PCR Applications in Inherited Disorders

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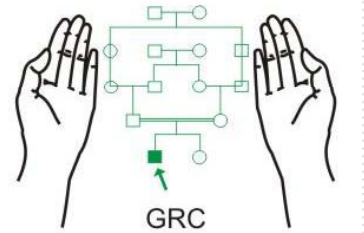
Genetics resource Centre (GRC)



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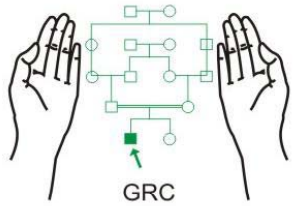
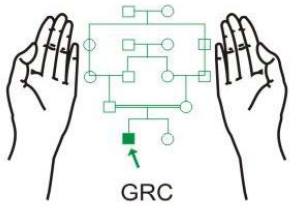


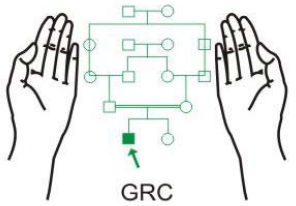
Genetic or Inherited Disorder?



Genetic Disorders

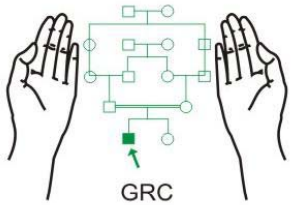
- **Chromosomal Defects**
  - Trisomies (Trisomy 21 Down's syndrome)
  - Monosomies (45,X Turner syndrome)
  - Deletions
- **Single Gene Defects**
  - Autosomal
    - Recessive (Thalassaema)
    - Dominant (Achondroplasia)
  - X-linked (Haemophilia)
- **Polygenic Defects**
  - Diabetes, IHD, Peptic Ulcer etc.

Diagnosis of Inherited Disorders

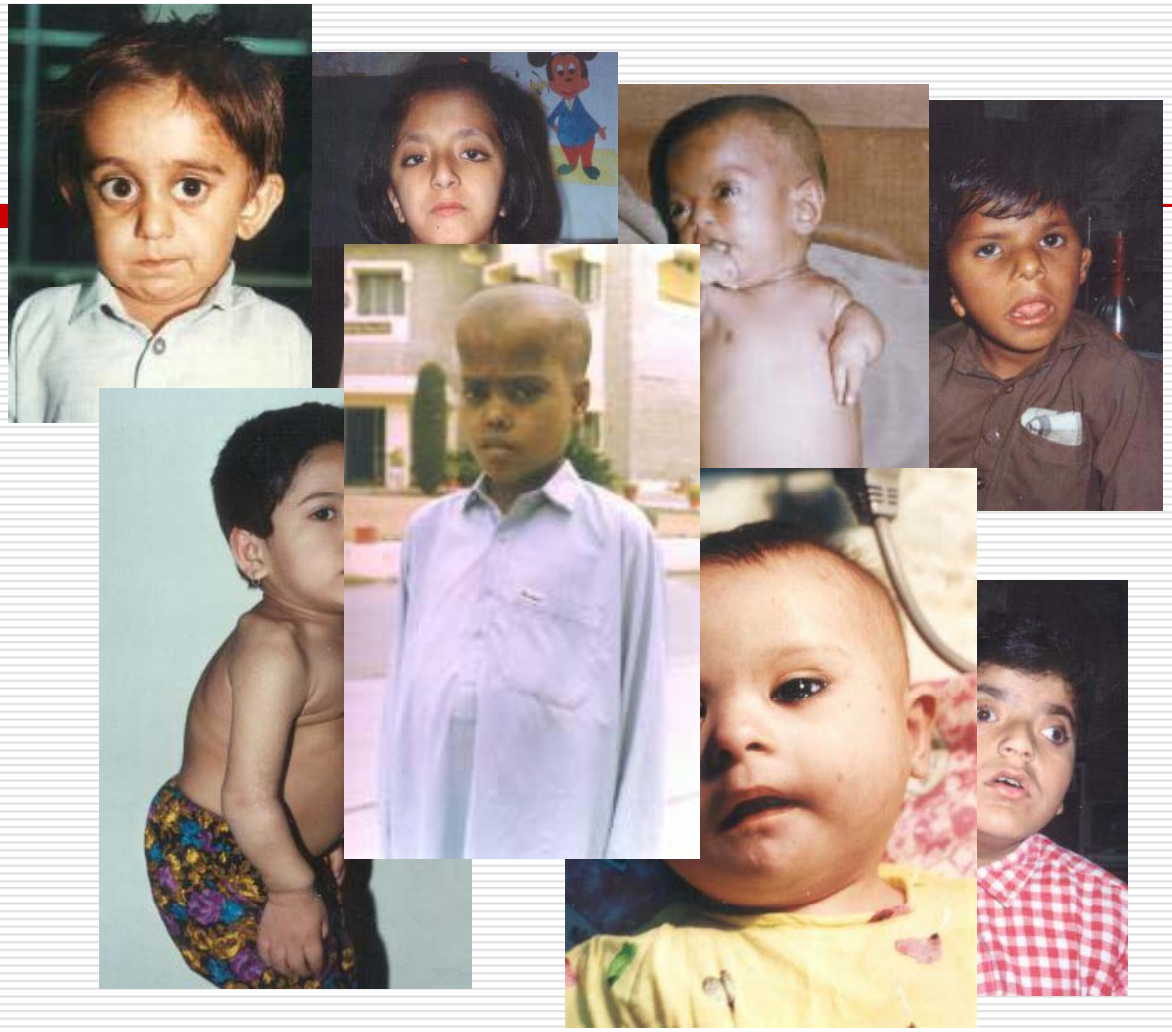


- Clinical presentation
  - Symptoms since birth
- Family history
  - Siblings
  - Cousins
  - Other family members
  - Distant relatives
- Consanguinity

Diagnosis of Inherited Disorders

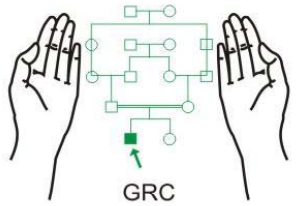


- Routine Tests
- Biochemical Tests
- Cytogenetics
- Molecular Genetics
- Other related investigations



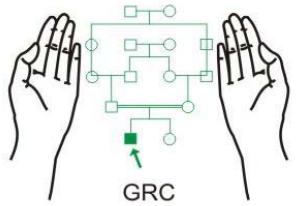
Of the 5000 known Genetic Disorders
2500 can be diagnosed by Genetic Analysis

Diagnosis of Inherited Disorders



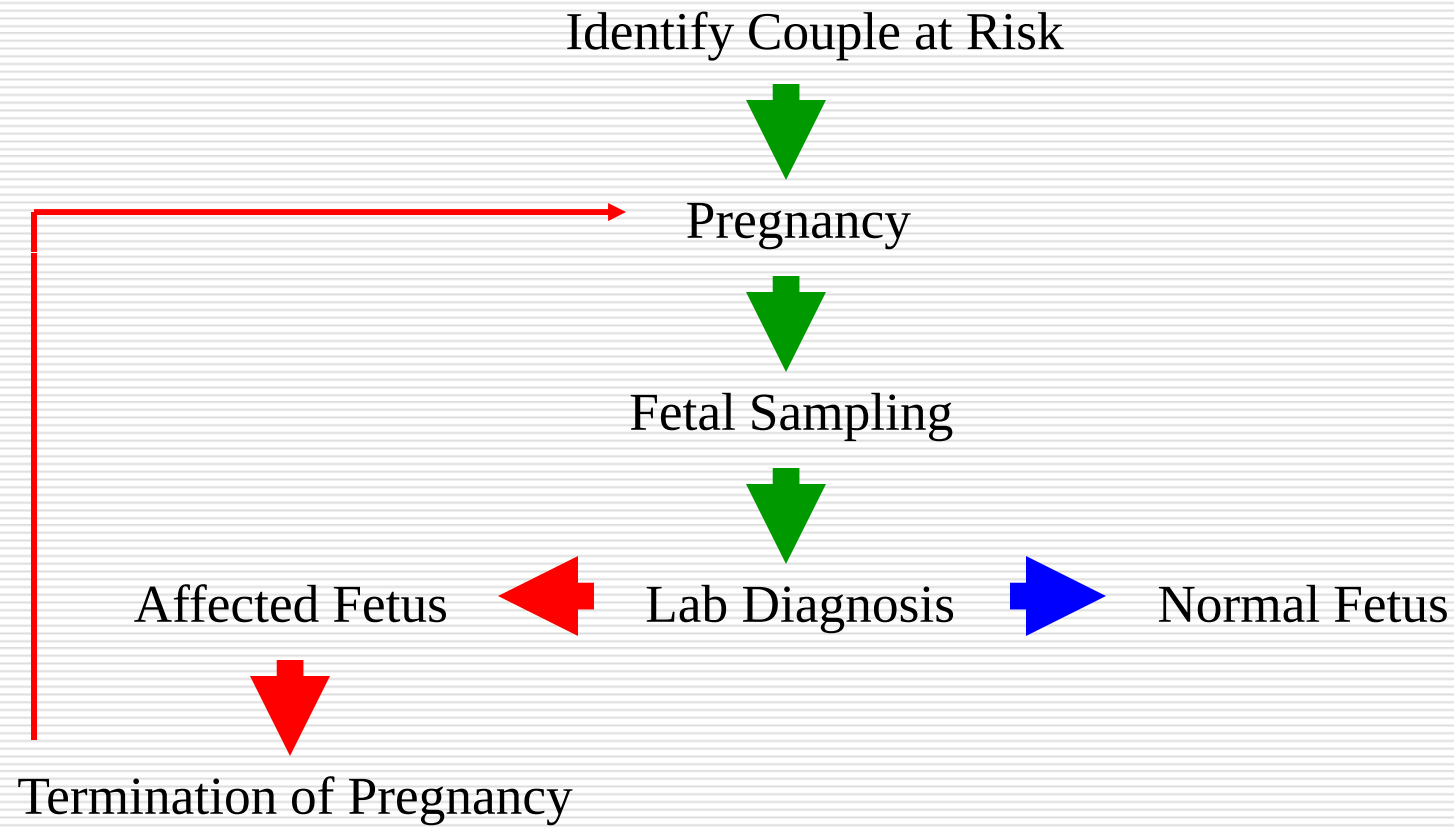
- Prenatal
- Postnatal
- Carrier screening

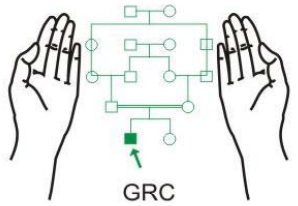
Genetic Diagnosis of Thalassaemia



- Prenatal Diagnosis
- Diagnosis in previously transfused patients
- Silent thalassaemia alleles
- Distinction between structural variants
- Thalassaemia intermedia
- α-thalassaemia
- β-Thalassaemia carriers in certain situations
- Rare thalassaemias

Prenatal Diagnosis



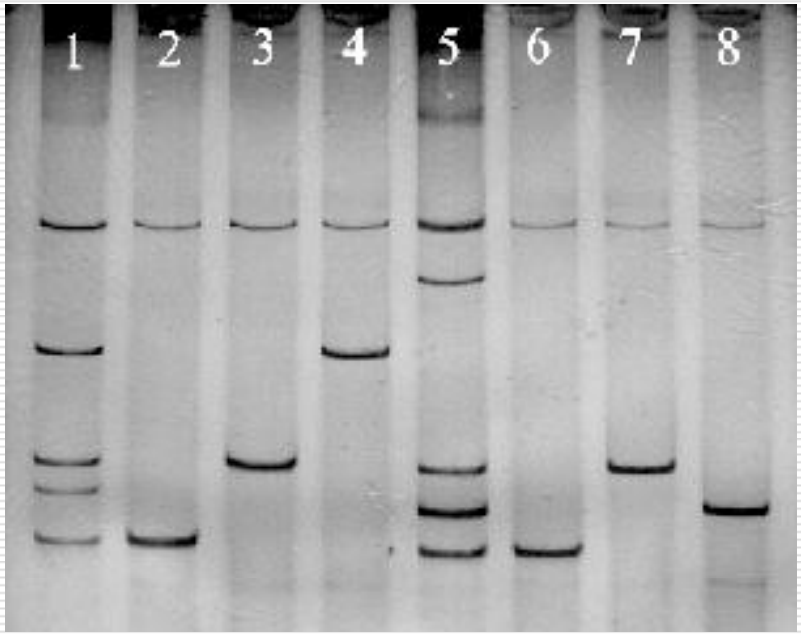


Mutation	Punjabi	Pathan	Sindhi	Baluchi	Mohajir	All
Common mutations						
IVSI-5 (G-C)	107 (27·2%)	27 (12·9%)	114 (43·9%)	131 (76·2%)	75 (41·4%)	454 (37·3%)
Fr 8–9 (+G)	146 (37·2%)	103 (49·1%)	29 (11·2%)	14 (8·1%)	23 (12·7%)	315 (25·9%)
Del 619 bp	14 (3·6%)	4 (1·9%)	36 (13·9%)	2 (1·2%)	29 (16·0%)	85 (7·0%)
Fr 41–42 (−TTCT)	36 (9·2%)	18 (8·6%)	16 (6·2%)	1 (0·6%)	11 (6·1%)	82 (6·7%)
IVSI-1 (G-T)	19 (4·8%)	4 (1·9%)	33 (12·7%)	2 (1·2%)	7 (3·9%)	65 (5.4%)
Uncommon mutations						
Cd 15 (G-A)	14 (3·6%)	13 (6·2%)	5 (1·9%)	9 (5·2%)	8 (4·4%)	49 (4·0%)
Cd 30 (G-C)	15 (3·8%)	1 (0·5%)	19 (7·3%)	3 (1·7%)	4 (2·2%)	42 (3·5%)
Cd 5 (−CT)	11 (2·8%)	16 (7·6%)	0 (0·0%)	1 (0·6%)	2 (1·1%)	30 (2·5%)
Fr 16 (−C)	6 (1·5%)	8 (3·8%)	6 (2·3%)	6 (3·5%)	3 (1·7%)	29 (2·4%)
Cap+1 (A-C)	9 (2·3%)	8 (3·8%)	0 (0·0%)	0 (0·0%)	3 (1·7%)	20 (1·6%)
Hb-E	3 (0·8%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	10 (5·5%)	13 (1·1%)
Cd 30 (G-A)	3 (0·8%)	2 (1·0%)	0 (0·0%)	2 (1·2%)	4 (2·2%)	11 (0·9%)
IVSII-1 (G-A)	6 (1·5%)	1 (0·5%)	0 (0·0%)	1 (0·6%)	2 (1·1%)	10 (0·8%)
Rare mutations						
−88 (C-T)	1 (0·3%)	2 (1·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	3 (0·3%)
IVSI-1 (G-A)	1 (0·3%)	0 (0·0%)	1 (0·4%)	0 (0·0%)	0 (0·0%)	2 (0·2%)
Fr 47–48 (+ATCT)	2 (0·5%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	2 (0·2%)
Fr 126–131 (−17 bp)	0 (0·0%)	2 (1·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	2 (0·2%)
Cd 39 (C-T)	0 (0·0%)	1 (0·5%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	1 (0·1%)
IVSI minus 25	0 (0·0%)	0 (0·0%)	1 (0·4%)	0 (0·0%)	0 (0·0%)	1 (0·1%)
Total	393 (100%)	210 (100%)	260 (100%)	172 (100%)	181 (100%)	1216 (100%)

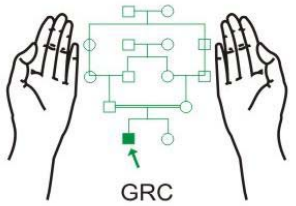
 British Journal of Haematology **94**: 476–482

(Suhaib Ahmed et al, Br J Haematol 1996)

Primer ID:	Mutations Pooled:	Amplified Product size:
AD-1	Fr 8-9 (+G) IVSI-5 (G-C) Fr 41-42 (-TTCT) IVSI-1 (G-T) Del 619bp	215 bp 285 bp 439 bp 280 bp 242 bp
AD-2	Cd 5 (-CT) Fr 16 (-C) IVSI-1 (G-T) Cd 30 (G-C) Cd 30 (G-A) IVSII-1 (G-A)	205 bp 238 bp 280 bp 280 bp 280 bp 634 bp
AD-3	Cd 15 (G-A) Cap+1 (A-C)	500 bp 567 bp

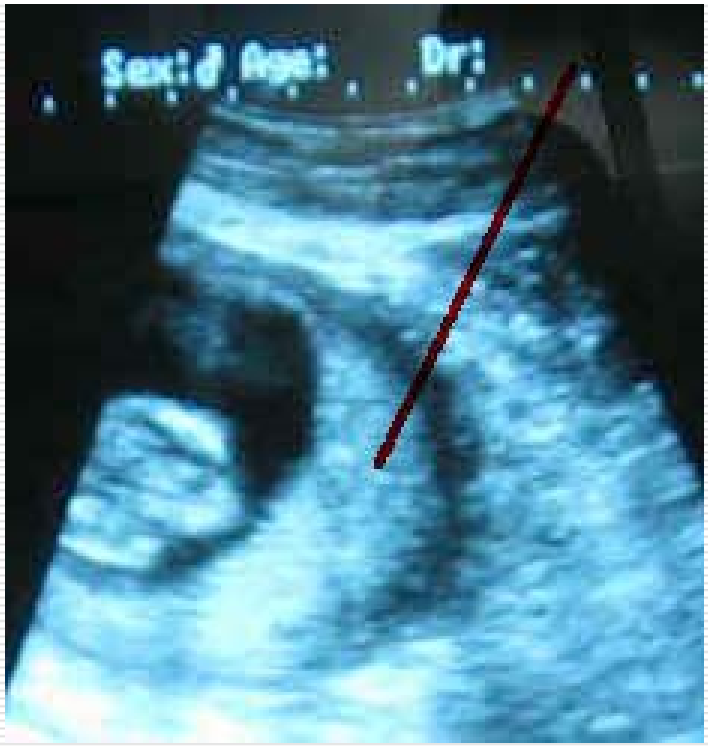


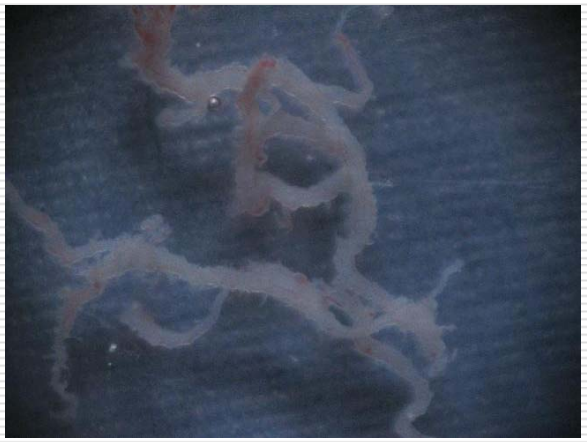
Fetal Sampling

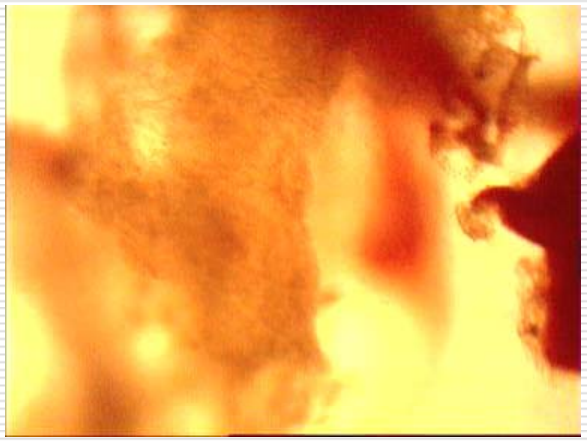


- Invasive techniques
  - Cord Blood Sampling
  - Amniocentesis
  - Chorionic Villus Sampling (CVS)
- Non Invasive Techniques
  - Fetal cells in maternal blood
  - Fetal DNA in maternal blood

Chorionic Villus Sampling (CVS)

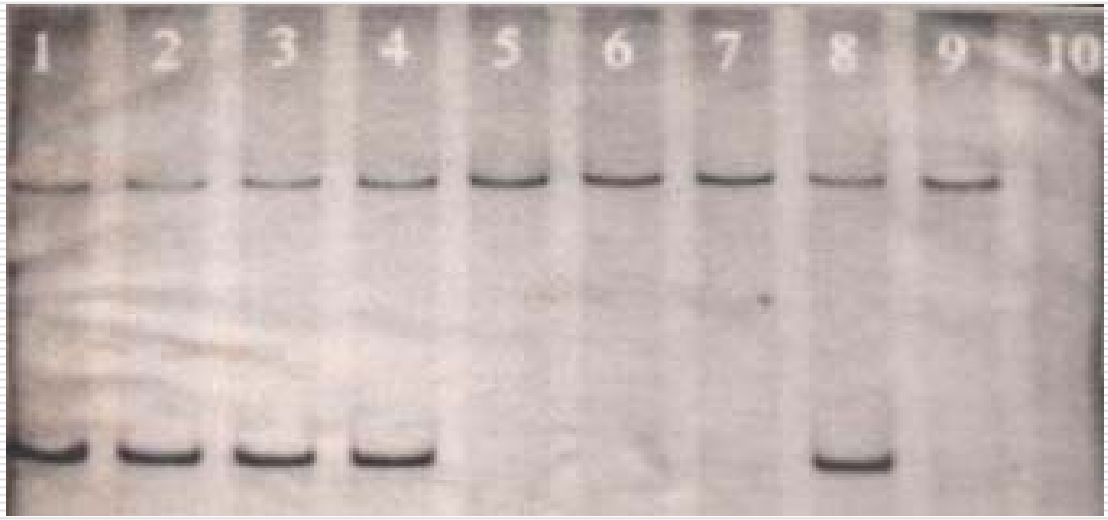




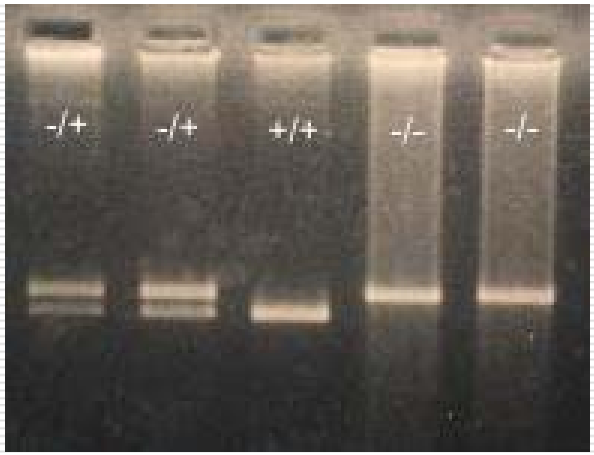


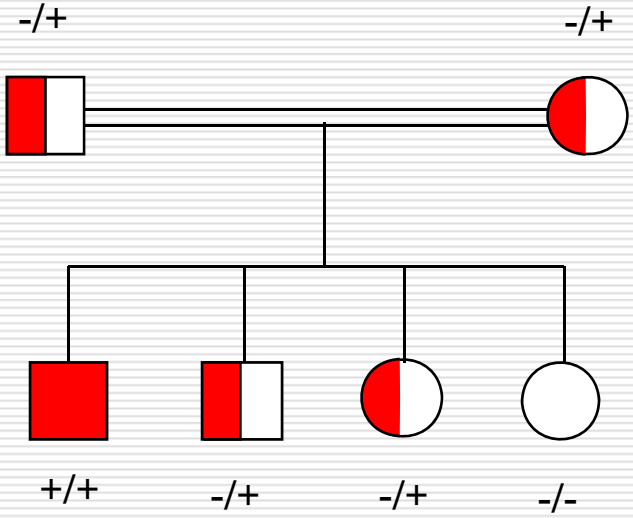
Prenatal Diagnosis by ARMS

- Mutation
  1. Father
  2. Mother
  3. CVS
  4. CVS
  5. -ve
- Normal
  6. CVS
  7. CVS
  8. +ve
  9. -ve
  10. Blank

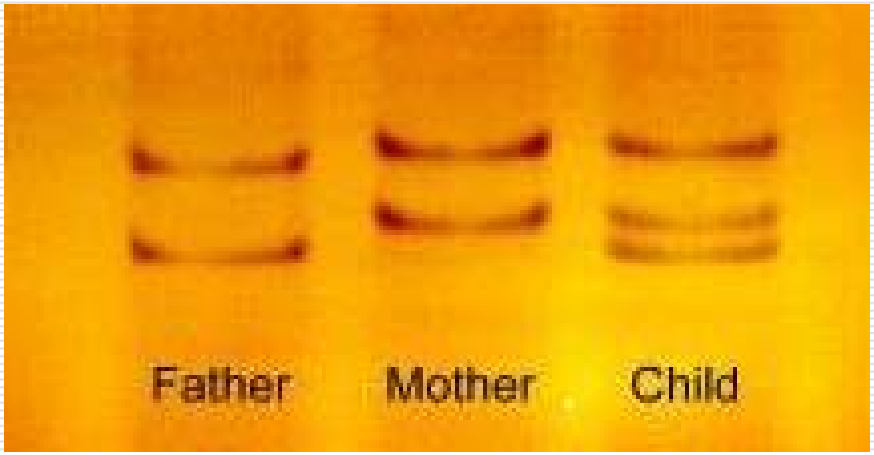


Prenatal Diagnosis by Linkage Analysis

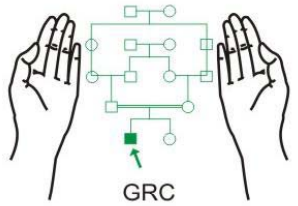




Prenatal Diagnosis
By Short Tandem Repeats (STR)

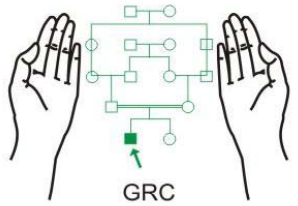


Misdiagnosis in PND (<0.5%)



- Maternal Contamination in Fetal Sample
- PCR Failure
- Clerical Mistakes
- Meiotic Crossover

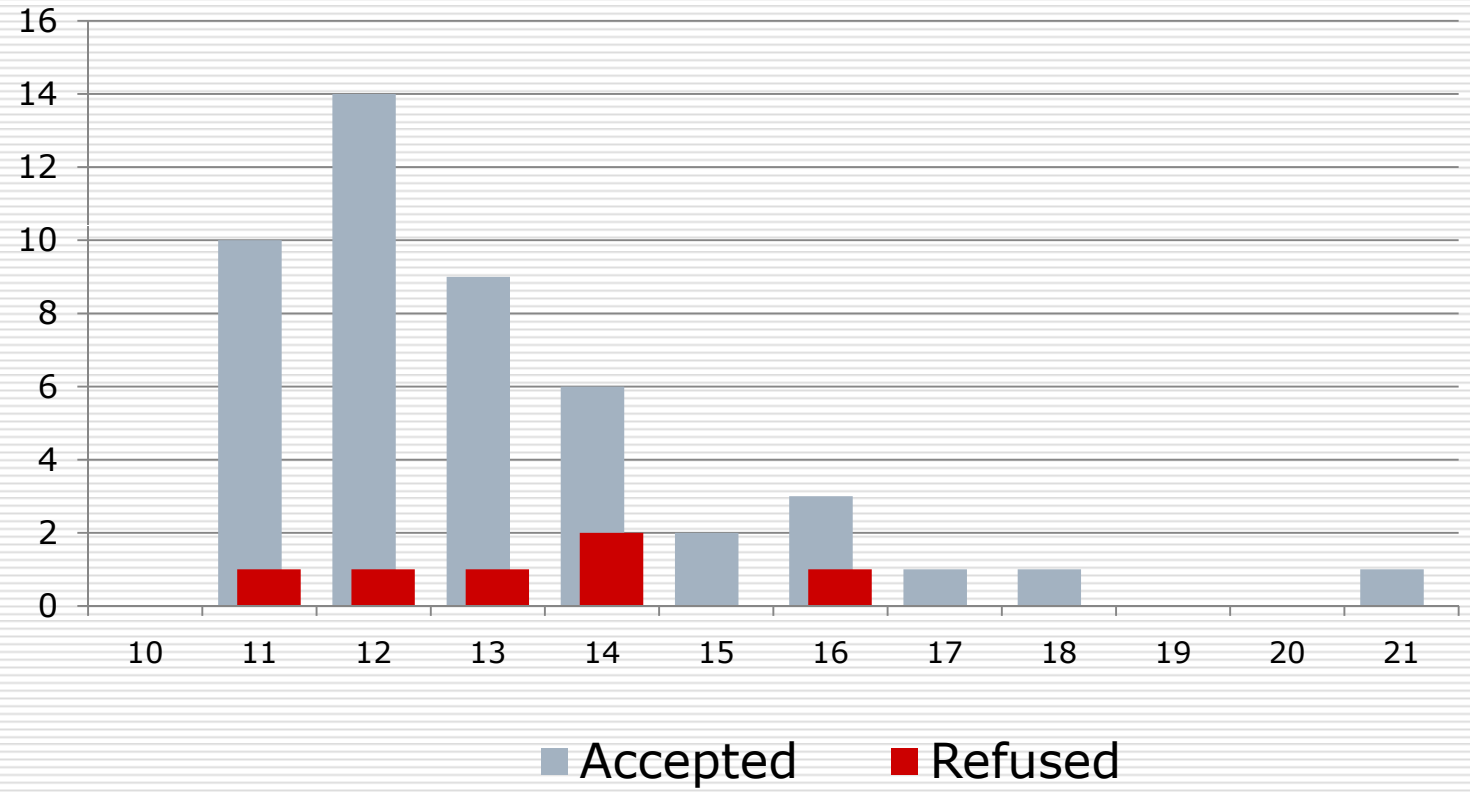
Prenatal Diagnosis



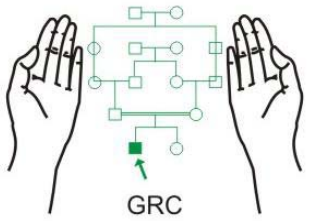
- Is the test required?
- CVS at 10-16 weeks gestation
- Average reporting time – one week
- Complications of CVS 1-2%
- Error rate – 0.5%

Termination of Pregnancy

Termination of Pregnancy



(S. Ahmed et al 2000)



Communicable diseases & Malnutrition

Genetic disorders

Present Health Priority

High infant mortality (90/1000)

Immunization

Primary Health Care

Nutrition

Fall in infant mortality (< 40/1000)

Genetic disorders

Commnicable diseases & Malnutrition

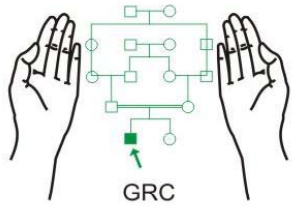
Future Health Priority

Treatment (very costly)

Prevention

(S. Ahmed 1998)

Prenatal Diagnosis at GRC



- **Chromosomal defects**
  - Trisomy 21 (Down syndrome)
  - Trisomy 13 and 18
  - Others
- **Single gene disorders**
  - Thalassaemia
  - Duchenne muscular dystrophy
  - Cystic fibrosis
  - Haemophilia
  - Others