Inheritance of von Willebrand’s disease in a colony of Doberman Pinschers

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Objective—To determine the mode of inheritance of von Willebrand’s disease (vWD) and perform linkage analysis between vWD and coat color or narcolepsy in a colony of Doberman Pinschers.

Animals—159 Doberman Pinschers.

Procedure—vWf factor antigen (vWF:Ag) concentration was measured by use of ELISA, and results were used to classify dogs as having low (<20%), intermediate (20 to 65%), or high (>65%) vWF:Ag concentration, compared with results of analysis of standard pooled plasma. Buccal bleeding time was measured, and mode of inheritance of vWD was assessed by pedigree analysis.

Results—von Willebrand’s disease was transmitted as a single autosomal gene defect. Results suggested that 27.04% of dogs were homozygous for vWD, 62.26% were heterozygous, and 10.69% did not have the defect. Most homozygous and some heterozygous dogs had prolonged bleeding times. Dogs with dilute coat colors (blue and fawn) were significantly overrepresented in the homozygous group, compared with black and red dogs, but a significant link between vWD and coat color was not detected.

Conclusions and Clinical Relevance—von Willebrand’s disease is transmitted as an autosomal dominant trait with variable penetrance; most dogs in this colony (89.3%) were carriers of vWD. Homozygosity for vWD is not likely to be lethal. Some heterozygous dogs have prolonged bleeding times. An association between dilute coat colors and vWD may exist. (Am J Vet Res 2000;61:115–120)

von Willebrand factor (vWF) is a multimeric glycoprotein that is crucial to normal hemostasis. It acts as an adhesive ligand that binds platelets and vascular subendothelium upon vascular injury,1 and as a carrier molecule and stabilizer for factor VIII.2 Decreased concentrations of functional vWF result in increased susceptibility to abnormal bleeding.3 The term von Willebrand’s disease (vWD) refers to a heterogeneous class of bleeding disorders characterized by abnormalities in vWF (quantitative, qualitative, or both). von Willebrand’s disease is the most prevalent coagulation disorder in humans (approx 1% of the population is affected4) and in dogs (identified in 54 breeds).5

The disease exists in acquired and hereditary forms; acquired vWD is associated with a variety of other conditions, such as hypothyroidism.6 Hereditary vWD is thought to be caused by a single autosomal gene,7 which differentiates it from the X-linked coagulation disorder hemophilia. The complex gene encoding human vWF has been localized to chromosome 12 and consists of 178 kilobases with 52 exons.8 Although the canine vWF gene has not been fully characterized, the complete cDNA sequence of canine vWF is now available in Genbank (accession L76227).

Three major types of vWD have been classified in humans and dogs. Type 1 vWD, the most common of the 3 types, is characterized by low vWF concentrations, with a proportionate reduction of all vWF multimers.9 Type 2 vWD is characterized by a selective decrease in the large vWF multimers, which are the most biologically active for hemostasis;9 there may or may not be qualitative abnormalities in these large multimers.10 Type 3 vWD, the least prevalent type of vWD, is characterized by undetectable concentrations of vWF10

The severity of vWD in humans and dogs varies with each affected individual.11,12 An individual’s vWD status can be determined by use of a combination of diagnostic tests. Plasma vWF antigen (vWF:Ag) concentrations can be measured with various immunoassays, such as ELISA,13 and an individual’s vWD status can be determined by use of this value. However, it is often difficult to accurately diagnose vWD solely on the basis of vWF:Ag concentrations, because concentrations may vary under certain conditions, such as pregnancy.11 Therefore, it is necessary to perform other tests, such as ristocetin cofactor assays, ristocetin-induced platelet agglutination, and activated partial thromboplastin time,14,15 to establish an accurate diagnosis. One of the simplest methods is to measure bleeding time (BT); buccal mucosal BT has been used in several studies to determine the severity of vWD in dogs (reference range, 1 to 4 minutes).16

von Willebrand’s disease is reported to be particularly prevalent in several breeds of dogs. Up to 70% of Doberman Pinschers may be carriers of Type 1 vWD.17 The mode of inheritance of vWD in this breed has not been fully determined. Some researchers suggest that the disease is transmitted in an autosomal dominant pattern with variable penetrance and that the dogs affected with Type 1 vWD are heterozygous, because homozygosity is lethal.1 Others propose that the disease is recessive, because heterozygotes are not clinically affected.18 Since 1978, the Stanford Center for Narcolepsy Research has maintained a breeding colony of narcoleptic and asymptomatic Doberman Pinschers for investigation into the pathophysiology of narcolepsy.19,20 Human narcolepsy is a disabling sleep disorder characterized by abnormal sleep patterns, cataplexy,
and dissociated manifestations of rapid-eye-movement sleep. Canine narcolepsy is a naturally occurring animal model of the human disorder; narcoleptic dogs have cataplexy and reduced sleep latencies. Narcolepsy in Doberman Pinschers and Labrador Retrievers is caused by mutations in the gene encoding a receptor for the novel neuropeptides, hypocretins/orexins, and it is transmitted as an autosomal recessive trait. The objective of the study reported here was to determine the mode of inheritance of vWD in this colony and to perform linkage analysis between vWD and coat color and vWD and narcolepsy.

Materials and Methods

Dogs—Medical records of 159 Doberman Pinschers (80 males, 79 females; age range, 1.5 to 98.2 months) were reviewed for vWF:Ag concentration. Of these, 127 (68 males, 59 females; age range, 1.5 to 96.4 months) belonged to families with complete information on vWF; other genetic traits, such as narcolepsy status and coat color, were recorded for parents and littersmates, thus permitting pedigree analysis. For measurement of BT, the study included 42 of the 159 dogs (29 males, 17 females; age range, 9.5 to 94.5 months). All dogs were housed in individual cages (1.0 m × 1.8 m) at the Stanford Department of Comparative Medicine Research Facility, maintained at constant conditions.

Measurement of vWF:Ag—Blood (4 mL) was collected from the cephalic vein of 139 dogs into blood collection tubes containing 0.129 M sodium citrate (9 parts blood:1 part sodium citrate). All dogs were drug-free and not in estrus or pregnant. Samples were centrifuged at 900 g for 10 minutes, and plasma was collected into plastic container tubes. Plasma was frozen within 1 hour after blood collection.

Measurement of Bleeding Time—Bleeding time was measured in 42 Doberman Pinschers using a platelet function analyzer (PFA-100; TRACUS, Tokyo, Japan). The PFA-100 uses a collagen/epinephrine stimulus and measures the time required for blood to clot in the presence of this stimulus. The study included 42 of the 159 dogs (25 males, 17 females; age range, 9.5 to 94.5 months). All dogs were housed in individual cages (1.0 m × 1.8 m) at the Stanford Department of Comparative Medicine Research Facility, maintained at constant conditions.

Results

The percentile distribution of vWF of all dogs was determined (Fig 1). The dips in distribution at 20 and 65% vWF suggested the existence of 3 groups in this population. We classified the dogs into L, I, and H vWF; using 20 and 65% as the cutoff values. With this classification method, 43 dogs (27.04%) were classified as L, 99 (62.26%) were classified as I, and 17 (10.69%) were classified as H. Mean ± SEM vWF:Ag concentrations for the L, I, and H dogs were 8.16 ± 0.57%, 38.24 ± 1.13%, and 88.41 ± 2.72%, respectively. Among the 42 dogs tested for BT, 11 were classi-
fied as L (vWF:Ag, 7.09 ± 0.71%), 28 were classified as I (vWF:Ag, 38.04 ± 1.81%), and 3 were classified as H (mean vWF: Ag, 97.00 ± 4.36%; Fig 2). Differences in mean BT of the 3 groups were significant (P < 0.001); mean ± SEM BT of the L group (5.35 ± 0.40 minutes) was significantly prolonged, compared with that of the I group (3.06 ± 23 minutes; P < 0.001) and the H group (2.30 ± 0.30 minutes; P = 0.004). It should be recognized that the I group contained some dogs with BT similar to that of the H group, as well as dogs with BT similar to that of the L group, and that some dogs in the I group had BT that were longer than the mean BT of the L group. Furthermore, a significant (P = 0.02) negative correlation was found between vWF:Ag concentration and BT (r = 0.56, df = 40).

Pedigree analysis based on vWF:Ag concentration in 127 dogs was performed (Fig 3). The dogs used in the pedigree analysis included 39 L, 76 I, and 12 H individuals. Matings in our colony were planned on the basis of narcolepsy status, although vWF:Ag concentration was also taken into account so that L × L crosses were avoided as much as possible. Furthermore, because of the low number of H dogs in our colony, H × H crosses have not been performed in the past 10 years. Five H × I crosses produced 14 pups,
L, I, and H categories were not detected. Differences among coat color groups and narcolepsy status within 118 AJVR, Vol 61, No. 2, February 2000 produced 53 pups, and 8 I H cross produced 3 pups, 10 I X I crosses produced 53 pups, and 8 I X L crosses produced 44 pups. The H X I crosses produced only H and I offspring, whereas L X I crosses produced L and I offspring (Table 1). Assuming that a single autosomal gene is responsible for the vWD trait, there were no significant differences between the expected and observed numbers of individuals in each group (L, I, and H; Table 1) and between males and females in any of the crosses. In addition, pedigree analysis clearly indicated that the trait was passed from sire to male offspring (Fig 3). Genotyping revealed that the 2 L dogs with normal BT (2.94 and 3.43 minutes) were homozygous for vWD. Two I dogs with prolonged BT (7.63 minutes), 1 I dog with BT within reference range (2.53 minutes), and 1 dog with BT within reference range (3.48 minutes) and classified as I (but with vWF:Ag concentration near the 65% cutoff point) were heterozygous for vWD (Fig 2).

Analysis of mean number of pups born per litter and mean number of neonatal deaths (observed only in the H X I, I X I, and I X L crosses) revealed that significant differences were not found between each cross (P = 0.69 and P = 0.91, respectively; Table 1), which suggests that cross type does not affect litter size or confer increased risk of neonatal death. Stillborn pups were observed in the H X L, I X I, and I X L crosses (Table 1), but differences in the numbers of stillbirths in each cross type were not statistically significant (P = 0.38). These results suggested that cross type did not confer increased susceptibility to stillbirths in pups.

Relationships between vWF:Ag concentration and other genetic traits were also analyzed. Of 136 dogs for which information on vWF:Ag concentration and coat color were available, 90 were black, 18 were red, 20 were blue, and 8 were fawn. Distribution of these dogs in the L, I, and H groups was determined (Table 2). Differences in distribution of coat colors among the 3 groups were not detected (P = 0.13). Because 2 alleles are reported to be responsible for coat color,20,21 the genotype of these dogs was approximated: black dogs were classified as having the genotype B-D-, red dogs were classified as bbD-, blue dogs were classified as B-dd, and fawn dogs were classified as bbdd. Based on this classification method, the various coat colors were further divided into subgroups, depending on whether they had the dominant B- or D- allele.20 Black and blue dogs were then grouped together as the B group, whereas red and fawn were grouped together as the B group. In addition, black and red dogs were grouped together as the D group, whereas blue and fawn dogs were grouped together as the D group. Using these groups, differences were not observed between the B and b groups (P = 0.60). However, the difference between the D and d groups was significant (P = 0.02). These results suggested that a genetic linkage or association may exist between vWD status and the diluted coat colors. Therefore, we performed linkage analysis on the relationship between vWD and coat color using the dogs used for the pedigree analysis. A significant linkage was not found between the D allele and vWD (LOD score –3.54 at 0% recombination; range, –0.81 to 0 at 5 to 50% recombination) and the B allele and vWD (LOD score –99.99 at 0% recombination; range, –0.79 to 0 at 5 to 50% recombination). We also performed linkage analysis on the relationship between vWD and narcolepsy using the same dogs; unlike vWD and coat color, significant differences between the narcolepsy genotypes and the vWD groups were not observed (P = 0.10; Table 2). As expected on the basis of distribution data, significant linkage between vWD status and narcolepsy was not

### Table 1—Expected and observed reproductive data (mean ± SEM) for matings between Doberman Pinschers with high (H), intermediate (I), or low (L) serum concentrations of von Willebrand’s factor. Percentages of expected offspring were calculated, assuming Mendelian inheritance of a single autosomal gene. Significant (P < 0.05) differences among mating groups or between expected and observed percentages for each mating group were not detected.

<table>
<thead>
<tr>
<th>Cross type (No. of litters)</th>
<th>No. of puppies born</th>
<th>No. of neonatal deaths</th>
<th>No. of stillbirths</th>
<th>Expected (%)</th>
<th>No. observed (%)</th>
<th>P value (cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L X I (8)</td>
<td></td>
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<td></td>
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<tr>
<td>Females</td>
<td>0.50</td>
<td>0.16</td>
<td>0.27</td>
<td>0.50</td>
<td>0.50</td>
<td>0.12</td>
</tr>
<tr>
<td>Males</td>
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<td>0.50</td>
<td>0.00</td>
<td>0.50</td>
<td>0.50</td>
<td>1.00</td>
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</tbody>
</table>

### Table 2—Percentage distribution of von Willebrand’s disease in 159 Doberman Pinschers with various genetic traits (coat color and narcolepsy genotype). Letters in parentheses indicate approximated genotype for coat color. Significant (P < 0.01) differences among coat color groups and narcolepsy status within L, I, and H categories were not detected.

<table>
<thead>
<tr>
<th>Coat color/variable</th>
<th>No. of dogs (%)</th>
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<tbody>
<tr>
<td></td>
<td>L I H</td>
</tr>
<tr>
<td>Black (B/D/D)</td>
<td>20 20 0</td>
</tr>
<tr>
<td>Red (bb/dd)</td>
<td>5 5 0</td>
</tr>
<tr>
<td>Blue (B/dd)</td>
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<tr>
<td>Fawn (bb/dd)</td>
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<tr>
<td>Narcoleptic</td>
<td>12 0 0</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>16 2 0</td>
</tr>
<tr>
<td>Control</td>
<td>10 0 0</td>
</tr>
</tbody>
</table>

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observed (LOD score, −0.48 at 0% recombination; range, −0.33 to 0 at 5 to 50% recombination). We also performed a linkage analysis of the B and D alleles and did not find significant linkage (LOD score, −7.21 at 0% recombination and 0 at 50% recombination).

**Discussion**

Type 1 vWD, the most common type of vWD in dogs, is characterized by a quantitative abnormality in vWF; vWF multimeric patterns are qualitatively normal. Doberman Pinschers are reported to be affected with Type 1 vWD, and as many as 70% are carriers. Type 1 vWD in Doberman Pinschers is transmitted as a single autosomal trait. Some researchers suggest that it is transmitted as a recessive trait, whereas others suggest that it is transmitted as a dominant trait and that all affected individuals are heterozygotes, because homozygosity is lethal. The Stanford Center for Narcolepsy maintains a database containing a large number of Doberman Pinschers, most of which were bred and born at our facility. This breeding colony provides a useful and unique opportunity to study inherited vWD in this breed.

A detailed report on the inheritance of Type 1 vWD in Doberman Pinschers was recently published by Moser et al. The study included 17 Doberman Pinschers, 9 mixed-breed adult dogs, and their 101 pups. The authors detected a trimodal distribution of plasma vWF concentration, which is typical for 1 locus with 2 alleles and 3 genotypes; the existence of 3 genotypes was further confirmed by pedigree analysis. In the study reported here, all 159 dogs were purebred Doberman Pinschers; 129 of these were born within a single breeding colony and had a homogeneous genetic background. As with the Moser study, we observed a trimodal distribution of plasma vWF:Ag concentration. Using the cutoff values of 20 and 65% vWF:Ag concentrations (Fig 1), the dogs were classified as either L, I, or H. Results of pedigree analysis validated the classification method used to determine the vWD status of the dogs and confirmed that vWD is transmitted as a single gene. Because the disease is transmitted from sire to male offspring (Fig 3), and because there were no differences between the expected and observed numbers of individuals of each sex, we concluded that the disease is transmitted as an autosomal trait. These results confirm that vWD in this colony of purebred Doberman Pinschers was transmitted as a single-gene autosomal trait. Therefore, on the basis of vWF:Ag concentration and pedigree analysis, the dogs in the L group are likely homozygous for vWD, the dogs in the I group are likely heterozygous carriers, and the dogs in the H group are likely unaffected with vWD. Furthermore, the fact that 1 individuals had intermediate vWF:Ag concentrations, compared with those of the dogs in the H and L groups, suggests that the vWF:Ag concentration depends on the number of functional alleles.

Although 3 groups were phenotypically distinguished by vWF:Ag concentration and pedigree analysis in our sample population, the genotype of some dogs with vWF:Ag concentration near the cutoff values (20 and 65%) could not be reliably determined solely by vWF:Ag concentration, as indicated by Moser et al. Recently, Shibuya et al isolated a polymorphic hexanucleotide microsatellite in the canine vWF gene corresponding to intron 40 in the human vWF gene. Using this microsatellite marker, Holmes et al identified 2 alleles (1 associated with normal vWF concentrations, and the other associated with normal and decreased vWF:Ag concentrations) and demonstrated that homozygosity for the former allele corresponds to normal vWF:Ag concentrations. However, the majority of dogs studied (95.6%) were heterozygous or homozygous for the latter allele, and the genotype of these dogs could not be specified by use of this marker. Brewer et al recently claimed that they had identified the DNA mutation(s) of type 1 vWD in Doberman Pinschers, and that genotyping for the mutation(s) is now commercially available. However, the precise location of the DNA mutation(s) and information on how the reliability of the genotyping was evaluated has not been disclosed at the time of submission of this paper.

In the study reported here, 27.04% of dogs were classified in the L group, 62.26% were classified in the I group, and 10.60% were classified in the H group; differences in BT between each group were significant (P < 0.001). In general, dogs in the L group had prolonged BT, compared with the I (P < 0.001) and H (P = 0.004) dogs, and the dogs in the I group had mean BT intermediate to those of the dogs in the L and H groups. These results suggest that some dogs with intermediate vWF:Ag concentrations are clinically affected with vWD, whereas some dogs with low vWF:Ag concentrations may not be clinically affected. Results of genetic testing further confirmed that dogs in the L group with normal BT were indeed homozygous, and all other dogs tested, regardless of BT, were heterozygous carriers. These results together suggest that vWD in this colony is likely to be transmitted as a single autosomal dominant trait with variable penetrance.

In the breeding colony described in this report, 89.3% of dogs were carriers of vWD; this number is much higher than another reported value of 70%. However, our colony consisted of dogs born from non-randomized matings that necessarily included a large number of backcrosses for the narcolepsy gene (12 homozygous narcoleptic × heterozygous narcoleptic crosses produced 95 pups). The vWF:Ag concentration of each dog was taken into account when matings were planned so that L × L crosses were avoided as much as possible. Thus, the prevalence of vWD in this population of Doberman Pinschers may not be representative of the general population.

Dodds' reported that homozygosity for vWD is usually lethal. The results of the study reported here suggest the existence of 3 groups within the population, which is consistent with the existence of 3 possible genotypes (homozygous, heterozygous carrier, and unaffected). That 27.04% of this population was classified as homozygous for vWD suggests that homozygosity is not lethal. This hypothesis is supported by a lack of significant differences among various breeding cross types for mean number of pups born per litter, mean number of neonatal deaths, and mean number of stillbirths.

The detection of large numbers of blue-coated Doberman Pinschers that were affected with vWD in our colony prompted us to question whether a link between coat color and vWD existed. Although the number of blue-coated dogs affected with vWD was not significantly
different from the number of affected dogs with other coat colors ($P = 0.13$), grouping together dogs with diluted coat color (ie, blue and fawn) and comparing them with the rest of the dogs grouped together (ie, black and red) revealed that dogs with diluted coat colors were significantly more likely to have vWD than those with dark coat colors ($P = 0.02$). These results suggest a possible genetic link or association between the 2 traits. Genetic linkage exists when genes on the same chromosome lie in close proximity to each other in such a way that they tend to cosegregate.26 The presence and number of genetic recombinations are crucial for genetic linkage analysis, so genetic linkage analysis should be performed on related individuals.26 Because the dogs in this study included a large number of related individuals, this population was ideal for genetic linkage analysis; however, linkage was not observed between vWF:Ag concentration and coat color in the families in our colony. Despite lack of evidence supporting a link between diluted coat colors and vWD (LOD score, $-3.54$ at 0% recombination, range $-0.81$ to 0 at 5 to 50% recombination; Table 3), it is possible that an association between the 2 traits exists.26 Miller22 suggested there is at least 1 allele associated with the color dilution locus (ie, the d allele) that confers increased susceptibility of dilute-colored Doberman Pinschers to color dilution alopecia, a disease characterized by loss of dilute-colored hair. Because hypothyroidism has been linked to alopecia27 and reduced vWF:Ag concentrations,28 it is possible that an association exists between coat color and hypothyroidism, which is transmitted as a single autosomal dominant trait with variable penetrance, with some heterozygotes manifesting clinical symptoms of vWD. Furthermore, a possible association exists between diluted coat color and vWD. An association between 2 genetic traits exists when the 2 traits occur together in several individuals at a higher frequency than could be accounted for by chance. In order to perform a reliable association study, the individuals in the sample must be unrelated, because having members of the same family would likely artificially inflate the prevalence of the 2 traits occurring together.26 In this sense, our colony was not suited for such an analysis. Additional field studies are thus required to substantiate this finding.

Our results suggest that our colony is affected with vWD, which is transmitted as a single autosomal dominant trait with variable penetrance, with some heterozygotes manifesting clinical symptoms of vWD. Furthermore, a possible association exists between diluted coat colors and vWD. Confirmation of this latter finding through association studies will be useful, because it would allow for straightforward identification of certain dogs that should be tested for their vWD status.

References


2. Simplate blades, Organon Teknika, Durham, North Carolina.

3. Genotyping was performed by VetGen Veterinary Genetic Services, Ann Arbor, MI.


