Detection of Intron22 Mutations in Iraqi Female Carriers in Wasit province with Hemophilia A

By Maysoon Mohammed Hassan

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Aims of study: Detection analyze the presence of intron twenty two mutations within the FVIII gene in ten HA Iraqi carriers families with significantly, there's a little risk for return even once the familial F8 mutation isn't known within the mother of the affected patient because of the probability of germ line mosaics within the community.

Keywords: hemophilia A, factor 8 gene, carriers, intron 22 mutations.

GJMR-F Classification: NLMC Code: WH 325

Strictly as per the compliance and regulations of:
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*Patients and Methods:* This study enclosed ten Iraqi carriers with Hemophilia A and five healthy members as control. This work exhausted medicine & science school laboratories also as Al. Karama Teaching Hospital from Nov, 2016 to January, 2017. These carriers antecedently diagnosed by case history, DNA testing.

*Results:* Throughout the screening for Inv22 (intron twenty two inversions) among the HA carriers, results showed four (40%) from ten carriers had this mutations.

*Discussion:* Our knowledge highpoint the importance of the analysis of Inv22 for their association with positive family history within the HA carriers and that we are continuous looking of Inv1 mutations.

*Conclusions:* Results specify the dangerous impact of positive family history and the consanguinity marriage. Carriers of with the haemophilia centre, and management procedures ought to be offered and determined. This result represents a step for genetic guiding. Knowledge of fetus gender is extremely valuable for facilitate management in labor and for diagnostic Procedure.

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The F8 gene encodes plasma protein VIII, a large plasma glycoprotein that functions within the clotting cascade as a cofactor for the factor IXa-dependent activation of factor X(12). The F8 gene contains twenty six exons, that vary long from sixty nine to 3,106 base pairs (bp) and twenty five introns spanning 186-kb ge-nomic DNA and mapped to the distal termination of the long arm of X-chromosome (Xq28). Intron sequences resemble to 177.9 kb, and are detached from the first transcript product throughout splicing to produce a mature F8 mRNA of roughly nine kilobyte long that predicts a precursor protein of 2,351 amino acids. Of the larger intron sequences, we have a tendency to found six that larger than fourteen kilobyte (introns one, 6, 13, 14, 22 and 25), with intron 22 the biggest at 32.8 kilobyte in distance (13), that intron twenty two inversion(Inv22) are the most public kind in about 40–45% of severe hemophilic cases and 2–5% of severe HA cases are littered with intron one inversion(Inv1)(14:15). Apparently, Rossiter et al. (1994) found that Inv22 originates preponderantly from male germ cells and hypothesized that the presence of a second X chromosome in female meiosis would hinder the intrachromosomal non-allelic pairing needed for Inv22(16). each inversions occur as a results of non-allelic meiotic intrachromosomal recombination between the int22h-1 region inside the F8 locus and either int22h-2 or int22h-3, in male germ cells (16). Int22h-1 recombines with the utmost telomeric copy of int22h that is usually reciprocally oriented to int22h-1 and in most of the cases its int22h-3. This int22h-1/int22h-3 recombination lds to inv22 sort I. In slight number of cases it was revealed that inversion was a results of 2 recombination events. Initial one was a recombination between the arms of the palindrome inv22h-2/ inv22h-3, that has been recognized as a publicnon-deleterious inversion polymorphism. That occasion changes the positions and orientations of int22h-2 and places it at the utmost telomeric and inverse position to inv22h-1. The second recombination between inv22h-1 and inv22h-2 end in inv22 sort II (17).Furthermore, it’s been expected that recombination between int22h-1 with equally leaning copy of either int22h, int22h-2 or int22h-3 could be accountable for massive hurtful deleterious deletions (Del22), and also probably non-deleterious duplications (Dup22), as opposite to the usual inversions (18).Inversions taking place in people with 2 duplications (Dup22), as opposite to the usual deletions (Del22), and also probably non-deleterious duplications (Dup22), as opposite to the usual inversions (18). The recombination between int1h-1 and int1h-2 replications from sister chromatids or homologous chro-matids and chromosomes, would cause dicentric chromosomes and acentric fragments and therefore shouldn’t cause feasible embryos. Both, the inv1 and inv22 stop the formation of full-length F8 messenger RNA (mRNA) and end in the lack of F8 proteins resulting in severe HA(21:14).

According to newest evidence, intron 22separating exons 22 and 23 (IVS22) comprises the occurrence of a bidirectional (CpG Island) that starts transcription of two expressed genes (nested genes, F8A and F8B). It’s a portion of a GC rich sequence of 9.5 kilobyte (int22h-1) that’s repetitive at 2 positions towards the Xq-telomere (int22h-2 and int22h-3) (20). Within the view of Youssoufian et al. (1986), these comments indicated that Cp Gdinucleotides are mutation hotspots. It had been assumed that methylated cytosines is also mutation hotspots as a result of 5-methylcytosine will impulsively deaminize to thymine, leading to a C-to-T transition in DNA (22). This CpG is land was related to a 1.8 kilobyte transcript raised to as A gene (F8A). The nested F8A gene was placed in opposed direction to that of F8 and contained no intervening sequences (23; 24). Freije and Schlesinger (1992) consequently showed that the X-chromosome contains 3 copies of F8A and its adjacent regions, one in intron 22 and two telomeric and just about five hundred kilobyte upstream to the F8 gene transcription begin site(25)while F8Btranscript of 2.5 kb, that originates from identical F8 intron22 CpG island as F8A and transcribes within the similar direction as F8.This CpG isl and seems to act a bidirectional promoter for the F8A and F8B genes, that are together expressed ubiquitously in various tissues . The different transcripts F8A and F8B start from inside122 bases of every begin point (24).

F8A gene was shown to code for a forty kDhuntingtin-associated protein, termed HAP40 and is supposed to be concerned within the aberrant nuclear localization of the huntingtin protein in Huntington disease (26). Lakich et al. (1993) revealed that intron 22 was uncommon in various respects. Containing 32.8 kb, it’s the largest intron within the F8 gene. Both disease-causing mutations and neutral polymorphisms seem de novo in every new generation. Given an assessed world population of 7 • 10^9 people and a mean mutation frequency of 10-8 per base pair and generation, it is clear that all changes companionable with life are seemed within the human population; that’s, all positions are going to be mutated over once (27).

This mutation (intron 22 inversion) occurred of roughly 4 × 10−6 per gene, per gamete, per generation (15, 28),(29). Inv22-positive patients presented higher risk for increasing inhibitors compare with patients resounding other severe mutations (30).

II. The Aims of Study

The purpose of this research in this study is to detection the existence of intron 22 mutations within the
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non-coding FVIII gene of ten sex-linked disorder (hemophilic Iraqi) carriers families by polymerase chain reaction (PCR) via Multigene Optimax (Thermo Cycler) device and direct sequencing for inversion analysis. Intron 22 primer with 1916 base pairs products demonstration in figures no.(1),(2),(3) by using different programs for these mutations analysis containing (NTI vector Pro., Clustal W method of MEGA4 pro., NCBI/ BLAST program, Chromos Pro, Mutation Surveyor).

Figure 1: PCR products of FVIII gene on 2% agarose gel at 70 voltages for one hour. Intron22.

Lane 1T: Lane-M-standard molecular weights: Lane 2, 3, 4, 5, 6, 7, 8, 9, 10, C, 11: Lane 12T. Gel was stained with Ethidiumbromide staining. *C for carriers; T for control

Figure 2: PCR products of FVIII gene on 2% agarose gel at 70 voltages for one hour. Intron22.

Lane-M-standard molecular weights: Lane 1T: Lane 2, 3, C. Gel was stained with Ethidiumbromide staining.
*C for carriers; T for control
Intron22 - Lane-M-standard molecular weights: Lane 1T: Lane 2, 3, C. Gel was stained with SYBER Green staining.
* C for carriers; T for control.

Collection of samples
This study has enclosed ten Iraqi carriers with classical hemophilia (hemophilia A) from unrelated families and five healthy members as control, were collected from Al-Karama teaching hospital, in Wasit province-kut-city. The age of carriers were ranged from twenty four to sixty four year

III. Methods
All samples study of hemophilia A completed in medicine, science college and of AL-Karama Teaching Hospital laboratories. These carriers formerly identified based on family history, DNA testing. And a few information like age, sex, relative state. After checking the extracted DNA for its purity and concentration, its being subjected to amplification to choose area of F VIII, which has intron 22 then Sequencing has been Conducted for intron22 for all carriers and control for molecular analysis that detection of mutation of commonest segment of FVIII gene. Figure (4) shown PCR product of intron 22 for carrier sand control.

IV. Carrier Detection
Approximately 10 percent of females with one F8 pathogenic variant and one normal allele have a factor VIII clotting activity under than thirty percent a bleeding disorder; mild bleeding can take place in carriers with low-normal coagulation factor 8activity (38).

In this study all carrier females are asymptotic because of the lyonization phenomenon and FVIII
activity is over fifty percent that genetic defects are known by family history assessment (39; 40).

Carrier testing by molecular genetic testing is feasible for utmost at-risk females if the pathogenic variant has been known within the family. Factor VIII clotting activity, or its ratio to von Willebrand factor level, isn't a reliable check for determinant carrier status: it will solely be suggestive if low, because factor VIII coagulation activity in plasma is augmented with pregnancy, aerobics exercise, oral contraceptive use, and chronic inflammation. Factor VIII coagulation activity in plasma is just about twenty five percent lower in people of blood group $O$ than in people of blood groups A,B, or AB and therefore the majority of obligate carriers, even of severe hemophilia A, have normal factor VIII clotting activities.

V. Results

a) DNA Isolation

The genomic DNA extracted from blood of Hemophilia A patients showed good single band when fractionated by gel electrophoresis as shown in figure no.(5) then checked for their purity and by using spectrophotometer device.

![Figure 5: Chromosomal DNA electrophoresis showing bands on 2 % agarose gel at 70 volt/cm² for 1 hour.](image)

Lane (1) – C, Lane 2 C, Lane 3 C, Lane 4 – C, Lane 5 C, Lane 6 C, Lane 7 C, Lane 8 C, Lane 9 C, Lane 10 C, Lane 11 T, Lane 12 T, Lane 13 T. Gel was stained with Ethidium bromide staining and using loading dye.

*C for carriers; T for control

b) DNA sequence analysis

Sequencing has been run for all the exons and intron 22 for all patients and control for process of determining the exact order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases (adenine, guanine, cytosine, and thymine) in a strand of DNA. The analysis of nucleotide sequencing was done by using NCBI/Blast computer program. Nucleotide sequences were translated into amino acid sequences also by using the Blast program. Each DNA sequence obtained was aligned with reference F VIII gene sequence that means reference Genomic DNA for intron22 then, same sequence being aligned with Mutation Surveyor software to check the normal variation and checking amino acid change.

The study was done for 10 hemophiliac carriers (mothers), and 5 control samples, to detect intron 22 inversion which responsible for hemophilia disease. All control samples were obtained from female gender. We found Inv22 mutations in 4 from 10 carriers. During the screening for Inv22 mutations among the HA carriers and controls, we did not found this mutation or gene abnormality in all controls. Family history and consanguinity state of haemophilia was recorded in some carriers. Percentage of Hemophilic carriers group data is depicted in (Table 1).
Mutations screening conducted throughout the study shows that most common mutations and located in, intron 22. Carrier no. 3 appears in this study aligned was regarded as first carrier detect with intron 22 inversion of the FVIII gene reveal with no family history and consanguinity state as showed in figures no(6,7).

Table 1: Percentage of Hemophilic Carriers Group Data

<table>
<thead>
<tr>
<th>Carriers sample no.</th>
<th>Mutation segment</th>
<th>Mutation\Genome</th>
<th>Mutation type</th>
<th>Family history</th>
<th>Consanguinity state</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intron 22</td>
<td>nill</td>
<td>-</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>2</td>
<td>Intron 22</td>
<td>nill</td>
<td>-</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>Intron 22</td>
<td>Inth22</td>
<td>Inversion</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>4</td>
<td>Intron 22</td>
<td>Inth22</td>
<td>Inversion</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>5</td>
<td>Intron 22</td>
<td>Inth22</td>
<td>Inversion</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>6</td>
<td>Intron 22</td>
<td>nill</td>
<td>-</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>7</td>
<td>Intron 22</td>
<td>Inth22</td>
<td>Inversion</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>8</td>
<td>Intron 22</td>
<td>nill</td>
<td>-</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>9</td>
<td>Intron 22</td>
<td>nill</td>
<td>-</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>10</td>
<td>Intron 22</td>
<td>nill</td>
<td>-</td>
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<td>negative</td>
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<td>Total</td>
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<tr>
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<td>No</td>
<td>Positive</td>
<td>Negative</td>
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<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>60%</td>
<td>40%</td>
<td>40%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Figure 6: DNA sequencing (forward) for carrier no. 3 detect with Inv22.
Figure 7: DNA sequencing (reverse) for carrier no. 3 detect with Inv22.

Carrier no. 7 appears in this study aligned was regarded as first carrier detect with intron 22 inversion of the FVIII gene reveal with positive family history and consanguinity state as showed in figures no(8,9).

Figure 8: DNA sequencing (forward) for carrier no. 7 detect with Inv22.
Becker et al. (1996) assessed the male: female ratio of mutation recurrence (k) to be 3.6. By use of the percentages of mutation origin in maternal grandfather to patients’ mother or to maternal grandmother, k values were directly estimated as 15 and 7.5, respectively. As each mutation type separately which an inversion of the gene presented a 10-fold-higher mutation rate in male germ cells. Although intron 22 segment in the non-coding regions of FVIII gene, intron 22 mutations intermittent the F8 mRNA between exon 22 and 23 with large inversion and translocation of nucleotides between these two exons.

Inversion of intron 22 (inv22) originates 50% of cases of severe HA and is a major risk factor for inhibitor development and The non-significant risk for developing inhibitors among inv22-positive patients agrees with the variety of genetic and non-genetic factors involved in such a complication. Other normal changes in genomes (normal variants) not indicated in all carriers VIII gene which all intron 22 involved have been aligned and compared the all possible variants.

**VI. Discussion**

The current study examined different properties of mutations carrying F8 haplotypes. This information was used to infer whether same mutations. Carrier females have a 50% chance of transmitting the F8 pathogenic variant in each pregnancy: sons who inherit the pathogenic variant will be affected; daughters who inherit the pathogenic variant are carriers. Affected males transmit the pathogenic variant to all of their daughters and none of their sons.

**Intron 22 Mutations Frequency Percentage**

In this study, four from ten Iraqi carrier females from ten unrelated families were had intron 22 mutationas showed in figure (10).
The mutation is forecast to impair attachment to the factor VIII (FVIII) carrier protein, von Willebrand factor, and thus increased clearance of FVIII from plasma. Clinical and molecular characterization of these carriers is essential to raise follow-up, genetic counseling and treatment of the disease (33). Increased risk are probable if the F8 pathogenic variant has been identified in a family member or if informative (family history) intragenic linked markers have been recognized which genetic counseling deals with genetic risk valuation and the use of family history and genetic testing to explain genetic status for family members. In this study six from ten carriers are with a hemophilia history (60%) which 3 from four carriers have (Inv22) mutations with positive family history represents a major factor for genetic predisposition lead to defective FVIII gene. Carrier no.3 appears in this study aligned was regarded as first carrier detect intron 22 inversion of the FVIII gene reveal with no family history and consanguinity state. There are several clarifications for a hemophilic carrier being identify with inv22 when there is no history of hemophilia in the family which about 30 percent of these cases arise from aspontaneous mutation.

1. The mother is a carrier of a new disease-causing mutation that occurred in one of the following ways:
   - As a “germ line mutation” (i.e., in the egg or sperm at the time of her conception so the mother is then the first person in the family to transmit hemophilia. Her children might be influenced either as carriers or as hemophiliacs (34). And thus show in every cell of her body and noticeable in her DNA). Ninety-eight percent of mothers of a simple case with an intron 22 inversion are carriers because most of these mutations arise in spermatogenesis.
   - As a somatic mutation (i.e., a alteration that arisen very early in embryogenesis, subsequent in somatic mosaics in which the pathogenic variant is current in some but not all cells and may or may not be obvious in DNA).
   - As germ line mosaics (in which some germ cells have the pathogenic variant and some do not, and in which the pathogenic variant is not evident in DNA from her leukocytes).

2. The mother is a carrier and has inherited the pathogenic variant either from her mother who has a new disease-producing variant or from her asymptomatic father who is mosaic for the pathogenic variant.

3. The mother is a carrier of a pathogenic variant that rose in a previous generation and has been send on through the family without manifesting symptoms in female carriers due to the ionization which hemophilia does certainly run in the family but there is no indication of it because no hemophiliac boys have been born (35:36).

General, the mother has an roughly 80% chance of being a carrier when her son is the first influenced individual in the family; however, the mother of a severely affected male with an intron 22 inversion has a 98% chance of being a carrier (37) and about 40% of carriers (four) under study with consanguinity marriage that one from four carriers have (Inv22) mutations with positive consanguinity marriage result in concentrated the bad gene copy. Figure no. (12) showed DNA sequencing for carrier no.7 detect with intron 22 mutation in factor 8 gene and represent positive family history and consanguinity state and Figure no.(11) below showed alignment of hemophilic carrier no.7 detect with intron 22 mutation and control of selected intron 22 sequence with the genomic DNA reference in deep details and represent positive family history and consanguinity state.
VII. Conclusion

Hence, present study indicated that detection of Intron 22 mutations in F8 gene is important in identifying females with genetic defects that lead to the birth of sons affected with hemophilia, a disease and females almost as carriers. This result represents a step for helpfully guide the direction of molecular study in genetic counseling and subsequent for facilitate management in labour and for prenatal diagnosis also for prevention of
the inhibitor development which inversion of intron 22 (inv22) is a major risk factor involved in such a complication. This knowledge represents a step. Most of cases are with a family history (60%) represent a major factor for genetic predisposition lead to defective FVIII gene and about 40% of carriers under study with consanguinity marriage result in concentrated the bad gene copy so this is highly suggestive that hemophilia disease is not uncommon. There is an obvious public ignorance about the role of heredity in many disorders in Wasit province.

References Références Referencias


