The molecular pathology of haemophilia B

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It is a great honour and privilege to deliver this Fourth Wellcome Trust Lecture on the subject of the molecular pathology of haemophilia B. Why study haemophilia, a rare inherited bleeding disorder, you might ask? After all, there are many commoner and more serious diseases of mankind. I refer to cancer or diseases of the circulation such as strokes, heart attacks, atherosclerosis or pulmonary embolisms. Let me remind you of the ‘genetic component’ of these commoner diseases. It is now abundantly clear that somatic mutations in specific oncogenes correlate with particular cancers. They are clearly one important factor in the cause of malignancy. And, turning to the circulatory disorders, we now know that inherited defects in the low-density lipoprotein receptors are present in patients with a genetic predisposition to atherosclerosis. Genetic factors, whether inherited or acquired by mutation during our lifetime, are therefore important contributory factors to these diseases, but are clearly not the only component. For example, other factors such as diet, or whether one exercises, or one’s hormone balance will be among the factors that affect the formation of atheromatous plaque, an early ‘indicator’ of circulatory problems. Clearly the medical profession has to take ‘genetic disease’ seriously if we are to learn more about it and eventually cure it.

In haemophilia, the subject of my lecture, we have none of these complications of a multifactorial cause of disease. Haemophilia, whether the A or B form, is caused by an inherited defect in a single X-linked gene, and the disease has the characteristic feature of all X-linked recessives in that it affects boys but not girls. Although unaffected, girls can act as carriers transmitting the disease to future generations.

I will suggest to you in this lecture that a study of haemophilia is therefore a good model for understanding the molecular basis of genetic disease as well as for devising better diagnosis and treatment, and for thinking about a cure. I have to admit that other model systems, such as the thalassaemias, have been well studied at the molecular level, but for studies on improved treatment and for cure by gene therapy, I suggest that a study of the haemophilias will provide an excellent if challenging model.

Historical perspectives

Haemophilia has been known since biblical times as an inherited bleeding disorder, since boys born into families known to have the disease were excluded from ritual circumcision as early as the third century A.D. (quoted by McKee, 1983). Clinical symptoms of the disease were very serious before modern therapy was introduced. Patients often died as the result of internal haemorrhage especially into muscles and joints, and often the bleeding could occur without any previous trauma. The pattern of inheritance of the disease, whereby males were affected whereas females were not (although they could transmit this disease to future generations), intrigued the 19th century biologists and was not adequately explained until the genetic basis of sex was understood. Until about 1950, it was not realized that there were two forms of haemophilia, as the clinical symptoms of both forms are similar and both have an X-linked, recessive, pattern of inheritance. The differential diagnosis of haemophilia A (classical haemophilia) and haemophilia B (Christmas disease) depends on laboratory tests of the clotting time of indicator plasma. The former, haemophilia A, is the commoner disease, occurring at a frequency of about 1 in 6000 males, whereas haemophilia B occurs in about 1 in 30,000 males (see Table I). In haemophilia A patients there is a defect in the clotting factor VIII(C), whilst haemophilia B patients are defective in clotting factor IX. These factors are both essential proteins in the middle phase of the intrinsic clotting cascade, summarized in a simplified form in Fig. 1.

Cloning the factor IX gene defective in haemophilia B

My staff and I in Oxford decided to embark on studies of haemophilia B rather than haemophilia A because the cloning of the relevant gene seemed technically simpler at the time we started in 1980. I don’t propose to detail the work on cloning and sequencing the messenger RNA and gene for factor IX as this information is available in the detailed...
The extrinsic pathway (activation of factor VII) e.g. Factor VIIa activation of X. From Austen & Rhymes (1975).

Table 1. Vital statistics of the haemophilias

<table>
<thead>
<tr>
<th>Name</th>
<th>Synonym</th>
<th>Chromosome</th>
<th>Detailed location</th>
<th>Frequency (male)</th>
<th>No. of U.K. patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemophilia A</td>
<td>Classical haemophilia</td>
<td>X</td>
<td>End of long arm (q28)</td>
<td>Approx. 1 in 6000</td>
<td>4500</td>
</tr>
<tr>
<td>Haemophilia B</td>
<td>Christmas disease</td>
<td>X</td>
<td>Near end of long arm (q27.1)</td>
<td>Approx. 1 in 30000</td>
<td>900</td>
</tr>
</tbody>
</table>

The clotting cascade

Fig. 1. The clotting cascade

The intrinsic pathway is shown from top left to bottom right. The extrinsic pathway (activation of factor X by activated factor VII) is also shown. This is a simplified version showing the main features, but it omits feedback loops and a step, e.g. factor VIIa activation of IX, interconnecting the two pathways. From Austen & Rhymes (1975).

acid; (4) the connecting peptide region, which is a region rich in cysteine residues and where there are two separate sub-regions showing homology to epidermal growth factor; (5) the activation peptide region, which is cleaved during the activation of factor IX. From Yoshitake et al., 1984; Yoshitake et al., 1985. It has eight exons, over 90% of the DNA is composed of introns, and there are several Alu repeat sequences within these introns.

The factor IX protein is in some ways more interesting. It is a multidomain protein whose catalytic region is preceded by five other ‘signalising’ domains. These are, from N- to C-terminus of the factor IX: (1) the pre-domain, part of precursor factor IX, of unknown function (or possibly a signal for membrane insertion into the endoplasmic reticulum during secretion); (2) the propeptide region, also part of precursor factor IX, of unknown function (or possibly a signal for the correct carboxylation of the glutamic acid residues in the third or ‘Gla’ region of the molecule); (3) the ‘Gla’ region at the N-terminus of the definitive factor IX protein where all 12 glutamic acid residues are modified to γ-carboxyglutamic acid; (4) the connecting peptide region, which is a region rich in cysteine residues and where there are two separate sub-regions showing homology to epidermal growth factor; (5) the activation peptide region, which is cleaved during the activation of factor IX. From Gilbert, 1978. The catalytic domain consists of the classical hexadecapeptide sequence around the active site serine (amino acid 365), -Gly-Asp-Ser-Gly-Gly-Pro-, and the histidine residue at amino acid 221 and the aspartic acid residue at amino acid 269. Fig. 2 shows there is some correspondence between exons and protein domains although there are important exceptions. There is good agreement in the correspondence between the exons of both the pre- and pro-peptide region and the connecting peptide region. The ‘Gla’ region of the protein is separated into two separate exons, although the majority of the region is in a single exon b, which also encodes the propeptide. The only real exception to the correspondence between exons and protein domains is in the carbohydrate region where there are two separate exons, g and h. Despite this subdivision, there is incontrovertible evidence that this protein domain has been conserved in evolution. Figs. 2 and 3 show there are at least five different types of processing events in the biosynthesis of factor IX, subsequent to its translation on the ribosome. There are two N-linked carbohydrate residues, 12 γ-carboxyglutamate residues and a single β-hydroxyaspartate residue at amino acid 64. The addition of carbohydrate and γ-carboxylation occurs during secretion, but the enzyme that modifies amino acid 64 has not yet been characterized. I will refer below to the other processing events which result in the sequential cleavage of the precursor signal and propeptide region.

Expression of recombinant factor IX

Haemophilia B patients require repeated intravenous injections of factor IX if haemorrhage is to be controlled or prevented. The existing drug is derived from pooled blood of donors, and a factor IX concentrate prepared. As factor IX occurs in relatively low amounts in plasma (5 μg/ml) a large pool is needed to provide enough to treat patients. Past the HTLV-III/LAV virus (the causative virus of acquired immune deficiency syndrome, ‘AIDS’) has been introduced in this way and haemophilia B patients have died from receiving contaminated blood. As pasteurization of factor IX has been introduced, and since screening of blood donors is now carried out for serum conversion by HTLV-III/LAV virus, these problems are less likely to occur in the future. Nevertheless, other viruses such as non-A, non-B hepatitis virus and coronavirus, known to be blood-borne, are still a risk for patients receiving blood products. For this reason, and because blood donors may not always be available, it is desirable to have an alternative source of genetically engineered factor IX. This should be free of human blood-borne viruses. Because of the extensive post-translational processing required for factor IX, and in particular because of the known requirement of γ-carboxylation in the ‘Gla’ region for enzymic activity, my own group in Oxford, as well as others,
have used expression in mammalian cells in an attempt to produce genetically engineered factor IX. A general scheme for this is shown in Fig. 4 in which a cDNA clone under the control of a viral promoter linked to a positive selectable marker ‘Neo’ is introduced into a mammalian cell and selection applied resulting in the isolation of permanent cell lines secreting factor IX into the medium in which the cells are grown. From such cloned cell lines, factor IX can be purified by the scheme shown in Fig. 5, and the material can then be characterized by Western blotting, by its clotting activity in vitro and by quantitative enzyme-linked immunosorbent assay (ELISA). Table 2 summarizes the five existing reports on the expression of recombinant factor IX. The first two reports are from my own laboratory in Oxford, and we observed a marked improvement when we used dog kidney cells rather than rat hepatoma cells as the host cell for our genetically engineered constructs. In both cell lines, we were able to isolate fully active factor IX, i.e. the ratio of clotting activity to antigen present was the same as is present in factor IX isolated from normal blood. In other studies (see Table 2) higher yields of recombinant factor IX were
Table 2. Properties and yield of factor IX in conditioned medium of transfected or viral-infected cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Promoter</th>
<th>Yield in medium (μg/ml)*</th>
<th>Specific activity (clotting activity - antigen concentration) as % plasma factor IX</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Rat hepatoma (H4-11-E-C3)</td>
<td>Mouse Molony Leukaemia virus LTR</td>
<td>0.001</td>
<td>100</td>
<td>Anson et al. (1985)</td>
</tr>
<tr>
<td>(2) Dog kidney (MDCK)</td>
<td>SV40 early</td>
<td>0.25</td>
<td>100</td>
<td>I. M. Jones &amp; G. G. Brownlee (unpublished work)</td>
</tr>
<tr>
<td>(3) Hampster kidney</td>
<td>Adenovirus 2 major late</td>
<td>1.4</td>
<td>50†</td>
<td>Busby et al. (1985)</td>
</tr>
<tr>
<td>(4) Human hepatoma (HEP G2) infected with vaccinia factor IX virus</td>
<td>Vaccinia 7K early</td>
<td>3.7</td>
<td>80†</td>
<td>de la Salle et al. (1985)</td>
</tr>
<tr>
<td>(5) Hampster ovary (CHO)</td>
<td>Adenovirus 2 major late</td>
<td>100</td>
<td>2†</td>
<td>Kaufman et al. (1986)</td>
</tr>
</tbody>
</table>

*Normal human plasma has 5 μg factor IX/ml.
†This activity is reported to be dependent on added vitamin K.

reported, varying from 1.4 to 100 μg/ml, but in all these other reports, the material was to a greater or lesser extent inactive in clotting activity. In addition, a fraction of the factor IX fails to bind quantitatively to barium salts, strongly suggesting that the γ-carboxylation is incomplete. Indeed, in the last report (Kaufman et al. 1986), where the highest yield of factor IX is produced, the authors estimate that only 2% of the material is biologically active.

It seems clear that one reason for the lack of factor IX activity in the highest yielding cell lines is that the activity of the vitamin K-dependent carboxylase, which is present in the microsomes, is insufficient to modify factor IX completely. Therefore we must either find other cells that contain sufficient carboxylase, or this enzyme itself has to be cloned, engineered and introduced into cells to compensate for the lack of sufficient endogenous material, if higher yields of biologically active factor IX are to be produced. Alternatively, methods for activating the carboxylase or for carboxylating material post-translationally will have to be devised. Such studies are presently hampered by the instability of the vitamin K-dependent carboxylase and the fact that this enzyme has not yet been purified to homogeneity.

Do these genetically engineered cell lines make sufficient factor IX to be the basis of a commercial process for isolating factor IX from this source, rather than from blood? I estimate the U.K. requirements of factor IX to be 100 g per annum. Even assuming active factor IX could be produced and purified without loss of yield from the very best producing material, we would require a culture of 50,000 litres. This seems unrealistically high and too costly to consider at present. I conclude that we need higher yielding cell lines secreting at least 1–5 μg of active factor IX into the conditioned medium for this recombinant medium to compete realistically with the present process of isolating factor IX from blood. Despite this I am hopeful that this development work will be achieved and will provide the basis for a commercial process in the near future.

Carrier and antenatal diagnosis of factor IX

When girls in known haemophilia B families reach childbearing age, they naturally wish to know the risk of having an affected son. Girls have, on average, a 50% chance of
Table 3. DNA polymorphisms of the factor IX locus

For Caucasian populations, total 'catch' is 80% of females.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Nucleotide position in gene</th>
<th>Frequency of rarer allele in Caucasians</th>
<th>Heterozygote frequency</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) BamHI RFLP</td>
<td>- 5.87</td>
<td>0.05</td>
<td>0.09</td>
<td></td>
<td>Hay et al. (1986)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P. R. Winship (unpublished work)</td>
</tr>
<tr>
<td>(2) DdeI RFLP</td>
<td>Deletion 5505-5554</td>
<td>0.24</td>
<td>0.37</td>
<td></td>
<td>Winship et al. (1984)</td>
</tr>
<tr>
<td>(3) XmnI RFLP</td>
<td>7076 (G → C)</td>
<td>0.29</td>
<td>0.41</td>
<td></td>
<td>Winship et al. (1984)</td>
</tr>
<tr>
<td>(4) TaqI RFLP</td>
<td>11111</td>
<td>0.35</td>
<td>0.45</td>
<td>In linkage disequilibrium</td>
<td>Giannelli et al. (1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Camerino et al. (1984)</td>
</tr>
<tr>
<td>(5) Mspl RFLP</td>
<td>?15994</td>
<td>0.20</td>
<td>0.32</td>
<td></td>
<td>Camerino et al. (1985)</td>
</tr>
<tr>
<td>(6) OPI</td>
<td>20422 (G → A)</td>
<td>0.33</td>
<td>0.44</td>
<td></td>
<td>McGraw et al. (1985)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Winship &amp; Brownlee (1986)</td>
</tr>
</tbody>
</table>

Carrying the defective gene, assuming they have inherited this from their mother. Traditional methods of carrier detection in such girls rely on the measurement of the concentration of factor IX in plasma which, on average, is half that in normal individuals. Unfortunately, the value of such measurements is limited because of the wide spread of actual values in both normal individuals and in carriers. Therefore, on average, methods based on this type of test are only 80–85% reliable. Gene probes offer the possibility of carrier detection by a DNA linkage analysis using naturally occurring DNA polymorphisms within or very close to the factor IX gene. They depend on a knowledge of the existence of suitable polymorphisms of the factor IX locus in the population as a whole, on the mother of the potential carrier being heterozygous and there being one affected person, preferably a sibling, to establish the genetic 'phase' (i.e. to deduce which of the polymorphic forms is linked to the disorder). Six polymorphisms are now known in the factor IX gene (see Table 3), five detected as restriction fragment length polymorphisms (RFLPs) and one by use of a pair of oligonucleotide probes. Because of linkage disequilibrium between five of the polymorphisms used, the 'catch' (i.e. the percentage of the total number of families where the mother is heterozygous, a necessary condition for a linkage diagnosis) is currently limited to 80% in Caucasian populations. This 'catch' is still too low and we require three or four more polymorphisms of the type already found, or the detection of a new highly variable polymorphic region near the factor IX gene, [such as the one that occurs (Weatherall, 1985) near the α-globin or insulin genes] in order to extend the total 'catch' to 95% or more, so that the majority of affected families seeking advice can be helped.

Fig. 6 illustrates an example of such an RFLP linkage analysis of a potential carrier taken from Winship et al. (1984). The person under investigation, III1, was shown to have the genotype a1a1 for the XmnI RFLP. Given the genotypes of her mother (a1a2), father (a2) and affected brother (a1), we concluded she must be a carrier. If she had been a12, we would have excluded her as a carrier. The chances of error in this indirect method are related to the
chance of recombination, occurring in the female oocyte at meiosis, between the actual disease mutation (somewhere in the factor IX gene) and the RFLP (also located and at a known position, nucleotide 7076), within the factor IX gene. Recombination probably occurs non-randomly in chromosomes and we do not know whether this is more or less frequent than average near the factor IX locus. For the purposes of argument, if we assume there is a possibility of three cross-overs per X-chromosome and that the X-chromosome is 200 \times 10^6 base pairs long, knowing that the maximum distance between the polymorphism and gene defect cannot be more than approx. 30 \times 10^6 base pairs, there will be an 1\% chance of the linkage being broken in the ratio of 30 \times 3 = 200 \times 10^6. This works out as 1 in 2 \times 10^3 times or as a 0.05\% chance of error. For such reasons, we concluded that carrier detection using a linkage analysis with intragenic RFLPs is probably at least 99.9\% accurate for factor IX (Winship et al., 1984). In the clinical situation where carriers and families will be counselled, we must remember that this is a probability estimate, and there might be a chance as more families are counselled that cases will arise in which linkage is broken and therefore a wrong diagnosis is made.

Antenatal diagnosis can be carried out on DNA isolated from the foetal chorionic villi in a known haemophilia B family using exactly the same methodology as for carriers. This allows the possibility of an early termination if the results indicate a need for this and if this is wished by the family. Alternatively, the diagnosis can also be used to reassure parents that a foetus is normal if the mother is known to be a carrier. So far there has been, to my knowledge, only a small demand for carrier and antenatal diagnosis in haemophilia B, but I suspect it is because it is not widely realized how fast scientific progress has been on this topic in the last 3 or 4 years. The much improved accuracy of the methodology, and the fact that almost 80\% of people can be helped at present, should lead to an increased demand.

**Molecular defects in haemophilia B**

Evidence of heterogeneity exists in haemophilia B as there is variation in the clinical severity of the disease in different families. There is also a corresponding variation in the laboratory tests of clotting activity and in the antigen concentration measured in samples of blood taken from different patients. We therefore expect a wide range of molecular defects and indeed a precedent for this exists in the variety of mutations already known in the haemoglobin disorders (Weatherall et al., 1984). Haemophilia patients may be conveniently subdivided into those in whom the protein is present as detected by immunological methods, referred to as antigen positive, and those in whom it is absent, referred to as antigen negative. The former might be expected to have point mutations in those regions of the gene coding for the factor IX protein and studies of such patients should pinpoint critical functional regions of the protein. The antigen-negative patients are likely to have mutations involving deletions of reasonably large regions of the factor IX gene, or having critical point mutations involved in the control of the biosynthesis of the messenger RNA or of the protein. A small subgroup of patients, of the antigen-negative type, are referred to as ‘inhibitors’ because these patients, in response to injection of factor IX protein in the course of therapy, produce specific anti-factor IX antibodies.

Only a few patients have been characterized fully at the molecular level by recombinant DNA methods (see Table 4), although I expect the results from many more to be available within the next few years as a number of studies are in progress in different laboratories. I include one factor IX antigen-positive patient, factor IX\textsubscript{ChapeliH}, whose defect had been previously characterized by a study of the abnormal protein.

One of the more interesting antigen-positive patients to be studied is the patient haemophilia B\textsubscript{Oxford1}, in which Bentley et al. (1986) found the causative defect to be a point mutation in the propeptide region. This patient was originally picked up as having an apparently longer factor IX protein from a study of a Western blot. By preparing phage lambda clones covering the entire factor IX gene from this patient, and by sequencing all the exons, Bentley et al. (1986) found a point mutation (Fig. 7) where there is a G → A change at residue 6365 resulting in the R(Arg) → Q(Gln) change. That this was the true mutation and not a cloning artefact was confirmed from the results of direct amino acid sequencing on protein isolated from the patient’s plasma. This showed that the abnormal factor IX protein was longer than normal by an 18 amino acid extension at its N-terminus. This suggests that in the normal maturation of factor IX the signal peptide cleaves the peptide bond between amino acid residues 18 and 19 before there is a second processing of the factor IX precursor protein by an unknown enzyme that cuts between arginine at 1 and tyrosine at +1. This latter step is blocked virtue of the R → Q change at –4 in the patient under investigation.

The result on haemophilia B\textsubscript{Oxford1} prompted a comparison of factor IX with the other vitamin K-dependent clotting factors and related proteins, and it has emerged there is considerable homology in their propeptide region shown in Fig. 8. From the proposed signal peptide cleavage site in human factor IX and this comparison, and a knowledge that signal peptides display elastase-like specificity, we have proposed the cleavage sites as shown in Fig. 8 for factor X, prothrombin and protein C. The reason

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**Table 4. Characterized molecular defects in haemophilia B**

<table>
<thead>
<tr>
<th>Group</th>
<th>Patient</th>
<th>Defect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen positive</td>
<td>Factor IX\textsubscript{ChapeliH}</td>
<td>Asp → Gly(47)*</td>
<td>Davis et al. (1984)</td>
</tr>
<tr>
<td></td>
<td>Factor IX\textsubscript{Oxford1}</td>
<td>Arg → His(145)*</td>
<td>Noyes et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>Haemophilia B\textsubscript{Oxford1}</td>
<td>Arg → Glu(−4)*</td>
<td>Bentley et al. (1986)</td>
</tr>
<tr>
<td>Antigen negative</td>
<td>Haemophilia B\textsubscript{Oxford1}</td>
<td>GT → TT, donor splice junction of exon 1</td>
<td>Rees et al. (1985)</td>
</tr>
<tr>
<td></td>
<td>Haemophilia B\textsubscript{Oxford2}</td>
<td>GT → GG, donor splice junction of exon c</td>
<td>P. R. Winship (unpublished work)</td>
</tr>
<tr>
<td>‘Inhibitors’ (a subgroup of antigen negative)</td>
<td>Four patients from independent pedigrees in U.K.</td>
<td>Partial and complete gene deletions; exact length of deletion not known</td>
<td>Giannelli et al. (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peake et al. (1984)</td>
</tr>
</tbody>
</table>

*The numbers in parentheses refer to the amino acid residues of the factor IX protein.*
why haemophilia B\textsubscript{Oxford} is inactive in the clotting cascade may be that it fails to bind factor X\textsubscript{a}, as preliminary results show that activation of the purified abnormal factor IX by factor X\textsubscript{a} \textit{in vitro} may be impaired (A. K. Bentley, D. J. G. Rees & G. G. Brownlee, unpublished work). We might expect a study of other antigen-positive patients to illustrate the importance of particular amino acid residues in factor IX, but nevertheless our ability to interpret these results is limited by a lack of knowledge of the three-dimensional structure of this molecule. It should also be said that the methods for finding mutations in patients are still somewhat laborious and there is a need to use improved methods, perhaps that suggested by Myers \textit{et al.} (1985), to aid progress in this field.

**Gene therapy or cure**

The ultimate cure for patients with inherited genetic defects would involve replacing their bad or inactive gene with a normal active gene giving a permanent supply of factor IX endogenously. As factor IX is not needed at 100\% of normal level, it is a favourable test model system as 10\% would suffice for a haemophiliac except when undergoing major surgery. We can envisage a methodology based on removing some tissue from a patient, dispersing the cells \textit{in vitro}, introducing recombinant clones efficiently, perhaps by the use of retroviruses, and then re-introducing factor IX producing cells back into the patient. The main problem in connection with gene therapy is that it is an untried procedure as compared with the present symptomatic treatments. This means that there is a need for animal experiments to test procedures and safety before any gene therapy is contemplated in humans (for a fuller review see Weatherall, 1985).

**Summary**

Haemophilia is a rare inherited disease of blood clotting known since biblical times. The rarer form (haemophilia B) occurs in about 1 in 30,000 males and there are about 900 patients in the U.K. at present. Biochemically, patients either lack or have a defective protein (called factor IX) which is needed for the clotting of blood in response to injury. Only males get the disease. However, females can carry the trait in a latent form and transmit the disease to their offspring. Untreated, the disease leads to internal bleeding.

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**Fig. 7. Nucleotide and amino acid sequence of the propeptide region of normal factor IX to show the point mutation in haemophilia B\textsubscript{Oxford}**

The base change of G to A in the patient's DNA at residue 6365 is marked by an arrow and changes an arginine (R) to glutamine (Q) at amino acid -4. The Ha\textsubscript{III} site in the normal factor IX is indicated by an overbar. From Bentley \textit{et al.} (1986).

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**Fig. 8. Comparison of the amino acid sequence of the propeptide region of factor IX with other sequenced vitamin K-dependent factors**

Identical amino acids are boxed. The point of signal peptidase cleavage for factor IX determined by protein sequencing is marked by an arrow. The potential sites of signal peptidase cleavage for factor X, prothrombin and protein C are also indicated. From Bentley \textit{et al.} (1986).
bleeding into muscles and joints and is life-threatening. In the U.K. and in countries with effective health care programmes, patients are treated by periodic injection of factor IX concentrate, a drug isolated from the pooled plasma derived from many blood donors. This drug replaces their own absent or defective factor IX and allow them to enjoy a relatively normal lifestyle.

I have reviewed recent studies on the molecular genetics of haemophilia B which started with the isolation of the gene coding the factor IX protein from normal individuals in 1984. Following this, it has been possible firstly to produce factor IX artificially in the laboratory from cloned copies of the messenger RNA of the factor IX gene. Secondly, it has been possible to improve the diagnosis of ‘carriers’. Carrier females often wish to know whether they are carriers or not before they have children. If they are positively identified as carriers, the risk and implications of having a haemophilic son can be discussed and therapeutic abortion considered. Thirdly, the exact molecular defect in patients can be studied. It has been known for some time that the clinical symptoms and biochemical defects vary in different patients. The exact molecular defect is now known in five cases, and many more are being studied.

There is a need for establishing diagnostic methods in the clinical environment so that better carrier diagnosis is available. The new methods are not as widespread as they should be and not all haemophilia B families are benefitting from the new methods. We also need a better factor IX drug produced from a non-blood source. Although other viral products may contaminate the existing drug, other viral products may contaminate the existing drug. Further research and development is necessary to improve the yield of genetically engineered factor IX for this to become a realistic option.

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