

Analysis of Intron 22 Inversion Mutation of Factor VIII Gene in the Patients with Hemophilia A in J&K State of India

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ABSTRACT: Objective Hemophilia A, an X-linked bleeding disorder, affecting 1 in 5 000 males is caused by heterogeneous mutations in factor VIII gene. Inversion mutation in intron 22 of F8C gene remains its leading cause. The aim of this study was to evaluate the frequency and distribution of the intron 22-inversion mutation in the patients and in the family members in the region.

Methods 29 hemophilia A patients from Jammu and Kashmir (20 severe, 8 moderate and 1 mild) were analyzed for intron 22-inversion mutation. **Results** 11 (38%) were positive for the distal type of inversion mutation. The mutation was found in 9/20 (45%) patients with severe factor VIII deficiency and 2/8 (25%) with moderate severity hemophilia A, whereas the patient with mild hemophilia A was found to be negative for inversion mutation. Evaluation of twenty-six female relatives from 11 families of inversion mutation positive patients identified one mother and one sister from one family to be the carrier, suggesting its origin in the mother. **Conclusion** The present study confirms the intron-22 inversion mutation in F8C gene as the major cause of hemophilia A in the population from Jammu and Kashmir with a higher frequency of inversion mutation in sporadic cases compared to the familial cases.

Key words: hemophilia A; factor VIII; inversion; intron 22

Hemophilia A was documented as a familial bleeding disorder in the fifth century^[1] and persists as the commonest hemorrhagic disorder, affecting 1 in approximately 5 000 males. The disease is caused by the deficiency of factor VIII, a co-factor of activated factor IX in the factor X activating complex of the intrinsic coagulation pathway^[2]. Factor VIII gene is located at the tip of the long arm of X-chromosome^[3], and it spans over 186 kb, comprised of 26 exons and 9 kb mRNA encodes a polypeptide chain of 2 351 amino acids^[4-6].

The molecular basis of Hemophilia A has been described in several patients and variety of mutations including gene deletion^[7-9], insertion^[10] and point mutations^[7,9,11,12] have been reported however, inversion in intron 22 remains the commonest mutation accounting for 45% ~ 55% of the severe Hemophilia A patients^[13-16]. Intron 22 is the largest intron in the factor VIII gene^[3] and also contains a CpG island, located downstream of exon 22. This CpG Island is a bi-directional promoter for two genes A and B associated with factor VIII

gene^[17,18]. Gene A which is intron-less and nested within intron 22 transcribe in the opposite direction to factor VIII gene whereas gene B transcribes in the same direction as the factor VIII gene; its first exon lies with intron 22 and is spliced to exons 23~26. Inversion in intron 22 of F8C gene resulting from an intra chromosomal homologous recombination on the X chromosome is mediated by the three copies of int 22 h, one located in intron 22 of the gene and the other two, 400 kb 5' and telomeric to the gene. Homologous recombination appears to occur between the intron 22 copy (int 22 h-1) and either the distal (telomeric, int 22 h-3) or proximal (int 22 h-2) copy. The recombination results in factor VIII exons 1~22, in addition to adjacent DNA becoming inverted and relocated some 400 kb from its normal position, with exons 23~26 remaining in their original location thus disrupting the coding sequences of the gene.

Intron 22-inversion mutation analysis in hemophilia A patients from the state of Jammu and Kashmir has been carried out for the first time. The

aim of study was to evaluate the frequency and distribution of the intron 22-inversion mutation in the patients and in the family members in the region.

1 Materials and methods

1.1 Patients

Twenty-nine patients with a diagnosis of hemophilia A were identified through Hemophilia Federation of India, Jammu Chapter and their blood samples (29 patients) were collected in an EDTA vacutainer after obtaining the informed consent from them. Of the 29 patients enrolled in the study, (Fig 1), 20 had severe, 8 moderately severe and 1 had mild diseases as determined by the factor VIII C measurements ($< 1\%$ severe, $1\% \sim 5\%$ moderate and $> 5\%$ mild). Blood samples from 26 female members from 11 families were also included in the study to aid in the carrier analysis.

1.2 Methods

Genomic DNA was isolated by method given by^[19]. For screening the inversion mutation, in each case, 8 μg of DNA was restriction digested overnight using BclI at 50°C overnight. Digested genomic DNA was subjected to electrophoresis for 54 h on 0.7% agarose gel, followed by its transfer to Hybond (Amersham) nylon membrane and subsequently hybridized with 0.9 kb EcoRI/SalI isolated probe from clone p486. 2^[20] labeled with [α -³²P]-dATP using random primer labeling kit. The membrane was washed at 65°C for various stringencies of washes with 1% SSC and 0.1% SDS buffers and exposed to phosphor imaging film (Fuji) for screening and finally exposed to X-ray film (Kodak) at -70°C for 4 d for confirmation.

2 Results

Of the 55 DNA samples (29 of the patients and 26 of the females belonging to 11 families) digested with BclI enzyme and hybridized with the factor VIII probe, a normal pattern of bands of 21.5 kb, 16 kb and 14 kb was detected in 18 patients and 24 females. In 11 patients and 2 females (mother and

sister from one family) distal type of inversion mutation in intron 22 of F8C gene was detected with the band pattern of 20 kb, 17.5 kb and 14.0 kb (Fig 2, 3). Of 11 patients positive for the inversion mutation, 9 had severe hemophilia A and 2 were moderately severe. DNA analysis of the females belonging to the affected family was carried out in order to establish the carrier status for the detected mutation.

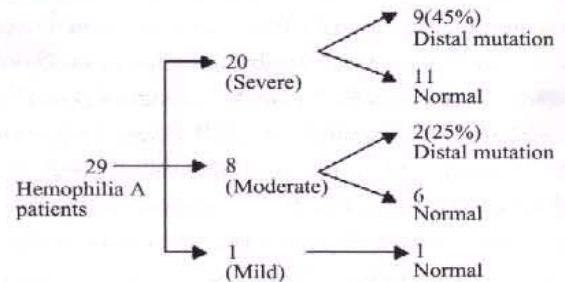


Fig 1 Inversion mutation in Patients with Hemophilia A

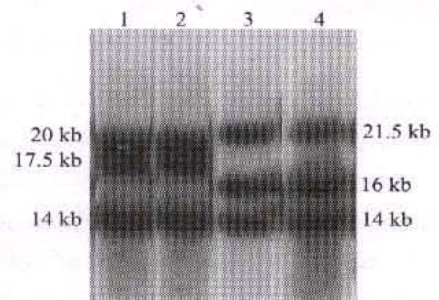


Fig 2 Southern blot analysis demonstrating the intron 22 inversion mutation in F8C gene. Lanes 1 and 2 showing the bands 20 kb, 17.5 kb and 14 kb characteristic of distal type of inversion mutation; lane 3 and 4 showing the 21.5 kb, 16.0 kb and 14.0 kb representing the pattern of bands in normal individuals

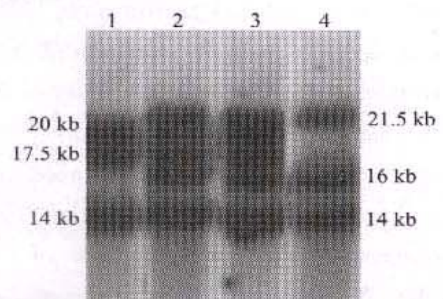


Fig 3 Southern blots demonstrating the inversion mutation in 1 family HAR. Lanes 1 (proband) distal type inversion, Lane 2 (Mother) distal type of inversion, lane 3 (Sister-01) Carrier of distal mutation, lane 4 showing the 21.5, 16 and 14 kb normal bands

3 Discussion

In the present study, frequency of the inversion mutation in intron 22 of factor VIII gene has been found to be 45% (9 of 20) in severe and 25% (2 of 8) in moderately affected hemophilia A patients. Several studies^[21-24] on intron 22 inversion mutation in severe hemophilia A cases reported nearly 45% whereas many other^[25-28] reported the frequency of intron 22 inversion mutation < 45%^[13,15,29] recorded slightly higher > 45% inversion mutation in their study samples. Thus study of the existing literature on intron 22-inversion mutation in hemophilia A shows the frequency of this mutation between 37% to 57%.

The present study, first of its kind in analyzing the inversion mutation status amongst hemophilia A patients of J&K recorded 45% mutation in severe and 25% in moderately affected patients. Our findings are therefore similar to the findings of^[21-28]. Therefore from the present study and the existing literature show that intron 22-inversion mutation of distal type remains the commonest mutation amongst severe as well as moderately affected hemophilia A patients. The frequency in severely affected and in moderately affected hemophilia A patients has been reported by^[25,29,30] and present findings are therefore similar to the earlier reports. The present work has been carried out for the first time in this region further analysis of the mutation in patients of the region would help in finding more information about the mutation affecting different populations of J&K State.

Direct detection of intron 22 inversions and family analysis revealed that this mutation appears to occur predominantly in male meiosis^[31,32]. Identification of the inversion mutation in the factor VIII gene could help in the direct diagnosis of the mutation especially families with sporadic history. However, the whole procedure is laborious and difficult in routine use. Linked polymorphic markers in the FVIII gene are better used for carrier analysis in the background of positive family history, provided

the affected male and enough family members are available. Even though, RFLPs and VNTRs present with significant limitations due to heterozygosity in certain cases, they still remain the primary approach for carrier analysis, prenatal diagnosis and genetic counselling. Inversion analysis is the simplest form of direct mutation detection technique available for diagnosis of hemophilia A patients. Data from our study and others^[16,22,33] favors the inversion analysis as a first direct choice to diagnose the FVIII gene defect in a high proportion of severely affected sporadic patients but appears to be of limited use in the identification of the female carriers in the family.

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4 References

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