Intron 1 inversion mutation among Turkish hemophilia A patients

İnanç Değer FİDANCI[1], Tufan AYDOĞDU[1], Hande ÇAĞLAYAN[1]

[1] Department of Molecular Biology and Genetics, Boğaziçi University, İstanbul, TURKEY

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ABSTRACT

Hemophilia A is an X-linked bleeding disorder resulting mostly from heterogeneous point mutations in the factor VIII (F8) gene. Small/large gene deletions, insertions and gross gene rearrangements underlie the molecular pathogenesis of the disease. Two large inversion mutations due to intrachromosomal recombinations between inverted repeats found in intronic sequences and upstream regions of the F8 gene result in severe hemophilia A. The intron 1 inversion mutation is responsible for about 2% of the cases in various populations. Herein, we report the establishment of the long polymerase chain reaction (PCR) intron 1 inversion mutation detection in our laboratory and a similar frequency of 1-2% among Turkish patients.

Key Words: Intron 1 inversion, Mutation detection, Hemophilia A.

ÖZET

Türk hemofili A hastalarında intron 1 inversiyon mutasyonu

Genellikle Faktör VIII (F8) geninde oluşan heterojen nokta mutasyonlarının neden olduğu hemofili A, kalıtımı X-kromozomuna bağlı olan kanama bozukluklarındandır. Hastalığın moleküler patogenezi ayrıca, küçük/büyük gen delesyonları, insersiyonlar ve büyük genomik değişimlere de bağlıdır. İntron dizilerinde ve F8 geninin yukarı bölgelerinde bulunan ters yönde dizi tekrarları arasında oluşan kromozom-içi rekombinasyonlar iki büyük inversiyon mutasyonundan sorumludur. Farklı toplumlarda, vakaların %2'sinden intron 1 inversiyon mutasyonlarının sorumlu olduğu gösterilmiştir. Bu çalışmada, laboratuvarımızda intron 1 inversiyon mutasyonunun saptanması için uygulanan polimeraz zincir reaksiyonu yöntemi ve benzer şekilde, Türk hastalarında da görülen sıklığın %1-2 olduğu rapor edilmektedir.

Anahtar Kelimeler: İntron 1 inversiyon mutasyonu, Mutasyon belirlemesi, Hemofili A.

INTRODUCTION

Hemophilia A is an X-linked recessive bleeding disorder characterized by qualitative and quantitative deficiency of factor 8 (F8) resulting from heterogeneous mutations in the factor 8 gene (F8C) located in the Xq28 region[1]. F8C gene is 186 Kb in length and has 26 exons. The organization of the gene is very complex with large introns and genes within introns, such as F8A and F8B in intron 22[2]. Mutations described in the F8C gene are mostly gene rearrangements, point mutations, large deletions and insertions [HAMSTeRS http://europium.mrc.rpms.ac.uk]. An inversion due to an intrachromosomal recombination, between F8A gene in intron 22 and two homologous copies 400 Kb upstream from the F8C gene, is responsible for about 45% of severe hemophilia A cases. It occurs mostly in the male germline[3]. A 1041-base pair sequence (int1h-1) of intron 1 was also found to be duplicated (int1h-2) and oriented in the opposite direction 140 Kb apart from the gene between C61A and VBP1 genes[4]. An intrachromosomal recombination between int1h-1 and int1h-2 results in an inversion mutation involving intron 1. The frequency of this rearrangement was found initially to be 5% in the British population[1]. Later studies indicate that the frequency is around 2% in the same population and also in others[4-8].

In this study, we report the establishment of the polymerase chain reaction (PCR) method to detect intron 1 inversion mutation in our laboratory and its frequency among Turkish patients.

MATERIALS and METHODS

Blood Samples

Blood samples of 112 Turkish hemophilia A patients provided by various hematology clinics were tested for intron 1 inversion mutation. Thirteen of these patients had severe phenotype and previously identified missense mutations and were negative for intron 22 inversion mutation. Among the remainder of the 99 patients who had variable phenotypes, 40 were negative for intron 22 inversion and point mutations by DGGE analysis and 59 were not tested for any type of F8 mutations. Thirty patients were known to develop inhibitors.

PCR Amplification

PCR amplifications of int1h-1 and int1h-2 regions were performed on 100 ng of genomic DNA with 1X Mg^{2+} Free Reaction Buffer, 2.5 mM Mg^{2+}, 0.5 mM of each dNTP, 200 ng of each oligonucleotide primer, 5% dimethyl sulfoxide and 2.5 U Taq DNA Polymerase (Promega). Thirty-five amplification cycles were performed at 94°C for 30 seconds, 55.5°C for 30 seconds, and 72°C for two minutes. Primer sequences used are given in Bagnall et al.[4].

RESULTS and DISCUSSION

Two PCRs each of which independently showed the presence of the inversion mutation, were performed for intron 1 inversion analysis. In the first reaction, primers specific for int1h-1 (9F, 9cR) plus the primer int1h-2F that recognizes int1h-2 were used in an amplification reaction that yielded a 1900 bp product from normal DNA and a 1300 bp product in the presence of inversion (Figure 1). In the second reaction, primers specific for int1h-2 (int1h-2F, int1h-2R) plus the primer 9F yielded a 1300 bp product from normal DNA and a 1900 bp product in the presence of inversion, whereas female carriers had both bands for the two PCR reactions (Figure 1).

Our results revealed only two patients with intron 1 inversion, which was also detected in their mothers (Figure 2). One of these patients had developed inhibitors and was negative for intron 22 inversion and point mutations. The other patient was not tested for any other type of F8 mutation. The absence of intron 1 inversion mutation in 13 patients with a single missense mutation and who were negative for intron 22 inversi-

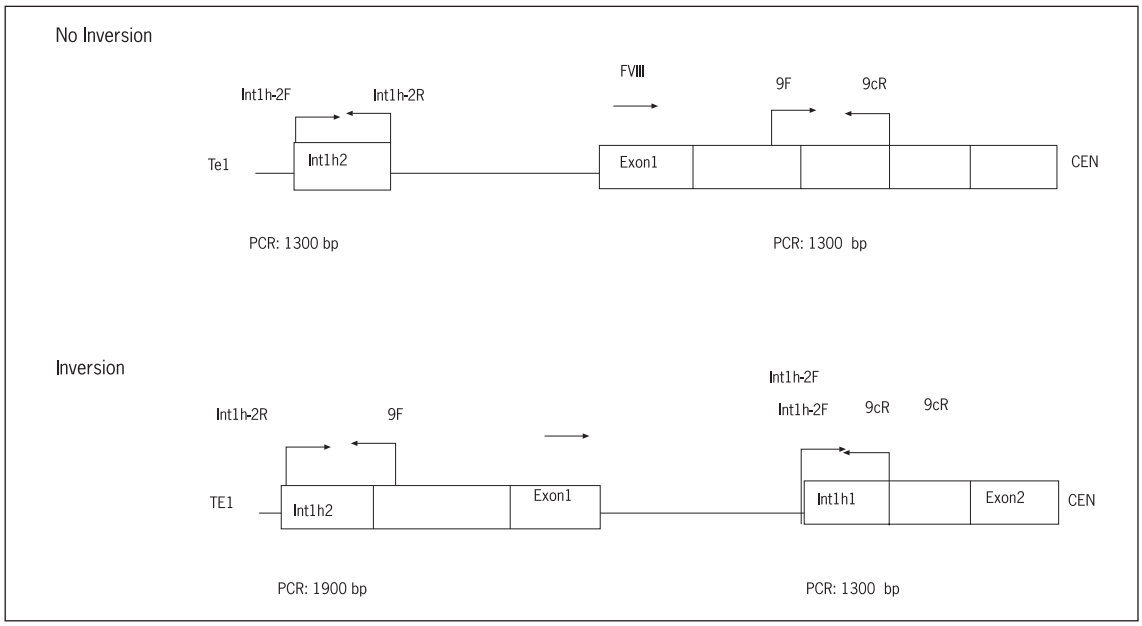

Figure 1. Schematic diagram of intron 1 inversion to show the primer site (Modified from Bagnall et al., 2002).

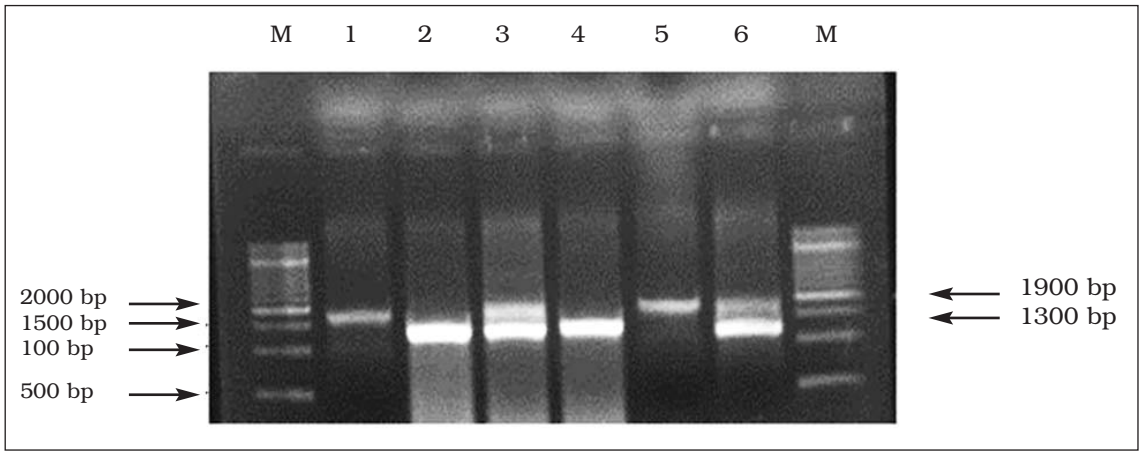

Figure 2. PCR pattern for int1h-1 and int1h-2 regions. Lanes 1, 2, 3 indicate the wild-type, inversion and carrier patterns, respectively, for int1h-1 region. Lanes 4, 5, 6 indicate the wild-type, inversion and carrier patterns, respectively, for int1h-2 region. M indicates 500 bp DNA ladder Marker.

on was a further step in supporting the pathological nature of these amino acid substitutions.

According to our data, the overall frequency of intron 1 inversion for our patient population was 1.8%. When previously identified 41 intron 22 inversion cases[9] and 21 other pathological mutations[10] among a total of 174 Turkish patients are considered together, the frequency of intron 1 inversion is even lower, approaching 1%. Therefore, our results confirm the lower frequency of intron 1 inversion mutation observed in other populations.

Despite its low frequency, detection of intron 1 inversion mutation is important in accurate hemophilia A diagnosis and also in the risk assessment of inhibitor development.

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Address for Correspondence:

Hande ÇAĞLAYAN, MD

Department of Molecular Biology and Genetics
Boğaziçi University School of Medicine
İstanbul, TURKEY

e-mail: *hande@boun.edu.tr*