1250

A POINT MUTATION AND A GENE DELETION OF FVIII GENE IN SEVERE HAEMOPHILIA A

GF FVIII GENE IN SEVERE HAEMOPHILIA A F.Bernardi(1), V.Bertagnolo(1), S.Bartolai(2), L.Rossi(2), F.Panicucci(2) and F.Conconi(1). Centro di Studi Biochimici sul Morbo di Cooley Università di Ferrara(1), Centro per lo Studio e la Terapia dell' Emofilia e delle Malattie Emorragiche Università di Pisa(2), ITALY

The presence of Factor VIII (FVIII) gene lesions has been investigated in 100 haemophilia A patients using cDNA (exons 14-26) probes for the 3'part of FVIII gene

In two related severe patients without inhibitor deletion removes the exon 26; the gene lesion has been confirmed with several restriction enzymes and been confirmed with several restriction enzymes and has been shown by densitometry of the autoradiogra-phic pattern in a woman of the same family. The complete deletion of the exon 26 has been described by Gitschier et al. in a patient with inhibitor. Thus the comparison of the end points of the two deletions could help to define the mechanism originating these gene lesions and the relation between gene lesions and the presence of antibody. In a patient with severe Haemophilia and without iphibitor a mutation removing the TagI site in the In a patient with severe Haemophilia and without inhibitor a mutation removing the TagI site in the exon 24 and originating an abnormal band of 4.2 Kb has been found. A C->T transition in this TagI site, originating a nonsense codon and a new HindIII site, has been reported by Gitschier et all interport site, originating a nonsense codon and a new Hindii site, has been reported by Gitschier et al in a patient presenting inhibitor. The DNA from our patient tested with HindIII shows a normal pattern thus indicating a C->T transition in the antisense strand. This mutation should cause an aminoacid strand. This mutation should cause an aminoacid change (CGA->CAA, Arg->Gln) possibly responsible for the FVIII inactivation but that does not remove the antigenic determinants present in the COOH terminal

part of FVIII. In addition the same mutation has been observed in an unrelated (as demonstrated by RFLPs analysis) Italian haemophilic patient confirming the observation of Youssoufian et al that TaqI sites are mutational hot spots in FVIII gene.

DNA POLYMORPHISMS IN OR LINKED TO THE FACTOR VIII GENE IN CHINESE. <u>Vivian Chan, V.W.S. Liu, A.C.K. Wong and</u> T.K. Chan. Department of Medicine, University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong.

78 unrelated X chromosomes from Southern Chinese 78 unrelated X chromosomes from Southern Chinese (56 normal and 22 haemophiliac) were studied. DNA was restricted by Bcl I, Bgl I or Taq I and hybridized to 3' factor VIII:C cDNA probe (5 kb, Chiron) or St 14.1 probe (3 kb, Oberle & Mandel) by standard techniques. The intragenic Bcl I polymorphic site was positive in 82%, while Bgl I polymorphic site was positive in all. Thus, 29.5% (2 x 0.82 x 0.18) of Chinese females carried the Bcl I polymorphism. As to the Taq I polymorphism in the closely linked DXS52 DNA segment carried the Bcl I polymorphism. As to the Taq I polymorphism in the closely linked DXS52 DNA segment, the incidences for the various alleles were : System I — allele (3) 10.2%, (4) 2.6%, (5) 2.6%, (6) 17.9%, (7) 21.8% and (8) 44.9%; System II —  $\alpha$  allele 56%,  $\beta$  allele 44%. Approximately 80% of females were heterozygous for two different alleles. Hence the Bcl I and Taq I polymorphisms can be used to track the defective factor VIII gene for carrier detection and prenatal diagnosis. Furthermore, their frequencies in the Chinese are different from those previously reported in other ethnic groups. previously reported in other ethnic groups.

FVIIT GENE LESIONS DETECTED TVO IN SEVERE AND MODERATE HAEMOPHILIA A V.Bertagnolo(1), S.Volinia(1), C.Legnani(2), <u>G.Rodorigo(2), V.De Rosa(2)</u> and F.Bernardi(2). Istituto di Chimica Biologica Università di Ferrara(1), Servizio di Angiologia Ospedale S.Orsola Bologna (2), Italy.

DNAs from 15 haemophilia A patients different families have been hybridized to from FVIII cDNA probes for the exons 14-26.

In a severely affected patient (FVIII:C 2%) the TaqI site of exon 24 is absent originating an abnormal band of 4.2 Kb. A C to T transition in the CG dinucleotide of the TaqI site (TCGA) is the probable gene mutation. Since the transition in the sense strand should originate an additional Hind III sense strand should originate an additional Hind III site, which is not detected in our patient, we infer that the mutation occurred in the antisense strand causing an aminoacid change (CGA  $\rightarrow$  CAA, Arg  $\rightarrow$  Gln). This is in accordance with the low activity of FVIII and with the absence of inhibitor. Infact Gitschier and with the absence of inhibitor. Infact distinct et al reported in a patient with a high titre of anti-FVIII antibody and with  $\langle 18$  FVIII activity a C -> T transition in the coding strand, originating a nonsense codon in the TaqI site of exon 24.

In the HindIII pattern from a moderately affected patient (FVII:C 4%) the fragment containing the exon 18 is 2.5 Kb in size (normal 2.6 Kb). Since the patterns with other restriction enzymes are indistinguishable from normal a small mutation originating a new Hind III site is likely. Both altered patterns have been detected in the patients' mothers.

Work supported by Ricerca Sanitaria Finalizzata Regione Emilia Romagna.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

EFFECT OF DIFFERENT FVIII/vWF-CONTAINING THERAPEUTIC PRODUCTS ON PLATELET ADHESION TO COLLAGEN. C. De Romeuf, C. Mazurier and M. <u>Goudemand</u>. Laboratoire de Recherche sur l'I Régional de Transfusion Sanguine, Lille, France l'Hémostase. Centre

In an attempt to predict by in vitro study the possible efficiency of various therapeutic products rich in  $\rm FVIII/vWF$  in the treatment of patients with von Willebrand's disease, we have compared their ability to support platelet adhesion to human non fibrillar collagen type I, III.

Platelet adhesion was studied in steady flow conditions at the wall shear rate of 1200 sec.<sup>-1</sup>, using a flat perfusion chamber containing collagen-coated coverslips.<sup>\*</sup>

Perfusates were samples of reconstituted blood made up from washed red cells to an hematocrit of 40 %, washed aspirin-treated and  $^{111}\text{In-oxine}$  labelled platelets to 2.10 $^{11}\text{/}1$  and a vWF-deficient plasma containing various amounts (0.25 to 2 U vWF:Ag/ml of plasma) of therapeutic product. The extent of platelet adhesion was evaluated by radioactivity counting and expressed in percentage (100 % representing the value obtained with normal plasma, 0 % being the value obtained with vWF-deficient plasma).

Cryoprecipitate enabled us to obtain 50 % platelet adhesion at a concentration lower than 0.5 U vWF:Ag/ml. Two virus-inactivated products derived from this cryoprecipitate were compared : The heated FVIII/vWF concentrate supported 50 %platelet adhesion at a concentration higher than 1.5 U vWF:Ag/ml whereas solvent-detergent treated concentrate failed to achieve 50 % adhesion at a 2 U vWF:Ag/ml. These results which may vary from one batch to another are correlated on the whole with the vWF multimeric composition.

In conclusion, provided that sufficient doses of vWF:Ag are infused, some virus-inactivated FVIII concentrates containing high molecular weight forms of vWF should be able to correct the bleeding time of patients with von Willebrand's disease.

\* Sakariassen K.S. et al., J. Lab. Clin. Med., 1983, 102, 522.