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Inherited platelet disorders

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Abstract

Objective – To present the latest information on inherited platelet disorders in domestic animals.
Data Sources – Research articles and reviews spanning 40 years available on PubMed.
Human Data Synthesis – Information regarding inherited platelet disorders in people is plentiful and often descriptions of human conditions have led to the identification of similar disorders in veterinary species. There are exceptions, however, in which specific inherited platelet disorders were first described in animals with subsequent identification in people.
Veterinary Data Synthesis – Many inherited platelet disorders have been documented in animals at the functional and molecular level and that information is presented in this review.
Conclusions – Much progress has been made in the past 20 years in the characterization of inherited platelet disorders in animals at the functional, biochemical, and molecular level. The study of inherited platelet disorders has greatly enhanced the understanding of platelet physiology and has led in some instances to the development of platelet inhibitory medications. Characterization of inherited disorders at the molecular level greatly facilitates diagnosis and identification of affected and heterozygous animals thus avoiding propagation of the defect by breeders. When used with available functional and biochemical diagnostic tests, it significantly enhances the quality of care and case management.

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Introduction

Platelets are the first line of defense in preventing blood loss due to vascular injury. Abnormal platelet function can result in platelet-type bleeding, typically characterized by mucocutaneous hemorrhage. Excessive bleeding during eruption of adult teeth is a classic clinical sign in affected dogs. Spontaneous hemorrhage in young adult and older animals is generally mild or insidious in nature but with time can be associated with development of iron deficiency anemia (personal observation). Development of anemia may be more rapid and acute if affected animals are also infected by hematophagous gastrointestinal parasites or other cell destructive parasites such as coccidia. Acute severe, life-threatening hemorrhage can occur with trauma or surgery. Hemorrhage into the spinal cord or brain is also possible and may manifest as paralysis or seizures. A combination of platelet dysfunction with thrombocytopenia can result in severe, acute hemorrhage. This review will describe inherited thrombopathias and thrombocytopenias reported in domestic animals.

Abbreviations

ACT	activated clotting time
ADAMTS13	a disintegrin and metalloprotease with thrombospondin repeats
ADP	adenosine diphosphate
APC3	adaptor protein complex 3
aPTT	activated partial thromboplastin time
ATP	adenosine triphosphate
CalDAG-GEFI	Calcium Diacylglycerol Guanine Nucleotide Exchange Factor I
CHS	Chediak-Higashi Syndrome
DDAVP	Desmopressin acetate
ECT	European Continental Type
FVIII-C	factor VIII-coagulant
GT	Glanzmann thrombasthenia
LAD-III	Leukocyte adhesion deficiency III
MHA	May-Hegglin anomaly
PAF	platelet activating factor
VWD	von Willebrand disease
VWF	von Willebrand factor

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Table 1: Intrinsic platelet disorders in dogs, horses, and cattle

Breed	Disorder	Mutation
Great Pyrenees[45–47]	Glanzmann thrombasthenia	IIb, exon 13, 14-bp repeat (premature stop)
Otterhound[48,49]	Glanzmann thrombasthenia	IIb, exon 12, GAC to CAC (D to H)
Oldenbourg[54]	Glanzmann thrombasthenia	IIb, exon 2 CGG to CCG (R to P)
Thoroughbred[50,52]	Glanzmann thrombasthenia	IIb, exon 2, CGG to CCG (R to P)
Quarter horse[50–52]	Glanzmann thrombasthenia (compound heterozygote)	IIb, exon 11, 10-bp deletion (premature stop) and exon 2, CGG to CCG (R to P)
Peruvian Paso[53]	Glanzmann thrombasthenia	IIb, exon 11, 10-bp del (premature stop)
Basset hound[70–72]	Thrombopathia	CalDAG-GEFI, exon 5, 3-bp del, (F del)
Eskimo Spitz[72,73]	Thrombopathia	CalDAG-GEFI, exon 5, 1-base duplication, frame shift (premature stop)
Landseer-ECT[72]	Thrombopathia	CalDAG-GEFI, exon 8, CGA to TGA (R to premature stop)
Simmental cattle[74–78]	Thrombopathia	CalDAG-GEFI, exon 7, CTC to CCC (L to P)
Japanese Black Cattle[40–42]	Chediak-Higashi	LYST, nucleotide 6065, CAT to CGT (H to R)
Grey Collies[32,33]	Cyclic hematopoiesis	AP3B1, exon 20, 1-base duplication, frame shift (premature stop)
German Shepherd[86]	LAD-I variant/LAD-III	Kindlin-3, 12-bp insertion (RRLP insertion in PH domain)
Greater Swiss Mountain Dog[61]	ADP response impaired	P2Y12, exon 2, 3-bp del (S del)
German Shepherd[88–92]	Procoagulant expression	Not known
Cocker Spaniel[43]	Selective ADP deficiency	Not known
Cavalier King Charles Spaniel[98]*	Macrothrombocytopenia	Beta1-Tubulin, exon 4, GAC to AAC (D to N)
Pug[106]	May-Hegglin anomaly	MYH-9, exon 38, CAG to AAG (E to K)

*Has also been documented in the following breeds: Chihuahua, Labrador Retriever, English Toy Spaniel, Labradoodle, Poodle, Shih Tzu, Maltese, Jack Russell Terrier, Havanese.

bp, base pair; del, deletion; F, Phenylalanine; D, Aspartic acid; H, Histidine; R, Arginine; L, Leucine; P, Proline; N, Asparagine; E, Glutamine; K, Lysine; PH, pleckstrin homology domain; IIb, platelet glycoprotein IIb gene; CalDAG-GEFI, calcium diacylglycerol guanine nucleotide exchange factor I gene; AP3B1, adaptor protein complex 3, beta-subunit gene; LYST, lysosomal trafficking regulator gene; ECT, European continental type. Landseer-ECT has been recognized as a breed in Europe since 1960 and is distinct from the Landseer-Newfoundland breed recognized in the United States.

Inherited Platelet Disorders

Inherited platelet disorders can be categorized into 2 main categories: extrinsic platelet disorders and intrinsic platelet disorders. Extrinsic platelet disorders are disorders in which the platelets are normal but a protein necessary for their function is either absent, reduced, or dysfunctional. The most common type of extrinsic platelet disorder is von Willebrand disease (VWD). Because fibrinogen is required for normal platelet aggregation, hypofibrinogenemia and dysfibrinogenemia may also result in an extrinsic platelet disorder. Disorders affecting fibrinogen, however, are much rarer than VWD and also potentially result in a coagulopathy as fibrinogen is a vital component of normal blood coagulation. Intrinsic platelet disorders involve the platelets directly. Intrinsic disorders may arise from abnormalities in platelet granules, membrane glycoproteins, signal transduction proteins, or proteins involved in platelet production from megakaryocytes. Several intrinsic platelet disorders have been described in animals (Table 1). Documentation of an intrinsic platelet disorder usually requires specialized techniques. Although these techniques, which include platelet aggregometry and flow cytometry, are effective at identifying affected animals, they usually do not reliably identify carrier states. Platelet aggregometry techniques should be completed within 4 hours of blood collection and so, usually require that the animal being tested be on the premises of the testing facility. Flow cytometry assays can be conducted on blood samples that are 24 hours old; however, the types of assessments are limited. Characterization of the molecular basis for recognized inherited platelet disorders greatly facilitates the identification of affected and heterozygous animals and eliminates the necessity of having animals on the premises of the testing facility. However, such testing requires fastidious assignment of animal identification with molecular diagnostic specimens.

Clinical Signs

Clinical signs in affected animals can vary considerably, reflecting differences in molecular basis and disease phenotype. Signs may be overt and nonspecific.

Excessive gingival bleeding during eruption of adult teeth may be the first indication that a platelet disorder exists. For older animals, periodic bruising or petechial hemorrhages may be the only clinical signs. Internal hemorrhage within the brain or spinal cord may result in seizures or paralysis. Unilateral or bilateral epistaxis occurs in some affected animals and may be either spontaneous or associated with treatment with platelet-inhibitory medications. Trauma or surgery can result in severe hemorrhage with either intrinsic or extrinsic platelet disorders. Chronic insidious blood loss through the gastrointestinal tract or urinary tract can result in severe iron deficiency anemia.

Von Willebrand Disease

The most common inherited bleeding disorder of dogs and people is VWD.[1,2] This disorder results from defective or deficient von Willebrand factor (VWF). VWF is a large, multimeric protein synthesized by endothelial cells and megakaryocytes that is important for normal platelet adhesion. Variably sized (small, medium, and large) VWF multimers circulate in plasma. The largest multimers of the protein are the most hemostatically active and are primarily stored within Weibel-Palade bodies within vascular endothelial cells.[3] VWF also acts as a carrier for factor VIII-coagulant (FVIII-C), providing stability for FVIII-C in circulation by preventing protease-related degradation.[3]

Human and canine patients with VWD are classified into 3 types based on the presence of quantitative or qualitative abnormalities in VWF. Type 1 VWD includes patients with an equal decrease in all sizes of multimers. This is the most common type of VWD in people and dogs. In people, this type accounts for 60–80% of all cases and in dogs accounts for greater than 95% of all cases. Type 2 includes patients with qualitative changes in multimers and decreases in only the large multimers. Type 2 VWD is the second most common type of VWD in people (20–40% of cases) but is rare in dogs. Type 3 (also called "severe Type 1") includes patients with no detectable VWF. Type 3 VWD is rare in people and in dogs. Almost all canine cases of VWD are Type 1. Only German Shorthair Pointers and German Wirehair Pointers have been described with Type 2 VWD.[4,5] Type 3 VWD has been described in Scottish Terriers, Shetland Sheepdogs, Dutch Kooikers, and a Chesapeake Bay Retriever.[6–12]

General signs include mucosal bleeding primarily manifested by gingival bleeding, epistaxis, and hematuria. Prolonged bleeding at tail docking, ear cropping, or dew claw removal are other common manifestations. VWF circulates as a complex with factor VIII-C, the protein deficient in hemophilia A. Factor VIII-C activity is usually reduced in severe forms of VWD; however, in most cases of VWD in dogs, reduction in factor VIII-C activity is mild-to-moderate[13] and coagulation screening tests, such as the activated partial thromboplastin time (APTT) and activated clotting time (ACT), are normal. Because VWF is important in mediating the adhesion of platelets to subendothelial surfaces, the clinical presentation is classical for signs observed with platelet disorders (mucosal or cutaneous hemorrhage). Dogs experiencing a bleeding diathesis in the absence of abnormal coagulation screening tests or thrombocytopenia should be tested for VWD.

At present, most assays are quantitative and involve an ELISA technique.[14] The Botrocetin assay[15] is a qualitative assay that has been developed; however, this assay is not readily available. A collagen-binding activity assay has been described for assessing VWF function.[16] Citrated plasma samples to be analyzed for VWF should be frozen immediately and sent frozen in plastic tubes to a veterinary diagnostic laboratory within 2 weeks of collection. Puppies should not have been vaccinated or received medication within 2 weeks of sampling. Molecular testing has become available for some breeds (Table 2). Information on genetic testing can be obtained at: http://www.vetgen.com.

Affected puppies may only experience bleeding problems following vaccination or surgical procedures. The administration of drugs known to alter platelet function should be avoided in dogs suspected or known to have VWD. Hemorrhagic crises can be arrested by the transfusion of fresh whole blood or plasma or cryoprecipitate.[17] Plasma or cryoprecipitate is preferred especially if cross-matching cannot be performed since these dogs may require repeated transfusions.[18] Desmopressin acetate (DDAVP), a synthetic analogue of vasopressin, has been administered intravenously in people to increase the concentration of circulating VWF.[19,20] This compound serves to release preformed high molecular weight (HMW) multimers of VWF from Weibel-Palade bodies within vascular endothelial cells.[3,21] A maximal response (2-fold or greater rise in VWF) is usually reached within 1–2 hours after a dosage of 0.3 μg/kg body weight. Equivalent responses were not seen in normal healthy dogs or in Doberman Pinschers with VWD even at dosages as high as 3 μg/kg body weight.[22] Although VWF concentrations did increase in 1 study, the proportion of HMW multimers was not increased after DDAVP administration.[23] In spite of the lack of observable rise in VWF antigen, the bleeding times of dogs with VWD did shorten to the normal range 2 hours after administration of DDAVP. This was likely due to localized release of VWF that could not be documented in

Table 2: Extrinsic platelet disorders in dogs, horses, and cats

Breed	Disorder	Mutation
Doberman[P*]	VWD type 1	Exon 43, TCG to TCA, enhancement of cryptic splice site (premature stop)
Quarter horse[25]	VWD type 2	Not known
Thoroughbred[26]	VWD type 2	Not known
German Shorthair Pointer[5]	VWD type 2	Exon 28, AAT to AGT (N to S)
German Wirehair Pointer[5]	VWD type 2	Exon 28, AAT to AGT (N to S)
Scottish Terrier[9]	VWD type 3	Exon 4, 1-bp del, frame shift (premature stop)
Dutch Kooiker[10]	VWD type 3	Intron 16, splice site gt to ga, alternative splicing (premature stop)
Shetland Sheepdog[P]	VWD type 3	Exon 7, 1-bp del, frame shift (premature stop)
Himalayan cat[24]	VWD type 3	Not known

*Bernese Mountain Dog, Coton de Tulear, Drentsche Patrijschond, German Pinscher, Kerry Blue Terrier, Manchester Terrier, Papillion, Pembroke Welsh Corgi, Poodle, and Stabyhoun have the same mutation as Doberman Pinschers. P, US Patent 6,074,832; bp, base pair; del, deletion; N, asparagine; S, serine.

peripheral blood samples. DDAVP may be useful in some dogs as a transient relief to a bleeding episode.

In contrast, VWD is rare in cats[24] and horses.[25,26] In 1 Quarter horse[25] and in 1 Thoroughbred[26] with VWD, the HMW multimers were decreased suggesting the presence of Type 2 VWD. Further characterization of VWD in cats and horses is lacking.

Acquired VWD can occur in animals with enhanced degradation of HMW multimers of VWF by ADAMTS13 (a disintegrin and metalloprotease with thrombospondin repeats), an enzyme that cleaves VWF under high shear conditions.[27] High shear conditions can be generated with mitral valve regurgitation and other types of heart valve disorders.[28,29]

Intrinsic Platelet Disorders

Storage pool disorders

Cyclic hematopoiesis

Cyclic hematopoiesis is an autosomal recessive disorder described in grey collies characterized by cyclic fluctuations in the number of circulating neutrophils, reticulocytes, and platelets.[30,31] Melanocytes are also affected in this disorder. The basis for the disease is a bone marrow stem cell defect resulting in neutropenic episodes occurring approximately every 12 days. Mortality is high; most puppies die prior to 6 months of age due to fulminating infection. Platelet numbers usually do not decline below the normal range and fluctuate between 300,000 and 700,000/μL. Platelet reactivity to collagen, PAF, and possibly thrombin is defective.[32] Platelet dense granules are absent. Clot retraction and platelet adhesiveness are impaired. A mutation in the gene encoding adaptor protein complex 3 (AP3) beta-subunit has been linked to this disorder. AP3 directs trans-Golgi export of transmembrane cargo proteins to granules.[33]

Chediak-Higashi Syndrome

Chediak-Higashi syndrome (CHS) is an autosomal recessive genetic disorder characterized by abnormal leukocyte, melanocyte, and platelet granulation.[34] Affected animals have partial oculocutaneous albinism, increased susceptibility to infection, and a bleeding diathesis as a result of impaired platelet function. Platelets of affected individuals lack discernable dense granules and are deficient in storage pools of adenine nucleotides, serotonin, and divalent cations. Studies of platelet ultrastructure indicate that CHS platelets do not form tight aggregates in response to adenosine diphosphate (ADP) in vitro. The disease has been identified in a line of Persian cats; all of the affected animals exhibited a "blue smoke" hair color and pale irises with the development of bilateral nuclear cataracts in several individuals. Affected cats experienced prolonged bleeding at incision sites and the development of hematomas following venipuncture.[35] CHS has also been diagnosed in Aleutian mink, 3 breeds of cattle, blue foxes, killer whales, and mice.[36–41] CHS in Japanese Black cattle, mice, and people has been linked to mutations in the lysosomal trafficking regulator (LYST) gene.[42] *LYST* encodes a 425-kDa cytoplasmic protein that may be involved with incorporation of proteins into lysosomal membranes. Molecular studies have not been reported in Brangus or Hereford cattle or other species affected with CHS.

Dense Granule Defect

A dense granule defect has been described in a family of American Cocker Spaniels. Platelet ^{14}C-serotonin uptake and retention was normal; Adenosine triphosphate (ATP) content was also normal. ADP content was low resulting in an increased ATP/ADP

ratio. Platelet number and morphology were normal suggesting a functional dense granule defect. Affected Cocker Spaniels outside this family have not been identified and molecular studies have not been conducted.[43]

Membrane receptor disorders

Glanzmann thrombasthenia

Glanzmann thrombasthenia (GT) is an intrinsic platelet disorder in which the glycoprotein complex IIb-IIIa (GPIIb-IIIa), also known as the fibrinogen receptor and as integrin α_{IIb}-β_3, is absent or reduced on the surface of platelets (Figures 1 and 2). In people, GT is categorized into 3 main types: Type I, Type II, and Variant.[44] In Type I, less than 5% of the receptor is detectable on platelet surfaces and clot retraction is absent. In Type II, 10–20% of the receptor is present on platelet surfaces and clot retraction is detectable but reduced. In Variant GT, the receptor is present but dysfunctional; clot retraction may or may not be detectable. The GPIIb-IIIa receptor, which undergoes a conformational change in response to stimuli such as ADP, collagen, and thrombin, binds fibrinogen and mediates platelet aggregation. Platelets of GT patients have markedly impaired platelet aggregation responses and clot retraction is usually markedly impaired to absent. The 2 subunits for the receptor (GPIIb and GPIIIa or αIIb and β3) are encoded by separate genes. Because both subunits must be synthesized and are present to form stable complexes on platelet surfaces, mutations in either gene can result in GT. GT has been well-documented in people; over 80 mutations have been documented in the gene encoding GPIIb and over 50 mutations have been identified in the gene encoding GPIIIa.[44] A database of causative mutations in people is available online at: http://sinaicentral.mssm.edu/intranet/research/glanzmann/menu.

All of the mutations resulting in GT in domestic animals thus far have been in the gene encoding GPIIb (Table 1). GT has been documented at the molecular level in Great Pyrenees,[45–47] Otterhounds (formerly referred to as Thrombasthenic Thrombopathia),[48,49] a Thoroughbred-cross,[50,51] a Quarter Horse,[50,52] a Peruvian Paso,[53] and an Oldenbourg.[54] GT has also been reported in a Standardbred in Australia[55] and in a Thoroughbred in Japan;[56] however, the molecular basis for GT was not determined in those reports. The mutations in Great Pyrenees and Otterhounds are distinct and both are located within gene sequences that encode highly conserved calcium-binding domains within GPIIb.[47,49] Great Pyrenees dogs are being tested sporadically by breeders and owners; mutation prevalence is unknown.

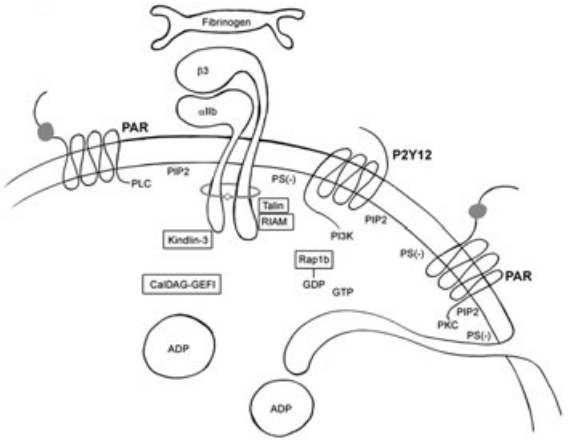

Figure 1: Illustration depicting an unactivated platelet. Prior to activation the fibrinogen receptor, integrin α_{IIb}-β_3, is in a low affinity state that does not bind fibrinogen. The low affinity state is maintained by a salt bridge between the α_{IIb} and β_3 tails that prevents them from moving apart. Major receptors that are involved in platelet activation include the ADP receptor P2Y12 and the thrombin protease-activated receptors (PAR). Dense granules contain components, including ADP, that are essential for providing a positive feedback mechanism for enhancing platelet activation. Calcium diacylglycerol regulated guanine nucleotide exchange factor I (CalDAG-GEFI), kindlin-3, Rap1b, Rap1-GTP-interacting adaptor molecule (RIAM), and talin are all important signal transduction components necessary for altering the affinity state of integrin α_{IIb}-β_3. In the unactivated platelet, Rap-1b is associated with guanosine diphosphate and is not active, and talin, RIAM, and kindlin-3 are not associated with the β_3 tail. Phosphatidylserine (PS) is primarily located on the inner membrane of the unactivated platelet.

Great Pyrenees dogs that are affected or are heterozygous for the mutation have been identified in Mississippi, Alabama, Florida, Missouri, Indiana, Illinois, Oklahoma, and Washington. Gene therapy studies aimed at the correction of GT in Great Pyrenees dogs as an animal model for human GT has been conducted at the Medical College of Wisconsin.[57]

Two distinct mutations have also been identified in horses. The Thoroughbred-cross, located in England, and the Oldenbourg, located in Canada, are homozygous for a single nucleotide change in exon 2 (CGG to CCG) resulting in the change of an arginine to a proline in a highly conserved area of GPIIb.[52,54] The Peruvian Paso, located in Idaho, is homozygous for a 10-base-pair deletion in exon 11 that includes the exon 11 and intron 11 splice site.[53] The deletion results in lack of splicing of the intron and the appearance of a premature stop codon that prevents synthesis of a stable protein. The Quarter Horse, located in Alabama, is a compound heterozygote and has both of the mutations described above.[50–52]

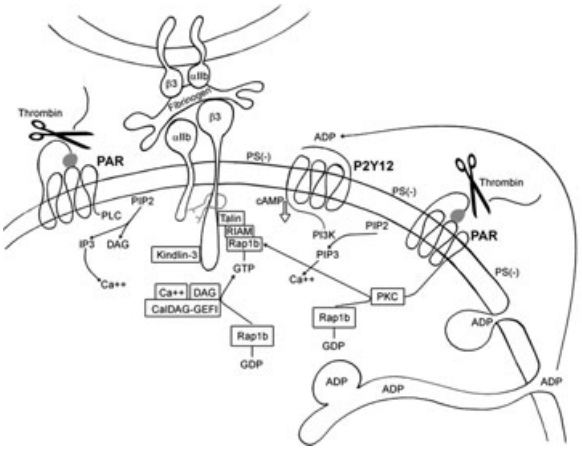

Figure 2: Illustration depicting an activated platelet. Platelet activation occurs when agonists, including ADP and thrombin, bind to specific receptors on the platelet surface. Thrombin cleaves protease-activated receptors (PAR) to generate a tethered ligand that binds to and activates the receptor. Thrombin- and ADP-mediated platelet activation lead to the activation of phosphoinositide-3-kinase (PI3K) and the generation of inositol triphosphate (IP3), a calcium-mobilizing agent, and diacylglycerol (DAG), via activation of phospholipase C (PLC). ADP binding to P2Y12 also results in lowering of cAMP levels that lowers the threshold for platelet activation. DAG and calcium bind to and activate calcium diacylglycerol guanine nucleotide exchange factor I (CalDAG-GEFI), which in turn facilitates the exchange of GDP for GTP on Rab-1b. Guanosine triphosphate bound Rap1b, RIAM, and kindlin-3 facilitate and enhance the binding of talin to the β_3 tail resulting in the elimination of a salt bridge and a change in conformation of integrin α_{IIb}-β_3 to a high affinity state that is capable of binding fibrinogen. Thrombin cleavage of PAR can also result in protein kinase C (PKC)-mediated activation of Rap1b that does not require CalDAG-GEFI, which is thought to explain why platelets from animals with CalDAG-GEFI thrombopathia respond to thrombin and have normal clot retraction. Calcium mobilization also results in the flip-flop of phosphatidylserine (PS) to the outer platelet membrane thus providing a negatively charged surface for assembly of coagulation factors. Granule release also occurs secondary to calcium mobilization. Granules may either bind to the open canalicular system (OCS) or directly to the outer membrane of platelets in species (cattle, horses) that do not have an OCS. Components released by granules, including ADP, serve to provide a positive feedback loop that further enhances platelet activation.

P2Y12 (ADP) Receptor

Platelets have 2 ADP receptors, P2Y1 and P2Y12.[58] P2Y1 is coupled to the heterotrimeric GTP-binding protein Gq and to phospholipase C-β activation. ADP binding to P2Y1 induces mobilization of cytoplasmic calcium and mediates shape change and an initial wave of rapidly reversible aggregation. P2Y12 is coupled to adenylyl cyclase through Gi, with activation resulting in the lowering of cAMP levels accompanied by progressive and sustained platelet aggregation (Figure 2). P2Y12 activation plays an important role in potentiating the effects of several agonists, including collagen and thrombin, via granule release of ADP invoked by these agonists. Eight distinct mutations have been identified in the gene encoding P2Y12 in people.[59] Clinical signs in affected people include easy bruising and excessive hemorrhage after trauma or surgery.[60]

Recently, a mutation in the gene encoding P2Y12 was identified in a Greater Swiss Mountain dog located in Alberta, Canada.[61] The veterinarian did not have access to a platelet function facility, thus platelet function was not evaluated using platelet aggregation techniques. Flow cytometric studies on platelets isolated within 24 hours of blood collection indicated adequate levels of GPIIb and GPIIIa; however, fibrinogen binding, documented using a receptor-induced binding site antibody,[62] in response to ADP was undetectable. Fibrinogen binding in response to convulxin and platelet activating factor (PAF) was comparable to 2 other samples sent as controls; control samples also bound fibrinogen in response to ADP. The mutation is predicted to eliminate a serine from the second extracellular loop of the P2Y12 receptor, a portion of the loop that is thought to play a role in ADP ligand recognition and binding.[63] The dog experienced abnormal hemorrhage after an ovariohysterectomy; prior to surgery there was no clinical evidence of a bleeding disorder. Subsequent evaluation of DNA collected from related and unrelated Greater Swiss Mountain dogs identified the mutation in multiple generations and in unrelated dogs, suggesting the mutation may be widespread in the breed.

Signal transduction disorders

Calcium diacylglycerol guanine nucleotide exchange factor I disorders

Platelet function disorders, likely due to problems with signal transduction, have been described in several dog breeds, including mixed-breed dogs.[64] Many of these disorders have not been evaluated at the molecular level. As observed in people,[65] signal-transduction disorders are probably the most common cause of inherited intrinsic platelet disorders in dogs and possibly other species. The molecular bases for the signal transduction-related platelet disorders in Basset hounds, Spitz, Landseers-ECT, and Simmental cattle are due to distinct mutations in the gene encoding calcium diacylglycerol guanine nucleotide exchange factor I (CalDAG-GEFI). CalDAG-GEFI is a GEF involved in activation of Rap1b (Figure 2), which in turn plays a role in eliciting the change in conformation of GPIIb-IIIa necessary for fibrinogen binding.[66,67] These disorders are characterized

functionally by a decreased to absent response to most platelet-activating agents, including ADP and collagen. Platelets of affected animals do respond maximally to thrombin; however, the kinetics are impaired. There is a lag phase of 3–6 minutes before maximal platelet aggregation occurs compared to normal platelets that are fully aggregated in response to thrombin within 2–3 minutes. Because affected platelets do respond to thrombin, clot retraction is normal in these disorders. The ability of thrombin to activate mutant platelets is either due to the ability of thrombin to bypass CalDAG-GEFI pathways in the activation of Rap1b or act via a pathway that is independent of Rap1b.[68,69]

Basset hound thrombopathia

Basset hound thrombopathia is a hereditary, intrinsic platelet disorder first described in 1979.[70] Affected dogs exhibit signs typical of quantitative or qualitative platelet defects including epistaxis, gingival bleeding, and petechiation. Affected basset hounds may present with weakness and rear limb paralysis secondary to spinal cord hemorrhage. The mode of inheritance for the defect is autosomal recessive; heterozygotes have no clinical signs. The platelet defect is characterized by impaired or absent platelet aggregation responses to all agonists except thrombin; the thrombin response is rate impaired but present.[71] Platelets of affected dogs have normal levels of membrane glycoproteins, including GPIIb and GPIIIa. The clot retraction test is normal in affected dogs. The mutation responsible for Basset hound thrombopathia is in the gene encoding CalDAG-GEFI.[72] (Table 1). Basset hounds experiencing platelet-type bleeds that are not thrombocytopenic should be tested for VWD and for Basset hound thrombopathia. Heterozygote prevalence, based on the testing of approximately 300 dogs, is 25–30% making this disorder as common or more common as VWD in this breed. Heterozygous dogs have been identified in the United States, Canada, Italy, and Germany.

Spitz thrombopathia

An intrinsic platelet disorder was identified in 2 Eskimo Spitz dogs that presented at the Auburn University Small Animal Hospital in the mid-1990s.[73] Both dogs were female and presented at separate times (approximately 18 mo apart) with histories of chronic epistaxis and gingival bleeding. One of the dogs had shifting leg lameness. Both dogs were anemic from chronic blood loss at the time of presentation. The platelets of the dogs did not aggregate in response to ADP, collagen, or PAF. The platelets did aggregate in response to thrombin but the response was rate impaired. The platelet aggregation pattern observed was identical to that described in Basset hounds with thrombopathia. The mutation causing Spitz thrombopathia is in the gene encoding CalDAG-GEFI.[72] The mutation is distinct from the mutation identified in Basset hounds (Table 1). Since the first report, no other affected or heterozygous Eskimo Spitz dogs have been identified. Whether lack of identification of other cases is due to low prevalence of the mutation in the population or lack of recognition of the disorder is not known.

Landseer-ECT thrombopathia

Landseers of European Continental Type (ECT) have been recognized as a distinct breed in Europe since about 1960. These are large black and white dogs that are derived from the Newfoundland breed and are distinct from American Landseers. An intrinsic platelet disorder had been documented in Landseers-ECT in the mid-1990s. Studies at the University of Utrecht indicated that the platelet disorder resembled that seen in Basset hounds and Spitz dogs with thrombopathia (personal communications). The mutation causing Landseer thrombopathia is in the gene encoding CalDAG-GEFI[72] and is distinct from the mutations described in Basset hounds and Spitz dogs (Table 1). Landseers-ECT have been tested at the molecular level in several European countries including Finland, Norway, Sweden, the Netherlands, Germany, Estonia, Austria, Lithuania, Poland, Belgium, Denmark, France, and Hungary. Heterozygote prevalence in Landseers-ECT, based on the testing of approximately 300 dogs, is approximately 12%.

Bovine thrombopathia

Bovine thrombopathia is an inherited platelet function defect identified in Simmental cattle in the United States and Canada.[74,75] Clinical bleeding varies from mild to severe and is exacerbated by trauma or surgery. Affected cattle have platelet aggregation responses similar to those described in Basset hounds, Landseers-ECT, and Spitz dogs with thrombopathia.[76,77] The molecular basis for Bovine thrombopathia is a distinct mutation in the gene encoding CalDAG-GEFI (Table 1).[78] Genetic testing is not being conducted and has not been mandated by breed organizations to determine the prevalence of this mutation in Simmental cattle.

CalDAG-GEFI thrombopathia summary

The finding of distinct mutations in the gene encoding CalDAG-GEFI in 3 breeds of dogs and in cattle, all resulting in clinical bleeding, illustrates the importance of this signal transduction protein in the pathways leading to the change in conformation of the fibrinogen receptor

necessary for normal platelet aggregation. Thrombin is able to bypass CalDAG-GEFI and induce platelet aggregation; thus animals with CalDAG-GEFI disorders have normal clot retraction. Mutations in the gene encoding CalDAG-GEFI are likely present in other breeds of dogs and in other species including people, cats, and horses. A CalDAG-GEFI mutation previously reported in people associated with severe bleeding, leukocytosis, and susceptibility to infection[79,80] has since been determined to be an insignificant polymorphism linked to a mutation in the gene encoding Kindlin-3.[81–83] None of the animals (dogs or cattle) documented to have CalDAG-GEFI mutations experienced leukocytosis or were prone to infections; clinical signs were solely characterized as a bleeding diathesis. To date, significant mutations have not been identified in the gene encoding CalDAG-GEFI in people.

Kindlin-3 Disorders (LAD-I Variant or LAD-III)

Leukocyte adhesion deficiency III (LAD-III), also known as LAD-I Variant, is a rare disorder characterized by failure of activation of β1-, β2-, and β3- type integrins expressed on hematopoietic cells.[84] Affected individuals have a bleeding diathesis similar to that observed with GT and also have persistently high leukocyte counts and are susceptible to infections. Several reports have implicated mutations in *KINDLIN3* in people as cause for an LAD-III phenotype.[81–83] Kindlin-3 is thought to enhance Talin-induced inside-out signaling mediated by binding of Talin to membrane proximal beta-1 and beta-3 tail motifs (Figure 2). Kindlin-3 likely also plays a role in integrin outside-in signaling.[85]

LAD III has been documented in a male German Shepherd Dog.[86] The dog presented at Washington State University College of Veterinary Medicine on numerous occasions starting at 6 months of age with complaints of lameness, abnormal bleeding, and infections, including deep pyoderma and pododermatitis, gingivitis, and cellulitis, often accompanied by fever. The affected dog developed profuse hemorrhage after a laceration on the lip and was euthanized at 6 years of age. A male sibling of the affected dog bled to death at 3 years of age. Assays of primary and secondary hemostasis and complete blood counts (CBC) were performed on multiple occasions. Assays of hemostasis included buccal mucosal bleeding times, coagulation screening assays (prothrombin time [PT], APTT), VWF antigen concentration, factor VIII-C activity, platelet number, platelet aggregation, and clot retraction. The buccal mucosal bleeding time (BMBT) was consistently prolonged and was usually greater than 8 minutes (reference 2.5 to 4.5 min). Coagulation screening assays were within reference intervals as were VWF antigen (122%) and factor VIII-C (115%). Platelet aggregation studies indicated a delayed reaction and diminished overall response to ADP and collagen when compared to a normal control. Clot retraction was also impaired. White cell counts over a 5-year period ranged from 24,000 to 187,440/μL (reference interval 5,800–11,700/μL). Flow cytometry studies, conducted at Auburn University and the University of Pennsylvania, indicated normal concentrations of GPIIb and GPIIIa on platelets, and CD11a, CD11b, CD11c, and CD18 on leukocytes. DNA sequences of coding regions for CalDAG-GEFI, GPIIb, and GPIIIa were comparable to sequences obtained from the canine genome. These findings ruled out the presence of a CalDAG-GEFI disorder, Glanzmann thrombasthenia, or LAD-I. A 12-base-pair insertion was identified in the coding region for *KINDLIN3* in the affected dog that was not present in the canine genome sequence or the control dog sequences.[86] This mutation is predicted to result in the insertion of amino acids RRLP within an alpha helix located in the kindlin-3 pleckstrin-homology domain. It is not known whether this mutation results in lack of expression of Kindlin-3 or lack of function.

Disorder of procoagulant expression

Scott syndrome

When platelets become activated, they express a negatively charged surface due to flip-flop of negatively charged phosphatidylserine to the platelet surface (Figures 1 and 2). The negatively charged surface is critically important in supporting the assembly of coagulation factors leading to generation of thrombin and conversion of fibrinogen to fibrin. Scott syndrome is a rare disorder described in people and in German Shepherd Dogs in which platelets do not express procoagulant activity.[87–89] Affected platelets are impaired in their ability to express phosphatidylserine or prothrombinase activity on their surface and microparticle release is impaired. Platelet function testing including aggregation and release, clot retraction, buccal mucosal bleeding times, platelet functional analyzer (PFA-100) analysis, and thromboelastography, are normal.[88,90] Flow cytometric techniques can detect impaired microparticle release.[90] Annexin binding is also impaired in this disorder.[91] Although this is a platelet disorder, clinical signs are more typical of a coagulopathy due to inability of the platelets to generate and support a surface for assembly of coagulation protein complexes. The molecular basis for this disorder is unknown in people or in dogs. Genome-wide studies are being conducted at Cornell University in an effort to locate the mutation causing this disorder.[92]

Inherited thrombocytopenia

BETA1-tubulin macrothrombocytopenia

Cavalier King Charles Spaniels (CKCS) commonly have macrothrombocytopenia characterized by large platelets and platelet numbers between 30,000 and 100,000/μL.[93–96] This thrombocytopenia is a congenital disorder and the inheritance has been classified as autosomal recessive.[97] However, dogs that are heterozygous for this disorder often have platelet numbers between 100,000 and 200,000/μL suggesting autosomal dominant inheritance. The disorder is not associated with abnormal hemorrhage; however, misdiagnosis as an acquired thrombocytopenia can lead to inappropriate case management and treatment. The molecular basis for the inherited macrothrombocytopenia in Cavalier King Charles Spaniels is due to a mutation in the gene encoding beta1-tubulin[98] (Table 1). The prevalence rate in CKCS is high; 90% or more of CKCS are either carriers or affected for the mutation. The description of the genetic basis for macrothrombocytopenia in CKCS led investigators in Japan to evaluate the gene encoding beta1-tubulin in a 7-year-old boy with macrothrombocytopenia of unknown cause. The investigators subsequently identified the first beta1-tubulin gene mutation in people associated with an inherited macrothrombocytopenia.[99]

CKCS also have a high incidence of mitral valve regurgitation[100] and while in some dogs the disorder is characterized by rapid development of heart failure secondary to volume overload, in many CKCS mitral valve regurgitation is well-tolerated until late in life. Considering that the beta1-tubulin isoform is up-regulated in cardiac tissue during volume overload,[101] it is interesting to speculate that up-regulation of mutant beta1-tubulin in affected CKCS may diminish or prevent increased microtubule polymerization and protect dogs from volume overload-associated ventricular wall rigidity and ensuing heart failure.[102,103] More studies are required to investigate this possibility.

The same mutation has been documented in other breeds of dogs, including Chihuahua, Jack Russell terrier, Labrador Retriever, English Toy Spaniels, Labradoodle, Poodle, Shi Tzu, Maltese, and Havanese, though the prevalence of the mutation is much lower in these breeds (personal observations). Norfolk Terriers[104] and Cairn Terriers (personal observations) have also been identified with what is likely an inherited thrombocytopenia; studies are on going to determine the molecular basis for the thrombocytopenia observed in these breeds. Inherited thrombocytopenia should be suspected in any animal with a persistently low platelet count in the absence of clinical bleeding and that is nonresponsive to treatment with steroids or antibiotics.

May-Hegglin anomaly

May-Hegglin anomaly (MHA) is a disorder characterized by macrothrombocytopenia and leukocyte inclusions due to mutations in the *MYH-9* gene that encodes nonmuscle myosin heavy chain IIA (NMMHCIIA).[105] The disorder has been well-documented in people; clinical manifestations are diverse and range from solely a macrothrombocytopenia to concurrent renal failure, deafness, and cataract formation. Epstein syndrome, Fechtner syndrome, and Sebastian platelet syndrome, all due to mutations in the *MYH-9* gene, have overlapping clinical manifestations that tend to be more severe than those described for MHA. The *MYH-9* gene is large and the more severe phenotypes are associated with mutations affecting the first 1,400 amino acids that function in the N-terminal head and neck regions of the protein. Macrothrombocytopenia alone is more likely observed with mutations affecting the more distal portion of the gene, exons 20–40 that encodes the C-terminal tail. Bleeding symptoms are mild to nondetectable but may be exacerbated with coexisting severe acquired thrombocytopenia. Recently an 8-year-old spayed female Pug was identified with MHA at the University of Tennessee College of Veterinary Medicine.[106] The dog had been treated with immunosuppressive drugs because of presumed immune-mediated thrombocytopenia; however, platelet numbers were not altered with drug treatment. Inclusion bodies, similar to those described in people with MHA were documented in neutrophils that prompted evaluation of the gene encoding MYH-9. A single nucleotide change was documented in Exon 38 at nucleotide position 5521 (C5521A) that would be predicted to substitute a lysine for glutamine at amino acid position 1841 (E1841K). The identical mutation has been documented as a cause for MHA in people.

Management and Treatment

Young growing animals with chronic blood loss must often be treated with iron injections to resolve iron deficiency anemia; oral iron is often not sufficient to replace the needs of the animal for growth and erythrocyte production. Clients who own animals with inherited platelet disorders should be taught how to evaluate the mucous membranes of their pets on a regular basis. They should visit their veterinarian at least every 3–4 months to monitor hematocrit and serum iron concentrations. Gastrointestinal blood loss is a common manifestation of inherited platelet disorders. Adult animals may require oral iron supplementation to prevent development of iron deficiency anemia. Control of acute severe hemorrhage due to an extrinsic platelet disorder requires

transfusion with plasma or cryoprecipitate. Whole blood transfusions should be reserved for patients in whom the hematocrit has dropped below 15%. Control of acute severe hemorrhage due to an intrinsic platelet disorder requires treatment with platelet-rich plasma or platelet concentrate. When considering transfusion therapy either in conjunction with an elective surgical procedure or to treat an on going hemorrhage, it is imperative to distinguish between the presence of an extrinsic or intrinsic platelet disorder.

Conclusion

While VWD is still considered the most common inherited platelet disorder in most species, several inherited intrinsic platelet disorders have been documented at the functional and molecular level in domestic animals. In some species and breeds, the inheritance of an intrinsic platelet disorder is as likely as inheritance of VWD. This becomes critically important when treatment modalities are being considered to arrest hemorrhage since plasma, cryoprecipitate, or whole blood transfusions are not likely to be sufficient for treatment of intrinsic platelet disorders. Inherited thrombocytopenias have become more widely recognized in dogs. Unlike in people, the inherited thrombocytopenias documented so far in domestic animals do not result in clinical bleeding and the greatest risk to affected animals is inappropriate treatment with antibiotics or steroids. Characterization of inherited platelet defects at the molecular level has greatly enhanced the ability to diagnose these disorders and in some cases may help to reduce or even eliminate disorders from some breeds. Characterization of inherited platelet disorders in domestic animals has also been beneficial in the development of treatment strategies and the development of novel platelet inhibitory medications.

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