CARRIER DETECTION AND PRENATAL DIAGNOSIS OF HAEMOPHILIA. PRESENT AND FUTURE STRATEGIES

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Accurate carrier detection of disease and effective early prenatal diagnosis where appropriate are the aims of any practical geneticist, since they represent the most effective forms of disease control. Haemophilia A and B are both X-linked diseases where the severity of disease runs true within families. As a result, meaningful genetic counselling can be given based on carrier assessment and, in severe cases, prenatal diagnosis can be offered. The most important aspect of this strategy is accurate carrier detection since this has the effect of not only eliminating normal females from further concern, but also of reducing the number of prenatal diagnostic procedures to only proven carriers.

1. OVERALL PROBLEM

It has been estimated that there are between 5 and 6 potential carriers of haemophilia for every haemophiliac, resulting in the UK for example, in there being 25-30,000 females at risk. Obligate carriers can be readily identified from family information being either daughters of haemophiliacs, mothers of two haemophilic sons born at separate births, or mothers of one haemophilic boy where there is a well documented haemophilic relative on the maternal side of the family. The remaining potential carriers can be divided into women who have a haemophilic relative on the maternal side, and women who have given birth to a haemophilic son without a family history of the disease. Some of the latter group will result from inheritance of a previously undetected haemophilic gene, and the remainder will result from new mutations. The precise carrier state of women with one haemophilic child and no family history can be difficult to assess and up to 30% of haemophilia families fall into this group. Recent reports suggest that of this 30%, a fifth may result from germinal or somatic mosaicism within the affected individual's mother, so additionally complicating carrier detection.

Key-words: Carrier detection; Haemophilia A; Haemophilia B; PCR amplification; Prenatal diagnosis; RFLP analysis.

Accepted for publication on May 17, 1990.
2. PHENOTYPIC ANALYSIS

2.1 Carrier detection

In X-linked genetic disease carriers will, by definition, have the ability to produce only half the normal level of gene product. This can be seen in the haemophilias where obligate carriers of haemophilia A and B have, in general, only 50% of the normal level of factor VIII or factor IX measured biologically: VIIIC or IXC. The same is true of immunological measurements (VIIIAg or IXAg) except in cases of cross-reacting material positive (CRM+) haemophilia where low levels of biological activity in the haemophiliac are accompanied by normal levels of immunologically detectable proteins. However, because of the large normal range of these factors in plasma and, more importantly, because of the process of lyonisation (random inactivation of the one of the X-chromosomes in females) a significant overlap between plasma factor levels found in normals and those found in obligate carriers is observed, resulting in up to a 20% misclassification rate despite the use of international and national standard plasmas for the factor VIII and factor IX levels. The situation can be improved for haemophilia A by also measuring the plasma level of von Willebrand factor (vWF), usually measured immunologically as vWF antigen or vWFAG. vWF and factor VIII have a close relationship in plasma and it is now known that vWF not only acts as a carrier of factor VIII, but also serves to protect inactivated factor VIII from proteolytic degradation. Thus, while the ratio of factor VIII to vWF is 1.0 in normal plasma, in carriers of haemophilia A it is closer to 0.5 and this ratio has been shown to be the best phenotypic discriminant for carrier detection. Complex statistical analysis of these measurements has been reported and is the subject of a WHO publication5. However, 5-10% of obligate carriers of haemophilia A still appear to be phenotypically normal given the best estimates of plasma factor VIII and vWF levels.

2.2 Prenatal diagnosis

Measurement of factor VIII or IX levels in fetal blood obtained at fetoscopy at 18-20 weeks gestation has provided, in skilled hands, a very effective prenatal diagnostic service for haemophilia A and B, as shown by the experience of the group at King’s College Hospital, London, UK6. In over 300 cases, no incorrect diagnoses have been made, and the quality of sample has meant that measurement of factor VIIIC alone has been used in all cases except one, where diagnosis based on VIIIAg was made. Results with haemophilia B are similar. While fetal blood sampling at fetoscopy has provided an efficient prenatal diagnosis service, the fact that sampling is only possible within the 2nd trimester at 18-20 weeks has resulted in termination of affected fetuses at a gestational age which many are finding increasingly unacceptable.

3. GENOTYPIC ANALYSIS

3.1 Introduction

It is clear that the ideal method for carrier detection and prenatal diagnosis is to identify the affected gene in an affected family member at the DNA level and to look for this gene in possible carriers and in fetuses at risk. The