

## vWD

### DNA Studies in Doberman von Willebrand's Disease.

#### The Mutation Discovered and a DNA Test Developed

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Our research team is very excited about our discovery of the mutation that causes von Willebrand's disease (vWD) in the Doberman Pinscher. Credit for the discovery must include my colleagues, Dr.'s Patrick Venta, Vilma Yuzbasiyan-Gurkan, and William Schall, of the College of Veterinary Medicine at Michigan State University, and to Dr. Jianping Li, who works in my laboratory at the University of Michigan as well as at VetGen LLC, and who did all the DNA sequencing. This discovery is a nice example of the productive cooperation between the two universities and the company mentioned, as well as four funding organizations that provided support, The Doberman Pinscher Foundation of America, Inc., The Orthopedic Foundation for Animals, the Morris Animal Foundation, and the American Kennel Club.

The mutation itself has some interesting aspects. For one thing, precisely the same mutation has occurred in some human patients with vWD. It is a little unusual to see mutations be identical across species. This shows how closely we are related to our canine brethren! Second, the mutation is of a type such that completely normal von Willebrand's factor (vWF) is made about 5-10% of the time. Technically, the mutation is called a splice site mutation, with alternative splicing occurring about 90-95% of the time. That jargon won't mean much to the average Doberman breeder or owner, but let me explain what is happening in layperson language. It may be useful for the Doberman fancy to understand the mutation to a certain extent, because its nature explains why it was so confusing to understand for a long time, and it also explains why affected Dobermans have a milder disease than, say, affected Scotties.

To try to understand the effects of this mutation, let's use an analogy common to general experience. Imagine that a freight train is supposed to go from point A to point B following a railroad track. There is a point where a sidetrack goes to point C. However, normally the train never goes to point C, because the switch to point C, connecting the track up to the main track, is never thrown. Then the switch breaks (this is the mutation) such that the lock holding the switch from connecting the track to point C is no longer effective. The switch can now jiggle back and forth, sending some trains to point B, and others to point C. As freight trains rumble towards the switch, 95% of the time it jiggles over and causes the train to end up at point C. This is useless because point C ends at a cliff. The trains rumble over the cliff and are never heard from again. A minority of the time, maybe about 5%, the switch jiggles the other way and the trains end up at their normal destination. So, only 5% of the freight is delivered.

This is exactly what happens in the Doberman affected animal. These animals have two

doses (two trains in the above example) of the mutated gene. Each gene is capable of making 5-10% of normal vWF (that is, going down the main track to point B), because the normal splice site is used a little. The 90-95% of the time the mutated splice site is used (going down the side track to point C), no useful vWF is produced. Since each of the two mutated genes is producing 5-10% of normal vWF the affected Doberman ends up with twice that, or 10-20% of normal vWF in their blood.

So, one of the mysteries of Doberman vWD that has puzzled scientists for years, how affected dogs can end up with a small amount of completely normal vWF, is cleared up by understanding this type of mutation. A second mystery is also cleared up. Doberman owners and breeders have had their dogs tested for vWF for years using the protein assay of vWF, and have often discovered low values in dogs without a bleeding history, even at surgery. The reason is, such dogs have 10-20% of normal vWF. If the bleeding stress isn't too great, the 10-20% of normal vWF that is present can prevent undue bleeding. Part of the time uneventful surgery fits that criterion, and unusual bleeding does not occur.

I hasten to add that this should not be taken to mean that vWD in the Doberman is clinically harmless. The literature is full of reports of Doberman's bleeding and dying from vWD. There are a number of factors, known and unknown, which will affect the clinical outcome in a given case. First coagulation factors, such as vWF, are consumed during blood clotting. The more the bleeding, from injury or surgery, the more the consumption, and the more likely the limited supply of vWF in an affected Doberman will be used up, leading to renewed bleeding, now from vWF deficiency. Second there is also variation in the amount of vWF in affected Dobermans. A dog with a 5% value is at greater risk than one with 15%. Of course, other factors, such as other coagulation and tissue factors that we aren't measuring, will certainly vary from one affected dog to another, and change the risk of bleeding up or down in a given situation.

The Doberman breeder and owner should view vWD as a significant health risk, and a fault, and strive to get rid of the mutated gene. The discovery of the mutation, and the recent development of a DNA test, now provides just that opportunity.

Another mystery about Doberman vWD that we now understand better is the actual frequency of vWD in Dobermans. Dobermans have been said to have a 70% plus frequency of this disease, but that is not correct. It's more on the order of 35% affected, with an additional large group being carriers, but free of any bleeding risk. The disease is an "autosomal recessive", which means that affected animals have two doses of the mutated gene, and a mild to moderate risk of bleeding, for reasons explained earlier. Based on very preliminary data, we believe the mutant gene has a frequency of about 0.6 (60% of the genes are mutant) which translates into about 36% of all Dobermans being homozygous affected (two doses of the abnormal gene and at risk for bleeding), 48% being carriers (one abnormal and one normal gene, no risk of bleeding), and 16% being homozygous clear (two doses of the normal gene). If the gene frequency turns out to be closer to 0.5, the frequencies for affected will be 25%, carriers 50%, and clear 25%. Of course, our small sample comes from a limited region of the country. The gene frequencies may vary some in different parts of the country, but the bottom line will remain the same. This is a very common disease and a very common mutant gene.

Carriers of the mutant vWD gene are at no risk of bleeding from vWD, but of course, will transmit the mutant gene to their offspring 50% of the time. Roughly, the ranges of vWF factor levels are 5 to 20% for affected, 30-100% for carriers, and 50-130% for homozygous normal. Note the major overlap between carriers and normals for vWF levels. This overlap accounts for the extreme unreliability of the vWF assay in trying to identify Doberman carriers of vWD.

The new DNA test for Doberman vWD is offered by VetGen LLC (3728 Plaza Drive, Suite 1, Ann Arbor, Michigan 48108; (313) 669-8440, (800) 4-VETGEN; Fax (313) 669-8441). It is very easy to do the test. You can order the test kit from VetGen by phone or letter. Each test kit costs \$5 and contains three soft brushes and instructions. Following the instructions, the dog owner brushes the inside of the dog's mouth. Some of the cells lining the inside of the mouth stick to the brush, and provide the DNA for the test. No blood is required. The brushes are replaced in their envelope and mailed back to VetGen. Each vWD DNA test costs \$135. VetGen will supply test results within two weeks of receiving the DNA.

Test results will come back as "clear," "carrier," or "affected." As stated earlier, clear means both vWF genes are normal, carrier means one is normal and one is defective, and affected means both genes are defective. It is important to realize that this DNA test is very different from the old protein-based factor assay. The DNA test is definitive and final, a lifelong, permanent determination of the vWD status of each dog tested as contrasted to the factor assay, in which the levels could change drastically over time. We can now say in hindsight that the old test probably correctly identified some affected Dobermans (values under 20), but it is completely unreliable for carrier detection.

What should a breeder do with the test results, once they are obtained, in terms of breeding decisions? The problem facing the Doberman breeder is that it appears that only 15 to 20% of Dobermans are clear of the vWD gene. If one breeds mostly clear to clear, it narrows the breeding pool so much that there is risk of losing some of the Doberman's genetic heritage, i.e., some of the genes determining valuable positive characteristics of the Doberman might be lost, or highly diluted. Therefore, as a first priority, we advise breeding clear to clear and clear to carrier, at least for the next two or three generations. Over time, as the frequency of clear dogs increases, it should be possible to breed mostly clear to clear, and to eventually eliminate the mutant vWD gene.

As a second priority, we suggest that it is reasonable to breed carrier to carrier, if an acceptable clear dog is not available for breeding. This type of mating will produce 25% clear, 50% carrier, and 25% affected, on average. The puppies should be tested and the affected puppies not used for breeding.

Breeding carrier to affected and affected to affected should be avoided if at all possible. The first breeding produces 50% affected on average, and the second produces 100% affected animals. In my opinion, there should be two initial objectives of the Doberman vWD breeding program. One objective should be to produce as few affected animals as possible, because each is a health risk. That doesn't mean we believe affected Doberman puppies should be put down. Most of them can live normal lives. If possible, we believe it

would be a good idea to neuter affected animals. The second objective of the breeding program should be to gradually reduce the gene and disease frequency. The kinds of breedings involving the mating of an affected, as listed at the first of this paragraph, tend to increase the disease gene frequency, whereas clear to clear and clear to carrier breedings tend to decrease frequency. [Click here for further information on Breeding Strategies.](#)

To further raise the awareness and standards of Doberman breeders, VetGen is helping the Orthopedic Foundation for Animals (OFA) establish a vWD registry for Dobermans. By registering the results of the vWD DNA test on their dogs, breeders stand to benefit at the point of sale when selling either carrier or clear puppies as established by the vWD DNA test.

In summary, Doberman pinscher breeders are now in the advantageous position of being able to begin eliminating one of the significant diseases in their breed, because of the discovery of the mutation producing vWD in this breed, and the development of a vWD DNA test by VetGen. The test is remarkably easy to get done, and is reasonably priced, considering that it is a definitive lifetime determiner of the vWD genetic type of the dog tested. We urge Doberman breeders to get their breeding stock tested, so that we can get on with eliminating this disease.

For further information, or to order test kits, contact VetGen at:  
3728 Plaza Drive, Suite 1, Ann Arbor, Michigan 48108  
800-4-VETGEN (800-483-8436) / fax 313-669-8441

As you know, VetGen is now offering DNA tests for von Willebrand's disease (vWD) in Scotties, Shelties, and Dobes, and Kristi, of VetGen, is beginning to post information about these tests. The responses indicate some confusion, particularly about the Doberman. You have asked me, as one of the investigators who discovered the mutation, to clarify the genetics and usefulness of the new DNA tests. This is a fairly long letter, and I will apologize for its length now, but I felt that much of what is presented is information that you and other interested parties will want. A key part of this message that you must understand is that we now know the precise DNA mutations and why and how they cause vWD in these three breeds.

So all past hypotheses and speculations in the Merck Vet Manual and elsewhere, which were based upon the old protein-based factor assay, are out the window. Ignore them—they are past history. Now that we have the mutations in Scotties, Shelties, and Dobes, we can speak from fact not speculation regarding these three breeds. We are working on other breeds as well, but we cannot promise the date at which we will find any of the other mutations (although, of course, we hope it will be sooner rather than later).

The bottom line of what is given below is as follows:

- (1) vWD in Doberman Pinschers is a true clinical disease in which affected animals are predisposed to have abnormally (and sometimes fatally) prolonged bleeding times.
- (1) The Dobe disease is recessively inherited, contrary to what some previous research had suggested in the past.

- (1) Carriers are unlikely to have bleeding problems but affected (that is, homozygous mutant) animals are at a significant risk of serious bleeding problems, if they undergo surgery or sustain moderate trauma. Penetrance is \*far\* less important than was inferred from the dominant, incompletely penetrant model.
- (1) If this one mutant gene was eliminated from the breed, vWD would become a very rare disease, indeed, in Dobs.

I will begin by describing the disease in two other breeds, because I believe that this will lead into the Dobe situation very well. Both the Scottie and Sheltie have the severe Type 3 form of the disease. The Sheltie may be a rare exception to the rule, that better than 99% of any simply inherited disease in a breed is caused by one mutation. In other words, while the major and most severe form of vWD in Shelties is Type 3, there is a possibility that a minor portion of the vWD problem is due to Type 1 vWD. This is according to data developed by Dr. Jean Dodds and her colleagues (Brooks et al., 1992—see below for complete references). We are working to see if this is true (it seems likely). Type 2 vWD, by the way, has only been seen in only two breeds of dogs, German shorthaired pointers and German wirehair pointers, so we will ignore it, in this letter. Both the Scottie and Sheltie Type 3 vWDs are caused by mutations that prevent \*any\* von Willebrand factor (vWF) from being produced. The technical term for these mutations are “single base deletions.” These diseases are recessive, so that both copies of the gene that a dog possesses must be mutant before the animal has a bleeding problem. Carriers almost never have bleeding problems (Johnson et al., 1988).

The Doberman pinscher mutation, on the other hand, is Type 1 but it is \*recessively\* inherited! Most human Type 1 vWD is inherited in a dominant, incompletely penetrant mode. There are two things that made the Dobe vWD appear to be inherited in a fashion like the human disease.

- (1) The Dobe mutation is what geneticists refer to as “leaky.” That is, the mutant gene makes a small amount of normal vWF protein. The amount made by each mutant gene is about 5% of the total normal amount. A normal gene would make 50% (so that two genes produce 100%).
- (1) The frequency of the mutant gene in the Doberman pinscher breed is greater than 60%! The parent of an affected animal can be also be affected, due to the high gene frequency (thus, the apparent dominant inheritance), but this is not always the case (thus, the apparent incomplete penetrance).

This result was easily (and understandably) misinterpreted as the dominant, incompletely penetrant mode of inheritance as seen in humans. In human genetics, it is assumed that each genetic disease is rare, and one would not expect the parents of “affecteds” to also be affected if the disease was recessive. With animals, rarity of a disease gene cannot always be assumed, as illustrated by Dobe vWD. Other researchers have also presented data and arguments that Dobe vWD is actually a recessive disease (Moser et al., 1996; Johnson et al., 1988). The identification of the mutation fully explains it. Homozygotes for the disease in Dobermans do \*not\* die in utero. The mode of inheritance with other breeds, such as the German shepherd dog, could still be dominant, incompletely penetrant. We simply are not certain of the inheritance pattern for other breeds at this time.

Dobe carriers should produce 55% of normal vWF, on average (5% from the mutant gene and 50% from the normal gene). However, other biological variables can affect the amount of factor found in the blood. These variables include thyroid hormone level, estrous, liver status (diseased or not), etc. Variation can also be produced by inappropriate handling of the blood sample or some variability in the protein-based tests themselves. These variations for concentration of the protein in the blood can make an animal appear to be a carrier on one day and homozygous normal (clear) on the next (which value does a breeder believe?). This is why the protein-based tests are not as useful as they might otherwise be. The DNA-based tests are completely different, because they detect the genetic change at the gene level, which does not vary. There are only three possible results from the DNA-based test. An animal is either clear, a carrier, or affected. Re-testing is pointless, because the result will always be the same for a given animal. So one test is good for the life of the animal. Incidentally, we have also set up the test so that it is noninvasive (you swab the inside of the dog's mouth with a small, soft brush), convenient (you can send the brush by regular mail—no need to refrigerate), and you can test at any age, even young puppies.

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Dobe \*carriers\* with abnormally long bleeding times are not common. Dodds, Johnson and Stokol et al. have all reported that animals do not usually bleed excessively when they have factor levels greater than 36% of normal (Dodds, 1982; Johnson et al., 1988; Stokol et al., 1995). Carriers will occasionally go below this level, but usually not by very much. Surveys of fairly large numbers of animals have been conducted, and the data appear to show the expected trimodel distributions for number of animals vs. factor concentrations (Dodds and Covey, 1981; Moser et al., 1996; Johnson et al., 1988; Stokol et al., 1995). By extrapolating the overlapping curves it can be seen that carriers do not dip into the danger range more than occasionally. Therefore, the fear that an animal who tests as a carrier might someday suddenly become a severe bleeder because of the dominant, incompletely penetrant scenario is completely negated. Occasional carriers might have bleeding times that are prolonged, but these are the exceptions. Clear animals will never bleed abnormally, due to hereditary vWD (the removal of the disease gene should be, after all, the eventual goal). Fortunately, even affected Dobs usually do not bleed spontaneously (unlike the case for Scotties, and perhaps Shelties). If they did, there probably would have been a stronger natural selective pressure to remove the disease gene. However, with surgery or moderate trauma, these dogs are at risk for serious bleeding problems (there are numerous reports in the scientific literature addressing this fact, and I am sure that there have been numerous anecdotal reports in this forum as well). So the disease and its causative gene are something that breeders should most certainly want to remove from their breeding programs. This will have to be done with care, however, because we do not believe that it is in the best interest of the breed to limit the gene pool by breeding only clear to clear. By following the guidelines that Kristi at VetGen posted previously (also available at <http://www.vetgen.com/>), it should be possible to allow the desirable genes to separate from the disease gene over a few generations, while at the same time preventing the occurrence of affected animals.

The mutation that we have found accounts for essentially all of the vWD seen in Dobermans. It is always possible that a rare mutation in combination with the common mutation

would cause a bleeder. However, this should be very rare, because the rate of occurrence of \*new\* mutations for most genes is between one in one hundred thousand to one in a million per generation (Crow, 1993 and references contained therein). If the mutation we have found is eliminated from the breed, von Willebrand's disease will also be eliminated from the breed (ignoring those one in a million new mutations that can never be prevented). The same is true for specific lines, as well.

Breed out the disease gene (which can now be detected with complete accuracy) and the disease will be gone from the line. The paper describing the Scottie mutation is nearly "in press" and the paper describing the Dobe mutation will be submitted very shortly. The paper describing the Sheltie mutation is currently "in preparation." Normally, as a scientist, I would prefer not to let people know about a result until it has come out in the scientific press. However, because of the intense interest shown by postings to various lists, and because the test is immediately useful, we felt that it would be worse to deny breeders access to such a test for perhaps as long as a year because of our desire to wait for scientific publication (one year from submission to publication is typical for scientific journals—unfortunately). We hope that you and others will recognize our good will in this decision. This situation will be a chronic dilemma for all researchers who discover mutations that are of breed-wide impact. If some portion of this letter needs clarification, please let me know and I will do my best to do so. The other primary investigators for this research are Vilma Yuzbasiyan-Gurkan, Ph.D. and William Schall, DVM at Michigan State University, and George Brewer, MD and Jianping Li in the Department of Human Genetics at the University of Michigan.

Sincerely,

Pat

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References:

Brooks, M., W.J. Dodds, and S.L. Raymond. Epidemiological features of von Willebrand's disease in Doberman pinschers, Scottish terriers, and Shetland sheepdogs. *Journal of the American Veterinary Medical Association* 200:1123-1127 (1992).

Crow, J.F. How much do we know about spontaneous human mutation rates? *Environmental and Molecular Mutagenesis* 21:122-129 (1993).

Dodds, W.J. Detection of genetic defects by screening programs. *AKC Gazette* pp. 56-60 (June 1982).

Dodds, W.J. and J.S. Covey. Canine von Willebrand's disease. *AKC Gazette* pp 53-55 (April, 1981).

Johnson, G.S., M.A. Turrentine, and K.H. Kraus. Canine von Willebrand's disease. A heterogeneous group of bleeding disorders. *Veterinary Clinics of North America: Small Animal Practice* 18:195-229 (1988).

Moser, J., K.M. Meyers, and R.H. Russon. Inheritance of von Willebrand factor deficiency in Doberman pinschers. *Journal of the American Veterinary Medical Association* 209:1103-1106 (1996).

Stokol, T., B.W. Parry, and P.D. Mansell. von Willebrand's disease in Dobermann dogs in Australia. *Australian Veterinary Journal* 72:257-262 (1995).