Hemophilia and von Willebrand’s Disease: Genetic Considerations

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ABSTRACT

Recent progress in the biochemical characterization of coagulation factors VIII and IX has greatly contributed to our understanding of the inheritance of hemophilia and von Willebrand’s disease and facilitated the recognition of carriers of these disorders. Factor VIII is a molecular complex which may be quantitated immunologically as factor VIII-related antigen. Within this complex reside the von Willebrand factor, absent in von Willebrand’s disease, and factor VIII procoagulant activity and antigen. Hemophilia is an X-linked disorder; female carriers may be recognized by a disproportionate increase in factor VIII-related antigen or procoagulant antigens in relation to procoagulant activity. Prenatal diagnosis of hemophilia has been accomplished by measurements of clotting activity and antigens in fetal blood.

Von Willebrand’s disease has been classified on the basis of laboratory abnormalities, the biochemical characteristics of the von Willebrand factor, and its patterns of inheritance. In the most commonly observed form, there is autosomal dominant inheritance, and most patients are heterozygotes. These individuals manifest variably prolonged bleeding times and concordantly reduced activities associated with factor VIII. Rarely, there is an autosomal recessive pattern in which the homozygotes have much more severe clinical disease, including hemarthroses. However, the biochemical defects in the von Willebrand factor appear to be quite diverse, defying any simple classification of this disorder.

Introduction

Individuals from families having members with congenital hemorrhagic disorders are anxious to learn whether or not their own offspring will be bleeders. To provide this information requires a precise definition of the hemorrhagic disorder and a knowledge of the inheritance of the particular condition. In recent years, great progress has been made in identifying the biochemical basis of hemophilia and von Willebrand’s disease, and with this information has come an understanding of the modes of inheritance of these coagulopathies.

Carrier Detection

The majority of severe hemophiliacs lack the clotting activity associated with
either factor VIII (anti-hemophilic factor or VIII:coagulant, VIII:C) or factor IX (Christmas factor). The genes for both these factors are borne on the X-chromosome.

According to Mendelian genetics, all of the daughters of a hemophiliac will be carriers of the disorder (figure 1). This means that they will inherit the defective X-chromosome from their fathers. In turn, they may transmit either the defective X-chromosome or their normal X-chromosome to their offspring. Thus, there will be a 50:50 chance for each of their pregnancies to produce either a hemophiliac son or a carrier daughter. The daughter of a hemophiliac is termed an obligatory carrier, whereas the sister of a hemophiliac is a potential carrier. Potential carriers are very desirous of knowing whether they are or are not carriers. Measurement of the concentration of the clotting factor in their plasma provides some useful information in this regard.

The X-chromosome in the appropriate somatic cells supplies the genetic information required for the synthesis of both factors VIII and IX by that cell. If the X-chromosome bears the stigma of factor VIII-type hemophilia, then the synthesis of factor VIII by those cells will be impaired. However, in the case of the carrier of hemophilia, there is also the normal X-chromosome to consider. The contribution of each X-chromosome in any given cell may be understood by reference to the Lyon hypothesis. This states, in essence, that in each somatic cell there is a random inactivation of one of the X-chromosomes and that this occurs on the basis of statistical chance. Thus, on a probability basis, in half of the somatic cells of a carrier the normal X-chromosome will predominate; in the other half, the defective X will predominate. Therefore, a carrier would be expected to produce half the normal amount of clotting factor, and measurement of the clotting factor concentrations in suspected carriers gives some indication of carrier status.

Figure 1. Inheritance of Factor VIII or Factor IX deficiency. $X = X$ chromosome bearing the affected gene; $XY =$ hemophilic male; $XX =$ carrier female.

Unfortunately, the situation is complicated by the fact that, firstly, clotting factor levels vary widely in a population, with a range of 50 to 200 percent of the average normal values; and secondly, values can fluctuate broadly in any given individual depending on such factors as exercise, fever, anxiety, hormonal status and drug intake. In consequence, isolated measurements of clotting factor concentrations provide definite evidence of carrier status in less than 50 percent of cases. Fortunately, in the case of carriers of factor VIII deficiency, another technique is available which provides the diagnosis in over 80 percent of cases.

 VIII:C circulates in the plasma as part of a large molecular complex. This complex can be recognized immunologically and is termed factor VIII-related antigen or VIIIIR:Ag. It is present in a 1:1 ratio with VIII:C in normal subjects; however, in carriers of VIII:C—deficiency hemophilia, the relative deficiency of VIII:C results in ratios of VIII:C to VIIIIR:Ag that are generally less than one and approach 0.5. Thus, the determination of both VIII:C and VIIIIR:Ag provides a powerful tool for distinguishing carriers of classical hemophilia. By using the method of discriminant analysis, Ratnoff and Jones have identified more than 90 percent of 87 obligate carriers at a level of certainty that would have misidentified only 5 percent of normal women as carriers.

Recently, 62 obligate carriers were examined by us of whom 23 were daughters of hemophilic men (paternal carriers) and 39 were mothers of hemophilic sons (maternal carriers). Surprisingly, it was found that the paternal carriers had significantly lower coagulant
activity than the maternal carriers but similar VIIIR:Ag. The differences in VIII:C could not be attributed to age or pubertal status. The VIII:C levels of paternal carriers whose fathers had severe hemophilia were lower than those whose fathers were more mildly affected. The results suggested that there is an unbalanced lyonization in the paternal carriers, with the father's X-chromosome having favored status at the expense of the normal maternal X-chromosome. This would mean that fewer cells would be capable of producing VIII:C and explain the lower values for VIII:C in the paternal carriers. Support for this hypothesis will come if it is discovered that a selective advantage of the paternally-derived X-chromosome occurs not only in hemophilia but also in other X-linked disorders.

Another technique for detecting carriers of factor VIII-type hemophilia has become available with the development of methods which specifically detect the procoagulant antigen of the factor VIII molecule. Using a neutralization technique, Muller et al found that approximately one-third of factor VIII-type hemophiliacs harbor biologically inactive procoagulant antigens in their plasma. The identification of these inactive antigens in the plasma of possible carrier mothers and sisters will provide another means of carrier recognition. Similarly, about a third of factor IX deficient hemophiliacs have non-functional factor IX antigens in their plasma. Their carrier relatives were found to have antigen levels that were disproportionately increased over activity levels provided the original factor IX activity ranged from 50 to 100 percent of normal. Higher coagulant activities were associated with proportionately elevated antigen levels making recognition of the carrier state impossible.

**Prenatal Diagnosis**

Methods for assaying non-functional coagulant antigens in the plasma of hemophiliacs permit the prenatal diagnosis of hemophilia. Fetal blood, obtained by fetoscopy, may be examined for either coagulant activity or antigen or both. Measurement of VIIIR:Ag on the same sample provides a way of assessing the effects of dilution on the content of inactive protein. The results of such studies are still of a preliminary nature, but Fine et al correctly predicted the outcome in six fetuses at risk for factor VIII-deficiency hemophilia. Mibashan et al measured the coagulant activities of factors VIII and IX in fetal blood and compared the concentration of factor VIII activity by assuming the VIII-procoagulant antigen as well. Two male fetuses at risk had normal values for activity and antigen and the pregnancies ended in the birth of normal boys. These results are very exciting, but many technical difficulties are yet to be overcome, including the safe collection of fetal blood and reliable measurements of coagulant and antigen activities in specimens that are usually extensively mixed with amniotic fluid, saline and other potentially interfering substances.

**Genetic Variants**

Several variant forms of hemophilia have been recognized. These have been classified on the basis of specific biochemical abnormalities of the clotting factor molecule. For example, patients with factor IX deficiency or "Christmas Disease" frequently are found to have factor IX molecules which are biochemically aberrant. Bertina recently reviewed this field and summarized the distinctive features of six of these variant factor IX's, reported in some 185 pedigrees. Factor IX Bm is able to compete with factor X for the factor VII-calcium-thromboplastin substrate and thus prolong the prothrombin time when ox brain is used as the source of thromboplastin. Factor IX Chapel Hill demonstrates defective activation by factor XIa, and factor IX Alabama shows impaired interaction with phospholipid. One of two newly described variants has
an increased molecular weight and abnormal electrophoretic mobility; the
other shows abnormal activation by factor XIa and prolongs the ox brain prothrombin time. The biochemical defect of factor IX Worcester has yet to be elucidated. Finally, there is the curious factor IX Leiden, whose coagulant activity is very low at birth but rapidly increases after puberty.

Von Willebrand's Disease

Von Willebrand's disease presents a much more complicated picture, since variation in this disorder appears to be the rule rather than the exception. Patients with this condition generally have a deficient or defective von Willebrand factor, a major constituent of the factor VIII-related antigen. The disorder may be classified on the basis of the results of laboratory tests, the biochemical characteristics of the affected hemostatic factor or the genetics of the hemorrhagic condition. All three of these approaches shall be considered.

The European Working Party on von Willebrand's disease has distinguished four typical patterns of this disorder. In Type 1, or the classical form, the bleeding time is prolonged, factor VIII coagulant activity and related antigen are reduced, and the ability of the patient's plasma to support the ristocetin-induced aggregation of platelets is impaired. Type 2 also has a prolonged bleeding time and reduced ristocetin cofactor activity, but VIII:C and the antigen may be normal. Type 3 has a prolonged bleeding time, normal VIII:C, but reduced antigen. Finally, in Type 4 the bleeding time may be normal but VIII:C, VIIIIR:Ag and ristocetin cofactor are all reduced.

Unfortunately, this classification fails to consider one other attribute of the von Willebrand factor; i.e., its ability to support the adhesion of platelets to the denudated vessel wall. This latter characteristic may be the most important in vivo function of the von Willebrand factor, but there is no simple, convenient laboratory test for its measurement.

Several biochemical abnormalities of the plasma von Willebrand factor have been reported. These include altered carbohydrate content, decrease in high-molecular species and increased mobility on crossed-immunoelectrophoresis. The latter technique is suitable for screening large numbers of patients. With the use of this method, it was possible for us to recognize an abnormal von Willebrand factor in some dozen members of a large Illinois kindred. While several of these subjects had the typical clinical and laboratory manifestations of von Willebrand's disease (Type 1 variety, vide supra), others were clinically silent. Their identification made possible a better definition of the inheritance of the disorder, and was in accord with the genetic scheme recently presented by Ingram.

According to this scheme, two types of inherited patterns are recognized: autosomal dominant and autosomal recessive. Patients with the autosomal dominant variety are generally heterozygotes, have moderately severe clinical bleeding, variably prolonged bleeding times and usually concordantly reduced factor VIII components. Our family fell into this pattern. In contrast, heterozygotes of the autosomal recessive form of the disease have a subclinical bleeding tendency and normal laboratory studies. The homozygous offspring of two heterozygotes, on the other hand, have severe clinical disease, including hemarthroses and a very prolonged bleeding time with marked reduction of all factor VIII activities. An example of a patient belonging to this category has been reported by Veltkamp and van Tilburg, and a similarly affected individual was observed by Bloom and Peake. However, this latter subject had an affected family member with the heterozygous form of the disorder! It therefore appears that von Willebrand's disease is a very heterogeneous disorder which may have variable clinical manifestations depending upon the bio-
chemical lesions induced in the von Willebrand factor molecule.

Finally, Hoyer et al.\textsuperscript{10} recently reported the prenatal studies of an infant, whose two parents had mild von Willebrand’s disease and was therefore at risk for the severe form of the disorder. Assays of the fetal blood for VIII:Ag and the procoagulant antigen were within normal limits, and the absence of von Willebrand’s disease was confirmed at the time of delivery. As the technique of fetal blood sampling improves and the measurements of coagulant proteins are standardized, the prenatal diagnoses of hemophilia and von Willebrand’s disease will become important adjuncts in the genetic counselling of families affected by these disorders.

References