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Genetics of Classic von Willebrand's Disease. I. Phenotypic Variation Within Families

By Connie H. Miller, John B. Graham, Lynn R. Goldin, and Robert C. Elston

In order to determine the phenotypic variability within families transmitting classic von Willebrand's disease (vWd), a single cross-sectional examination was made of two large seven-generation vWd kindred living in the Carolinas. A total of 181 subjects were examined at least once. Fifty-eight were classified as "affected," 24 in the smaller kindred of 418 persons and 34 (2 of whom were homozygous) in the larger kindred of 750 persons. Detailed histories were taken, and bleeding time (BT), coagulant factor VIII (VIII:C), factor-VIII-related antigen (VIIIR:Ag), and Willebrand factor (VIIIR:WF) were determined on all subjects. Affected persons were present in several generations in each kindred, and the affected state (defined as having a value outside the normal range in one or more of the four tests in the direction expected in vWd) was transmitted by both sexes to both sexes. Segregational analysis showed the pattern of inheritance to be consistent with an autosomal dominant mode, and both kindred appeared to be transmitting classic vWd. The abnormal gene was found to have a highly variable expression, 11 of the 16 possible combinations of normal or abnormal results for BT, VIII:C, VIIIR:Ag, and VIIIR:WF being observed among the affected persons. Penetrance of the abnormal gene was incomplete, 11 of the 26 presumably heterozygous transmitters having all F-VIII-related activities within the normal range. Two years after the initial study, a subsample of one kindred was reexamined twice, 5 mo apart. The phenotype of the affected state was found to be highly stable over time; 77% of those restudied were assigned the same classification as originally. Among the 5 of 22 who were phenotypically reclassified on second or third test, no changes in genotypic designation were required, since their genotypes could be correctly determined from genetic data.

VON WILLEBRAND'S DISEASE (vWd) is a hemorrhagic disorder that appears to be inherited, in an irregular fashion, as an autosomal dominant trait. Clinical expression is variable,[1] and the condition is about one-fifth as prevalent as hemophilia A.[2] The mutant gene has long been known to have multiple phenotypic effects, the association of prolonged bleeding time (BT) and reduced coagulant factor VIII (VIII:C) having been recognized 25 yr ago.[3] Recently, subjects with vWd have been found to have two other phenotypic abnormalities. First, there is reduction in a (presumably single) plasma factor required for two phenomena involving platelets: (1) adherence to glass surfaces[4] and (2) agglutination in the presence of the antibiotic ristocetin.[5] The factor in normal plasma responsible for these functions is referred to as Willebrand factor (VIIIR:WF).

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Second, it has been discovered that an antigen of plasma detected by precipitation with a heterologous antibody to human factor VIII is reduced in vWd.[6] This activity is often designated factor-VIII-related antigen (VIIIR:Ag). There is fairly high correlation (circa 0.70) between levels of VIIIR:Ag and VIIIR:WF in normal populations, and there is a widespread belief that a single substance is responsible for both the antigenic and platelet-related activities. Because reductions of VIII:C, VIIIR:Ag, and VIIIR:WF occur together in vWd, a hereditary trait believed to be monogenic, the three have been referred to collectively as the F-VIII-related activities.

Assays for VIIIR:Ag and VIIIR:WF have improved the diagnosis of vWd in recent years, but they have also emphasized the phenotypic variability of the condition. A patient showing a clear prolongation of BT and a significant reduction in plasma levels of VIII:C, VIIIR:Ag, and VIIIR:WF may be said to have classic vWd. However, a quantitatively normal result with one or more of the four tests is often seen in patients who show abnormalities of the other F-VIII-related activities. This has been seen so frequently that the literature contains numerous reports of "vWd variants." More recently, a different class of vWd variants has been described in which there are clearly qualitative alterations of F-VIII.[7-15]

Table 1 illustrates the range of variant forms of vWd that have been observed when only quantitative variation among the four tests is considered. A binary notation has been used in the table, plus (+) implying a value within the normal range and minus (−) implying a value outside it, the normal range being whatever had been so stated by the authors of each report. This procedure produces 16 combinations of (+) and (−) for the four tests, of which three are classic vWd, hemophilia A, and normal. The last two columns record the variants reported in the literature.[7-25] It will be noted that all but 2 of the 16 possible variants, types 7 and 9,

Table 1. Variants of vWd in the Literature*

Variant Type	Bleeding Time	VIII:C	VIIIR:Ag	VIIIR:WF	Report of a Relative With Classic vWd	References
1	−	−	−	−	Classic vWd: yes	Numerous
2	−	−	+	−	Yes	8-15
3	−	−	−	+	Yes	16
4	−	+	−	−	Yes	16,17
5	+	−	−	−	Yes	11,16-21
6	−	−	+	+	No	9,13
7	−	+	−	+	No	None
8	−	+	+	−	Yes	7,11,20,22,23
9	+	−	−	+	No	None
10	+	+	−	−	Yes	16
11	+	−	+	−	Yes	11,24
12	+	+	+	−	No	7
13	+	−	+	+	Hemophila A: yes	25
14	+	+	−	+	No	11
15	−	+	+	+	Yes	11
16	+	+	+	+	Normal: no	None

*A binary system (+ = normal, − = abnormal) is used to indicate the result of each test. Decision in each instance was based on the opinion of the author concerning the range of normal for his test, his laboratory, and his controls.

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have been reported either in single individuals with vWd or in families. Furthermore, in connection with 11 of the 16 reported variants, it has been stated that the variant individual has had at least one relative with classic vWd. This suggests that some of the variants in the literature represent phenotypic heterogeneity within families transmitting classic vWd, presumably reflecting variable expression of the vWd gene.

Most studies of vWd have been studies of unrelated patients or studies of small families. This type of study tends to accentuate differences among unrelated persons and to obscure phenotypic variability among persons having the same gene. Since study of a large group of persons possessing the same abnormal gene would appear to be a better method of defining the range of phenotypic variability, we chose to study two large families transmitting classic vWd. Our hypothesis is that each affected person within a kindred is expressing the same abnormal allele. Individual study of each family, therefore, provides an opportunity to assess variability under conditions in which environmental differences are minimal and genetic backgrounds are similar. At the same time, comparison of the two families provides a means of assessing variability under conditions in which environments, genetic backgrounds, and origins of the mutant genes are different. Although one family contains two homozygous individuals,[26,27] both families seem to show the same type of inheritance.

This report will describe the families, the method of study, and the range of phenotypic variability. A second report will describe how it is possible to assign the heterozygous genotype (i.e., diagnose vWd) with confidence using discriminant analysis, a method that allows all the data to be combined into a single likelihood.

SUBJECTS

Family 1

The members of family 1 (Fig. 1) are descendants of five brothers and sisters (generation II) born between 1843 and 1853. Most now live in the mountainous area between Greenville, South Carolina, and Asheville, North Carolina. This family shows vertical distribution of a bleeding state suggestive of autosomal dominant inheritance, since both males and females are affected and transmit. No consanguineous marriages have occurred. Clinical severity ranges from none to moderate among those regarded as affected. The proband (IV-89) was the subject of transfusion studies in 1966 that demonstrated nonreciprocal in vivo complementation between subjects with vWd and hemophilia A.[26] She fulfills all the criteria for classic vWd. (This family is recorded as HGAR3 in Dr. Elston's registry of large families used for linkage studies.)

Family 2

Family 2 (Fig. 2) was ascertained twice through two severely affected individuals (V-37 and V-138). Each was the product of a consanguineous union, and both have long been believed to be homozygous.[27] They were diagnosed independently and were not aware that they were related. Each is the offspring of a second-cousin marriage; the four parents are second cousins, being descended from the same ancestral pair, and the probands are third cousins. Their parents do not consider themselves to be "bleeders," and although they have been examined many times, they have not displayed the phenotype defined below as "affected." The complete pedigree, which contains seven generations and 750 individuals, has been condensed into a workable size of 349 persons (Fig. 2). Living family members are concentrated in Wilkes County, North Carolina, a rather isolated mountainous area. It seems likely that the two kindred are genetically independent, since they reside at different ends of the Blue Ridge Mountains, and there is evidence of genetic separation for at least seven generations. (This family is recorded as HGAR4 in Dr. Elston's registry.)

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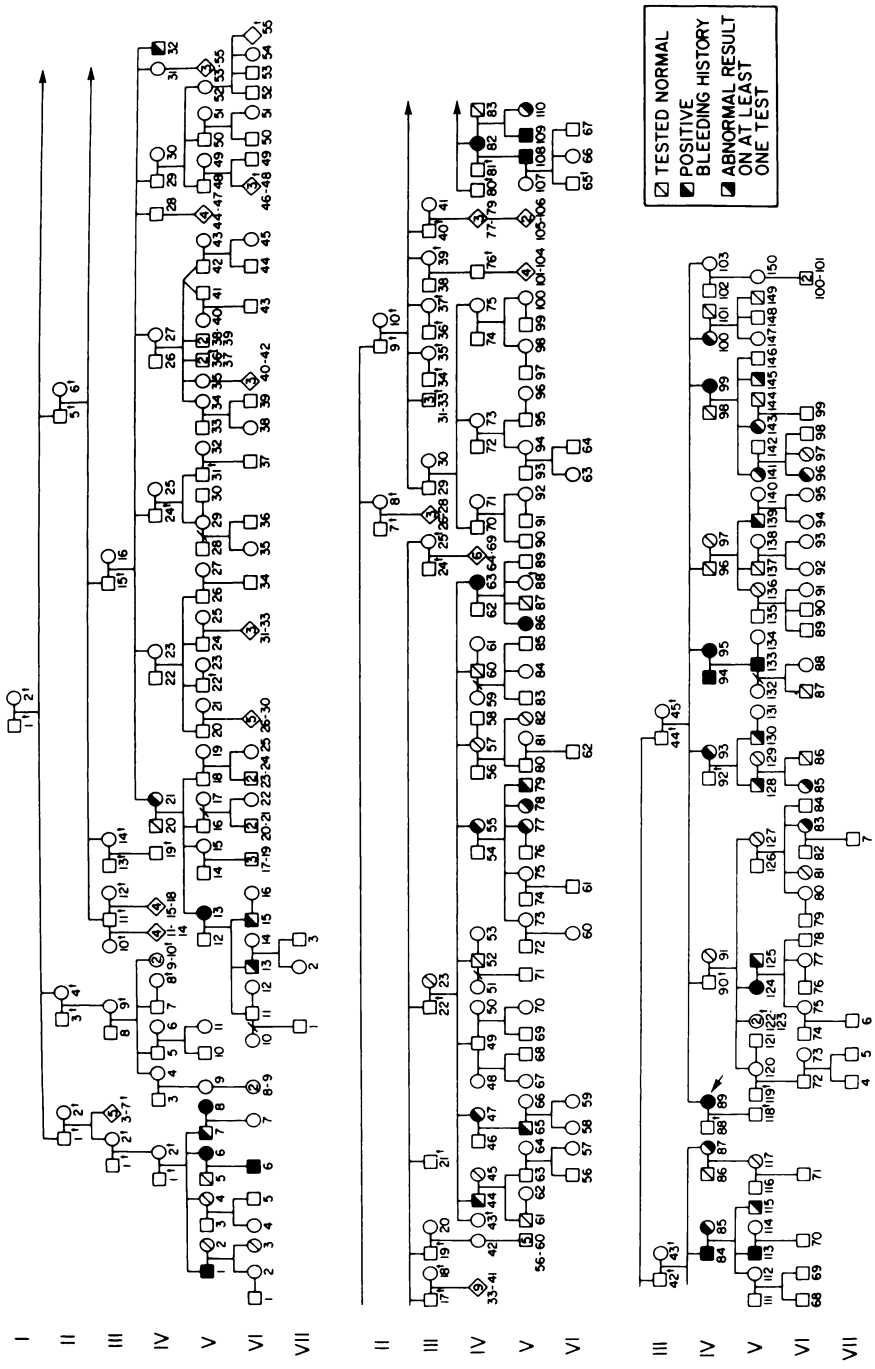

Fig. 1. A seven-generation kindred with classic vWd containing 418 persons. Proband (—) was IV-89. Family members scored as symptomatic (symbols with tops darkened) or affected (symbols with bottoms darkened) are present in generations IV, V, and VI. Note male-to-male transmission (IV-84 to V-113).

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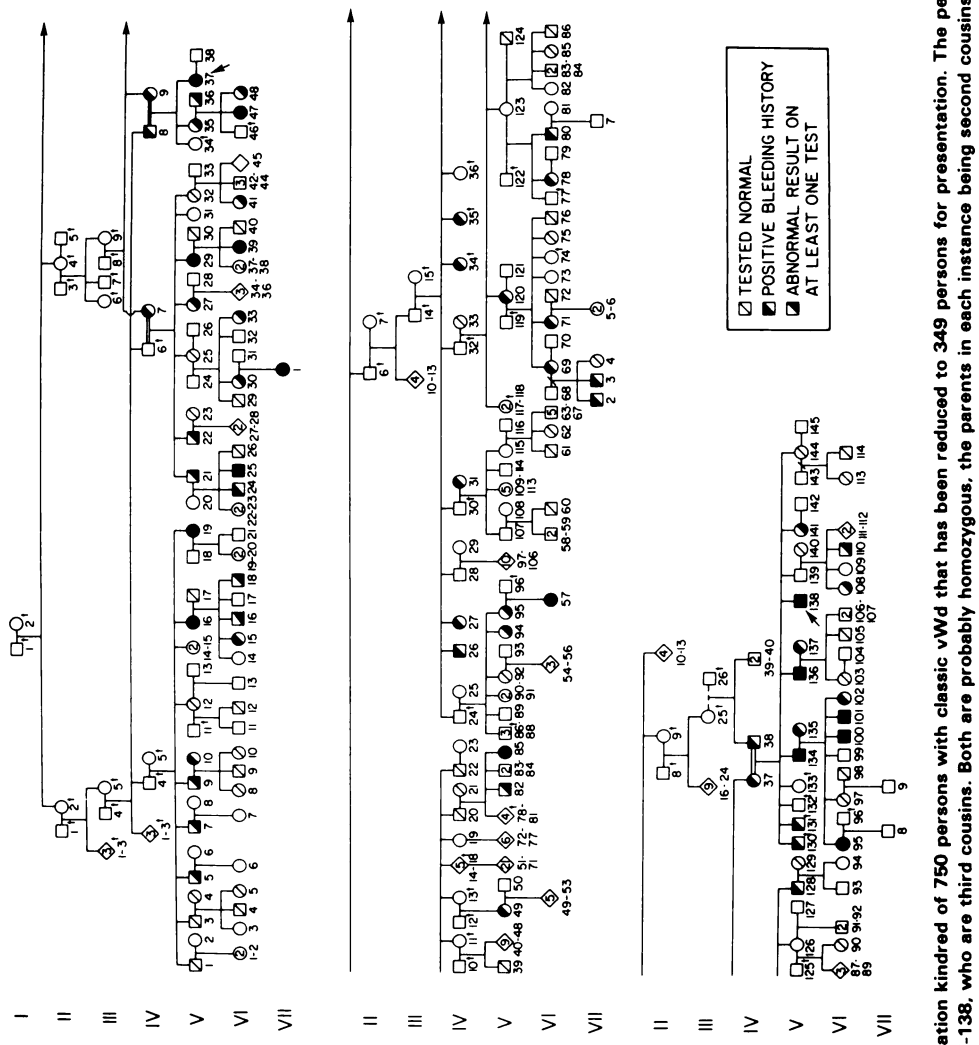

Fig. 2. A seven-generation kindred of 750 persons with classic vWd that has been reduced to 349 persons for presentation. The pedigree was ascertained twice through V-37 and V-138, who are third cousins. Both are probably homozygous, the parents in each instance being second cousins and in the main line of descent. Another consanguineous mating (IV-6 × IV-7) appears not to have produced homozygous offspring. Note male-to-male transmission (V-21 to VI-25).

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MATERIALS AND METHODS

During a field trip in 1976, data were collected on 75 members of family 1 and 106 members of family 2, since both families reside more than 150 miles from Chapel Hill. Similar field trips were made twice in 1978 to reexamine 22 members of family 2. Blood was obtained by a two-syringe technique described previously[28] in which the second syringe contained citrate and the first was discarded. Plasma was prepared by centrifuging the citrated whole blood at 1000 rpm for 20 min at room temperature. Siphoned plasma was aliquoted, "snap-frozen" in alcohol-CO_2, and transported to the laboratory in dry ice. All samples were stored at −70°C in a Revco freezer until assayed, and all assays were performed by one of us (C.H.M.).

The age and position in the pedigree were established for each person, and answers to a standardized set of questions regarding bleeding history, reproductive history, and recent ingestion of drugs were obtained. Aspirin and compounds containing aspirin were specifically inquired about, and venipunctures and BTs were not done on persons who had recently ingested aspirin. BT determination was performed on each individual at the time of blood sampling by the method of Ivy,[29] using a sterile disposable lancet (Medipoint blood lancet, A. H. Thomas Co.) that produces a skin incision 2 mm wide and 3 mm deep. Informed consent was obtained in writing from the subject or a guardian for all procedures carried out on each subject.

Clinical Symptoms

Clinical symptoms were classified as to severity using a predetermined scheme:

None: no unusual bleeding, or mild bleeding on only one occasion.

Mild: more than one episode of bleeding not requiring transfusion and not of a life-threatening nature.

Moderate: one or more episodes of bleeding either requiring transfusion or of a life-threatening nature.

Severe: chronic atraumatic bleeding, often requiring treatment.

VIII:C was assayed by a modification of the partial thromboplastin time method of Langdell et al.,[30] as described previously.[28]

VIIIR:Ag was measured by Laurell's quantitative immunoelectrophoresis technique, as described elsewhere.[28]

VIIIR:WF was measured by means of a macroscopic ristocetin aggregation assay using fixed washed platelets, as described by Reisner et al.[31,32]

Since the samples collected in the home were centrifuged at room temperature and at 1000 rpm, frozen while probably containing platelets, and then thawed, it was essential to determine if the factor-VIII-related activities of such plasma differed significantly from those of plasma prepared in the laboratory by high-speed centrifugation in the cold. A sham experiment was performed using five laboratory staff members of evaluate the field procedures. The laboratory personnel were bled exactly as described for our subjects. Each sample of citrated blood was divided in half, one portion being spun at 1000 rpm for 20 min at room temperature and other being spun at 10,000 rpm for 20 min at 4°C. Plasmas were withdrawn, frozen at −70°C, and stored overnight. Then the factor-VIII-related activities were determined on all samples. The results are shown in Table 2.

It can be seen that the VIII:C and VIIIR:WF levels were somewhat higher in the 10,000-rpm plasma, whereas the VIIIR:Ag level was somewhat higher in the 1000-rpm plasma; however, none of the differences was statistically significant.

The control for all of the assays was a pool of normal plasma prepared from 10 healthy normal donors. Potential donors were first screened for VIII:C, VIIIR:Ag, and VIIIR:WF using our laboratory standard, and individuals having extremely high or low values were eliminated. Then plasma was prepared from each of 10 acceptable donors, blood being obtained in two plastic syringes, the second syringe containing 0.11-*M* sodium citrate at a 1:8 ratio and the first syringe being discarded. The samples of citrated blood were centrifuged at 2000 g for 15 min at 4°C, and the plasma was pipetted off. Each plasma sample was then recentrifuged at 25,000 *g* for 30 min at 4°C. Platelet-poor plasma was pooled by mixing an equal volume from each of the samples. The normal pool was then aliquoted into 1-ml lots, frozen, and stored at −70°C in a Revco freezer. Once thawed, a control sample was discarded. By repeated test and elimination of outliers, the process of producing normal pools provided a cohort of volunteers whose F-VIII activities were reasonably stable and close to the mean of the pool. These were called on in preparing new pools.

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Table 2. Comparison of Slow and Fast Centrifugation on F-VIII-Related Activities

Activities	Means ± SD (n = 5)	Difference Between Means (d)	Pooled Standard Error of Differences ($S\bar{x}$)	t*	p
VIII:C					
1,000 rpm	151.4 ± 11	+10.6	6.50	1.63	>0.10
10,000 rpm	162.0 ± 24				
VIIIR:Ag					
1,000 rpm	161.2 ± 47	−23.4	23.38	1.017	>0.30
10,000 rpm	137.4 ± 14				
VIIIR:WF					
1,000 rpm	107 ± 8	+13.0	9.22	1.4	>0.20
10,000 rpm	120 ± 8				

*$t = \frac{d}{S\bar{x}}$.

Each normal pool prepared during the course of the study was assayed before use and at the end of its period of use for VIII:C by means of a lyophilized reference plasma (Hyland Laboratories) that had been assessed against an international standard. All assays obtained on individual subjects were adjusted to the international standard by the following method. A correction factor was obtained for each working pool by comparison with the reference plasma. Then the unitage of the working pool was converted into international units (IU). The values on our subjects that were used in the calculations and recorded in the tables are therefore in international units per deciliter (equivalent to percentage of the international standard). The adjustments were made by the following method. A working pool, for example, that tested at 80% of the Hyland reference plasma (which in turn had tested at 110% of the international standard) was regarded as being 88% (80% of 110%) of the international standard or 88 IU/dl. A subject's plasma having 50% of the working pool could be said to have 44% of the international standard or 44 IU/dl.

Since the reference plasma had not been standardized for VIIIR:Ag or VIIIR:WF, no attempt was made to use it to correct the value of the pool for these assays. However, each new pool of normal plasma was tested against the last previous one for VIIIR:Ag and VIIIR:WF, the intention being to use a correction factor, if necessary, to maintain constancy. Since the values of VIIIR:Ag and VIIIR:WF for all normal pooled plasmas differed only very slightly, no corrections were needed.

Paternity was examined in each mating using a battery of more than 30 polymorphic genetic markers. The markers included blood groups, serotypes, and erythrocyte and serum enzymes and were carried out in Dr. Elston's laboratory. They were available for our purposes as the by-product of a linkage study of vWd being carried out jointly with Dr. Elston's group that will be published separately.

Descriptive statistics, including mean, standard deviation, and skewness, were calculated by standard methods in original units and after square-root and logarithmic transformations. Significance of skewness was tested for by reference to standard tables,[33] and outlying values contributing significantly to skewness were eliminated from the study.

RESULTS

It became apparent early that heterogeneity of both clinical and laboratory results was causing a problem in classification. This heterogeneity was epitomized by (1) some subjects who had completely discrepant test results and symptoms, (2) some subjects who were entirely normal phenotypically but who had produced affected progeny and could be assumed to be heterozygous, and (3) some persons who could not be classified because, although they might have received the mutant gene, they were phenotypically normal and had not produced affected children. These difficulties led us to define five categories of subjects, categories that are

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overlapping and not mutually exclusive. It is necessary to keep them clearly in mind as our results are described:

1. *Unrelated normals*. These were 33 spouses of members of the kindred; they were unrelated to the kindred genetically and were assumed not to possess the vWd allele. This group provided the data for calculating the limits of normal for the tests used in defining the affected.

2. *Affected persons*. These were 58 members of the two kindred having one or more tests falling outside the 95% range of the tests on the preceding group of unrelated normals, in the direction expected in vWd. Some, but not all, persons who scored as affected were also symptomatic. All were assumed to be heterozygous for the segregational analysis, except for the two probands of family 2, who were excluded because they were almost certainly homozygous.

3. *Symptomatic persons*. These were the subjects judged from detailed history and questioning to have mild or moderate symptoms of bleeding. Only the two homozygotes were judged to have severe symptoms. Some having symptoms were also classified as affected on the basis of laboratory tests.

4. *Transmitters*. These were 26 persons from the two kindred who were in the main line of descent and had had one or more descendants who scored as affected. They were assumed to be heterozygous, regardless of their own phenotypes. Some were symptomatic, whereas others were asymptomatic; independently, others could be scored as affected or unaffected. Since they could be considered to be heterozygotes on genetic evidence, independent of their own laboratory tests, they were used as a reference group to test the efficacy of the various methods of assigning the heterozygous genotype.

5. *Others*. These were members of the kindred of uncertain status.

Normal Ranges

Thirty-three nonconsanguineous spouses were tested at the same time as members of the kindred. This population was collected to be used for calculating the range of normal for each test, since they could be assumed not to have a vWd allele, but to be subject to similar environmental effects as those who did. Their test data are displayed in Table 3.

Three spouses were eliminated from the reference group. Incomplete data were available on one (VI-98), and another (IV-45) had all F-VIII activities greater than 200%, which skewed the distributions. The third was IV-94 of family 1. His VIIIR:Ag of 39% and VIIIR:WF of 40% not only skewed these distributions but also suggested that he might have vWd, despite his negative family history.

The 30 spouses who remained served as normal controls, and their assays provided the ranges of normal. Their assays for VIII:C, VIIIR:Ag, and VIIIR:WF were transformed logarithmically to eliminate skewness, and the transformed values were used to calculate 95% ranges (± 1.96 SD) for each bioassay. A similar range was calculated for BT in original units (minutes), and the 95% ranges for the four tests on these 30 persons of both sexes were used to define the limits of normal for the study. The 95% range, rather than the 99% range (3 SD), was chosen, since using 3 SD as the cutoff would have increased the number of false negatives (affected persons scored as normal), already expected to be high because of reduced penetrance. The 95% range was used in a one-tailed test, which implies that only

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Table 3. Unrelated Normal Spouses

Subject	Age	Sex	BT (min)	VIII:C (U/dl)	VIIIR:Ag (U/dl)	VIIIR:WF (U/dl)	Symptoms
Family 1							
III-23	74	F	1.5	263	196	104	None
IV-20	68	M	1	208	122	91	None
IV-45†	54	F	5.5	214	371	208	None
IV-83	63	M	1.5	86	121	151	None
IV-85	48	F	9	172	112	135	Mild
IV-86	59	M	5	203	111	82	None
IV-91	63	F	1	149	176	149	None
IV-94†	65	M	4	91	39*	40*	Mild
IV-97	52	F	9	169	153	173	None
IV-98	55	M	3.5	221	63	90	None
IV-101	55	M	8	109	148	81	None
V-2	37	F	3	159	126	134	None
V-5	46	M	4	88	74	102	None
V-8	32	F	10.5*	129	70	80	Moderate
V-125	44	M	7.5	147	147	148	Mild
V-129	43	F	3.5	138	105	125	None
V-144	29	M	3.5	104	114	82	None
Family 2							
IV-21	68	F	4.5	178	158	286	None
IV-31	71	F	7	70	139	105	Moderate
IV-33	67	F	7.5	118	58	53	None
V-4	44	F	4	150	109	74	None
V-10	36	F	3.5	105	102	76	Moderate
V-17	42	M	6.5	71	84	214	None
V-23	47	F	8	78	95	81	None
V-30	42	M	4.5	112	168	170	None
V-36	40	M	6	57*	81	90	None
V-124	48	M	2.5	119	85	89	None
V-129	31	F	4	98	101	70	None
V-135	39	F	7	108	180	110	Moderate
V-137	40	F	6	81	92	102	Mild
V-140	36	F	5	88	67	75	None
VI-72	29	M	5.5	124	100	260	None
VI-98†	29	M	7	—	—	—	None

*Value outside the 95% range in the vWd direction.

†Not included in reference group used for establishing the 95% range and for calculating the discriminants.

2.5% of normals are expected to lie outside it in the direction being used to score for vWd. When the data were retransformed into original units, the normal ranges were 58%–253% for VIII:C, 58%–208% for VIIIR:Ag, 52%–217% for VIIIR:WF, and 1–9 min for BT. A value was scored as abnormal (–) if it was less than 58% for VIII:C, 58% for VIIIR:Ag, or 52% for VIIIR:WF or was greater than 9 min for BT.

Summary of Laboratory Data

The results for 24 members of family 1 and 34 members of family 2 with an abnormality in at least one of the four tests are shown in Tables 4 and 5. An asterisk following a value indicates that it is outside the 95% range in the direction expected

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Table 4. Affected Members of Family 1

Subject	Age	Sex	BT (min)	VIII:C (U/dl)	VIIIR:Ag (U/dl)	VIIIR:WF (U/dl)	Symptoms
IV-63	33	F	10*	119	107	69	Mild
IV-82	58	F	12.5*	26*	38*	16*	Moderate
IV-84	54	M	8	25*	28*	21*	Moderate
IV-87	52	F	1.5	75	50*	52*	None
IV-89†	69	F	>15*	27*	34*	23*	Moderate
IV-95	62	F	>15*	84	82	93	Moderate
IV-99	56	F	11*	84	69	81	Mild
V-1	49	M	5.5	31*	47*	<6*	Mild
V-6	41	F	10*	61	66	32*	Moderate
V-13	48	F	5	79	38*	39*	Mild
V-78	8	F	—	74	93	47*	None
V-86	15	F	6	98	119	50*	Mild
V-110	15	F	4.5	54*	67	124	None
V-113	25	M	7	37*	44*	41*	Moderate
V-115	13	M	8.5	39*	69	79	None
V-124	44	F	4	61	52*	60	Moderate
V-128	46	M	5.5	87	80	50*	None
V-130	47	M	5	131	65	46*	None
V-133	38	M	13*	30*	48*	46*	Moderate
V-141	33	F	6.5	87	56*	106	None
V-145	25	M	6.5	61	44*	44*	None
VI-6	17	M	9.5*	25*	34*	20*	Mild
VI-83	19	F	5.5	66	54*	59	None
VI-85	20	F	4.5	110	72	48*	None

*Values outside 95% ranges of normal controls in vWd direction.
†Proband.

in vWd. Since it was reasonable to assume as a first approximation that a member of the family with a significantly reduced F-VIII-related activity carried the vWd allele, such subjects were classified as affected. The lower halves of the symbols in Figs. 1 and 2 have been darkened to indicate those who have been scored as affected.* The frequency of symbols so darkened emphasizes the large number of such persons in the kindred.

Analysis of Clinical Symptoms

A detailed questionnaire was completed on each of 181 family members. The degree of bleeding symptoms presumably referable to vWd is indicated by blackening of the upper portions of symbols in Figs. 1 and 2 and is also recorded in the last column in Tables 4 and 5. Thirty-five members of family 1 and 41 members of family 2 reported mild or moderate bleeding and were scored as abnormal in the figures. A solid symbol in the figures indicates someone scored as being both affected and symptomatic, there being 14 of this type in family 1 and 16 in family 2. An attempt to relate a single abnormal test or combination of tests to the severity of bleeding was unsuccessful.

*V-108 and V-109 of family 1 have been scored as affected and used in the segregational analysis because they have been patients at Chapel Hill on several occasions and their phenotypes are unambiguous. Their data are not included in Table 2, however, because they were unavailable at the time of this study.

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The lack of correlation between symptoms and tests is apparent in Tables 4 and 5 and is exemplified by (1) the 6 persons (other than the 2 homozygotes) who scored affected on all four tests (3 had moderate symptoms and 3 had mild symptoms) and (2) the 27 persons with only one abnormal test (18 had no symptoms, 6 had mild symptoms, and 3 had moderate symptoms). Also, 7 of the 30 unrelated normal spouses (23%) used to establish the normal ranges for the tests were scored as symptomatic. Since genetic classification of heterozygotes by symptoms alone proved to be impossible, we considered whether or not a genetic hypothesis could be tested by using the persons scored as affected.

Variations of the Affected Phenotype

Examination of the laboratory data for those scored as affected disclosed additional heterogeneity. Table 6 summarizes the results of testing the 54 affected

Table 5. Affected Members of Family 2

Subject	Age	Sex	BT (min)	VIII:C (U/dl)	VIIIR:Ag (U/dl)	VIIIR:WF (U/dl)	Symptoms
V-5	38	M	10*	99	82	77	None
V-7	32	M	7	56*	53*	89	None
V-9	42	M	3	83	84	23*	None
V-16	44	F	4	52*	48*	42*	Mild
V-19	57	F	6	55*	87	48*	Moderate
V-21	58	M	15*	129	129	100	None
V-22	52	M	7	84	74	33*	None
V-27	61	F	7	106	49*	15*	None
V-29	42	F	6.5	85	35*	36*	Moderate
V-35	38	F	9	62	42*	19*	None
V-37†	33	F	>30*	<1*	<3*	<6*	Severe
V-82	45	M	4	84	58	24*	None
V-85	42	F	10.5*	45*	27*	18*	Mild
V-94	43	F	4	145	68	45*	None
V-95	38	F	3	54*	34*	38*	None
V-134	49	M	>15*	80	73	48*	Moderate
V-136	46	M	14*	63	100	126	Moderate
V-138†	40	M	>30*	<1*	<3*	<6*	Severe
V-141	38	F	6	56*	83	118	None
VI-16	22	M	5.5	57*	80	68	None
VI-18	17	M	6.5	87	53*	27*	None
VI-25	29	M	9	82	66	41*	Mild
VI-30	33	F	2	82	82	38*	None
VI-33	27	F	2.5	119	85	50*	None
VI-39	20	F	7	55*	51*	32*	Mild
VI-41	29	F	3	80	54*	61	None
VI-47	12	F	10.5*	54*	28*	12*	Mild
VI-57	12	F	6.5	114	65	14*	Mild
VI-95	19	F	8	48*	45*	48*	Mild
VI-100	14	M	—	73	44*	45*	Mild
VI-101	12	M	8.5	69	36*	32*	Mild
VI-108	16	F	7	—	28*	68	None
VI-110	12	M	9	—	35*	43*	None
VII-1	10	F	4.5	116	94	49*	Mild

*Test value outside 95% ranges of normal controls in vWd direction.
†Probands.

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Table 6. Distribution of Abnormal Values by Type Among Affected Persons

Abnormality	Family 1 No.	Family 1 Percentage of Affected	Family 2 No.	Family 2 Percentage of Affected	Total No.	Total Percentage of Affected
Increased BT	8	35	8	26	16	29
Decreased VIII:C	9	39	12	39	21	39
Decreased VIIIR:Ag	13	56	15	48	28	52
Decreased VIIIR:WF	14	61	24	77	38	70
Total subjects	23*		31*		54	

*Family 2 in Table 5 included 34 affected persons. Full data were not available on three (VI-100, VI-108, and VI-110); so they have not been included in Tables 6, 7, and 8. One member of family 1 (V-78) was also omitted for the same reason.

members of the two families for whom complete data were available, and it is arranged to show the frequency of each abnormal test. It will be noted that increased BT was the least common abnormality and decreased VIIIR:WF the most common abnormality among those scored as affected; yet only 70% had this most frequently occurring abnormality.

The frequencies at which sets of abnormal test results occurred in the same 54 affected persons are shown in Table 7. Only 8 affected persons (15%) showed the classic vWd phenotype, i.e., all four tests abnormal, whereas 52% were abnormal for a single test. The reason for the apparent discrepancy between Tables 6 and 7 (70% abnormal for VIIIR:WF and 52% abnormal for only one test) is that many of those with abnormalities of VIIIR:WF also had other abnormalities.

The diverse pattern of laboratory results for the affected persons is shown in Table 8. Here the binary scoring system introduced in Table 1 is used to classify those scored as affected by our criteria. It will be noted that 10 different quantitative variants were present among the 54 affected members of the kindred, including variant 1, the classic phenotype, 15% being of this type. It should also be noted that among the affected persons there were four with variant 13, i.e., all tests normal except for reduction in VIII:C, the hemophilia A phenotype. Not shown in the table, but alluded to earlier, is the fact that known heterozygotes exhibiting variant 16 (i.e., normal) were also seen. Specifically, there were 11 persons, including the four parents of the two homozygotes, all of whose values were within the 95% ranges of the unrelated normals, and for genetic reasons they must be regarded as false negatives.

Table 7. Distribution of Sets of Abnormal Tests Among Affected Persons

Abnormality	Family 1 No.	Family 1 Percentage of Affected	Family 2 No.	Family 2 Percentage of Affected	Total No.	Total Percentage of Affected
Abnormal on all 4 tests	4	17	4	13	8	15
Abnormal on 3 tests	3	13	4	13	7	13
Abnormal on 2 tests	3	13	8	26	11	20
Abnormal on only 1 test	13	57	15	48	28	52
Total subjects	23*		31*		54	

*Family 2 in Table 5 includes 34 affected persons. Full data were not available on three (VI-100, VI-108, and VI-110); so they have not been included in Tables 6, 7, and 8. One member of family 1 (V-78) was also omitted for the same reason.

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Table 8. Combination of Test Results Among Affected Persons

Variant Types	BT	VIII:C	VIIIR:Ag	VIIIR:WF	Family 1		Family 2		Total	
					No.	Percentage of Affected	No.	Percentage of Affected	No.	Percentage of Affected
1	–	–	–	–	4	17	4	13	8	15
5	+	–	–	–	3	13	4	13	7	13
8	–	+	+	–	1	4	1	3	2	4
9	+	–	–	+	0	0	1	3	1	2
10	+	+	–	–	2	9	5	16	7	13
11	+	–	+	–	0	0	1	3	1	2
12	+	+	+	–	4	17	9	29	13	24
13	+	–	+	+	2	9	2	6	4	7
14	+	+	–	+	4	17	1	3	5	9
15	–	+	+	+	3	13	3	10	6	11
Total no. affected					23*		31*		54	

*Family 2 in Table 5 includes 34 affected persons. Full data were not available on three (VI-100, VI-108, and VI-110); so they have not been included in Tables 6, 7, and 8. One member of family 1 (V-78) was also omitted for the same reason.

Segregational Analysis

Determining whether or not vWd is being transmitted as an autosomal dominant trait is not easy, since the trait is less than fully penetrant and is of variable expressivity. The reduced penetrance in our kindred is demonstrated by the 11 of 26 transmitters who did not score as affected. The variable expressivity is documented by the data in Table 8 showing that 10 of 15 possible phenotypic variants of vWd were observed. To establish a phenotype reasonably indicative of the heterozygous genotype for genetic purposes, we tested the hypothesis of autosomal dominant transmission of the affected state by examining the frequency of affected persons (one or more abnormal tests) among the progeny of matings where the parent in the main line of descent was also affected. (A mating in which the unrelated spouse scored as affected, IV-94 × IV-95 of family 1, was excluded, along with the two matings producing homozygotes, since their parents did not score as affected.) An autosomal dominant hypothesis predicts that from this mating type there will be a 1:1 ratio of affected persons to unaffected persons, unrelated to the sex of the transmitting parent or the child. Thirty such matings produced 83 children, and the results are shown in Table 9.

It will be noted that male-to-male transmission occurred in both families and that there are no empty cells in the matrix not explicable on the grounds of random variation. Only 41 of the 83 children could be classified, but a 22:19 ratio of affected to unaffected was observed among those who could be classified ($p > 0.50$). Also, the sex ratio among the affected and unaffected was not abnormal ($p > 0.20$). Therefore, autosomal dominant inheritance of the affected state, as defined earlier, could not be rejected.

Stability of the vWd Phenotype Over Time

The phenotypic variability described earlier made it essential to assess the stability over time of the phenotypes assigned in the first test. This was accomplished by restudying a subsample of family 2. This group was reexamined twice, in March and August of 1978. The retested group consisted of 22 persons

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Table 9. Transmission of the Affected State in Matings of Affected by Unaffected

Matings		Offspring						
		Affected		Not Affected		Unknown		
Male × Female	(No.)	Males	Females	Males	Females	Males	Females	Total
Family 1								
Affected × unaffected	(7)	3	0	1	2	4	5	15
Unaffected × affected	(10)	6	3	3	2	6	3	23
Family 2								
Affected × unaffected	(6)	3	1	4	5	3	4	20
Unaffected × affected	(7)	2	4	1	1	6	11	25
Totals	(30)	14	8	9	10	19	23	83
		22		19		42		

Ratio of affected to unaffected: expected 1:1, observed 22:19, $\chi^2 = 0.22$, $p > 0.50$. Ratio of affected males to affected females to unaffected males to unaffected females: expected = 1:1:1:1, observed = 14:8:9:10, $\chi^2 = 1.09$, $p > 0.20$.

classified on first testing as follows: four unaffected transmitters; five affected transmitters; five affected persons; three spouses (normal); five unaffected persons. Nine were male and 13 were female.

Table 10 shows the results obtained for the 22 subjects on all three occasions. They are grouped by the category into which they were classified on first testing. An superscript dagger following a value indicates that the value is outside the 95% range of normal for the test in the vWd direction; a value in parentheses is a value obtained on second or third testing that was different in its relationship to the 95% cutoff than the value obtained on first testing; an asterisk indicates an individual who changed class on retesting, from affected to unaffected or vice versa. It will be observed that only 18 of 140 retests (13%) were different in relation to the 95% cutoff than they were on the first test, whereas 5 of the 22 subjects (23%) changed class. Two of these subjects (IV-37 and IV-38) shifted from unaffected transmitters to affected transmitters; two others (V-21 and V-36) shifted from affected to unaffected; the fifth subject (V-134) shifted from affected to unaffected to affected. It should be noted that three of the five shifted on second testing, the last two (IV-37 and IV-38) shifting only when tested a third time.

DISCUSSION

The purpose of this study was to try to define the phenotypic variability within families transmitting classic vWd. In order to do so, it was necessary to study a population all of whom possessed the same abnormal allele. We were fortunate to have two suitable and cooperative families available, each spanning seven generations and genetically independent for at least 200 yr.

When developing the protocol for this study, we had to decide whether to sample a large number of subjects during a reasonably short period of time by drawing blood at a few home collection points or to obtain fewer samples over a longer period of time under laboratory conditions. Since a large sample size was judged to be the most critical aspect of the study, we opted for home collection under conditions we had previously found to be reliable. However, no other compromises were made. Very careful controls and standards were established, and all tests were carried out by one person; careful statistical evaluations were made. The data in Table 2 indicate that our method of home collection was acceptable.

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Table 10. Repeated Tests on a Subsample of Kindred 2

	BT (min)			VIII:C (U/dl)			VIIIR:Ag (U/dl)			VIIIR:WF (U/dl)		
Subject	1976	1978a	1978b	1976	1978a	1978b	1976	1978a	1978b	1976	1978a	1978b
Unaffected transmitters												
IV-7	7.5	8.5	—	243	160	—	88	100	—	98	75	—
IV-8	5	4.5	4	137	125	85	66	87	64	70	65	73
IV-37†	3	8	(10*)	118	157	94	89	101	90	72	98	123
IV-38†	4.5	9	(11.5*)	141	93	159	75	108	116	70	74	110
Affected transmitters												
V-21†	15*	(4.5)	(4)	129	144	96	129	175	153	100	122	98
V-29	6.5	5	—	85	72	—	35*	30*	—	36*	32*	—
V-35	9	3.5	5	62	91	77	42*	30*	32*	19*	28*	45*
V-95	3	4.5	4.5	54*	(84)	(81)	34*	(90)	46*	38*	23*	29*
V-134†	>15	(8.5)	(7)	80	181	84	73	72	72	48*	(92)	40*
Affected												
V-85	10.5*	(6.5)	—	45*	45†	—	27*	37*	—	18*	24*	—
VI-39	7	5.5	—	55*	(89)	—	51*	(74)	—	32*	48*	—
VI-47	10.5*	(5.5)	(5)	54*	32†	43*	28*	8*	19*	12*	<6*	16†
VI-57	6.5	8	5.5	114	100	104	65	59	(46*)	14*	43*	42*
VI-101	8.5	6.5	6	69	103	98	36*	28*	36†	32*	42*	52*
Spouses												
V-4	4	7.5	—	150	84	—	109	91	—	74	92	—
V-36†	6	3.5	3	57*	(85)	(67)	81	91	112	90	83	104
V-135	7	5.5	8	108	143	124	180	119	125	110	150	111
Unaffected												
V-3	5	—	6	110	—	92	94	—	118	169	—	87
VI-5	3.5	—	6.5	73	—	61	92	—	77	97	—	85
VI-26	6	—	6.5	69	—	95	89	—	99	75	—	114
VI-97	8.5	5.5	—	109	116	—	185	214	—	138	129	—
VI-99	4	7.5	5.5	136	106	85	111	149	96	120	149	169

*Test value outside the 95% range of normal in the vWd direction; parentheses indicate value on repeat test that is on opposite side of cutoff than on first test.

†Individual who scored differently on retesting.

Family 2 appeared at first glance to manifest an example of the recessive form of vWd, as described by Veltkamp and van Tilburg,[34] since the disorder in the probands was very severe and the parents did not have abnormal test values. However, two other relatives (V-85 and VI-47) did have the phenotypic characteristics of dominant vWd, and the family clearly demonstrated the classic type. Since the data in Tables 4 and 5 show the phenotypes observed among members of the two families to have been very similar, we are treating them as essentially the same, and we believe that our general conclusions apply to all kindred transmitting classic vWd. However, it will be necessary to do similar studies of other large kindred to verify this conclusion.

The fact that family 2 does not represent an example of recessive vWd but of classic vWd means that we have not yet encountered the recessive form of the disease in the 181 patients on record at Chapel Hill. This may imply that vWd in America differs from vWd in Europe,[11,34] although most of our patients are of European extraction. Alternatively, our experience may imply that there has been a "founder effect" in America, i.e., Americans with vWd have sprung from relatively few mutated genes, all of which are of the dominant type. However, the phenotypes

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of the severely affected homozygotes in family 2 and their parents are very similar to those of the recessive homozygotes who have been observed in Europe. Thus there is another possibility, i.e., that more extensive study of the recessive kindred in Europe might show that they are really of the classic type. Had we seen only the probands in family 2 and their parents and not the many other members of their large family, we might have concluded that this kindred was also of the recessive type.

One of our objectives was to determine if quantitative variants occur within families ascertained through probands having classic vWd. The variants in question are those individuals who show abnormalities of some but not all of the tests for F-VIII-related activities. Using a binary scoring system (+, −) for a set of four tests, there are $2^4 = 16$ possible combinations. Setting aside the classic vWd phenotype, there are 15 possible quantitative variants. We observed 11 of these among the affected persons in our two kindred, and only 13 have been reported in the literature. The heterogeneity within our families suggests that many of the variants that have been reported individually or in small kindred represent chance observations of phenotypic heterogeneity for classic vWd, a speculation made earlier by Meyer and colleagues[17,22] about the variant "vWd type B" reported by Firkin et al.[23]

A major problem in our study was classifying our subjects. Symptoms were not very useful, since 23% of the normal controls were also classified as symptomatic by our method, and some family members with histories of bleeding were normal with all tests. The discrepancy between a history of bleeding and the presence of normal tests seemed more common in the older subjects, but where age significantly affected the levels of the F-VIII activities, they increased with age. (Our experience with the effects of age on F-VIII will be discussed at greater length in the second report in this series). Three of 33 unrelated spouses had abnormal test results, but 2 spouses (V-8 and V-36) had a single barely abnormal value each and could be explained away as false positives. One spouse (IV-91) with two abnormally low F-VIII-related activities may well have been heterozygous for vWd, despite his negative family history. Eleven of 26 individuals who clearly transmitted the vWd gene, being in the main line of descent and having had affected descendants, were normal by all tests. Thus the classic vWd gene in our material was pleiotropic and only 58% (15 of 26) penetrant when an abnormality of at least one test in a single set of tests was used to identify the heterozygotes.

It was not possible to determine the mode of inheritance in the kindred on the basis of symptoms; so we turned to the phenotype of the affected state, meaning a person having one or more abnormal tests. The hypothesis of autosomal dominance of the affected phenotype could not be rejected. Although the argument is circular, the fact that our cutoffs (95% range in a one-tailed test) produced a typical monogenic mendelian ratio suggests that they were not inappropriately chosen.

An interesting feature in both kindred was the occurrence of subjects who might have been misdiagnosed as carriers of hemophilia A or as having mild hemophilia (Tables 4, 5, and 8). The possible carrier females included V-141 in family 2 and V-110 in family 1. The former had an VIII:C level of 56% and an VIIIR:Ag level of 83%, and the discriminant procedure used in our laboratory for identifying hemophilia A carriers[35] produced for her a likelihood ratio (LR) favoring carrier-ship of 333:1. An LR of 333:1 implies that had she happened to have been the

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daughter of a carrier of hemophilia A with a genetic probability of 0.50, the final probability favoring carriership would have been greater than 99%. However, she was not a member of a hemophilia A kindred, and the genetic probability of carriership in her case was the random chance of encountering a carrier anywhere in the population, i.e., four times the mutation rate ($4 \times 1.3 \times 10^{-5} \sim 5 \times 10^{-5}$). When this probability was combined with her likelihood ratio of 333:1, the final probability that she was a carrier of hemophilia A was only 0.017. Similarly, V-110 of family 1 had a final probability of only 0.003 when the appropriate genetic probability was used. We can conclude, therefore, that these women are very probably not carriers of hemophilia A, but the occurrence of their data sets emphasizes the importance of correct use of genetic information when attempting to identify hemophilia carriers, a matter discussed in detail elsewhere.[36]

The problem was somewhat different with two males, VI-16 of family 2 and V-115 of family 1. The first man (VI-16) can be regarded as normal, since his VIII:C level was only 1% below the normal range and his other tests were within the normal range.* However, V-115 of family 1 was more puzzling. His VIII:C of 39% was 20% below the cutoff, and his other tests were normal. Yet his father (IV-84) and brother (V-113) clearly had classic vWd, with abnormally low values for VIII:C, VIIIR:Ag, and VIIIR:WF. Seen in isolation, he might have been regarded as hemizygous for a hemophilia A allele that had had a minimal effect, since we have recently seen a man in an X-linked kindred of mild hemophilia with an VIII:C of 38%.[37] However, in the context of his family, we can conclude that V-115 manifests a hemophilia-A-like variant of vWd. He calls to mind another man with an VIII:C level of about 40% first seen in 1953 who, in the context of those times, was believed possibly to have been hemizygous for an X-linked hemophilia A gene.[38] Seen again last year, his VIII:C was 42%, his VIIIR:Ag was 73%, and his VIIIR:WF was 66%. Only his VIII:C was outside the cutoffs for our study, but his other F-VIII-related activities were on the low side. In light of the present study, we think it is as likely that he is heterozygous for vWd as hemizygous for "sub-hemophilia."

An important question concerns the stability of F-VIII-related activities over time, since they might be stable or might fluctuate together or separately. This is an important matter both from the standpoint of correct diagnosis of individual patients and from the genetic point of view, e.g., testing modes of inheritance or determining linkage relationships. Therefore we restudied a significant subsample of family 2 and discovered a surprising amount of phenotypic stability. Seventy-seven percent of the phenotypes established on first testing were unchanged. In four of the five that changed, the BT shifted from one side of the cutoff to the other, not a surprising event in view of the observations made many years ago by Pitney and Arnold on a patient whose BT fluctuated between 3 min and more than 30 min during a period of 1 yr.[39] Two of those whose classifications shifted during restudy were the parents of a homozygote, and they can be assumed to be heterozygous. Another whose classification shifted had affected children, and still another was

*He is scored in Fig. 2 as affected and is so counted in Table 7. This is because we must apply our cutoffs without exceptions. For genetic purposes this is acceptable, because there will be both false positives and false negatives to balance each other. For clinical purposes, this is, of course, unsatisfactory.

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scored abnormal twice, on first and third testings. The spouse who was barely abnormal on 1 of 12 tests probably represented a false positive diagnosis. Thus, restudy confirmed our classification directly in 77% and did not change the classifications of those who showed differences on retesting, since their genotypes had already been established independently with genetic evidence. These findings are in some respects similar to those of Abildgaard et al.,[40] who also studied patients serially. They reported that the vWd phenotype was stable over time in some kindred but variable in others. We found that it was stable in some members of a kindred but variable in other members of the same kindred. Unfortunately, their results were published only in abstract form and are not available for detailed comparison with ours.

It must be emphasized that the practical problem of assigning genotype to an individual for diagnosis and treatment is entirely different from that of assigning genotype for the testing of a genetic hypothesis. In the individual case a probabilistic decision must be made on the basis of all the facts, and an action (therapy) must be taken that may directly affect life. In the genetic case, a certain amount of error can be accepted within a group of decisions, since there is the opportunity for the false positive diagnoses to balance the false negative diagnoses. Since errors of both types are expected with overlapping distributions of test results, the problem is to make the errors symmetric. Since 26 of our subjects, the transmitters, had affected persons as descendants and may reasonably be assumed to be heterozygous, even though a large minority were not scored as affected by the usual tests, it is obvious that we need better diagnostic methods than those that are currently available.

In our restudy we managed, on the third try, to classify as affected 2 persons who had been classified as unaffected on first and second testing but who had had affected children and were almost certainly heterozygous. We therefore agree with Abildgaard et al.[40] that restudy is an important method of correctly identifying heterozygotes, but this may require several retestings. Although our restudy procedure, with its interval of 2 yr between first and third testings, was suitable for our genetic studies, it is not very practical in patient care. What is required is a procedure that works on single tests. We have found that the heterozygous genotype can be assigned probabilistically to certain persons by discriminant analysis, even when the values for all tests lie within the normal ranges. For example, in family 2, the four presumably heterozygous parents of the homozygous probands (V-37 and V-138) who could not be scored as affected on first testing, and 2 of whom were scored as affected only with the BT on third testing, scored as heterozygotes when discriminant analysis was applied to the first set of test results. A description of the discriminant procedure we used in assigning their genotypes and the results of its application are the subjects of the second report in this series.

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