Gene deletion in an Italian haemophilia B subject

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SUMMARY DNA from 20 Italian haemophilia B patients was analysed by the Southern blotting technique and hybridisation to a factor IX cDNA probe. A large deletion of factor IX gene was detected in one patient with antibodies to the infused factor; the EcoRI pattern of the other 19 subjects examined was normal.

Haemophilia B is an inherited X linked disease¹ caused by a heterogeneous² functional deficiency of factor IX, a glycoprotein involved in the intrinsic pathway of blood coagulation.³ The recent cloning of DNA fragments coding for human factor IX⁴⁻⁸ has allowed the identification of a genetic lesion in haemophilia B^{9,10} and the detection of an intragenic restriction enzyme polymorphism potentially useful in informative families for antenatal diagnosis.^{10,11}

We studied the DNA isolated from 20 Italian males carrying haemophilia B using a cDNA probe for factor IX.⁸ A large gene deletion was detected in one subject, while the DNA patterns of the other haemophilia B patients were indistinguishable from normal.

Subjects and methods

Blood samples from 20 Italian haemophilia B patients were obtained by standard procedures. DNA preparation and Southern blotting technique were as previously described.¹² DNA was digested with the appropriate restriction enzymes under the conditions indicated by the supplier (Boehringer, Mannheim).

Thyroglobulin genomic probe was a generous gift from Dr E Avvedimento.

Hybridisation was performed in accordance with the Gene Screen transfer membrane method (New England Nuclear, Boston). Excess probe was washed off by incubation with 0.06 mol/l Tris-HCl (pH 8), 0.3 mol/l NaCl, 0.002 mol/l EDTA, and 1% SDS at 60°C for one hour, followed by incubation in 0.03 mol/l NaCl, 0.006 mol/l Tris (pH 8), and 0.0002 mol/l EDTA for one hour at room temperature.

Autoradiographs were developed after three to six days of exposure.

Results

The DNA from the 20 haemophilia B subjects under study were digested with EcoRI restriction enzyme and after Southern blotting hybridised to a factor IX cDNA probe (for which we thank Dr G G Brownlee).⁷ Fig 1 shows the autoradiographic frag-

FIG 1 Southern blots obtained from the DNA of three haemophilia B patients and one normal control. DNA was digested with EcoRI restriction enzyme and hybridised to factor IX cDNA probe. 1, 2, 3: haemophilia B patients; N: normal control.

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FIG 2 Southern blots obtained from the DNA of case 1 of the previous figure and of a normal control. DNA was digested with *Bam*HI (B), *Eco*RI (E), *Hind*III (H), *Taq*I (T), and *Xba*I (X) restriction enzymes and hybridised to factor IX cDNA probe (a) and to a thyroglobulin probe (b).

ments obtained in three haemophilia B subjects and one normal subject. The patterns of cases 2 and 3 are indistinguishable from normal.

Similar results were obtained in the other 17 haemophilia B subjects investigated. However, case 1 did not show any hybridisation with the radioactive probe used.

The DNA of case 1 was analysed further using *Xba*I, *Taq*I, *Hind*III, and *Bam*HI restriction enzymes and the same cDNA probe. The results obtained (fig 2a) confirm that the DNA of this patient fails to hybridise with factor IX probe.

In order to assess the presence of hybridisable DNA in the Southern blots of case 1, the *Eco*RI digest was additionally tested with a thyroglobulin gene probe (fig 2b). Like the normal control, patient 1 showed the thyroglobulin specific 2 kb band. The DNA of case 1 also gave normal results when hybridised with a γ globin gene probe (data not presented).

Discussion

The DNA of an Italian subject carrying haemophilia B, digested with several restriction enzymes, did not show hybridisation fragments when tested with a factor IX cDNA probe. As the cDNA used hybridises to all factor IX exons, the findings obtained indicate that the DNA coding regions were deleted and, since the gene for factor IX is 34 kb in length,⁸

this is the smallest extent of the deletion, provided that more complex rearrangements are not present.

Large and heterogeneous structural changes in the factor IX gene have already been described by Giannelli *et al*⁹ and Peake *et al*¹⁰ in the United Kingdom. Neither in the British patients so far described nor in this Italian patient are the end points of the DNA lesion known. DNA sequences 3' and 5' to the factor IX gene will be needed to determine the precise extent of the lesion, which, as in thalassaemia,¹³ could be very extensive and generated through a similar molecular mechanism.^{13 14}

Taken together, previous published reports and the present data indicate that the majority of the gene defects causing haemophilia B are due to small deletions or point mutations, impairing gene expression or the structure-function relationship of the molecule, as in haemophilia B Chapel Hill.¹⁵

As in the British patients, our patient has antibodies to the factor IX concentrate administered for therapeutic purposes. The other 19 haemophilia B patients considered, whose hybridisation pattern with factor IX cDNA probe was normal, do not produce antibodies to the infused factor IX. These data support the hypothesis of Giannelli *et al*⁹ that patients with a gross gene defect, leading to the absence of immunologically recognisable factor IX, develop antibodies to the factor, which make its infusion inefficient.

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