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HERITABILITY OF PLASMA VON WILLEBRAND FACTOR ANTIGEN CONCENTRATION IN GERMAN WIREHAIRD POINTERS

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SUMMARY

We applied quantitative genetic analyses to a population of German Wirehaired pointer dogs affected with type 2 von Willebrand disease. Plasma von Willebrand factor (vWF) protein concentration measured as vWF antigen (vWF:Ag), clinical history, and pedigree data were compiled for 331 dogs over a 5-year test period. Eight dogs had histories of abnormal bleeding and had markedly decreased plasma vWF:Ag concentrations (<1%). Four per cent of the dogs were inbred, with an average inbreeding of 2.52%. The estimated heritability of plasma vWF concentration was 0.52. We found a major gene effect on vWF concentration. Using a single gene locus model and two different prediction methods, the upper threshold value for the aa genotype was less than 1% vWF:Ag, and the optimal threshold value for discrimination between the AA and Aa genotypes was between 68% and 72% vWF:Ag. Our analyses indicate that phenotype, assigned on the basis of a single vWF:Ag determination, is heritable and can be applied for selective breeding in a von Willebrand disease test programme.

Keywords: von Willebrand factor, canine von Willebrand disease, type 2 vWD, heritability.

INTRODUCTION

Von Willebrand disease (vWD) is the most common hereditary bleeding disorder of dogs and human beings (9, 16). The bleeding tendency of vWD is caused by a lack of von Willebrand factor (vWF), a large, multimeric plasma protein required for normal platelet adhesion at sites of vessel injury (22). The vWD phenotype in dogs, as in human beings, can be classified on the basis of the plasma vWF concentration and the vWF multimer structure (9, 22). Type 1 vWD is characterized by a low vWF concentration but a normal structure, type 2 by low vWF concentration and abnormal vWF multimer structure, and type 3 by a complete absence of plasma vWF. Measurement of plasma vWF concentration is therefore necessary for a definitive diagnosis of vWD. Quantitative assays of plasma vWF concentration [vWF antigen (vWF:Ag)] have also been used as screening tests for carrier detection of canine vWD in family studies and breed surveys (6, 8, 14). The measurement of vWF:Ag concentration to predict genetic status assumes that this phenotypic measure is heritable, and hence that the selection on the basis of parental vWF:Ag concentration will influence the vWF:Ag values of the offspring. Temporal variation (13), pregnancy and hormonal factors (15,

18), and variation due to sampling artifacts (12, 13) have been reported to influence the results of vWF:Ag assays. We sought to determine the heritability of vWF:Ag concentration. We tested the hypothesis that, regardless of environmental factors, a major gene effect could be demonstrated to account for much of the observed variation in plasma vWF:Ag concentration within a breed. For this study, we evaluated vWF:Ag data collected from a population of German Wirehaired pointers affected with type 2 vWD (5).

MATERIALS AND METHODS

Study Population and Sample Collection

In response to the discovery of a clinically severe form of vWD in GWHP, a North American German Wirehaired pointer breed registry, the Verein Deutsche Drahthaar, set up a voluntary testing programme. A database was developed consisting of clinical history, age, sex, registry number, pedigree, and results of plasma vWF:Ag assay (4). Referral veterinarians submitted citrate plasma samples (1 part 3.8% citrate: 9 parts blood) to the Comparative Coagulation Laboratory for determination of plasma vWF:Ag. Plasma samples were considered valid for assay if they were non-haemolyzed, contained no grossly visible clot fragments, and were shipped on refrigerant cold packs with a transit time of <72 hours. Owners and breeders were instructed to test non-pregnant bitches and were encouraged to test entire litters with dams and sires. Pedigree information and valid vWF:Ag data for parents and offspring submitted over a 5-year test period (1991 to 1996) were compiled for analyses. This interval was early in the test programme and represented the period in which there was the greatest participation among kennels and the largest number of dogs were tested each year.

von Willebrand Factor Assay

Canine plasma vWF:Ag was measured in an enzyme-linked immunosorbent assay (ELISA) (1), modified by the use of a monoclonal anti-canine vWF capture antibody. Results are reported as percentage of a pooled plasma standard (prepared from 15 clinically healthy dogs) with an assigned value of 100% vWF:Ag. The interassay coefficient of variation for this assay is 4%, and the lower limit of detection is <0.1% vWF:Ag^a.

a Quality control data, Comparative Coagulation Section laboratory.

b Sas User's Guide v. 6.09, Sas Inst. Inc., Cary NC.

c MDTFREML Manual, USDA-ARS, US Meat Animal Research Center, Clay Center, NE.

Data Analyses

Age, sex, registration number, vWF:Ag concentration, dam and sire registration numbers, and dam and sire vWF:Ag concentration (if known) for each dog were compiled for descriptive statistics and quantitative genetic analyses.^{b,c} Linear models of ANOVA were used to test for sex and age

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effects on vWF:Ag concentration and for an age quadratic effect on vWF:Ag concentration. An animal model was used to estimate the heritability of vWF:Ag (11). This estimation procedure was based on a derivative free restricted maximum likelihood algorithm. The effect of genotype on plasma vWF:Ag concentration was calculated using the major gene index (11). As a result of this analysis, a one locus genetic model with three genotype classes (i.e. aa, Aa, AA) was assumed, and two different methods (17, 24) were then applied to the data set to determine a threshold vWF:Ag value for each of the three possible genotypes.

RESULTS

Descriptive statistics

The mean, standard deviation, and range of vWF:Ag concentration and age were determined for 331 German Wirehaired pointers (168 males and 163 females; Table). No significant differences were found between the vWF:Ag concentration of males and females, and there were no demonstrable effects of age on vWF:Ag concentration ($P > 0.05$). Eight dogs (6 males, 2 females) had a clinical history of one or more episodes of abnormal bleeding. All of the clinically affected dogs had $< 0.5\%$ vWF:Ag.

Descriptive Statistics for 331 German Wirehaired Pointers.

Variable		Males	Females	All Dogs
vWF:Ag* (%)	mean	84	82.1	83
	range	0-220	0.1-147	0-220
	sd	34.6	29.9	33.5
Age (years)	mean	0.96	1.3	1.15
	range	0.1-10	0.1-10.6	0.1-10.6
	sd	1.6	2.2	1.9

* vWF:Ag = von Willebrand factor antigen as a percentage of that in a pooled canine

standard plasma containing 100% vWF:Ag.

Heritability of vWF:Ag

Data from 494 dogs (313 of the tested dogs having complete pedigrees and 181 of their relatives) were used to build an additive genetic relationship matrix for estimation of heritability (11). The model did not include fixed effects of sex or age, because these parameters were found to have no significant effect on vWF:Ag concentration. The heritability estimate of vWF:Ag concentration in this study population was 0.52. Twenty inbred dogs were found in the data set (20/494; 4.05%). The average inbreeding for these dogs was 2.52%. The regression of vWF:Ag concentration on the inbreeding coefficient for these dogs ($R = -0.246$) indicated that there was no evidence of an inbreeding depression on vWF:Ag concentration (17).

Determination of major gene effect

The major gene index ($MGI_{(\alpha)}$) was calculated using the following equation (11):

$$MGI_{(\alpha)} = \sum [(z_o - (z_f + z_m)/2)^\alpha] + \sum [(|z_o - z_f| |z_o - z_m|)^{\alpha/2}]$$

Where z_o corresponds to the phenotypic value (i.e. vWF:Ag concentration) of an offspring whose parents have values z_f and z_m . The value of α is a free parameter set by the researcher, and then the equation is repeatedly solved for each assigned α . The assigned values of α and each corresponding

MGI for the data set were: 0.5, 1.009397; 0.75, 1.035742; 1.00, 1.067169; 1.5, 1.127408; 2.00, 1.168816

All of the calculated $MGI_{(\alpha)}$ values were high (> 1.0) and increased with successive values of α . These criteria satisfy the requirements for a major gene segregating among all genes contributing to variations in plasma vWF:Ag (11).

vWF:Ag threshold estimates for a one locus genetic model

Pedigree analyses in a previous study (5), and the results of MGI in this study indicated that vWD in GWHP is likely to be a single gene defect. Two different methods were then used to determine threshold values of vWF:Ag for the three genotype classes [homozygous wild-type (AA), heterozygous (Aa), homozygous mutant (aa)]. In the first method (24), based on the assumption of Hardy Weinberg equilibrium, we examined the vWF:Ag concentration frequency distribution and found that no dogs had values between 0.3% and 5%, and that all dogs having values of 0.3% or lower had a clinical history of abnormal bleeding. Using a vWF:Ag concentration of 0.3% or lower as threshold for the aa genotype, we found the aa frequency to be 8/313 (0.0256). The aa genotype frequency was considered a fair estimate of q^2 , therefore $q = 0.16$ and $p = 0.84$. With these values, the AA frequency was 70.56% (p^2), Aa was 26.88% ($2pq$), and aa was 2.56% (q^2). The 313 observations were sorted in ascending order with respect to vWF:Ag concentration and the predicted genotype frequencies were applied, resulting in a vWF:Ag threshold value for the Aa and AA genotypes of between 71% and 72% vWF:Ag.

In a second approach for determining genotype threshold (17), the aa genotype frequency was again assigned a value of 0.0256 but the threshold vWF:Ag concentration for the Aa genotype was now allowed to vary from 39 to 100%. At each threshold, a genetic model was applied to explain the observed variation in vWF:Ag concentration. The goal of these calculations was to find threshold values of vWF:Ag for the Aa genotype that maximized the coefficient of determination (R^2). The range of threshold vWF:Ag concentrations with a high R^2 (64-75% vWF:Ag) was then refined to a single value by fitting a regression equation using estimated R^2 as the dependent variable, and threshold as independent explanatory variable. The equation was solved with a resultant final value for vWF:Ag (that maximized R^2) of 68.1%.

The estimated threshold vWF:Ag value obtained for the first method (71.5% vWF:Ag) and for the second method (68.1% vWF:Ag) were similar. Differences between the two methods in threshold vWF:Ag values for genotype prediction may be due in part to sampling bias, and to likely selection pressure against the aa genotype (dogs that expressed a bleeding tendency).

DISCUSSION

In this study we examined the heritability of plasma vWF:Ag concentration in GWHP. The analytic approach is one frequently used for selection based on parental traits such as milk production or litter size (7, 10), but is appropriate for evaluating any quantitative phenotypic variable in a population of parents and offspring (11).

Limitations of the study design included a voluntary, rather than random, selection process for submission of samples. Samples were collected prospectively from dogs throughout North America, with inclusion of dogs bred in 58 different kennels in North America and Europe. Only 4% of the dogs studied were inbred (with average inbreeding of 2.5%), and

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we believe conclusions derived from the study population are generally applicable to the breed overall. To minimize assay and sample variables, we used a sensitive ELISA method to measure vWF:Ag concentration and uniform criteria for sample inclusion from the single breed population. In this clinical study, we found that vWF:Ag concentration had a relatively high heritability, and a major gene effect was readily demonstrable as contributing to the observed variation in vWF:Ag concentration. With this single-gene defect model, application of two independent methods of threshold determination to the data set resulted in a similar cut-off vWF:Ag concentration (68-71%) in GWHP for predicting genotype Aa (vWD carrier) versus AA (vWD clear). Although a threshold of approximately 70% vWF:Ag appears to be optimal in this breed, we found that non-genetic factors also influence the phenotypic expression of vWF:Ag. Previous studies in this (and other breeds) also suggest that no discrete vWF:Ag cut-off concentration can unambiguously identify carrier and clear dogs when their vWF:Ag concentrations are near the threshold (4, 14, 19). Definitive assignment of genotype for dogs testing near vWF:Ag thresholds can be accomplished through pedigree review and progeny tests (4, 21), and ultimately through application of breed-specific molecular genetic tests as they become available (20, 25).

The regulation of vWF gene expression is complex and not fully understood. Our study indicates that age and sex are not responsible for the observed variation in vWF:Ag. Quantitative analyses of vWF:Ag concentration in different breed populations may provide further insight into the genetic (and non-genetic) factors that influence vWF:Ag concentration. For example, obligate carriers of type 3 vWD in the Dutch Kooiker breed were found to have unexpectedly high vWF:Ag levels (23). Some of the variation in vWF:Ag levels obtained in different studies might be caused by assay variables such as method, antibody source, reference standards, and assay coefficient of variation, or sampling variables such as collection and processing techniques. Nevertheless, canine vWD is proving to be molecularly heterogeneous (3, 20, 25). Different vWF mutations (or mutations in vWF regulatory regions) may explain the variation in vWF gene expression and resultant vWF:Ag concentrations found in different breed-variants of vWD.

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