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Molecular Analysis of Hemophilia A Mutations in the Finnish Population

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Summary

We have examined the Finnish hemophilia A population for factor VIII gene mutations. This study included 83 unrelated patients and revealed 10 mutations associated with hemophilia. Using cloned cDNA, genomic, and oligonucleotide probes, we have identified three classes of mutations: five mutations causing the loss of *TaqI* restriction sites, a point mutation resulting in a new *TaqI* site, and four partial gene deletions. Although exons 5 and 6 were involved in three of the four partial gene deletions, the extent of the DNA lost differs in each case. The fourth deletion was located entirely within intron 1 and segregated with the disease in a large hemophilia pedigree. There was no history of hemophilia in eight of the 10 families. The origin of the mutation was determined in six of these pedigrees, two of which showed evidence for maternal mosaicism.

Introduction

Hemophilia A is an X-linked coagulation disorder caused by the lack of functional factor VIII protein. The factor VIII gene spans 187 kb and contains 26 exons, although intron sequences account for 95% of the gene (Gitschier et al. 1984). The large number of de novo cases and the clinical heterogeneity of the disease suggest that a wide spectrum of molecular defects may result in hemophilia. Recent molecular analysis of the factor VIII gene of patients with hemophilia from genetically diverse populations has substantiated this hypothesis (Antonarakis et al. 1985; Gitschier et al. 1985; Youssoufian et al. 1987). These studies have primarily detected partial gene deletions and point mutations involving DNA sequences recognized by the restriction enzyme *TaqI*. We report an analysis by Southern blotting of all the available Finnish hemophilia A patients and their relevant family members. An object of this investigation was to determine whether a relatively isolated population would present a diverse collection of mutations or show some common molecular events. We observed a variety of different genetic defects, a result which supports the theory that new mutations have a substantial impact on the incidence of hemophilia A (Haldane 1935). The availability of cooperative multigeneration families for analysis allowed us to determine the origins of some mutations, including two examples of maternal mosaicism.

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Methods

Southern Blot Analysis of DNA

Genomic DNA was extracted from peripheral blood, and Southern blotting was performed as described elsewhere (Kunkel et al. 1977; Page and de la Chapelle 1984).

Segments of factor VIII cDNA were cloned into a set of Gemini vectors (Promega), and fragments were isolated from these plasmids. The two pools of fragments used for initial screening of Southern blots were termed probe 1 and probe 2. Probe 1 is composed of the following fragments: 450-bp *ClaI*/*Hin*dIII (exons 1–3), 1,258-bp *Hin*dIII (exons 8–14), and 3,750-bp *TaqI* (exons 14–18). Probe 2 consists of the following fragments: 650-bp *Hin*dIII (exons 4–7), 576-bp *DraI* (exons 18–23), 400-bp *DraI*/*Eco*RI (exons 24–25), and 1,950-

bp *Eco*RI/*Dra*I (exon 26). In certain cases blots were rescreened with the appropriate exon fragments, as described in the figure legends. DNA probes were labeled to high specific activity by the random-priming method (Feinberg and Vogelstein 1983). Southern blots were hybridized and washed as described elsewhere (Gitschier et al. 1985).

Polymerase Chain-Reaction

Various regions of the factor VIII gene were amplified from patient DNA with appropriate oligonucleotide primers (table 1) (Saiki et al. 1985; Kogan et al. 1987). Five hundred nanograms of DNA were amplified on a Perkin Elmer Cetus thermal cycler in a 50-μl reaction by using DNA polymerase from *Thermus aquaticus* (Perkin Elmer Cetus) under conditions specified by the manufacturer. Each amplification consisted of an initial 5-min, 94°C denaturation step followed by 30 cycles of 15-s, 94°C denaturation, 1-s, 60°C annealing, and extension at 72°C for varied times depending on the length of the region to be amplified.

Allele-specific Oligonucleotide Hybridization

Sets of 15-mer oligonuclotide probes representing the normal and two most likely sequences for the *Taq*I site mutations were synthesized (table 1). Fifty picomoles were end-labeled with ^{32}P-γATP. Twenty percent of the patients' amplified DNA was denatured in 0.4 M NaOH, 25 mM EDTA, heated for 2 min at 95°C, and dotted onto Hybond™ nylon filters (Amersham). Filters were then baked for 1 h, prehybridized 1–2 h at 37°C, hybridized 4–12 h under the same conditions with the appropriate labeled oligonucleotides, and washed with 3 M tetramethylammonium chloride at 50°C as described elsewhere (Wood et al. 1985; Levinson et al. 1987).

Direct Sequencing of Amplified DNA

DNA from patient 1308 was amplified using primers 13.1 and 13.9 (table 1) to yield a 255-bp fragment. The amplified DNA was purified on a 5% minipolyacrylamide gel; the specific fragment was electroeluted, extracted with phenol/chloroform, and ethanol precipitated. A sample of 0.3 pmol of the purified DNA was sequenced directly using T7 DNA polymerase (Sequenase®; U.S. Biochemical) as described elsewhere (Wong et al. 1987; Gitschier et al. 1988).

Coagulation Assays

Factor VIII coagulation activity was measured by the activated partial thromboplastin time method (Hardisty and MacPherson 1962). Factor VIII inhibitors were screened by the Bethesda method (Kasper et al. 1975).

Results

Screening for Mutations by Southern Blot Analysis

DNA samples from 83 unrelated Finnish patients with hemophilia A were digested with *Taq*I and *Sst*I and screened successively with factor VIII cDNA probes 1 and 2. This strategy results in unique bands for most of the 26 factor VIII exons, enhancing the detection of small changes in gene structure. In analyzing the Southern blot results, we made use of previously reported *Taq*I and *Sst*I restriction maps of the factor VIII gene (Gitschier et al. 1984, 1985).

Figure 1 shows a composite of the Southern blots revealing the 10 mutations detected by this screening.

Table 1

Oligonucleotide Sequences for Amplification Primers and Probes

	Primers	Probes
Exon 18	15.1 CAAGCTTTGGCTACATAATGGATACA 15.2 CTGGATAGAGATTGTACAGTG	AAGGATTCGATGGTA
Exon 23	13.1 GTCTTCTTTGGCAATGTGGATTC 13.5 CTACTTAAGTCACAGCCCATCAACTC 13.9 TTGTGTCCTGATACCGGGAACCCCTC	TATTGCTCGATACAT
Exon 24	16.1 GGTTGCAGCATGCCATTGGG 16.2 CTGAGGTCTCCAGGCATTAC	AAAGCTCGACTTCAC
Exon 26	6.5 GTTTTTCAGGGAAATCAAGACTC 6.6 GTGCTGCAGTGGCCACCCTCAG	CTACCTTCGAATTCA

NOTE.—Sequences for all primers and probes are 5′ to 3′ as shown. Some of the primer sequences incorporate restriction sites at the 5′ end. Shown are the normal sequences with the *Taq*I restriction site underlined. Nonsense probes substitute TTGA and missense probes substitute TCAA.

Nine of the 10 were observed by screening Southern blots of *Taq*I-digested DNA with probe 2. One mutation (in patient 1067) was found by using probe 1 on a Southern blot of DNA digested with *Sst*I. Five (in patients 785, 818, 1060, 1281, and 1286) appeared to be due to loss of *Taq*I sites in four sites already shown to be hot spots for mutation (Gitschier et al. 1985; Youssoufian et al. 1986). Partial gene deletions accounted for the defect in four other patients (1059, 1067, 2213, and 2253). Finally, in one patient (1308), a slight decrease in molecular weight of one *Taq*I fragment suggested a small deletion. As described below, the mutations in these 10 patients were further analyzed by hybridization of allele-specific oligonucleotides and exon-specific probes and, in one case, by sequencing amplified DNA.

Analysis of TaqI Site Mutations

Mutations were found in four *Taq*I sites. In each of these cases, a larger fragment appeared, reflecting the loss of a *Taq*I site (fig. 1*A*). In patients 785 and 818, loss of the *Taq*I site in exon 24 results in the absence of the 1.4-kb exon 23–24 band and in the appearance of a 4.1-kb band. In patient 1060, the exon 26 9.2-kb band is shifted to a 12-kb band. In patient 1281, a mutation in the *Taq*I site of exon 23 results in the replacement of the 1.4-kb band by a 3.8-kb band. In patient 1286, the *Taq*I site mutation in exon 18 causes the 2.2-kb band to shift to 5.9 kb.

A number of mutations that alter *Taq*I sites have been observed, and generally they occur by a C-to-T transition that results in either a stop codon (TGA) or a glutamine missense mutation (CAA) (Gitschier et al. 1985); Youssoufian et al. 1986; Levinson et al. 1987). To precisely characterize the mutations in the Finnish population, a set of three 15-mer oligonucleotide probes was synthesized for each of the four affected *Taq*I sites (table 1). The sequences of these probes were identical to either the normal sequence or those corresponding to the two predicted mutations. DNAs from the five patients were amplified with the appropriate primers for exons 18, 23, 24, and 26 (table 1), and were dot blotted in sets of three on nylon filters (fig. 2). Each set was screened with one of the three probes. Under identical stringent conditions, three patient samples (785, 818, and 1286) hybridized only to the appropriate missense probe, two patient samples (1060 and 1281) hybridized only to the nonsense probes, and normal samples always hybridized only to the normal probe. The three patients with missense mutations in exons 18 and 24 exhibited mild to moderate hemophilia, whereas the two affected males with nonsense mutations showed clinically severe disease. The results are summarized in table 2.

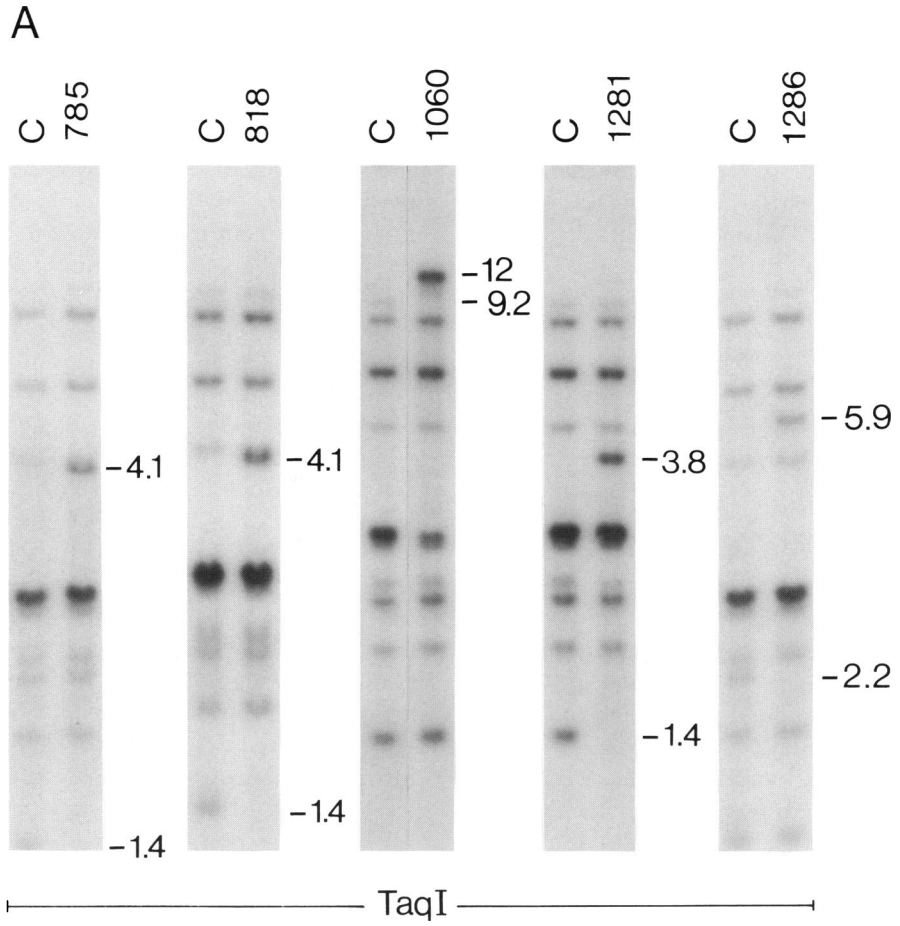
Analysis of Deletions

Partial factor VIII gene deletions were detected in four patients (fig. 1*B*). Hybridization of probe 2 to Southern blots of DNA digested with *Taq*I and *Sst*I revealed loss of all or part of exons 5 and 6 in three cases, as shown in figure 3. Patients 1059, 2213, and 2253 were missing a faint 2.7-kb band (corresponding to exon 6) on the *Taq*I blot. In patient 1059 a new weak 3.2-kb band appeared. To determine the extents of the deletions, the *Taq*I and *Sst*I blots were then screened with a probe containing only exons 4–7. The *Taq*I-digested DNA from patient 1059 showed a loss of a 2.4-kb band corresponding to exon 5 and of the 2.7-kb exon 6 fragment. A novel 3.2-kb band was observed. In the *Sst*I-digested DNA from this patient, the exon 5–6 (6.8-kb) band and exon 7 (11.6-kb) band were replaced by a single 13-kb band. A 6.6-kb band, representing exon 4, is still seen on the *Sst*I blot. When these data are compared with the normal restriction map of factor VIII, the results suggest that a 10-kb deletion removed all of exon 6 in patient 1059. Similarly, the *Taq*I and *Sst*I digests on DNA from patient 2213 showed that exon 6 is deleted. However, in this case the new junction fragments on the *Taq*I and *Sst*I blots, 1.7 and 4.1 kb, respectively, indicate a deletion of 3–6 kb. In both patients 1059 and 2213, exons 5 and 7 appear to be intact. More precise mapping was not done, because of the lack of single-copy genomic clones in this region. In patient 2253, the bands corresponding to exons 5 and 6 are completely deleted on both the *Taq*I and *Sst*I digests and no new junction-fragment bands appear. This indicates that a 2.5--10-kb deletion has removed both exons 5 and 6.

The deletion in the fourth patient, 1067, was detected on a *Sst*I blot using probe 1 (fig. 1*B*). In this case, a new 6-kb band was present. When this blot was rescreened with a probe corresponding only to exons 1–3, the 3-kb exon 1 band was found to be missing and the 6-kb band remained (data not shown). This new 6-kb band contained the exon 1 sequences and was the result of a deletion in the first intron. Using other single-copy probes 5′ and 3′ to exon 1, we determined that the deletion is about 7 kb and lies completely within intron 1.

Analysis of a New Point Mutation

The Southern blot of *Taq*I-digested DNA from pa-



tient 1308 showed a band slightly smaller than the 1.4-kb fragment corresponding to exons 23–24 (fig. 1 and 4). This result was consistent with a small deletion of part of exon 23 or 24. However, DNA amplification with primers 13.1 and 16.2 revealed a normal-size 1.4-kb band, suggesting that a new *Taq*I site was generated by the mutation. To test this possibility, amplified DNAs from the patient and a normal individual were digested with *Taq*I and run on a 8% minipolyacrylamide gel (fig. 4). A 55-bp band was seen in the patient's sample but not in the control, confirming the presence of a new *Taq*I site. The amplified DNA was sequenced, and a T-to-C transition was found at position 6555 in exon 23. This mutation causes a substitution of serine for leucine at amino acid 2166. Patient 1308 had less than 1% factor VIII activity and clinically severe hemophilia.

Pedigree Analysis

Hemophilia affected multiple generations in two of the 10 families in whom mutations were identified in the present study. In seven cases, the hemophilia was an isolated occurrence, and in the remaining one family only two sons were affected. We have performed Southern blot analysis of all members of these eight families, to determine the origin of the mutations and to provide carrier determination. Details of the pedigree analyses are summarized below and in table 2.

In cases 1060 and 1281, the mutation is only present in the patient, and thus the mutation appears to have been introduced into the maternal gametes. In two families, 785 and 1286, the mother was found to carry the mutation on the chromosome she inherited from her unaffected father, indicating that the mutation may have originated in his gametes. We were unable to determine the origin of the mutation in family 1059, since this abnormal allele was present even in the maternal grandmother. Similarly, we could not determine the origin of the exon 5–6 deletion in patient 2253. Densitometry measurements of Southern blot band intensities of exons 5 and 6 as compared with the uninvolved exons

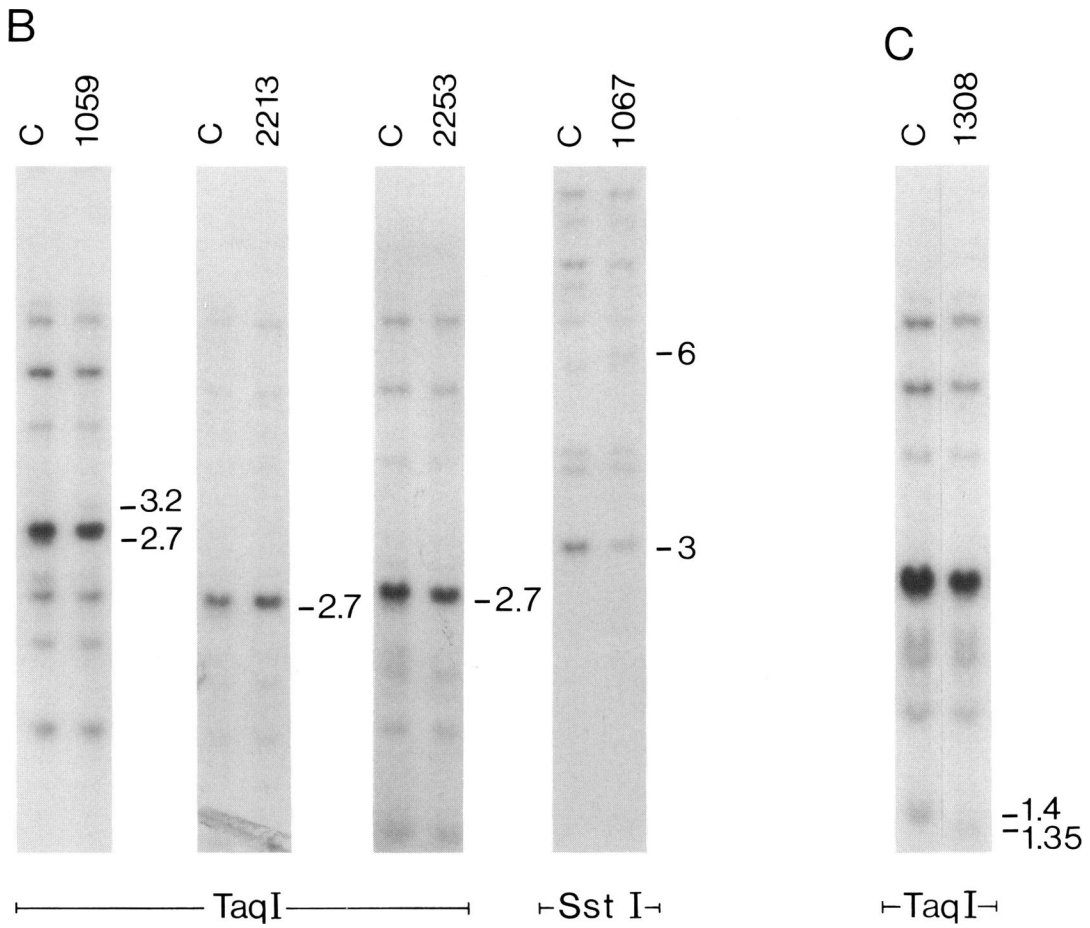


Figure 1 Southern blot analysis of 10 hemophilia DNA samples. Five micrograms of patient DNA was digested with *Taq*I or *Sst*I, run on 0.8% agarose gels, and blotted onto nitrocellulose filters. The blots were hybridized to either probe 1 or probe 2 as described in the text. Sizes of the altered bands are indicated. Lanes with patient samples are identified by the corresponding numbers. Adjacent lanes with control DNA are indicated by C. Panel *A*, the results for patients with *Taq*I restriction-site mutations. Patients' and control DNAs were digested with *Taq*I and screened with probe 2. Panel *B*, deletion mutations detected by digesting patient and control DNAs with *Taq*I or *Sst*I and screening with probes 2 and 1, respectively. Panel *C*, DNA from patient 1308 that was digested with *Taq*I and screened with probe 2.

4 and 7 indicated that the mother carried the deletion. No further analysis could be made, since the grandparents are deceased, and the polymorphic markers were uninformative.

There was evidence for maternal mosaicism in the two remaining pedigrees. As shown (fig. 1 and 3), patient 2213 had a partial deletion of exons 5 and 6, which results in abnormal bands on both *Taq*I and *Sst*I blots. As noted elsewhere (Gitschier et al. 1989), his mother has a normal pattern on these blots, whereas his sister's DNA contains bands associated with the deletion. From the results of these blots, we conclude that this family is an example of maternal germ-line mosiacism, although maternal somatic mosaicism cannot be excluded with certainty.

A second example of mosaicism was noticed by screening a *Taq*I blot of patient 1308 and his mother (fig. 4). As already described, this patient has a point mutation in exon 23 resulting in a new *Taq*I site. On the *Taq*I Southern blot, the abnormal band is also present in the mother's sample, although, surprisingly, at a lower intensity than the expected heterozygous level. On densitometric analysis of the autoradiogram the signal of the aberrant band was 10% of that of the normal allele, suggesting that she is a somatic mosiac. This conclusion was supported by examination of the *Taq*I-digestion products of the mother's amplified DNA. Like the patient, the mother's sample also produced the 55-bp fragment, although much decreased in intensity. On the basis of relative intensities of the bands on the South-

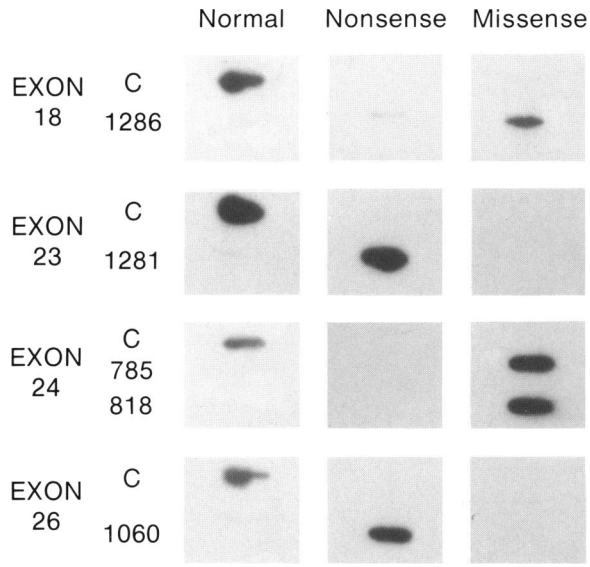


Figure 2 Allele-specific oligonucleotide hybridization. Patient and control (C) DNAs were amplified with *Taq* polymerase as described in Methods, using the appropriate exon-specific primers (table 1). The amplified DNA was dotted onto nylon filters, hybridized with ^{32}P-end-labeled oligonucleotides representing normal, nonsense, and glutamine missense sequences, and washed with 3 M tetramethylammonium chloride at 50°C.

ern blot and polyacrylamide gels, we estimate that 10% of the mother's peripheral blood cells are heterozygous for the mutation.

Discussion

The present report presents the results of a study of factor VIII gene mutations by Southern blot analysis of all available hemophiliacs in the Finnish population. This study was made possible through the help of the Finnish Red Cross Blood Transfusion Center, which maintains a file of all diagnosed hemophiliacs living in Finland. Of the 112 families contacted, 82 families and one individual hemophiliac became part of this study. We detected a total of 10 mutations representing 12% of the patients studied. Table 2 summarizes the findings for the patients in whom mutations were identified.

Mutations affecting *TaqI* sites in four different exons were detected in five of the 83 hemophiliacs. All of these genetic changes, except for the exon 18 missense mutation, have been previously reported elsewhere. Recently, another unrelated patient with this identical exon 18 mutation was found (H. Kazazian, personal commu-

Table 2

Summary of Factor VIII Mutations in the Finnish Population

Patient	Clinical Profile	Mutation	Genetic History and Origin of Mutation
785	Moderate, Ab negative	Exon 24; missense arg2209 to gln	Sporadic; *de novo* in mother; mutation occurred on grandpaternal chromosome
818	Moderate, Ab negative	Exon 24; missense arg2209 to gln	Familial; large hemophilia pedigree
1059	Severe, Ab negative	Complete deletion of exon 6; estimated size 10 kb	Sporadic, but mutation present in mother, aunt, and grandmother
1060	Severe, Ab negative	Exon 26; nonsense arg2307 to stop	Sporadic; *de novo* in patient
1067	Severe, Ab negative	Partial deletion of intron 1; estimated size 7 kb	Familial; large hemophilia pedigree
1281	Severe, Ab negative	Exon 23, nonsense arg2147 to stop	Sporadic; *de novo* in patient; patient shares *XbaI* allele with unaffected brother
1286	Mild, Ab negative	Exon 18; missense arg1941 to gln	Only two sons affected; *de novo* in mother; mutation occurred on grandpaternal chromosome
1308	Severe, Ab negative	Exon 23; new *TaqI* site	Sporadic; maternal somatic mosaicism
2213	Severe, Ab negative	Complete deletion of exon 6; estimated size 3–6 kb	Sporadic; presumptive maternal germ-line mosaicism
2253	Severe, Ab positive	Complete deletion of exons 5 and 6; estimated size 2.5–10 kb	Sporadic; mother is carrier by densitometric analysis; origin could not be determined

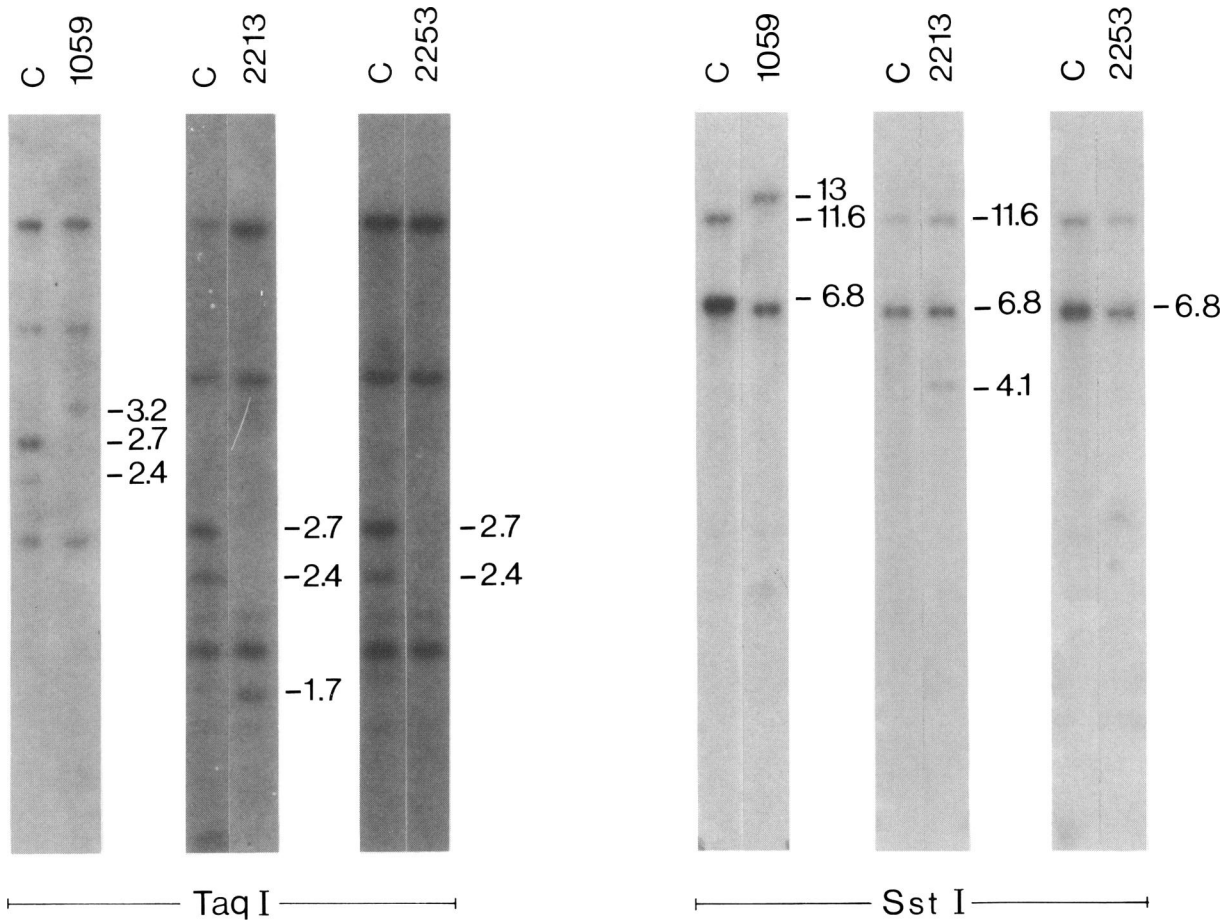


Figure 3 Southern blot analysis of patients with deletions of exons 5 and 6. DNA was digested with *TaqI* and *SstI*, and the blots hybridized to a probe containing exons 4–7. Fragment sizes are shown. Lanes are as described in Fig. 1.

nication). In this study, two unrelated patients (785 and 818) have identical missense mutations in exon 24. RFLP haplotype analysis using *BclI* and St14 and DX13 polymorphisms proved that these patients were unrelated (data not shown). The two patients with nonsense mutations have severe hemophilia, whereas the three with missense mutations have mild or moderate disease.

In general, these results are consistent with findings from other studies. However, the clinical profiles and factor VIII activity measurements of the two patients (785 and 818) with exon 24 missense mutations differ from those described by Youssoufian et al. (1988*a*) for two unrelated patients with the same mutations. In that study, both patients were found to have <1% factor VIII activity and clinically severe hemophilia A. Patient 785 was clinically evaluated as a moderate case of hemophilia, and the factor VIII activity levels were assayed three times, with resulting values of 2%, 3%, and 5%. Patient 818 and the other two hemophiliacs in his family were diagnosed as having moderately severe disease. Factor VIII activity was measured a total of seven times in these three patients without prior factor VIII replacement. Five tests showed significant activity (2%–5%), whereas <1% was measured in two tests. The reason for this inconsistancy in the laboratory factor VIII assays is unknown. Thus, the discrepancy between the clinical profiles in our patients and those of patients of Youssoufian et al., may be due to variability in laboratory assays and clinical description. Alternatively, other factors in addition to the precise nature of the mutation may play a role in the clinical picture of this disease.

Three of the four detected deletions, although differing in the extent of the genome missing, were from the exon 5 and 6 region of the factor VIII gene. Our results differ from those in other studies, where deletions vary

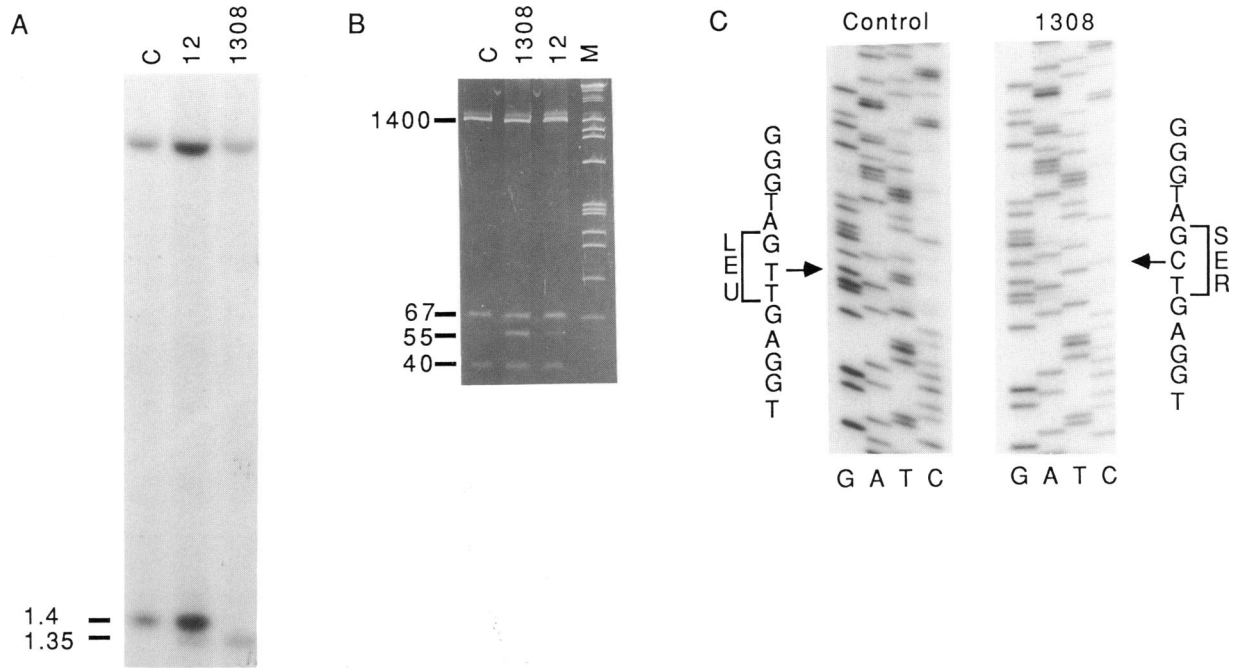


Figure 4 Analysis of patient 1308 and his family. Panel *A*, *Taq*I-digested DNA electrophoresed on a 1.6% agarose gel. The resulting Southern blot was screened with a probe containing exons 21–24. The 1.4-kb band corresponds to exons 23–24. Lane C contains DNA from a normal control individual, lane 12 contains DNA from the mother, and lane 1308 contains DNA from the proband. Panel *B*, DNA from a normal control (C), patient 1308, and his mother (12) were amplified using primers 13.1 and 16.1 to yield a 1.4-kb fragment containing exon 23, intron 23, and exon 24. Thirty microliters of the amplification reaction was digested with *Taq*I, and the digestion products were run on an 8% polyacrylamide gel. Lane M contains *Hin*dIII-digested λ DNA and *Hae*III-digested φX174 DNA. Panel *C*, Sequencing data establishing the T-to-C transition mutation in 1308. DNAs from a normal control and patient 1308 were amplified with the 13.1 and 13.9 primers (table 1), yielding a 255-bp fragment that contains exon 23 and 125 bp of intron 23. The amplified DNA was directly sequenced with primer 13.1 as described in Methods.

widely in both location and size and do not show a preference for certain sites in the gene (Antonarakis et al. 1985; Gitschier et al. 1985; Youssoufian et al. 1987, 1988*b*). We were unable to conclude whether this apparent clustering of deletions in the Finnish population is a meaningful finding or simply a chance event.

A surprising result was the finding of a deletion totally within an intron that is associated with severe hemophilia A. The 7-kb intron 1 deletion was found to segregate with the disease in a large hemophilic pedigree (1067), suggesting that this defect is related to the hemophilia in this family. Three other reported mutations in factor VIII introns have involved changes in *Taq*I sites. In one case, the altered sequence could form a new donor splice site (Youssoufian et al. 1988*c*), but in the other two cases no mechanism could be identified (Gitschier et al. 1985). It is difficult to prove that this intron mutation is the cause of the disease, and therefore we can only speculate that the deletion causes abnormal splicing of factor VIII mRNA, resulting in severe hemophilia in this family. Another possibility is that intron 1 contains important information for transcription initiation, as has been demonstrated for pro-α1(I) collagen (Rossouw et al. 1987).

Patient 1308 was shown by DNA sequencing to have a T-to-C point mutation in exon 23, causing a substitution of serine for leucine at position 2166. This substitution has occurred in one of the two conserved C domains required for factor VIII activity. One explanation for the deleterious effect of serine is that it could alter the protein's thermal stability because it is smaller and more hydrophilic than leucine. The genetic evidence that this mutation is the cause of severe hemophilia in patient 1308 is quite strong, since both the mutation and the hemophilia arose de novo.

Hemophilia occurred sporadically in 33 of the 83 families in the present study. We had the opportunity of investigating the origin of the mutation in the eight sporadic cases in which the mutation was identified. In two cases, the origin could not be traced. In two

additional cases the mutation first appeared in the patient, and in two other cases it first appeared in the mother. One possibility is that these mutations were isolated meiotic events and arose in the gametes giving rise to the affected individual. However, given the recent evidence for mosaicism in X-linked disorders (Bakker et al. 1987; Darras and Francke 1987; Lanman et al. 1987; Gitschier 1988), it is also possible that the mutations occurred before meiosis, during parental embryonic development. Indeed, in the remaining two Finnish families with sporadic hemophilia, we found evidence for maternal mosaicism, indicating that, at least in these cases, the mutations arose during the mother's embryogenesis or development. In the family of patient 2213 both the affected individual and his sister carried the abnormal gene, whereas this mutant allele was not observed in the mother. The best explanation for this unusual inheritance is the occurrence of a mutational event in the maternal germ line leading to gonadal mosiacism. In the second instance (patient 1308), the hemophiliac was the only child with the mutation, but only a small percentage of the carrier mother's leukocytes also had the mutation. This latter case is the fourth report of maternal somatic mosaicism for hemophilia A (Annette et al. 1988; Gitschier 1988; Higuchi et al. 1988). However, in contrast to previous reports of mosaicism in X-linked disorders, this case is associated with a point mutation rather than a deletion. The mechanisms and implications of mosaicism are elegantly presented by Maddalena et al. (1988), who report evidence for somatic mosaicism for ornithine transcarbamylase deficiency, and are reviewed by Hall (1988). Thus, in our population, mosaicism was detected in one-third of the cases in which the origin of the mutation was traced. Although the sample size is small, the results emphasize that mosaicism is an important consideration in pedigree analysis and counseling.

The population group analyzed in the present study differs from those previously reported. Finland has a population structure that is characterized by a small number of founder families followed by almost total isolation and very little later immigration. An excessive occurrence of some rare autosomal recessive diseases and the relative lack of others is a genetic consequence of this isolation (Nevanlinna 1972; Norio 1981). Our data support the hypothesis that hemophilia A is a heterogeneous disorder, stemming from a variey of mutations even in relatively homogeneous populations. It is of interest that these Finnish families also showed the same mutations of *TaqI* sites described for hemophiliacs from diverse genetic backgrounds. This spectrum of genetic events appears to be due to the significant incidence of new factor VIII gene mutations continually introduced into any given population.

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