Importance of Molecular Genetic Analysis in Iranian Families with Congenital Bleeding Disorders

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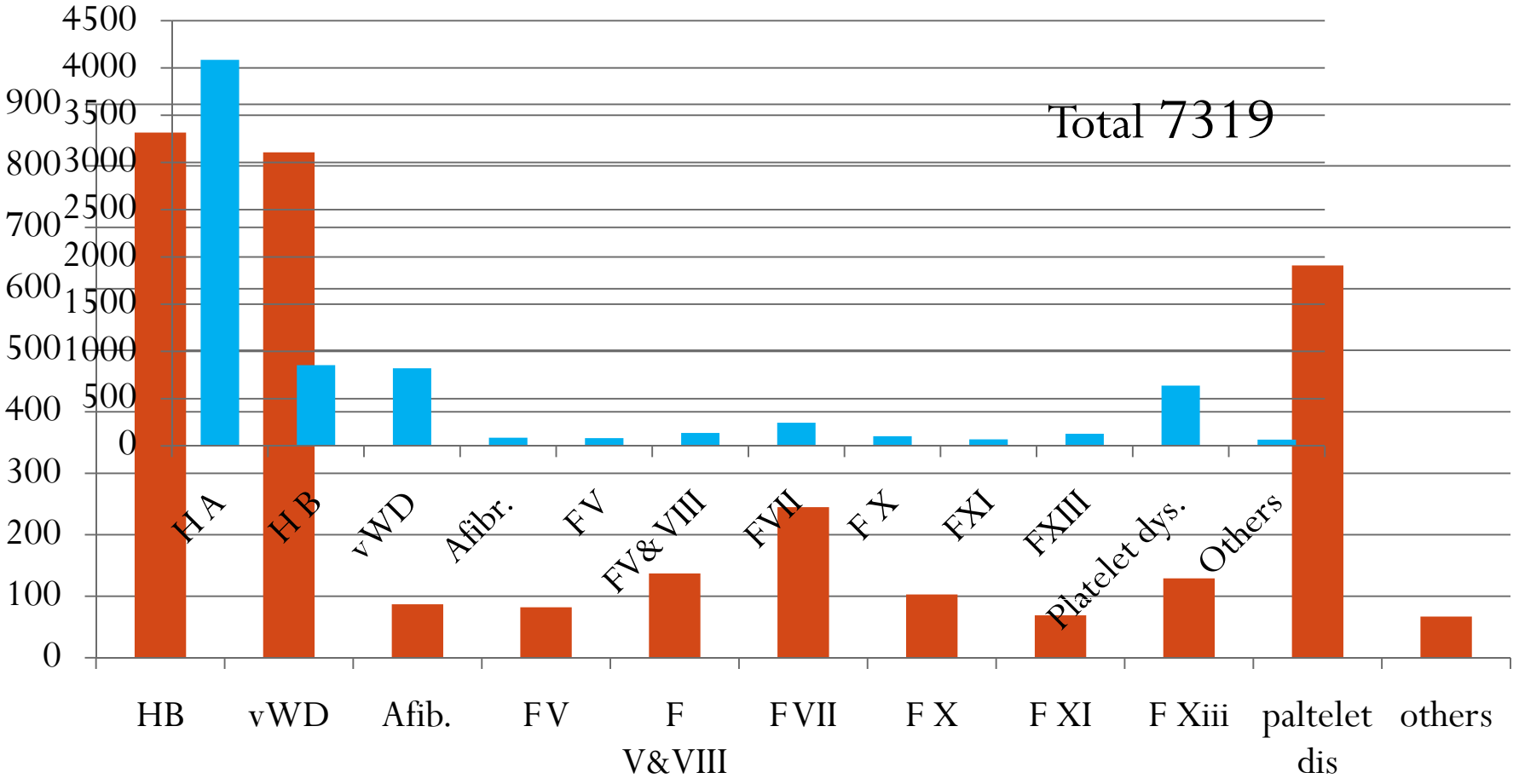
What are congenital Bleeding Disorders?

- **Hemophilia A and B. the most common inherited coagulation disorders, X-linked**
- **Other deficiencies of coagulation factors I, II, V, VII, V+VIII, X, XI , XIII and platelet disorders and VWD, autosomal recessive**

Prevalence of Bleeding Disorders in Iran

Source: CHCC database 6/11/2008



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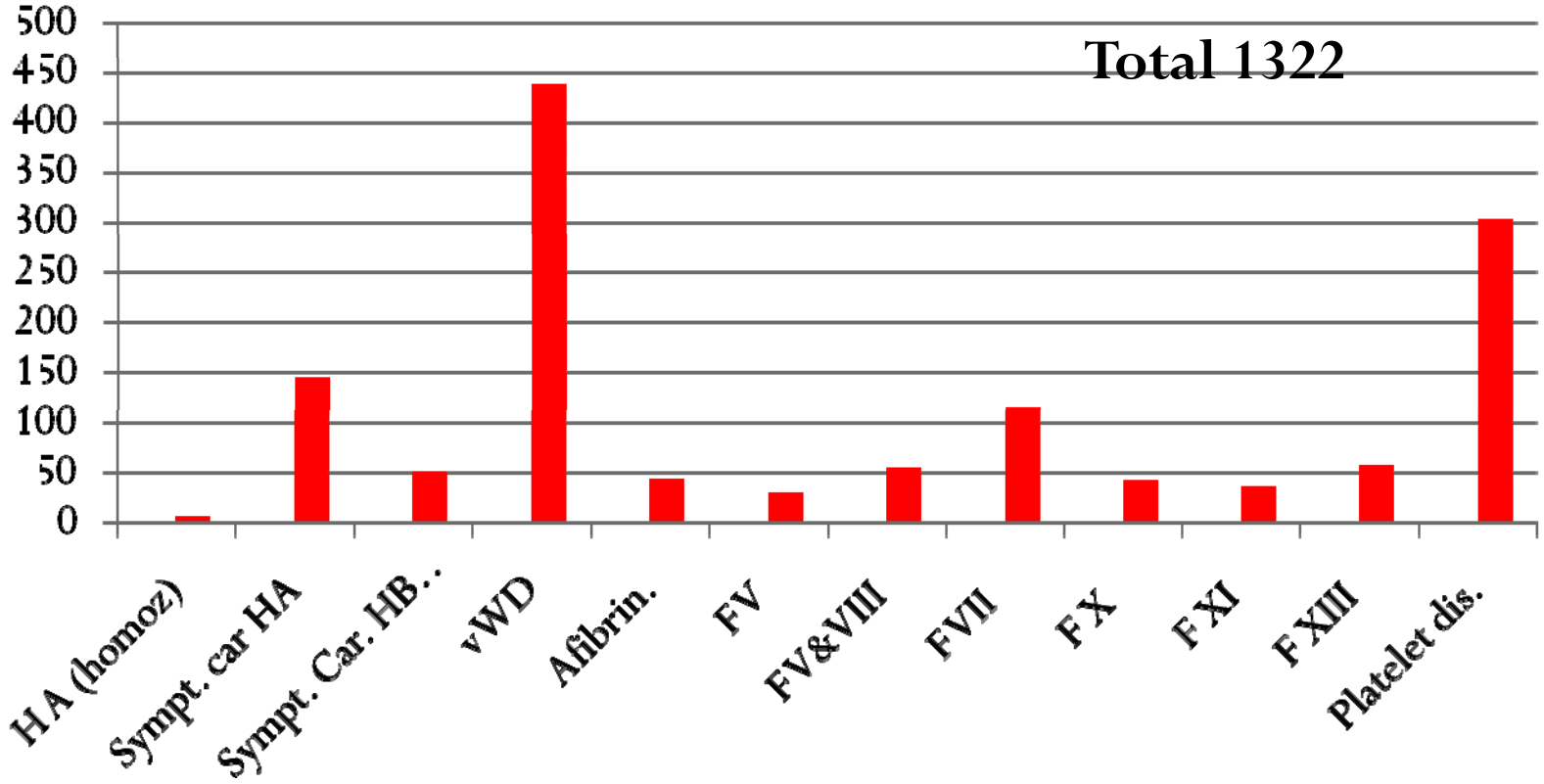
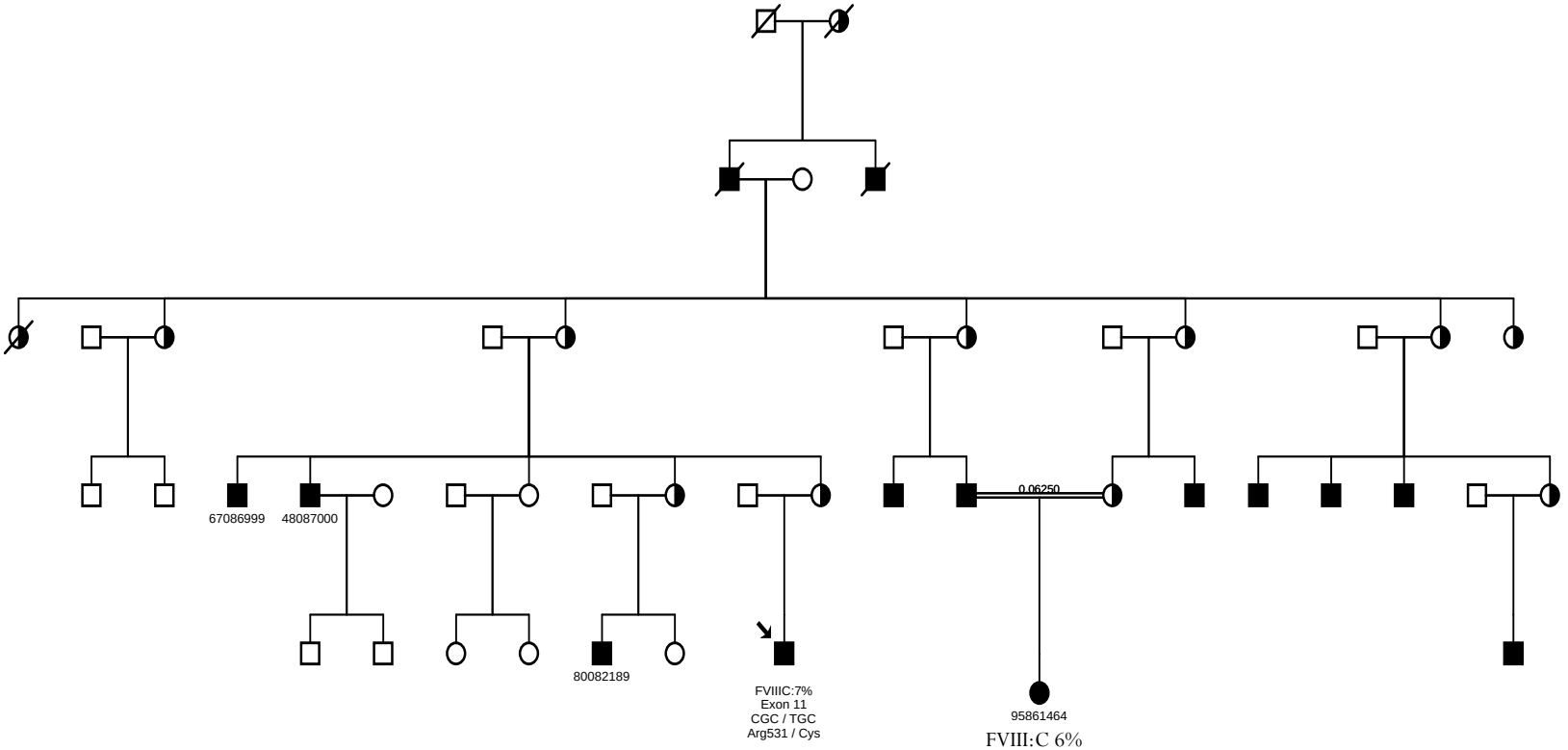
Prevalence of Bleeding Disorders in Iranian women

Source: CHCC database 6/11/2008



Why consanguineous marriage is favored?

- Easy acceptance of genetic disorder or carrier status in close relatives
- No religious and social differences
- Family economic considerations
- Parental pressure for marriage



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Challenges for Hemophilia Care

- Diagnostic facilities
- Professional medical personnel
- Providing costly, safe and efficacious therapy
  - Timely and equitable distribution of blood products

Difficulties of Hemophiliacs in developing countries

- Damaged joints associated with crippling arthropathy
- Frequent hospitalization
- Blood born infectious diseases
- Grim educational and career prospect
- Isolation and no engagement with peers, community
- Impact on relationships and marriage
- Drug dependency

Difficulties of Hemophilia within the family

- Mothers; impaired self-esteem, guilt, fear, anxiety, sadness,
  - Marriage (vulnerable situation)
  - Society in-laws pressure
  - Future pregnancies
  - Abortion
- Sisters; carrier-ship affects sisters lives
  - Anxiety re giving birth to unhealthy sons
  - Stigma (less desirable for marriage)
  - Risk of bleeding (menorrhagia)
- Fathers;
  - Little awareness of hemophilia
  - Financial stress
  - Temptations; want healthy sons, remarriage, leaving family

What is comprehensive Hemophilia Care?

- multidisciplinary approach
  - Hemophilia specialist
  - Nurse
  - Physical therapist
  - Psychologist
  - Dentist
  - Orthopedist, rehabilitation specialist
  - Coagulation laboratory
  - Clinical geneticist
    - Extensive pedigree, family history
    - DNA mutation analysis
    - Genetic counseling

What is the purpose of genetic analysis?

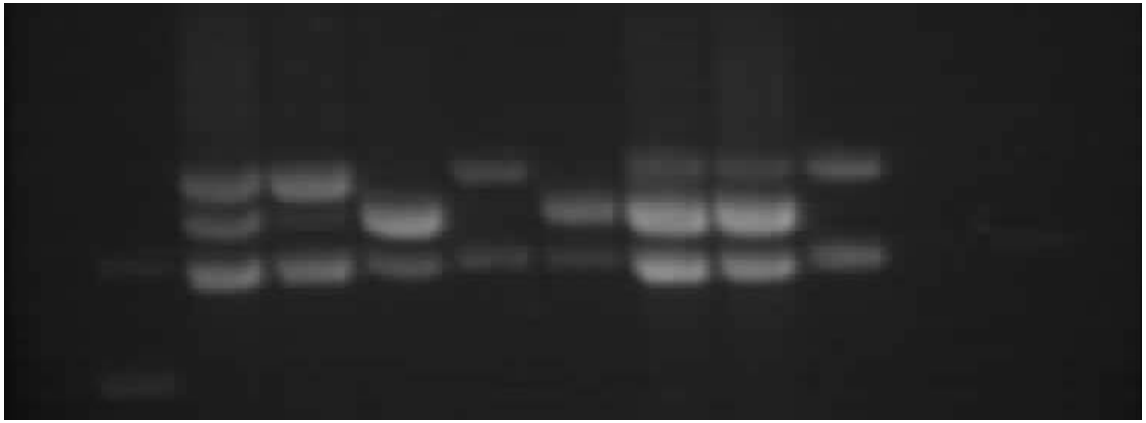
- Identify the causative mutation in individuals with hemophilia and other congenital bleeding disorders
- Determine the carrier status within the family
- Identify an affected fetus early in pregnancy
- Prediction the possibility of alloantibody formation

Strategies for mutation detection

- Inversions intron I and 22 for severe HA
- Prescreening of each exon with PCR-CSGE method (multiplex-PCR)
- Sequencing for abnormal fragments
- Linkage analysis for HA , HB
- Since Dec. 2008, direct sequencing

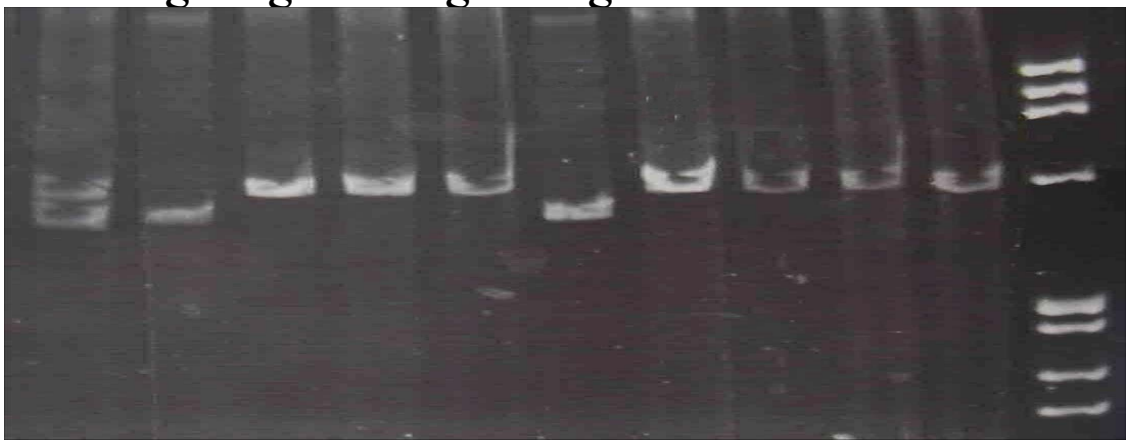
PCR detection of factor VIII intron 22 inversion

12 kb
11 kb
10 kb



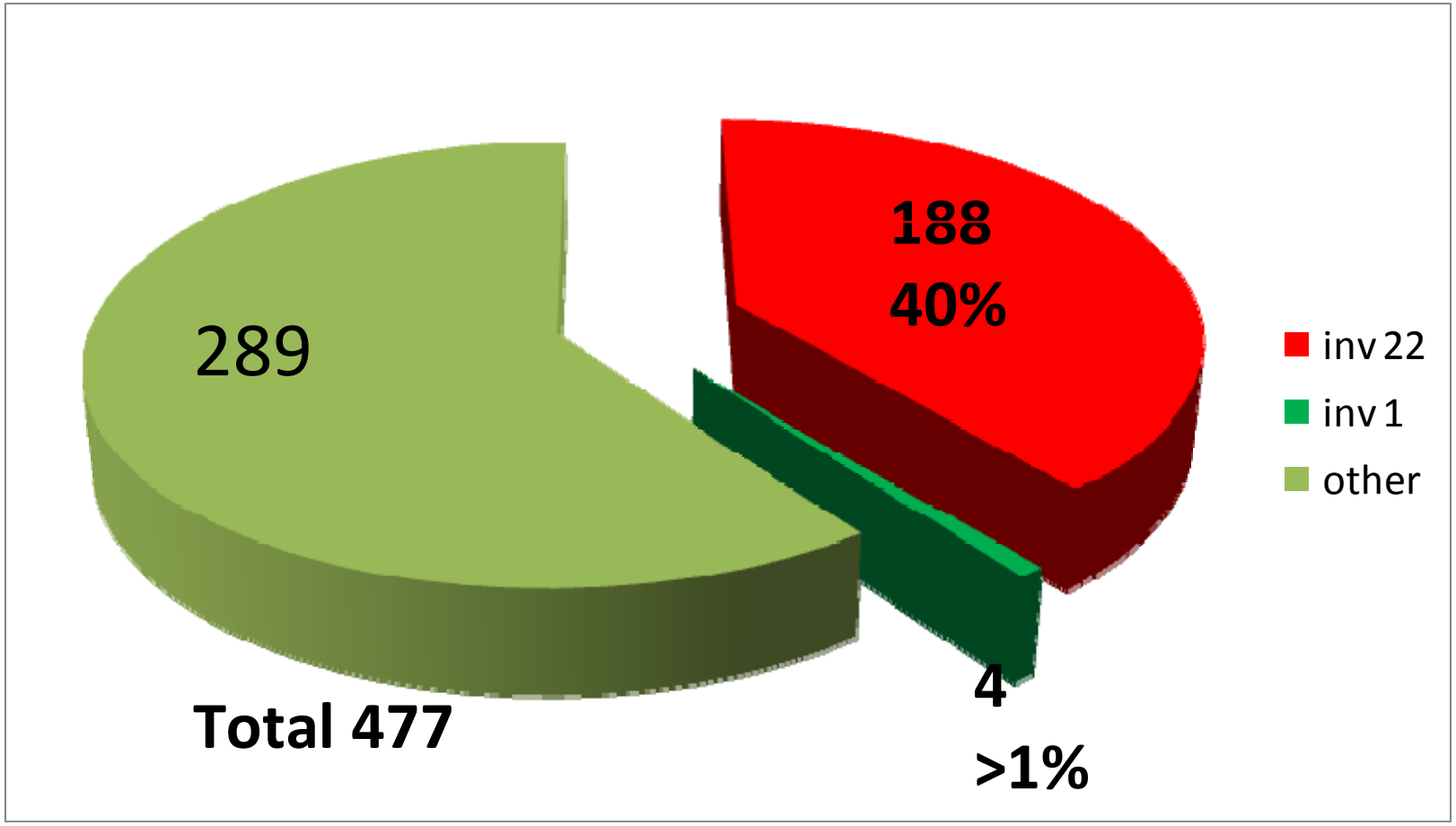
Long range PCR agarose gel

559 bp
487 bp

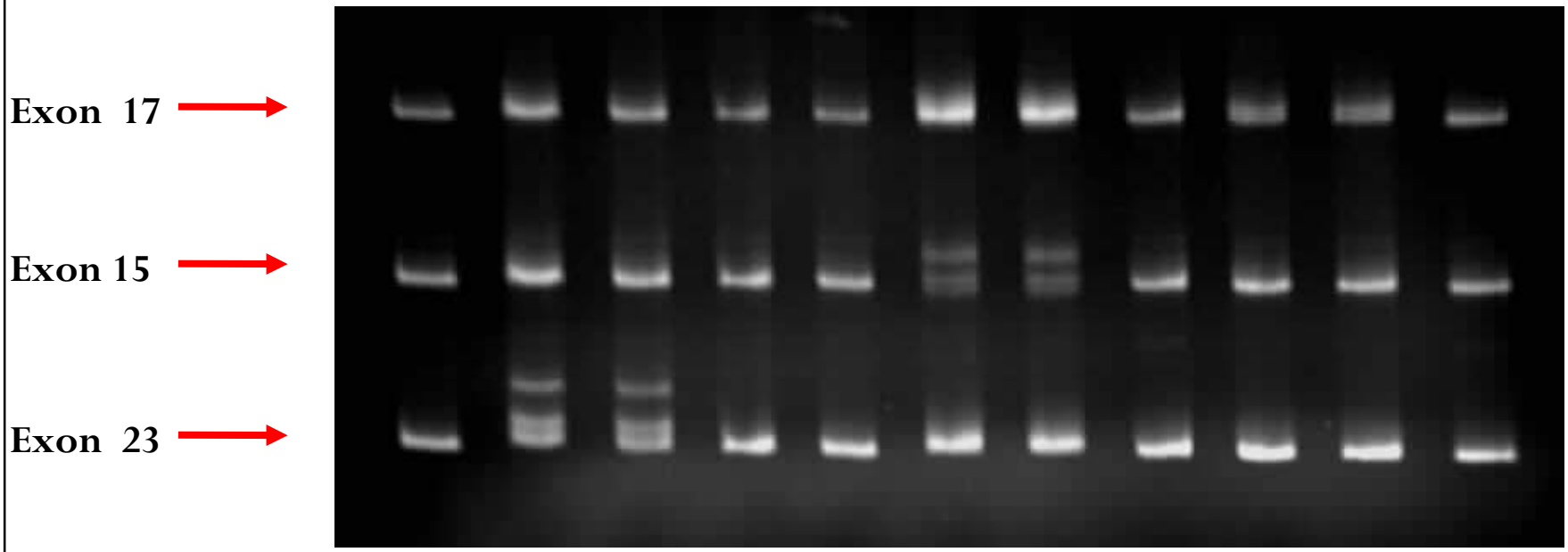


Inverse PCR polyacrylamide gel

Rearrangements detected in severe Iranian HA

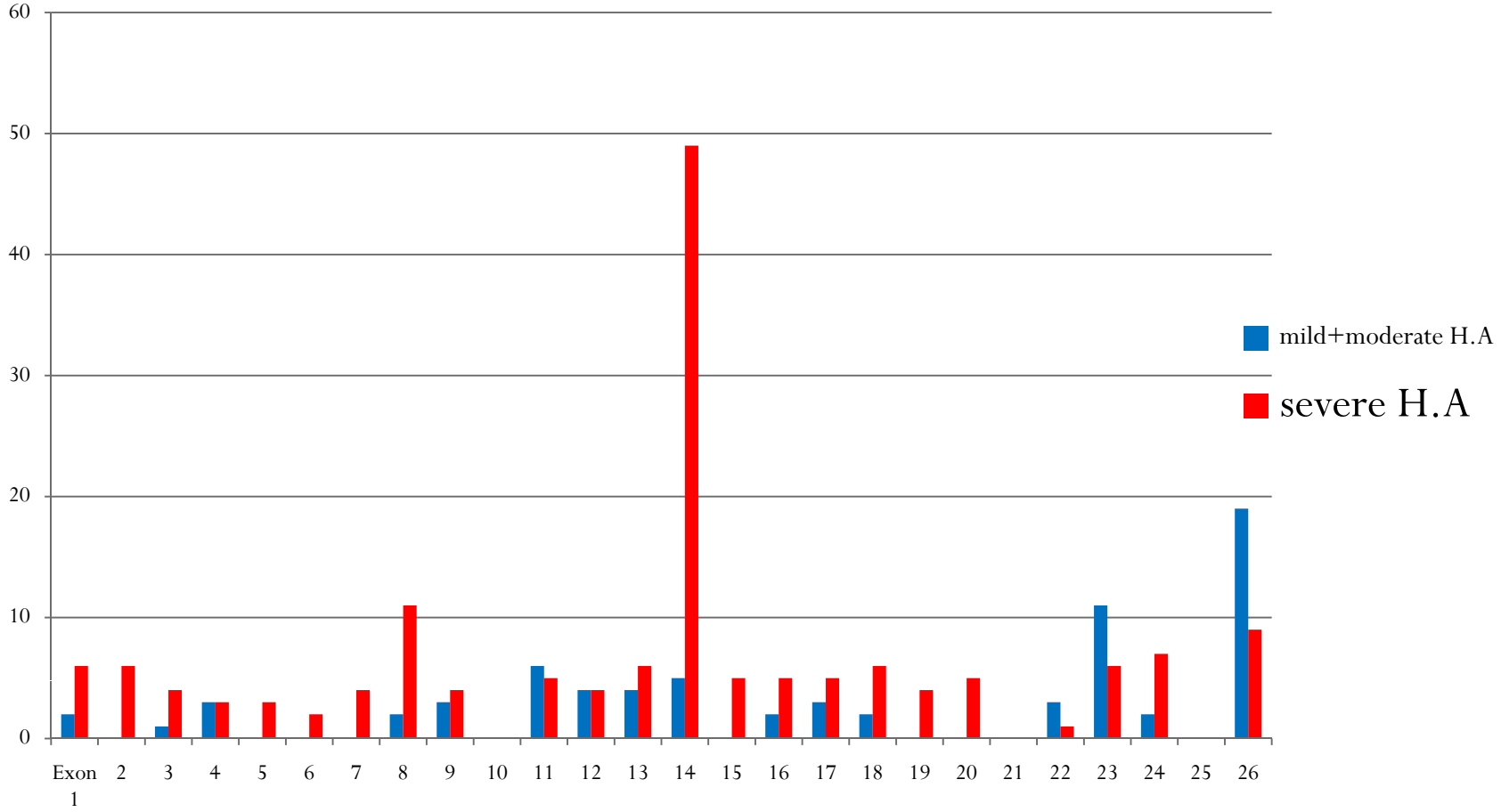


Typical band migration differences by a Multiplex CSGE gel



Only exons with abnormal band pattern were sequenced using an automated ALF Express Sequence instrument

Mutations detected in HA



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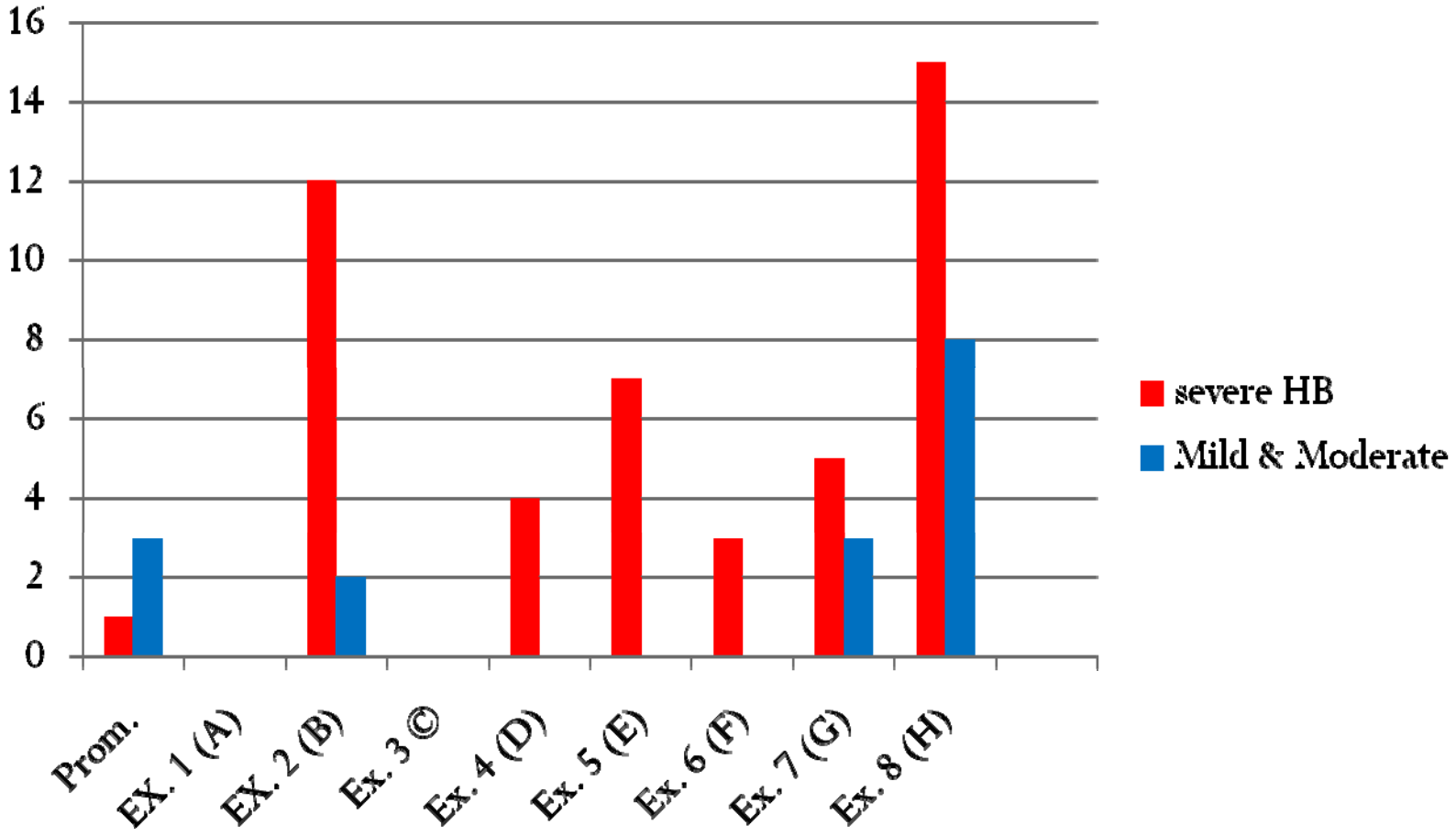
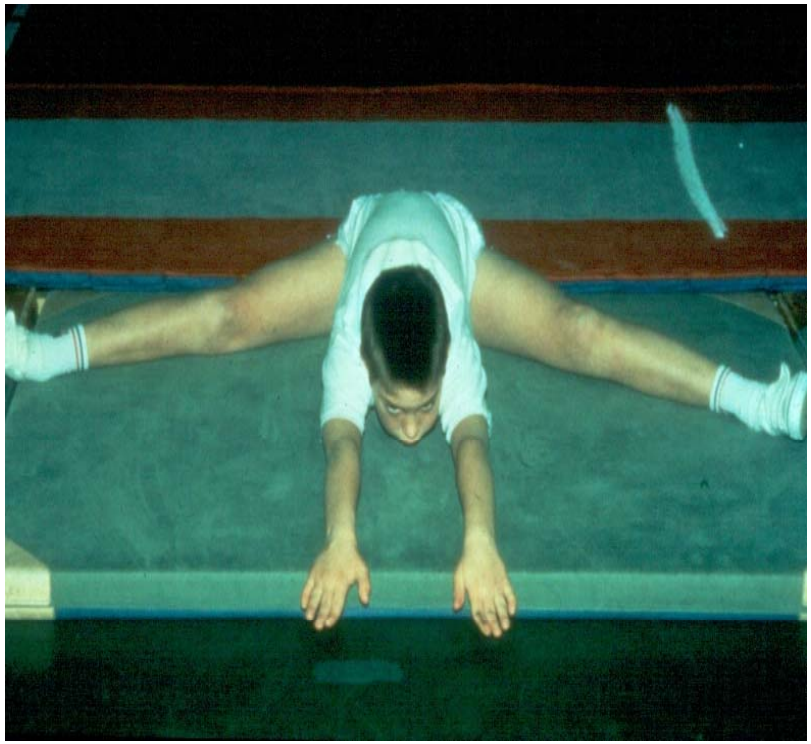
Mutations detected in HB

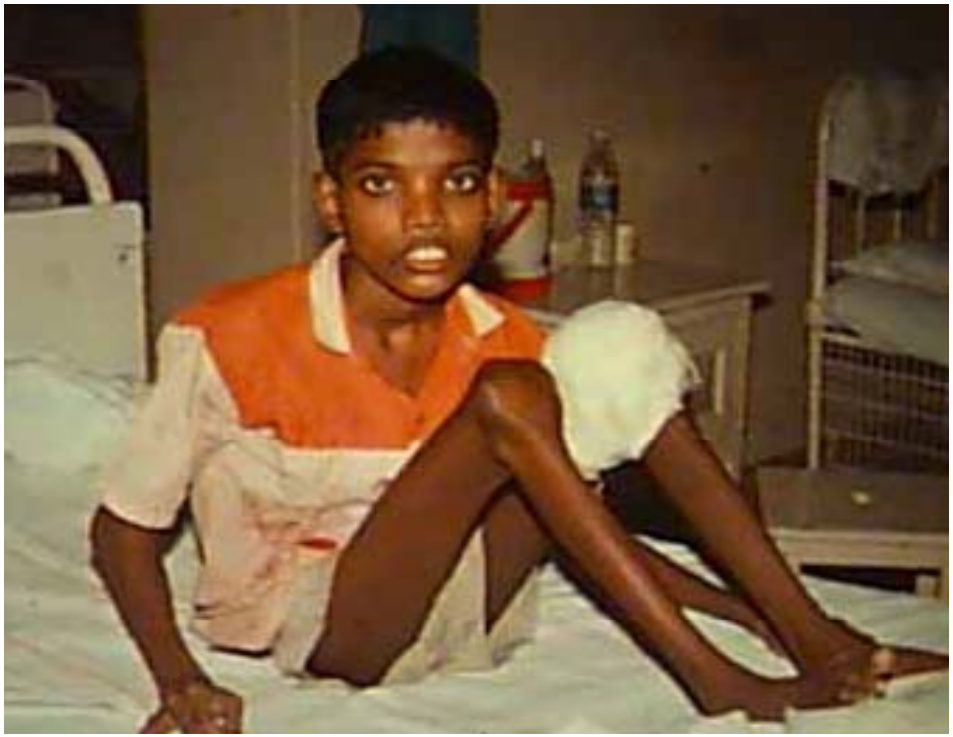


Providing Genetic Services in the CHCC

- Genetic counseling 4396 cases
- Consent and sample 1776
- HA & HB families 545
- FVII, FXIII, FX, FXI, BS. Type 2 vWD 233
- Identified mutations 492
- Identified female carriers HA/B 669
- Identified females not carriers HA/B 395
- PND (CVS)
  - Hemophilia A 32
  - Hemophilia B 4
  - FX (severe) 3

Where to be born with Hemophilia?





Conclusions

- **Establishment of a national reference genetics laboratory for congenital bleeding disorders, expertise in genetic analysis is essential for developing countries**
- **Carrier needs should be addressed**
  - Establishment of genetic counseling services within the hemophilia centers
  - Identification of obligate carriers (sporadic 30%)
  - Identification of probable carriers with a comprehensive family history

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