

A DELETION INVOLVING INTRON 13 AND EXON 14 OF FACTOR VIII GENE IN A HAEMOPHILIAC WITH ANTI-FACTOR VIII ANTIBODY

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Summary A deletion mutation in the factor VIII gene of a severe haemophiliac patient was found along with a high level of factor VIII inhibitor in the blood plasma among seventy Japanese haemophilia A patients. The 6 kbp long deletion involved a region from somewhere between *Pst*I and *Sst*I sites at nucleotide positions 2659 and 2991 of exon 14 and intron 13, respectively (nucleotide positions were defined as in Wood *et al.*, 1984).

Key Words factor VIII gene, deletion, haemophiliac, inhibitor

INTRODUCTION

Haemophilia A (classical haemophilia) is one of the most abundant inherited bleeding disorders caused by genetic deficiency of the coagulation protein, factor VIII. The clinical severity in patients with haemophilia varies mainly due to different levels of factor VIII activity among different families. Moreover, about 10% of patients with severe haemophilia have high levels of antibodies against factor VIII, so-called factor VIII inhibitor. The genetic heterogeneity of haemophilia patients suggests that many different molecular alterations are involved in the resultant malfunctional factor VIII gene in haemophiliacs. Several point and deletion mutations in the factor VIII gene were observed in Caucasian population (Gitschier *et al.*, 1985, 1986; Antonarakis *et al.*, 1985; Youssoufian *et al.*, 1986, 1987a, 1987b; Higuchi *et al.*, 1987; Bernardi *et al.*, 1987; Matthews *et al.*, 1987).

We report of an example of deletion extending from intron 13 to exon 14 of factor VIII gene in a Japanese patient with haemophilia A and high level of inhibitor.

CASE REPORT

The patient (24 years of age, male) showed a severe haemophilia A, having less than 1% of factor VIII coagulant activity assayed by one stage method (Hardisty and Macpherson, 1962) and less than 0.1% of factor VIII coagulant antigen assayed by IRMA (Peake *et al.*, 1979). His typical bleeding episodes were the repeated intracranial bleedings. At the age of 17 years, inhibitor against factor VIII developed, and the maximal titers were 74 Bethesda units/ml. He has no siblings but a normal son. His mother was diagnosed previously to be a carrier (30% factor VIII coagulant activity).

METHODS

Chromosomal DNA was isolated from leukocytes and digested with *TaqI*, *EcoRI*, *BamHI*, *KpnI* and *SstI* restriction endonucleases. Gel electrophoresis, radioisotope labeling and Southern blotting were performed as previously described (Maniatis *et al.*, 1982). DNA probes used for Southern analysis are shown in Fig. 1. Briefly, (1) probe A, 1.7 kbp long, delineates exons 1–12, (2) probe B, 4.7 kbp long, delineates exons 14–26, (3) probe C, 1.8 kbp, contains the remaining portion of exon 26, (4) probe D, 0.37 kbp, contains the portion of exon 14 between *EcoRI* and *PstI* sites at positions 2290 and 2658, respectively, (5) probe E, 2.5 kbp, contains another portion of exon 14 between two *PstI* sites at positions 2659 and 5163. The probes A, B and C are contained in pSP64, Ap^R (Antonarakis *et al.*, 1985), kindly provided by Dr. Lewis, Genetics Institute, whilst probes D and E are contained in pHSG398, Cm^R (Takeshita *et al.*, 1987).

RESULTS

Autoradiographs of Southern blots of normal and patient genomic DNA samples are shown in Fig. 2. The DNA samples digested with *TaqI* were hybridized to probe A, and those digested with *TaqI*, *EcoRI*, *BamHI* or *KpnI*, were hybridized to probes B and C. Probe A detected several fragments of 8.0 kbp (exons 3–4), 4.3 kbp (exon 7), 2.4 kbp (exon 11), 2.2 kbp (exon 1), 1.8 kbp (exons 7–9) and 1.4 kbp (exon 2) in length. The size of these fragments were all normal (data not shown).

The use of probes B and C revealed fragments of different size in patient DNA when digested with *TaqI*, *EcoRI*, *BamHI* and *KpnI*. In normal subject, *TaqI* digestion showed 9.2 kbp (exon 26), 5.9 kbp (exon 14 and exons 20–22), 2.8 kbp (exons 15–18, exons 24–25 and exon 26), 2.4 kbp (exon 23), 2.2 kbp (exons 18–19) and 1.4 kbp (exons 23–24) fragments (Gitschier *et al.*, 1985). In patient, the major 5.9 kbp fragment that corresponds to exon 14, was replaced by a smaller fragment of 4.9 kbp. In *EcoRI* digestion of patient DNA, the major fragment of 4.8 kbp (exon 14)

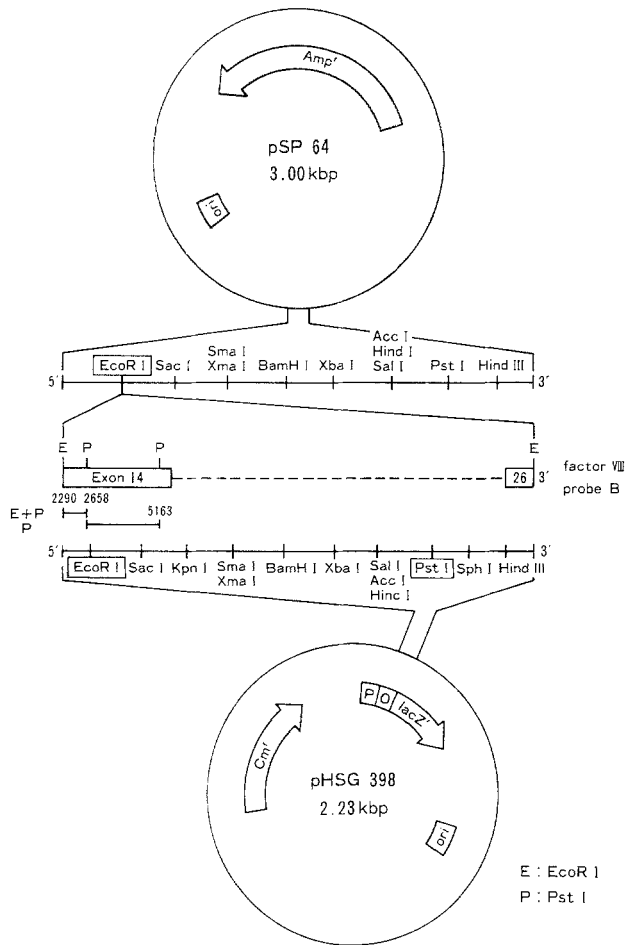


Fig. 1. Probes D and E. Probe B inserted in pSP64 was restricted with either *EcoRI* + *PstI* or *PstI*. Subfragments inserted into pHSG398, were cultured and screened. Probes D and E were obtained by gel electrophoresis.

was undetectable. *BamHI* digestion showed a new fragment and a normal-sized 0.6 kbp fragment of exon 14. *KpnI* digestion also showed a new fragment.

Probe D detected no band in patient DNA by *BamHI* digestion, and probe E detected a new fragment of 5.9 kbp by *EcoRI* digestion in addition to the 3.2 kbp fragment by *SstI* digestion (Fig. 3). Disappearance of the 4.8 kbp major fragment of exon 14, when digested with *EcoRI* and hybridized with probes B and C, was caused by an overup of the 5.9 kbp abnormal fragment derived from exon 14 and 5.9 kbp fragment from exon 26.

From these results, patient factor VIII gene has a partial deletion of approximately 6 kbp to 5' side from a site between *PstI* site (2659) and *SstI* site (2991) in

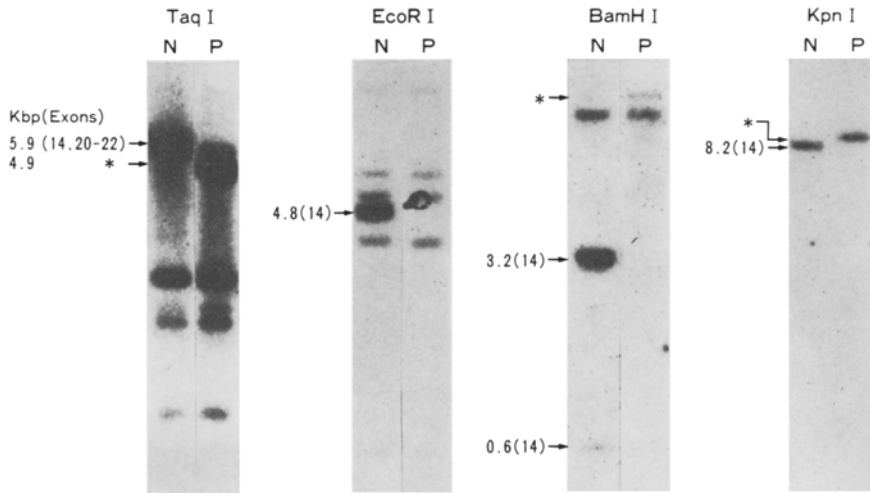


Fig. 2. Southern blots of normal (N) and patient (P) DNAs detected by factor VIII cDNA probes B+C. Asterisks show abnormal fragments in patients DNA.

exon 14, according to the restriction map of factor VIII gene (Gitschier *et al.*, 1984; Wood *et al.*, 1984).

DISCUSSION

It was clear by the use of cDNA probes for factor VIII DNA that a severe Japanese haemophilia A patient with high titers of inhibitor has a intragenic deletion of factor VIII gene. From Southern blot analysis and the restriction map of factor VIII gene (Gitschier *et al.*, 1984, 1985; Wood *et al.*, 1984), gene deletion in the patient DNA includes a part of exon 14, accounting for approximately 6 kbp in length.

So far, more than 10 cases of gene deletion have been reported in Caucasian haemophilia A patients (Table 1). As far as the gene deletion of exon 14 is concerned, a small deletion of 2.5 kbp or 2.0–2.5 kbp was not involved in the occurrence of an antibody (inhibitor) against factor VIII (Youssoufian *et al.*, 1987a; Higuchi *et al.*, 1987). However, a large deletion of 12–14 kbp containing exon 14 caused inhibitor (Higuchi *et al.*, 1987). On the other hand, it was reported that several nonsense mutations in exons of factor VIII gene resulted in the development of inhibitor (Gitschier *et al.*, 1985; Higuchi *et al.*, 1987; Matthews *et al.*, 1987; Mikami *et al.*, 1988). Therefore, it will be important to characterize the association between gene defects and epitope specificity of inhibitor against factor VIII.

Factor VIII gene has 186 kbp in length including 26 exons and exon 14 encodes a large part of factor VIII amino acid sequence, called B domain (Gitschier *et al.*, 1984), including thrombin cleavage sites in both ends. This domain connects A₂ and A₃ domains that play important roles for factor VIII coagulant activity (Vehar

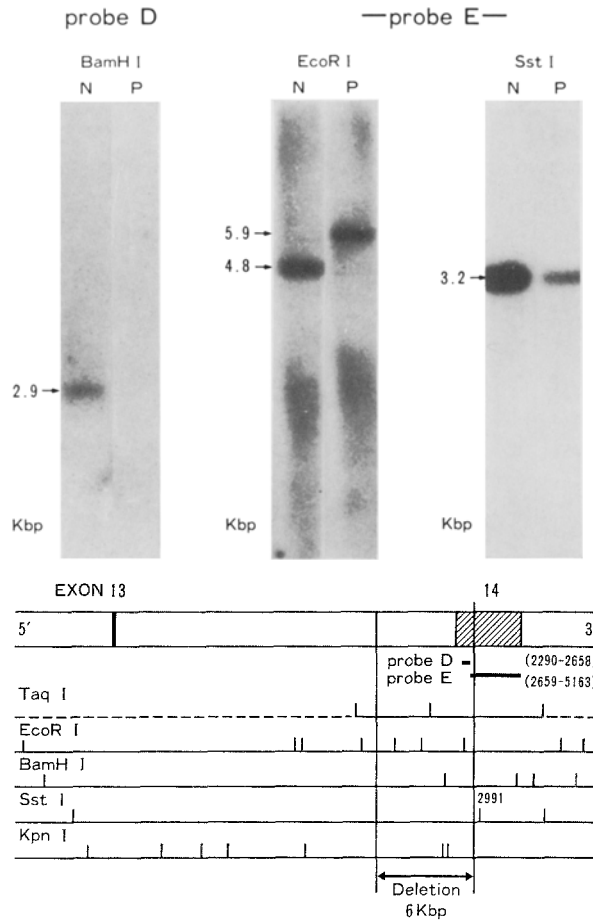


Fig. 3. Southern blots by exon 14 probes (probes D and E) and a local restriction map of factor VIII gene.

et al., 1984). Toole *et al.* (1986) demonstrated that factor VIII cDNAs deficient in B domain polypeptide, when inserted into mammalian cell expression vector, directed the synthesis of biologically active factor VIII. The patient, whose DNA had deletion in the region of exon 14, shows neither factor VIII coagulant activity nor autologous antigen specificity. In summary, we examined 64 Japanese haemophilia A patients and 6 cases with inhibitor. Among these, one case of gene deletion was detected.

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Table 1. Gene deletion of factor VIII in haemophiliac patients.
All cases except for a mild form (*) reported are severe form.

	Exon	Deletion	Inhibitor
Gitschier <i>et al.</i> (1985)	23-25	39.0 kb	(+)
Gitschier <i>et al.</i> (1985)	26	21.9 kb	(-)
Antonarakis <i>et al.</i> (1985)	11-22	80 kb	(+)
Youssoufian <i>et al.</i> (1987)	6	7 kb	(-)
Youssoufian <i>et al.</i> (1987)	14	2.5 kb	(-)
Youssoufian <i>et al.</i> (1987)	24-25	7 kb	(-)
Youssoufian <i>et al.</i> (1987)	23-25	16 kb	(-)
Youssoufian <i>et al.</i> (1987)	22*	5.5 kb	(-)
Higuchi <i>et al.</i> (1987)	1-5	>35 kb	(-)
Higuchi <i>et al.</i> (1987)	3	1.2-1.7 kb	(-)
Higuchi <i>et al.</i> (1987)	7-9	15-20 kb	(+)
Higuchi <i>et al.</i> (1987)	14	2.0-2.5 kb	(-)
Higuchi <i>et al.</i> (1987)	14	12-14 kb	(+)
Higuchi <i>et al.</i> (1987)	26	≥1.8 kb	(-)
Bernardi <i>et al.</i> (1987)	26		(-)
Bernardi <i>et al.</i> (1987)	26		(-)
Casarino <i>et al.</i> (1986)	1-26	>210 kb	(-)
Din <i>et al.</i> (1986)	23-26		(+)
Camerino <i>et al.</i> (1986)	15-18	15 kb	(+)
Youssoufian <i>et al.</i> (1987)	26	2 kb	(-)

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