

# ANALYSIS OF HIND III MARKER OF FACTOR VIII GENE AND ITS APPLICATION FOR CARRIER DETECTION

*by*

Dr Saima Bugvi

Dr. Saqib Mahmood

Dr. Tahira Tasneem



# Hemophilia A

- X-linked recessive haemorrhagic disorder
- caused by molecular defects in gene encoding coagulation factor VIII (FVIII)
- X- linked disorder it affects males almost exclusively
- Females are the carriers of the disease
- Carrier detection and prenatal diagnosis of HA is important for preventing the birth of children with HA



## LINKAGE ANALYSIS OR INDIRECT GENE TRACKING

- Is the method most commonly used to determine the female carrier status
- Indirect gene tracking is accomplished by polymorphic markers
- Hind III is the commonly used polymorphic marker which can be analyzed by PCR



- Genetic polymorphisms represent natural variations in the genome sequence.
- These are special segments of DNA that are located very close to the gene on the same chromosome
- These segments nearly always travel with the gene when it is passed from parent to child .these segments of DNA are called polymorphic markers.



- There is considerable ethnic and geographical variations in the allele frequencies of these polymorphisms.
- There is need to establish the allele frequency for various factor VIII gene markers in our population.



- Females with a normal karyotype (46,XX) have two copies of each polymorphism located on the X-chromosome.
- For a given polymorphic locus within these genes a female may have identical alleles (homozygous) or
- she may have one allelic variant in one gene and a different allelic variant in the other (heterozygous).
- Key requirement for linkage analysis is the heterozygosity of the polymorphic marker in the mother of index case .



# Objective of the Study

- To determine the allele frequency and heterozygosity rate *or* (informativeness) of HindIII marker.
- Usefulness of HindIII marker in carrier detection and prenatal diagnosis in our population



# RESULTS

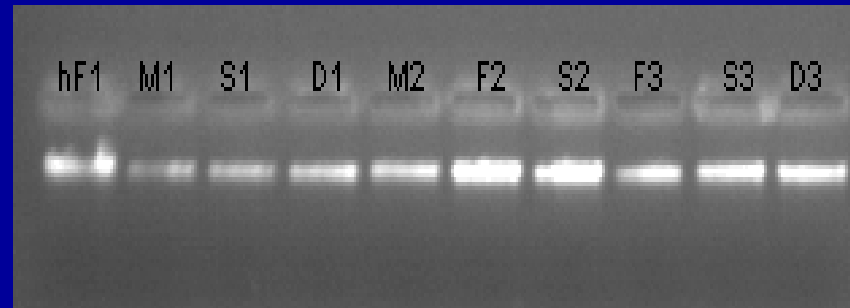
Total Subjects 95

(42 males )

(53 females)

from 20 unrelated families of  
Hemophilia A





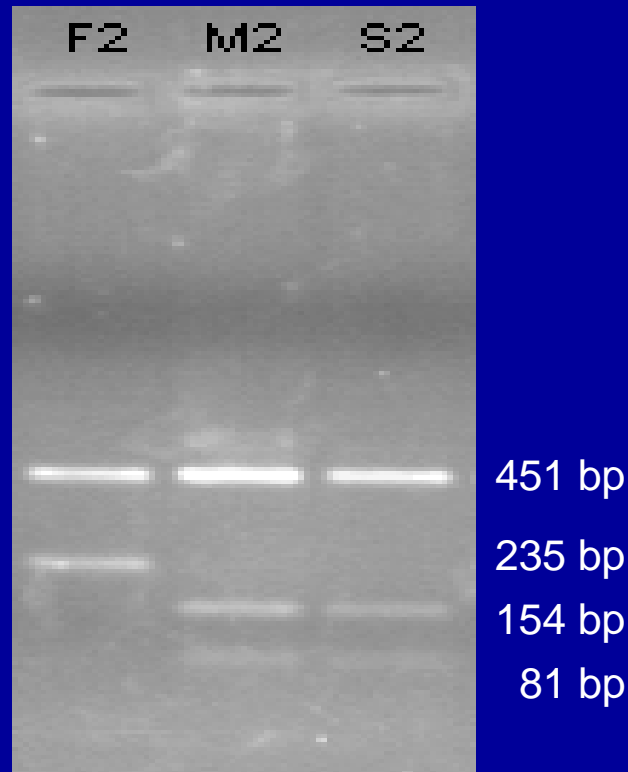
Result of gel electrophoresis to check the quality of genomic DNA after extraction



# Hind III Marker Primer Sequence

Primer designation	Sequence 5'-3'	PCR product Size/bp
Hind III forward	5'TTGGCGAGCATCTACATGCT3'	Product =686bp 451bp 235bp (- allele)
Hind III reverse	Reverse primer 5'CCATTCCCAGGGGAGTCT3'	154bp + 81bp (+ allele) 235bp + 154bp + 81bp (- and + alleles)





Photograph to illustrate positive+ and negative- alleles  
restriction always produce a 451 bp band in addition to  
235bp(-ve) allele and/or 154 & 81+ve allele



# ALLELE FREQUENCY OF INTRON 19

POLYMORPHIC SITE	ALLELE	NUMBER	FREQUENCY
INTRON 19 HIND III	+	60/147	0.41
	-	87/147	0.59



# Expected heterozygosity

Allele1/allele2=0.24

Allele2/allele1=0.24

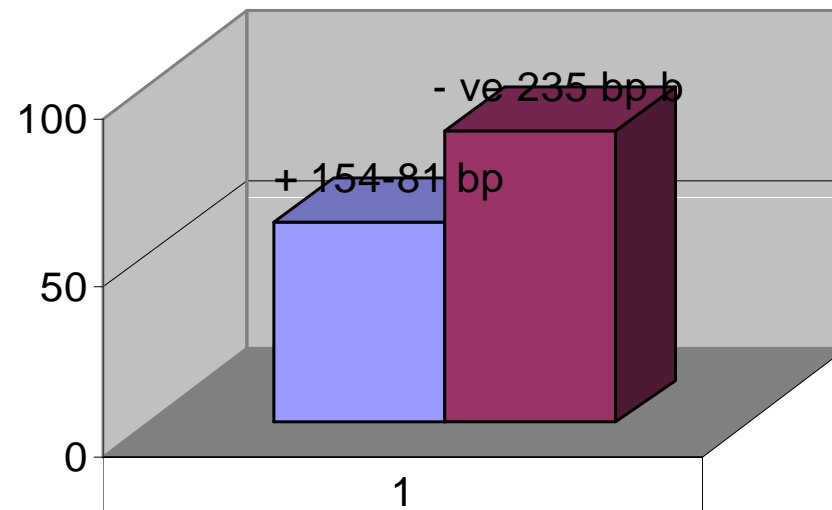
0,24+0.24=0.48 OR

48% of females may be expected to be heterozygous at this locus.



## Allele Frequency

■ + 154-81 bp ■ - ve 235 bp b



■ + 154-81 bp

60

■ - ve 235 bp b

87



# OBSERVED HETEROZYGOSITY (INFORMATIVENESS) OF INTRON 19

TOTAL NUMBER OF FEMALES	HETEROZYGOUS	
	NUMBER	PERCENTAGE
52	24	46 %



# Detection of Carriers in a Family with Hemophilia A using HindIII Marker



Carrier

M- Mother is heterozygous

Sa- Affected son has inherited +allele

Da- Daughter a is carrier, has inherited the diseased allele

Db/Dc-Daughters b &c are non carriers



# Comparison of Allele frequencies of Hind III marker in different populations

	<b>Populations</b>	<b>Positive alleles</b>	<b>Negative alleles</b>
1	Whites	0.25	0.75
2	Chinese	0.24	0.76
3	American blacks	0.78	0.22
4	Pakistanis	0.41	0.59



# Conclusion

- HINDIII RFLP marker is suitable for carrier detection in local population
- We recommend its use in combination with other informative marker to maximize diagnostic efficiency.



THANK YOU

