers and informative family members. Effective linkage studies in homogeneous populations, like most canine breeds, are likely to require a series of highly polymorphic intragenic and linked extragenic markers in order to define informative haplotypes. At present, intragenic markers have been identified in the canine genes for factor VIII (Mellersh 1997), factor IX (Gu and Ray 1997), and von Willebrand factor (Shibuya et al. 1994).

Direct detection of causative mutations is the most specific method of carrier detection. The development of a PCR-based methodology to identify mutations provides a relatively rapid and inexpensive screening test (Millar et al. 1991). Direct detection of mutations will be most useful for breeds with a high prevalence of a single disease phenotype caused by a single gene defect. It may be impractical however to identify causative mutations in every affected family for heterogeneous or sporadic diseases caused by mutations in large, complex genes.

Table 7. List of inherited bleeding disorders identified in the 20 most popular breeds

Rank[a]	Breed	Bleeding disorders[b]				
1	Labrador retriever	hem A	hem B	vWD		
2	Rottweiler	hem A	hem B			
3	German shepherd	hem A	hem B	vWD		
4	Golden retriever	hem A	hem B			
5	Beagle	hem A	hem B	fVII def.		
6	Poodle	hem A	vWD	fXII def.		
7	Dachshund	hem A	vWD			
8	American cocker spaniel	hem A	hem B	vWD	f II, X def.	plat. dysfct.
9	Yorkshire terrier	hem A				
10	Pomeranian	vWD				
11	Shih Tzu	hem A	hem B	vWD		
12	Chihuahua	hem A				
13	Boxer	hem A	fII def.			
14	Shetland sheepdog	hem A	vWD			
15	Dalmatian	hem A				
16	Miniature schnauzer	hem A	vWD			
17	Siberian husky	hem A				
18	Miniature pinscher	vWD				
19	Pug	hem A				
20	Doberman pinscher	hem B	vWD			

[a] Rank listing in descending order by number of total AKC registered individuals for 1996.
[b] Bleeding disorders referenced in Tables 2–6: hem A = factor VIII deficiency; hem B = factor IX deficiency; vWD = von Willebrand disease; fII def. = factor II (prothrombin) deficiency; fVII def. = factor VII deficiency; fX def. = factor X deficiency; fXII def. = factor XII deficiency; plat dysfct. = platelet dysfunction.

Regardless of test method, the clinical application of molecular tests for carrier detection requires accurate phenotypic diagnosis to ensure screening for the relevant disease within that breed or line. The occurrence of new mutations and sporadic disease complicates development of carrier detection strategies for many breeds (Table 7). It is difficult to calculate accurate estimates of disease prevalence of hemostatic disease within a breed. There are many selection biases, including those related to disease phenotype and those related to interpersonal dynamics of breed clubs and associations. Ultimately, the reduction in disease prevalence of canine inherited bleeding disorders will require education and cooperation between veterinarians and breeders to encourage the appropriate use and interpretation of biochemical and molecular tests.

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